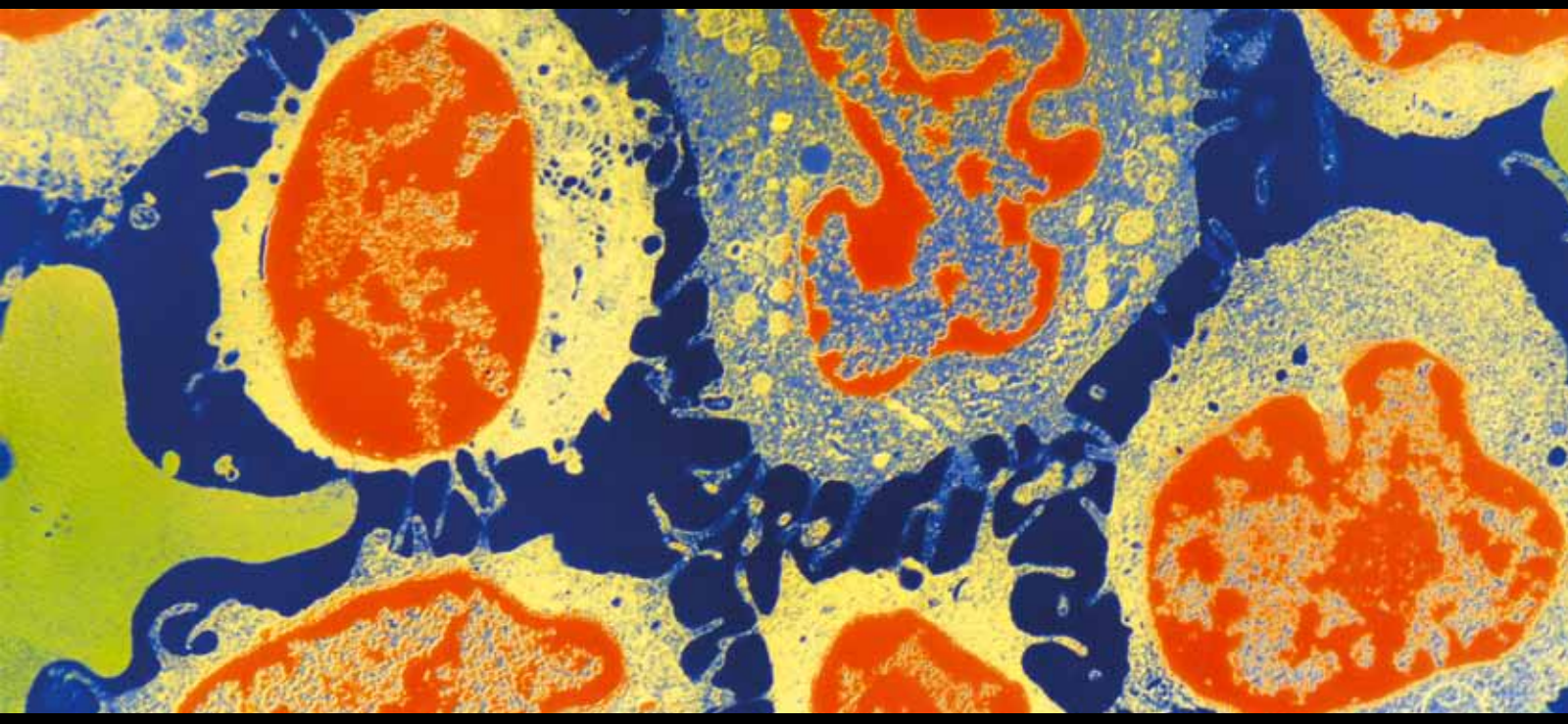
Journal of Oncology

Update on Anti-EGFR Targeted Therapy

Guest Editors: Daniel Chua, Peter Fasching, Brigette Ma, Sumitra Thongprasert, and Lori Wirth



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Editorial Board

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Editorial

Update on Anti-EGFR Targeted Therapy

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Targeted therapy has evolved recently as an important treatment modality for cancer, and the most extensively studied pathways for targeted therapy are those related to epidermal growth factor receptor (EGFR). EGFR is widely expressed in different tumor types and strong expression is associated with higher risk of recurrence/metastasis, poorer survival, and resistance to chemotherapy/radiotherapy. Both monoclonal antibody against EGFR and/or tyrosine kinase inhibitors are now in use clinically for treatment of advanced cancer. With the increasing usage of these agents and the availability of more new agents in the future, knowledge on anti-EGFR therapy is highly relevant to the current state of cancer therapy and to new research directions in the field of oncology.

The focus of this special issue is on the clinical applications of approved or investigational anti-EGFR therapy in solid tumors with emphasis on efficacy, toxicity, response, assessment, multimodality treatment, prognostic factors and predictive markers. The review by A. Harandi et al. provides a comprehensive and updated overview of the frequently used EGFR inhibitors and summarizes clinical efficacy data of these agents and their associated toxicity and management. This review clearly shows the impact of anti-EGFR therapy on cancer treatment since improved treatment results can be seen with the use of anti-EGFR therapy in many common cancers. One of these cancers is colorectal cancer and the approval of anti-EGFR monoclonal antibodies in the treatment of metastatic colorectal cancer has expanded the armamentarium against this disease. H. Loong et al. in their review summarize the historical progress and recent clinical developments of anti-EGFR therapies in the treatment of metastatic CRC and discuss the novel strategies of targeting the EGFR pathway to improve efficacy as well as ongoing research in identifying specific molecular predictors of response.

While the approved use of drugs like the dual kinase inhibitor Lapatinib represents significant advances in the clinical management of breast cancer, confirmatory studies must be considered to foster the use of anti-EGFR therapies including safety, pharmacokinetics, and clinical efficacy in this common cancer. The article by J. Flynn et al. reviews the mechanism of anti-EGFR therapy in breast cancer and summarizes recent advances including the development of improved high-throughput analyses for identifying novel anti-EGFR activity, as well as advances in DNA/RNA-microarray technology for classification purposes, which will contribute to the overall understanding and development of targeted therapy in treatment of breast cancer.

Cancers of the esophagus and stomach present a major health burden worldwide. The impact of cytotoxic agents on the disease has been modest. EGFR pathway has also been implicated in pathophysiology of esophageal and stomach cancers. Recently EGFR inhibitors have been explored in patients with esophageal and gastric cancers, and the results are summarized by T. Dragovich and C. Campen in their review. It also appears that tumors of the distal esophagus and gastroesophageal junction are more sensitive to EGFR blockade than distal gastric adenocarcinoma.

Similar to cytotoxic agenst, drug resistance remains an important issue and research field in targeted therapy. The article by J. Rolff et al. studied the impact of different resistance markers at protein and mRNA level in patient-derived nonsmall cell lung cancer xenografts in response to erlotinib and other cytotoxic agents. The results suggest

that the expression levels of multidrug resistant proteins and mRNA do not play an important role in the drug resistance of nonsmall cell lung cancer. Increased transforming growth factor-β (TGF-β) expression and EGFR amplification accompany the emergence of highly aggressive human carcinomas, and cooperative signaling between these two growth factor/receptor systems promotes cell migration and synthesis of stromal remodeling factors that in turn regulate tumor invasion, neo-angiogenesis and inflammation. R. Samarakoon et al. studied the role of plasminogen activator inhibitor-1 in carcinogensis and explored its potential role as a novel therapeutic target in combination with EGFR inhibition.

We hope that this special issue will stimulate interests and new researches in the development of anti-EGFR therapy in cancers, particularly the development of personalized medicine based on predictive biomarkers so that therapy can be tailored and optimized in every patient.

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Review Article

Clinical Efficacy and Toxicity of Anti-EGFR Therapy in Common Cancers

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Recommended by Daniel Chua

Epidermal growth factor receptor (EGFR) is a cell surface molecule and member of the ErbB family of receptor tyrosine kinases. Its activation leads to proliferation, antiapoptosis, and metastatic spread, making inhibition of this pathway a compelling target. In recent years, an increasing number of clinical trials in the management of solid malignancies have become available indicating the clinical efficacy of anti-EGFR monoclonal antibodies and oral small molecule tyrosine kinase inhibitors (TKIs). This review addresses frequently used EGFR inhibitors, summarizes clinical efficacy data of these new therapeutic agents, and discusses their associated toxicity and management.

1. Introduction

Epidermal growth factor receptor (EGFR), a member of the ErbB family of receptor tyrosine kinases, is a cell surface molecule whose activation leads to an intracellular signaling cascade affecting invasion, apoptosis, and angiogenesis [1, 2]. Members of the EFGR family receptors (erb1/EFGR, erb2/HER2, erb3/HER3, and erb4HER4) are composed of extracellular ligand binding domains. When ligands bind to these domains, receptor dimerization and autophosphorylation of intracellular tyrosine kinase domains occur. Autophosphorylation activates the downstream signaling pathways ras, raf, mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (Pl3K), Akt, and the signal transduction and activator of transcription (STAT) pathways. This downstream signaling leads to activation of cell growth, proliferation, and survival of cells [3, 4]. Binding of the EGFR by inhibitors leads to a disruption in proliferation resulting in apoptosis. Immunological effects, such as cell-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC), also contribute to their mechanism of action [5].

Drugs targeting EGFR in malignancies were initially developed in the 1980s, which lead to the development of anti-EGFR monoclonal antibodies and small molecule EGFR tyrosine kinase inhibitors (TKIs) [6–9]. EGFR is overexpressed in many solid tumors and this over expression correlates to advanced stage and a worse prognosis [10]. In the last few years, numerous clinical trials have proven the clinical efficacy of EGFR-targeted therapies in the management of several cancers, including breast, colon, pancreas, head and neck, renal, gastrointestinal stromal tumors (GISTs), and lung carcinomas. Since these agents are now commonly used, clinical presentation of associated toxicities and their management are important to recognize. Therefore, this review discusses commonly used EFGR inhibitors currently approved by the US Food and Drug Administration (FDA). A summary of clinical data in support of these agents and commonly encountered toxicities and management are discussed.

2. Anti-EGFR Agents Efficacy

2.1. Erlotinib. Erlotinib is an oral agent that reversibly binds to the intracellular tyrosine kinase domain of the HER1/EGFR thus blocking phosphorylation and inhibiting signal transduction [11]. Initially studied in nonsmall cell lung cancer (NSCLC), phase II data showed a response rate (RR) of 12% in patients previously treated with platinum-based chemotherapy [12, 13]. The National Cancer Institute

of Canada Clinical Trials Group (NCICCTG) then developed a phase III trial comparing erlotinib to placebo in patients with advanced NSCLC who had prior failure of first- or second-line chemotherapy. This study showed that erlotinib when compared to placebo had a higher overall (O)RR, median duration of response, progression-free survival (PFS), and overall survival (OS) (Table 1). There was also a greater reduction in cancer-related pain, cough, and dyspnea as well as improvement in physical function in those treated with erlotinib [14]. As a result, erlotinib is a useful treatment option presently utilized in the management of NSCLC. In another large phase III randomized trial of previously untreated advanced NSCLC, the combination of carboplatin and paclitaxel with or without erlotinib was evaluated. The results were not as favorable and showed no difference in ORR or OS [11]. EGFR gene mutations are being investigated as a predictor of efficacy with erlotinib in NSCLC. Recently presented at the American Society of Clinical Oncology (ASCO) Annual Meeting, a phase II trial of erlotinib in previously untreated NSCLC patients with mutations of the tyrosine kinase domain of EGFR was evaluated. In this trial, 37 of 297 tumors screened had mutations in the tyrosine kinase domain (25 with exon 19 deletion, 11 with L858R mutation). Responses occurred in 100% of exon 19 deletions and in 75% of those with the L858R mutation [15].

HER1/EGFRs are also overexpressed in pancreatic tumors conferring a worse prognosis. This led to an NCIC trial comparing gemcitabine in combination with erlotinib or placebo in patients with locally advanced or metastatic pancreatic adenocarcinomas. This trial showed a minimal but statistically significant increase in OS favoring the gemcitabine/erlotinib combination. Although statistically significant, the absolute increase in median survival was only 2 weeks [16].

2.2. Gefitinib. Gefitinib, an orally bioavailable EGFR TKI, was the first targeted drug to be approved for NSCLC. The Iressa Dose Evaluation in Adjuvant Lung Cancer (IDEAL1 and IDEAL2) trials were phase II nonrandomized studies investigating the efficacy of gefitinib monotherapy in NSCLC patients previously treated with a platinum agent [17, 18]. Based on objective responses, stable disease, and symptomatic improvement, gefitinib received accelerated approval by the FDA in 2003. In 2005, the Iressa Survival Evaluation in Lung Cancer (ISEL) trial, a phase III randomized study, evaluated gefitinib versus placebo in previously treated NSCLC [19]. Although there was a significantly higher response rate seen with gefitinib, the study did not show a significant difference in OS. As a result, the FDA restricted the use of gefitinib to patients enrolled in clinical trials or deriving benefit from ongoing treatment. Other randomized phase III trials, assessing gefitinib given concurrently with chemotherapy as well as gefitinib maintenance did not show improvements in OS [20–22]. Recently, the 33rd European Society for Medical Oncology Congress released results of a large-scale randomized phase III trial (IRESSA Pan-Asia study [IPASS]) [23]. In 1,217 patients, the study compared gefitinib monotherapy to carboplatin/paclitaxel (C/P) in chemonaive never- or light-exsmokers with advanced NSCLC with adenocarcinoma histology (Table 2). Gefitinib was superior in PFS, ORR, toxicity, and quality of life (QOL) compared to combination chemotherapy. However, OS and symptom improvement were similar between the two groups. PFS was longer for gefitinib than C/P in EGFR mutation positive patients and longer with C/P in mutation-negative patients.

2.3. Cetuximab. Cetuximab is a chimeric monoclonal IgG1 antibody that binds to the EGFR subsequently blocking phosphorylation of the receptor [24]. Cetuximab, initially approved for the treatment of metastatic colon cancer, has made a significant difference in the management of patients with this disease. A phase III trial comparing cetuximab monotherapy to best supportive care (BSC) showed improved OS and QOL in patients with colorectal cancer who had previously failed or had contraindications to fluoropyrimidine-, irinotecan-, and oxaliplatin-based therapies (Table 2) [25]. A subsequent randomized phase III trial compared cetuximab monotherapy to cetuximab plus irinotecan in refractory metastatic colorectal cancer (mCRC). This study was reserved for patients who had documented disease progression on a prestudy irinotecan regimen. The combination therapy arm showed significantly improved ORR, median time to progression (TTP), and disease control [26]. Another similar randomized phase III trial evaluated irinotecan monotherapy to cetuximab plus irinotecan in patients with mCRC previously failing oxaliplatin and/or a fluoropyrimidine who were irinotecan naïve. The combination therapy arm yielded improved ORR, PFS, and QOL, but similar OS to the cetuximab-only treated patients. This lack of OS difference may have been due to posttrial therapy since a large number of patients assigned to irinotecan eventually received cetuximab [27]. Most recently, the combination of irinotecan and 5-fluorouracil (FOLFIRI) with or without cetuximab in the first-line treatment of mCRC was evaluated. Cetuximab in combination with FOLFIRI significantly increased ORR and PFS [28].

The combination of EGFR and vascular endothelial growth factor-(VEGF-) targeted agents was also evaluated in a randomized phase III study of capecitabine/oxaliplatin (CapOx) plus bevacizumab with or without cetuximab in mCRC. Unfortunately, the combination chemotherapy, bevacizumab, and cetuximab resulted in a significant decrease in PFS compared to bevacizumab and CapOx alone with no difference in OS [29]. Therefore, it was concluded that cetuximab and bevacizumab should not be used concomitantly with chemotherapy.

At last year's ASCO annual meeting, data revealed that KRAS gene mutation conferred resistance to treatment with cetuximab and panitunimab. In contrast, "wild-type" or normal KRAS mutation status was found to be a predictive marker for cetuximab and panitumumab efficacy. In a retrospective analysis of 5-fluorouracil/leucovorin/oxaliplatin (FOLFOX) in combination with cetuximab, KRAS wild-type status was found to be associated with a significantly higher ORR and longer PFS when compared to mutant KRAS in EGFR-positive patients [29]. Similarly, when EGFR-positive patients with untreated mCRC were treated with FOLFIRI

Table 1: Selected clinical trials of erlotinib. *NSCLC,* Non-small cell lung cancer; *OS,* overall survival; *ORR,* overall response rate.

Malignancy	Regimen	Number of patients	Results	Comments
NSCLC	Erlotinib vs. placebo [14]	731 pts Stage IIIB/IV NSCLC after failure with first-line or second-line chemotherapy	Erlotinib: ORR (8.9%) OS (6.7 mo) Placebo: ORR (<1%) OS (4.7 mo)	Significant improvement in OS ($P < .001$)
NSCLC	Carboplatin, Paclitaxel +/− Erlotinib [11]	1059 pts Previously untreated stage IIIB/IV NSCLC	Erolotinib: ORR (21.5%) OS (10.6 mo) Placebo: ORR (19.3%) OS (10.5 mo)	No difference in ORR or OS with the combination of Erlotinib and chemotherapy
Pancreatic cancer	Gemcitibine, Erlotinib vs. placebo [15]	569 pts Unresectable, locally advanced or metastatic pancreatic cancer	Erlotinib: OS (6.2 mo) Placebo: OS (5.9 mo)	One year survival was greater with erlotinib plus gemcitabine (23% vs. 17%; $P = .023$)

Table 2: Selected clinical trials of gefitinib. *NSCLC,* Non-small cell lung cancer; *OS,* overall survival; *ORR,* overall response rate; C/P, carboplatin/paclitaxel; *PFS,* progression free survival; *EGFR,* epidermal growth factor receptor.

Malignancy	Regimen	Number of patients	Results	Comments
NSCLC	Gefitinib vs. placebo [19]	1692 pts Second-line or third-line treatment for patients with locally advanced or metastatic NSCLC	Gefitinib: OS (5.6 mo) Placebo: OS (5.1 mo)	No significant improvement in OS ($P = .087$) Subgroup analysis showed significantly longer survival in never-smokers and Asian patients
NSCLC	Gefitinib vs. Carboplatin/paclitaxel (C/P) [23]	1,217 pts Previously untreated stage IIIB/IV NSCLC, never- or light ex-smokers, adenocarcinoma histology	Gefitinib: ORR (43%) OS (18.6 mo) C/P: ORR (32%) OS (17.3 mo) $P = .0001$	No OS difference PFS longer for gefitinib than C/P in EFGR mutation positive patients ($P < .0001$) PFS longer with C/P in mutation negative patients ($P < .0001$)

with or without cetuximab, KRAS mutational status was predictive of response; wild-type KRAS was associated with improved ORR and prolonged PFS. Based on these studies as well as data with panitumumab, KRAS testing and verification of wild-type status are now required before treatment with these agents in colorectal cancer [29–31].

EGFR is also upregulated in squamous cell carcinoma (SCC) of the head and neck. The use of cetuximab to treat this disease has significantly benefited these patients. Initial phase II data revealed activity of single agent cetuximab in recurrent and/or metastatic SCC of the head and neck in those failing to respond to platinum-based therapy. As a single agent, the RR was 13% with a disease control rate of 46% [32]. The combination of cetuximab with chemotherapy has also been found to be effective. Platinum-based chemotherapy, fluorouracil, with or without cetuximab as first-line treatment of metastatic or recurrent SCC of the head and neck showed that the cetuximab combination yielded a higher ORR and OS [33]. A similar phase III trial was conducted addressing PFS with cisplatin monotherapy versus cisplatin with cetuximab in patients with recurrent and/or metastatic SCC of the head and neck. This included patients with documented progression during prior cisplatin therapy. This study did not show a significant difference in PFS or OS; however, there was a significant difference in RR favoring the cetuximab/cisplatin arm [34]. Cetuximab with radiotherapy versus radiotherapy alone was also studied in a separate phase III trial. The addition of cetuximab to radiotherapy significantly prolonged PFS, median OS, and duration of locoregional control in patients with locoregionally advanced head and neck cancer [35].

In pancreatic adenocarcinoma, trials with cetuximab have not shown significant clinical benefit. Cetuximab with gemcitabine or gemcitabine alone was evaluated in a large multi-institutional phase III trial in pancreatic adenocarcinoma. The addition of cetuximab did not significantly improve ORR, PFS, or OS [36–38].

At the plenary session of ASCO 2008, data was released on the treatment of NSCLC with cetuximab in combination with cisplatin/vinorelbine (CV) compared to CV alone. Only

patients with EGFR detectable by immunohistochemistry (IHC) were randomized. Cetuximab plus CV demonstrated an OS advantage. A modest survival benefit of one-to-two month(s) was seen depending on histology. This was the first trial to demonstrate an OS advantage of an EGFR-targeted agent in combination with platinum-based chemotherapy in NSCLC [39].

EGFR gene copy number detected by fluorescent in situ hybridization (FISH) has been shown to be useful in selecting NSCLC patients for treatment with cetuximab. Patients with advanced-stage NSCLC were enrolled into a phase II trial evaluating sequential or concurrent chemotherapy (carboplatin plus paclitaxel) with cetuximab. The ORR, disease control rate, PFS, and OS were significantly higher in the FISH-positive versus FISH-negative patients [40]. Further investigation on the accuracy of FISH-positive EGFR status is needed to evaluate its prognostic value in NSCLC.

2.4. Panitumumab. In contrast to cetuximab, panitumumab is the first fully human EGFR monoclonal antibody. It is an immunoglobulin (Ig)G2 antibody that binds to the extracellular portion of the EGFR thus inhibiting phosphorylation and activation of the intracellular kinases [41]. Efficacy of panitumumab has been evaluated in EGFR-expressing metastatic colorectal adenocarcinomas with disease progression following oxaliplatin, irinotecan, and fluropyrimidine-containing chemotherapy regimens. An initial phase II multicenter trial included patients with progressive mCRC treated with panitumumab monotherapy; patients were stratified into two groups based on EGFR staining intensity. As a single agent, panitumumab response and disease stabilization were seen irrespective of EGFR staining intensity [42]. This led to a phase III trial comparing panitumumab monotherapy to BSC alone. Efficacy was evaluated in patients with 1% or greater EGFR tumor staining by IHC and disease progression while on or within 6 months of the most recent chemotherapy. Panitumumab yielded a significant reduction in PFS when compared to BSC; however, there was no significant difference in OS (Table 3) [43]. Patients in the BSC arm were subsequently allowed to crossover to the panitumumab arm if disease progression was documented during the study. The crossover patient population yielded comparable results with prolonged PFS after panitumumab treatment [44]. This has led to the approval of panitumumab for treatment of EGFR-positive, metastatic colorectal carcinoma with disease progression following chemotherapy [41]. As mentioned earlier, wild-type KRAS is required for panitumumab efficacy in patients with mCRC [31].

The combination of EGFR- and VEGF-targeting antibodies was also found to lack benefit in the case of panitumumab. In the Panitumumab Advanced Colorectal Cancer Evaluation (PACCE) study, patients with untreated mCRC were randomized to FOLFOX or FOLFIRI based on investigator or patient choice. This combination was given with panitumumab plus bevacizumab or bevacizumab alone. The combination of FOLFOX/panitumumab/bevacizumab resulted in higher mortality compared to FOLFOX/bevacizumab alone. The primary endpoint of median PFS was also shorter in the panitumumab arm. Based on the results of the interim analysis, the study was stopped and panitumumab was discontinued in both the FOLFOX and FOLFIRI arms [45]. Similar to cetuximab, this trial with panitumumab argues against the combined use of these agents with bevacizumab in mCRC.

2.5. Sorafenib. Sorafenib is a novel multikinase inhibitor with antiangiogenic and proapoptotic activity targeting EGFR as well as multiple kinases including Raff/MAPK-ERK kinase, VEGFR-2, VEGFR-3, and PDGFR-β [39]. It is approved for use in the treatment of renal cell carcinoma (RCC) and hepatocellular carcinoma (HCC).

RCC is characterized by the loss of the von hippel landau (VHL) gene, which leads to dysregulation of the VEGFR, PDGFR-β, transforming growth factor-alpha (TGF-) α, EGFR, and Raf pathways promoting angiogenesis, lymphangiogenesis, tumor cell growth, and survival. Furthermore, RCC frequently displays EGFR immunoreactivity. Membranous and/or cytoplasmic EGFR immunostaining in RCC was present in 123 of 132 (93%) primary and 49 of 53 (92%) metastatic samples with extensive immunoreactivity present in 83% of primary and 74% of metastatic tumors [46].

In previously treated patients with metastatic RCC, the activity of sorafenib was demonstrated in two randomized trials. In the largest of these studies, a randomized phase III trial of metastatic cytokine refractory RCC, significant response and improvement in PFS was demonstrated (Table 4). At ASCO 2007, a final analysis of survival was presented. There was no statistically significant improvement in OS; survival benefit was likely obscured since one half of the patients originally assigned to placebo had switched to sorafenib [47].

EGFR is frequently expressed in human hepatoma cells; in fact, EGF is one of the mitogens required for the growth of hepatoma cells. At the ASCO meeting in 2007, data was released showing the efficacy of sorafenib in HCC. The Sorafenib HCC Assessment Randomized Protocol (SHARP) Trial was a large phase III double-blind placebo-controlled study evaluating the efficacy of sorafenib versus BSC in patients with advanced HCC who had not received previous chemotherapy. Patients receiving sorafenib had a three-month median survival benefit compared to placebo. Importantly, sorafenib was the first active treatment that has been proven to confer a survival benefit and to show promise as a standard treatment for advanced HCC [48]. In another phase III randomized trial of sorafenib in Asia-Pacific patients with HCC, results mirrored those of the SHARP trial. Despite Asia-Pacific patients having more advanced disease based on the Eastern Cooperative Oncology Group performance status (ECOG PS), a significant OS advantage with sorafenib was confirmed [49]. A North American phase II randomized trial of doxorubicin with sorafenib versus doxorubicin with placebo in 96 Child-Pugh A patients was published in abstract form and presented at the 2007 European Cancer Organization Conference (ECCO). Median TTP was two months longer in the combination arm, but did not reach

Table 3: Selected clinical trials of cetuximab. *EGFR*, epidermal growth factor receptor; *BSC*, best supportive care; *OS*, overall survival; *PFS*, progression free survival; *ORR*, overall response rate; *mCRC*, metastatic colorectal cancer; *FOLFIRI*, 5 Flourouracil/Folinic Acid and Irinotecan; *CapOx*, capecitabine/oxaliplatin; *SCC*, squamous cell carcinoma; IHC, Immunohistochemistry; *CR*, complete response; *PR*, partial response; *FISH*, fluorescent in situ hybridization.

Malignancy	Regimen	Number of patients	Results	Comments
mCRC	Cetuximab vs. BSC [17]	572 pts IHC EGFR+ mCRC Previously treated with chemotherapy	Cetuximab: PR (8%) SD (31.4%) BSC: PR (0%) SD (10.9%)	Cetuximab was associated with a significant improvement in OS ($P < .001$) Cetuximab: OS (6.1 mo), BSC: OS (4.6 mo)
mCRC	Cetuximab, Irinotecan vs. Cetuximab monotherapy [18]	329 pts mCRC with progression after Irinotecan-based chemotherapy	Cetuximab, Irinotecan: ORR (22.9%) Cetuximab: ORR (10.8%)	No difference in OS Median time to progression: Cetuximab, Irinotecan (4.1 mo), Cetuximab (1.5 mo)
mCRC	Cetuximab, Irinotecan vs. Irinotecan [19]	1298 pts EGFR+ mCRC	Cetuximab, Irinotecan: ORR (16.4%) PFS (4.0 mo) Cetuximab: ORR (4.2%) PFS (2.6 mo)	No significant difference in OS, but large number of pts receiving Irinotecan eventually got cetuximab
mCRC	FOLFIRI +/− Cetuximab [20]	1,217 pts EGFR+ mCRC First-line treatment	FOLFIRI + Cetuximab: PFS (8.9 mo) ORR (46.9%) FOLFIRI alone: PFS (8 mo) ORR (38.7%)	15% relative risk reduction of progression
mCRC	CapOx, bevacizumab +/− Cetuximab [21]	775 pts Previously untreated mCRC	CapOx, bevacizumab: ORR (40.6%) PFS (10.7 mo) Cetuximab arm: ORR (43.9%) PFS (9.8 mo)	Cetuximab combination was worse in PFS No difference in OS
mCRC	FOLFOX +/− Cetuximab [22]	337 pts 134 pts wild-type KRAS 99 pts mutant KRAS	Wild-type KRAS response with FOLFOX + Cetuximab (ORR 61%, PFS 7.7 mo) Mutant KRAS response with FOLFOX + Cetuximab (ORR 33%, PFS 5.5 mo)	Cetuximab only benefits patients with wild-type KRAS (HR 0.448, $P = .0009$)
mCRC	FOLFIRI +/− Cetuximab [23]	1,217 pts 348 pts wild-type KRAS 192 pts mutant KRAS	Wild-type KRAS response with FOLFIRI + Cetuximab (ORR 59%, PFS 9.9 mo) Mutant KRAS response with FOLFIRI + Cetuximab (ORR 36%, PFS 7.6 mo)	Cetuximab only benefits patients with wild-type KRAS and reduced risk for disease progression by 32% ($P = .017$)

Table 3: Continued.

Malignancy	Regimen	Number of patients	Results	Comments
SCC of the Head and Neck	Platinum (cisplatin or carboplatin), fluorouracil +/− Cetuximab [25]	442 pts Untreated recurrent or metastatic SCC of the head and neck	Platinum, fluorouracil, Cetuximab: ORR (36%) PFS (5.6 mo) Platinum, fluorouracil: ORR (20%) PFS (3.3 mo)	Median OS was significantly improved in the Cetuximab arm (10.1 mo vs. 7.4 mo), $P = .04$
SCC of the Head and Neck	Cisplatin, Cetuximab vs. Cisplatin [26]	117 pts Recurrent/metastatic SCC of the head and neck	Cisplatin, Cetuximab: ORR (26%) Cisplatin ORR (10%)	No significant improvement in OS or PFS Enhanced response for patients with EGFR staining less than 80% by IHC
SCC of the Head and Neck	Radiation, Cetuximab vs. Radiation alone [27]	424 pts Locoregionally advanced SCC of the head and neck	Radiation, Cetuximab: PFS (17.1 mo) OS (49 mo) Radiation alone: PFS (12.4 mo) OS (29.3 mo)	OS benefit favoring Cetuximab arm ($P = .03$) Incidence in grade 3 or higher side effects, including mucositis, did not differ significantly between the groups
Pancreatic cancer	Cetuximab, Gemcitabine vs. Gemcitabine alone [30]	735 pts	Cetuximab, Gemcitabine: ORR (14%) PFS (3.5 mo) OS (6.4 mo) Gemcitabine alone: ORR (12%) PFS (3 mo) OS (5.9 mo)	The addition of Cetuximab did not significantly improve ORR, PFS, or OS
NSCLC	Cisplatin, Vinorelbine +/− Cetuximab [31]	1,125 pts Only pts with EGFR detected by IHC were randomized	Cisplatin, Vinorelbine, Cetuximab: Median OS (11.3 mo) Cisplatin, Vinorelbine: Median OS (10.1 mo)	OS significantly improved in Cetuximab arm ($P = .04$)
NSCLC	Sequential or concurrent carboplatin and paclitaxel with cetuximab [32]	229 pts EGFR by FISH assessable in 76 pts (positive in 59%)	FISH-positive: CR/PR (81%) Median PFS (6 mo) FISH-negative: CR/PR (55%) Median PFS (3 mo)	Median OS superior in FISH-positive (15 mo vs. 7 mo), $P = .04$

statistical significance. However, OS was significantly longer for the combination of sorafenib/doxorubicin compared to the doxorubicin only arm (13.7 vs. 6.5 mo) [50].

Sorafenib has also recently demonstrated significant activity in the treatment of iodine-refractory thyroid carcinoma. In a phase II trial of 30 subjects, most of the patients (80%) showed a clinical benefit from this agent. Ninety-five percent of individuals with available thyroglobulin levels showed a rapid response in thyroglobulin levels with a mean decrease of 70%. These results represent a significant advance in both response and PFS over studies in the past utilizing chemotherapy [51].

2.6. Sunitinib. Like sorafenib, sunitinib is an oral small molecule TKI that inhibits cellular signaling by targeting EGFR, VEGFR, PDGFR-β, fetal liver tyrosine kinase receptor (FLT-3), and c-Kit, a stem cell factor receptor [52]. This ultimately targets both angiogenesis and tumor cell proliferation causing tumor shrinkage and cell death. Sunitinib is currently approved for the treatment of RCC as well

Table 4: Selected clinical trials of panitumumab. *mCRC,* metastatic colorectal cancer; *BSC,* best supportive care; *OS,* overall survival; *PFS,* progression free survival; *ORR,* overall response rate; *SD,* stable disease; *FOLFOX,* 5 Flourouracil/Folinic Acid and oxaliplatin; *FOLFIRI,* 5 Flourouracil/Folinic Acid and Irinotecan.

Malignancy	Regimen	Number of patients	Results	Comments
mCRC	Panitumumab vs. BSC [35]	463 pts Pts with progression after standard chemotherapy	Panitumumab: ORR (10%) PFS (13.8 weeks) BSC: ORR (0%) PFS (8.5 weeks)	No significant improvement in OS
mCRC	Panitumumab monotherapy after disease progression with BSC [36]	176 pts Pts with progression of disease in BSC arm of Panitumumab vs. BSC trial [35]	Panitumumab: ORR (11.6%) SD (33%) Median PFS of 9.4 weeks	Results comparable to initial study
mCRC	FOLFOX or FOLFIRI with Bevacizumab +/− Panitumumab [37]	823 pts	FOLFOX, Bevacizumab, Panitumumab: Median PFS (9.5 mo) OS (19.3 mo) FOLFOX, Bevacizumab: Median PFS (11 mo) OS (20.6 mo)	Panitumumab in combination with FOLFOX and bevacizumab was associated with a shorter PFS and increased toxicity

as gastrointestinal stromal tumors GISTs. Like RCC, EGFR expression in GISTs had been validated in a recent article in which tissue microarray samples of 33 GISTs were surveyed by IHC. EGFR expression was identified in 8 of those samples [53].

The antitumor activity of sunitinib was initially shown in two phase II trials of metastatic RCC patients who had failed previous cytokine therapy [54, 55]. This led to a large phase III trial comparing sunitinib to interferon-alpha (IFN-α) as first-line therapy (Table 5). Sunitinib showed superior activity in ORR and in PFS, including patients with good, intermediate, and poor risk features. Furthermore, OS was significantly longer in the sunitinib arm, despite significant patient crossover from the IFN-α arm to sunitinib [56]. Sunitinib was also found to be superior in QOL compared to IFN-α [57].

Sunitinib has also shown significant activity in metastatic and/or unresectable GIST following imatinib failure. In a phase III randomized trial comparing sunitinib to placebo in imatinib refractory GIST patients, time to tumor progression and PFS was 4-fold longer in patients on sunitinib compared to placebo; partial response (PR) and stable disease (SD) were also significantly longer in the sunitinib arm. Patients in the placebo arm were subsequently allowed to crossover to the Sunitinib arm if disease progression was documented during the study. The crossover patient population yielded comparable results. Despite the crossover, OS favored patients initially treated with sunitinib [58].

2.7. Lapatinib. Activation and overexpression of oncogenes encoding trans-membrane receptor tyrosine kinases of the EGFR family, including EGFR (ErbB1) and HER2/neu (ErbB2), play an important role in the development of breast cancer [58]. Lapatinib is an orally active 4-anilinoquinazoline TKI of both HER2/neu (ErbB2) and EGFR (ErbB1). It inhibits the autophosphorylation sites on the receptors, thereby blocking the downstream signaling pathways of HER2 and EGFR.

Lapatinib has shown activity for the treatment of advanced HER2/neu positive metastatic breast cancer refractory to trastuzumab (Table 6). Unlike trastuzumab, lapatinib seems to have activity against brain metastases [59–61]. Primary and secondary resistances have been seen in patients with HER2-positive breast cancers who had been treated with trastuzumab both in the metastatic and adjuvant settings [53, 62–65]. Potential mechanisms of resistance may be related to signaling through other receptors such as EGFR or IGFR-1 [66]. Lapatinib, being a small molecule TKI, interacts with intercellular domains and does not require full receptor activity. In a phase III study of HER2-positive advanced or metastatic breast cancer refractory to anthracyclines, taxanes, and trastuzumab, lapatinib plus capecitabine showed a significant advantage over capecitabine monotherapy with respect to ORR and PFS with a nonsignificant trend toward longer OS [67]. In another phase III randomized trial, the combination of paclitaxel with lapatinib compared to paclitaxel monotherapy in patients with HER2-positive cancer was evaluated. Patients in this trial were not treated with prior trastuzumab. The study showed a statistically significant advantage in ORR and TTP, but not in OS. Enrollment for this study came from countries with limited HER2 testing. Only 91 of 580 patients were HER2-positive on central testing, with retrospective analysis revealing benefit limited to FISH-positive or IHC 3+ tumors [68].

Table 5: Selected clinical trials of sorafenib. *PFS*, progression free survival; *OS,* overall survival; *PR*, partial response; *CR*, complete response; *HCC*, hepatocellular carcinoma; *RCC*, renal cell carcinoma; *TBRR,* Tumor burden reduction rate.

Malignancy	Regimen	Number of patients	Response rate	Comments
RCC	Sorafenib vs. placebo [41]	903 Resistant to standard therapy	Sorafenib: Median PFS (5.5 mo) PR (10%) Placebo: Median PFS (2.8 mo) $P < 0.01$ PR (2%)	The OS showed reduced risk of death compared with placebo but the results were not statistically significant
HCC	Sorafenib vs. placebo [42]	602 pts No previous therapy	Sorafenib: Median OS (10.7 mo) Placebo: Median OS (7.9 mo)	The median OS was significantly longer in patients who received Sorafenib HR (0.69), $P = .0006$
Metastatic thyroid carcinoma	Sorafenib monotherapy [45]	36 pts Metastatic, iodine-refractory thyroid carcinoma	PR in 7 pts (21%) SD in 20 pts (59%)	Significant anti-tumor activity with overall clinical benefit rate (PR + SD) of 80%

Table 6: Selected clinical trials of sunitinib. *GIST,* gastrointestinal stromal tumor; *PFS*, progression free survival; *OS,* overall survival; *PR*, partial response; *CR*, complete response; *TTP,* time to progression; *m RCC*, metastatic renal cell carcinoma; *QOL,* quality of life.

Malignancy	Regimen	Number of patients	Response rate	Comments
RCC	Interferon vs. Sunitinib [48]	750 pts Previously untreated mRCC	Sunitinib: Median PFS (11 mo) ORR (31%) OS (26.4 mo), $P = 0.051$ Interferon: Median PFS (5 mo) ORR (6%) OS (21.8 mo)	Sunitinib provides superior QOL compared with IFN-α in mRCC patients.
GIST	Sunitinib vs. placebo [50]	312 pts After progression or intolerance to imatinib	Sunitinib: TTP(6.3 mo) Placebo: TTP(1.5 month)	Sunitinib significantly improved TTP with a 67% reduced risk of progression.

3. Anti-EGFR Agent-Associated Toxicity

EGFR is expressed on nearly all normal cells, particularly those of epithelial origin such as skin, liver, and gastrointestinal tract, but not on hematopoietic cells [69]. As a consequence, the most commonly encountered toxic effects from these agents are rash and diarrhea. Along with other toxicities, recognition and management of associated adverse effects with anti-EGFR agents will result in improved clinical outcomes, patient compliance, and QOL.

3.1. Skin Toxicity. When EGF was initially discovered, it was named for its ability to increase growth and keratinization of skin epithelium [69]. EGFR was found to be expressed in the human skin within keratinocytes, the follicular epithelium, sweat and sebaceous glands, and in capillaries of the dermis [70–73]. For this reason, the most common toxicity of EGFR-targeted agents involves the skin and adnexal structures resulting in a rash and less commonly nail toxicity.

EGFR is expressed on hair follicles and sebaceous glands and the binding of this receptor by inhibitors leads to a disruption in proliferation, resulting in an immunological reaction with skin inflammation, folliculitis, and rash [72, 73]. The most commonly seen skin reaction with EFGR inhibitors is a follicular acneiform eruption, also termed acne-like rash or folliculitis. EGFR-associated rash differs from acne in that there are no comedones or blackheads. The incidence of an acneiform-like skin rash has been reported to occur in about 85% of cetuximab-treated patients [74, 75]. Symptoms typically appear within two weeks after starting treatment. This is mainly located on the face (nose, cheeks, nasolabial folds, chin, forehead, and in a perioral distribution). Other locations include the shoulders and upper part of the back and chest. The rash tends to improve over time even with continued use and does resolve fully after cessation of therapy. In 35% of patients, dry itchy skin of the arms and legs can occur, which can potentially become secondarily infected by *Staphylococcus aureus* or *Herpes simplex* infection [76].

There have been numerous studies showing a direct correlation between the severity of a rash with response and OS. In fact, the greatest benefit in survival of cetuximab-treated patients is seen in those with a grade 3 rash [27, 75, 76]. The degree of skin toxicity has been classified by

Table 7: Selected clinical trials of lapatinib. *MBC,* metastatic breast cancer; *PFS*, progression free survival; *OS,* overall survival; *TTP,* time to progression.

Malignancy	Regimen	Number of patients	Response rate	Comments
MBC	Capecitibine +/− Lapatinib [59]	324 pts HER2-positive MBC that had progressed with chemotherapy (anthracycline, a taxane, and trastuzumab)	Capecitabine, Lapatinib: TTP (6.2 mo) ORR (24%) Capecitabine monotherapy: TTP (4.3 mo) ORR (14%)	Non-significant trend toward improved OS favoring lapatinib Fewer pts in the lapatinib arm developed brain metastases as the first site of progression (13 vs. 4%)
MBC	Lapatinib, Paclitaxel vs. Paclitaxel monotherapy [60]	580 pts 55% received prior chemotherapy or hormonal therapy No pts received prior traztuzumab	Lapatinib, Paclitaxel: ORR (60%) Median TTP (8 mo) Paclitaxel monotherapy: ORR (36%) Median TTP (6 mo)	Improved clinical outcome was seen with the combination without a significant change in side effect profile No difference in OS, but majority of pts were not properly tested for HER2

Table 8: Management of anti-EGFR-associated rash and common terminology criteria for adverse events v3.0 (CTCAE), National Cancer Institute.

CTC Grade	Rash	Management
1	Macular or papular eruption or erythema Asymptomatic	Topical antibiotic agents (metronidazole, erythromycin, and clindamycin lotion) Corticosteroid cream if an extensive inflammatory component exists
2	Macular or papular eruption or erythema Symptomatic covering <50% of body	Anti-inflammatory oral antibiotics (minocycline or doxycycline) Corticosteroid cream if an extensive inflammatory component exists
3	Macular or papular eruption or erythema Symptomatic covering >50% of body	Anti-inflammatory oral antibiotics (minocycline or doxycycline) Oral corticosteroids EGFR therapy should be held until the acute inflammatory phase has resolved
4	Generalized exfoliative, ulcerative, or bullouis dermatitis	Anti-inflammatory oral antibiotics (minocycline or doxycycline) Oral corticosteroids (Medrol-dose pack) EGFR therapy should be held until the acute inflammatory phase has resolved

The National Cancer Institute Common Toxicity Criteria version 3.0 (Table 7). Prospective trials are needed to further investigate the correlation between anti-EGFR therapy and rash to elucidate the validity and clinical implications of this association.

Recommendations in the management of EGFR-associated rash have been limited by the lack of clinical trials evaluating rash therapies (Table 8). This has led to treatment recommendations based on the clinical experience of dermatologists and oncologists familiar with EGFR-associated rash. In general, preventive measures are essential and include avoidance of soaps, limiting shower time, use of lukewarm water, and liberal use of skin moisturizers and emollients. Beneficial topical treatment approaches for a grade 1 rash include the use of antibiotic agents (metronidazole, erythromycin, and clindamycin lotion) as well as local corticosteroid cream if an extensive inflammatory component is present. For grade 2 reactions, anti-inflammatory oral antibiotics (minocycline or doxycycline) should be used since secondary infections are common. In the event of a grade 3 rash, oral corticosteroids and antibiotics should be utilized and EGFR-targeted therapy should be held until the acute inflammatory phase has resolved [77, 78].

Rash and hand-foot syndrome, characterized by redness, ulceration, and dysesthesia of the palms and soles, are the most common adverse events associated with sorafenib and sunitinib. Hand-foot syndrome associated with these agents occurs in 20–30% of patients, with less than 10% experiencing grade 3 or higher toxicity. It rapidly resolves with drug discontinuation and topical emollients and moisturizers are used to prevent and diminish toxicity. Other associated adverse events seen with sorafenib and sunitinib include diarrhea, hypertension, fatigue, and hematologic cytopenias [79, 80].

Anti-EGFR therapy can also result in nail toxicity, which can occur in 10–15% of patients after 4–8 weeks of therapy. It can progress into a paronychia like cracking reaction, a painful and difficult to treat side effect [74]. Some individuals require several months for complete healing after cessation of therapy [71]. Hair disorders are also commonly seen with these agents. Since EGFR signaling plays a vital role in the initiation of hair growth, interruption of EGFR signaling can result in disorganized hair follicles leading to follicular necrosis and alopecia [81, 82].

Nimotuzumab, which is marketed under the name of BIOMAb$_{\text{EGFR}}$, is a recombinant humanized IgG1 monoclonal antibody targeting EGFR. It has been approved for SCC of the head and neck and glioma in a number of countries. In the clinical trials thus far, nimotuzumab has not been associated with skin toxicity. Further clinical trials in different cancers are currently ongoing [83].

3.2. Gastrointestinal Toxicity. The use of oral anti-EGFR TKIs has been found to be strongly associated with gastrointestinal toxicities including diarrhea and hepatotoxicity. The pathophysiology of anti-EGFR-induced diarrhea is thought to result from excessive chloride secretion inducing a secretory diarrhea [84]. In large randomized trials, oral TKIs erlotinib and lapatinib have been found to cause diarrhea in 40–60% of patients with approximately 10% experiencing grade 3 or 4 toxicity [11, 14]. The reported incidence of diarrhea associated with sorafenib and sunitinib has been 20–40% [79, 81]. Diarrhea induced by oral TKIs can be managed by lowering the dose and rarely involves treatment interruption. Loperamide is a useful therapy decreasing intestinal motility.

Hepatic toxicity with asymptomatic elevations of transaminases and hyperbilirubinemia is commonly associated with oral TKIs. The mechanism of action is thought to be direct targeting of hepatocytes that overexpress EGFR with potential induction of chronic hepatitis with active necrosis. The overall incidence of hepatotoxicity with oral anti-EGFR TKIs has been reported at around 10% (2% grade 2 or 3) [11, 14, 79, 80]. These compounds can be continued with mild hyperbilirubinemia and should be discontinued with grade 3 or 4 toxicity. Concurrent TKIs and hepatotoxic drugs should be used with caution.

3.3. Pulmonary Toxicity. Interstitial lung disease related to gefitinib therapy has been well reported, with a worldwide incidence estimated at 1% [85, 86]. In a small series from Japan, the incidence of ILD in 112 patients receiving gefitinib was estimated at 5.4% [87]. Four deaths occurred, all being in current or former smokers, with pre-existing pulmonary fibrosis being a significant risk factor. The adverse pulmonary effects of erlotinib are less well known, but cases of fatal ILD have been reported [88, 89]. A phase 3b trial has been initiated to examine the efficacy and safety of erlotinib in advanced NSCLC with disease progression after chemotherapy. From a total of 229 patients, one (0.4%) interstitial lung disease-like event was reported [90].

3.4. Cardiac Toxicity. Cardiac toxicity with anti-EGFR TKIs has been reported. It is clear that cardiotoxicity with TKIs is not a "class effect," since it does not occur with all known agents. Sunitinib caused a decline in left ventricular ejection fraction (LVEF) below 50% in 11% of the patients [91]. In a phase III trial comparing sunitinib to IFN-α, 10% of patients had declines in LVEF after a median duration treatment of 6 months [56]. Sorafenib has induced acute coronary syndromes, including myocardial infarction. In the RCC study comparing sorafenib to placebo, 2.9% of sorafenib-treated patients had a myocardial infarction, compared to 0.4% in placebo-treated patients [47]. The cardiac toxicity of lapatinib was analyzed in 3.558 patients treated in 18 phase I–III clinical trials; 598 received prior anthracyclines and 759 had been given trastuzumab in the past [92]. Lapatinib was associated with a decline in LVEF in 1.6% of patients (58 of 3,558). The mean LVEF decrease was 18.7% and was mostly asymptomatic (1.4% asymptomatic and 0.2% symptomatic). Of the seven with symptomatic LVEF decrease, cardiotoxicity resolved in all but one patient.

3.5. Allergic Reactions. Allergic and anaphylactoid reactions are associated with cetuximab and, less often, panitumumab administration [26, 75]. Severe reactions are observed in approximately 3% of patients following cetuximab administration, with a fatal outcome in 0.1% of patients [93, 94]. Up to 90% of severe reactions associated with cetuximab occur within the first few minutes of the first dose [95]. The decision to rechallenge or discontinue treatment after a reaction occurs depends on the severity of the reaction. In the case of anaphylactic reactions, further therapy with cetuximab is contraindicated. Mild-to-moderate hypersensitivity reactions can be managed by temporary infusion interruption and resuming at a slower infusion rate. Management of severe reactions must include immediate interruption and treatment with epinephrine. Corticosteroids, antihistamines, bronchodilators, and oxygen also might be required.

Hypersentivity reactions to cetuximab might correlate with the development of specific antihuman IgE antibodies [96]. On the contrary, no antihuman antibodies have been detected with panitumumab. The incidence of hypersensitivity reactions with panitumumab in multiple trials has been approximately 3%, with severe reactions accounting for approximately 1% [97]. The successful use of panitumumab after severe hypersensitivity reactions to cetuximab has been reported, but requires further investigation [98].

4. Conclusions

Therapeutic agents in clinical practice targeting the EGFR pathway have made great advances in the treatment of malignancy. EGFR activation is associated with proliferation, antiapoptosis, and metastatic spread, making this pathway a compelling target. Numerous large clinical trials have shown clinical evidence of anticancer activity with these new agents resulting in improved tumor response and patients' survival. Several other anti-EGFR agents are in development, giving hope to future advances in therapy. An awareness and proper management of associated toxicities can increase patient compliance, QOL, and overall treatment success. Ongoing and future research will expand the applications of anti-EGFR therapy, elucidate optimal combinations and sequences, discover pathways of resistance, and continue to benefit cancer patients.

References

[1] M. B. Sporn and G. J. Todaro, "Autocrine secretion and malignant transformation of cells," *The New England Journal of Medicine*, vol. 303, no. 15, pp. 878–880, 1980.

[2] D. S. Salomon, R. Brandt, F. Ciardiello, and N. Normanno, "Epidermal growth factor-related peptides and their receptors in human malignancies," *Critical Reviews in Oncology/Hematology*, vol. 19, no. 3, pp. 183–232, 1995.

[3] A. Citri and Y. Yarden, "EGF-ERBB randomize: towards the systems level," *Nature Reviews Molecular Cell Biology*, vol. 7, no. 7, pp. 505–516, 2006.

[4] N. E. Hynes and H. A. Lane, "ERBB receptors and cancer: the complexity of targeted inhibitors," *Nature Reviews Cancer*, vol. 5, no. 5, pp. 341–354, 2005.

[5] M. B. Khazaeli, A. F. LoBuglio, J. W. Falcey, et al., "Low immunogenicity of a chimeric monoclonal antibody (MoAB), IMC-C225, used to treat epidermal growth factor receptor-positive tumors," in *Proceedings of the Annual Meeting of the American Society of Clinical Oncology*, New Orleans, La, USA, May 2000, abstract no. 207a.

[6] N. Normanno, C. Bianco, A. De Luca, M. R. Maiello, and D. S. Salomon, "Target-based agents against ErbB receptors and their ligands: a novel approach to cancer treatment," *Endocrine-Related Cancer*, vol. 10, no. 1, pp. 1–21, 2003.

[7] S. V. Sharma, D. W. Bell, J. Settleman, and D. A. Haber, "Epidermal growth factor receptor mutations in lung cancer," *Nature Reviews Cancer*, vol. 7, no. 3, pp. 169–181, 2007.

[8] R. Zandi, A. B. Larsen, P. Andersen, M.-T. Stockhausen, and H. S. Poulsen, "Mechanisms for oncogenic activation of the epidermal growth factor receptor," *Cellular Signalling*, vol. 19, no. 10, pp. 2013–2023, 2007.

[9] H. Masui, T. Kawamoto, J. D. Sato, B. Wolf, G. Sato, and J. Mendelsohn, "Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies," *Cancer Research*, vol. 44, no. 3, pp. 1002–1007, 1984.

[10] J. Mendelsohn, "Targeting the epidermal growth factor receptor for cancer therapy," *Journal of Clinical Oncology*, vol. 20, supplement 18, pp. 1S–13S, 2002.

[11] R. S. Herbst, D. Prager, R. Hermann, et al., "TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer," *Journal of Clinical Oncology*, vol. 23, no. 25, pp. 5892–5899, 2005.

[12] M. Hidalgo, L. L. Siu, J. Nemunaitis, et al., "Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies," *Journal of Clinical Oncology*, vol. 19, no. 13, pp. 3267–3279, 2001.

[13] R. Pérez-Soler, "Phase II clinical trial data with the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib (OSI-774) in non-small-cell lung cancer," *Clinical Lung Cancer*, vol. 6, supplement 1, pp. S20–S23, 2004.

[14] F. A. Shepherd, J. Rodrigues Pereira, T. Ciuleanu, et al., "Erlotinib in previously treated non-small-cell lung cancer," *The New England Journal of Medicine*, vol. 353, no. 2, pp. 123–132, 2005.

[15] L. Paz-Ares, J. M. Sanchez, A. García-Velasco, et al., "A prospective phase II trial of erlotinib in advanced non-small cell lung cancer (NSCLC) patients (p) with mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR)," *Journal of Clinical Oncology*, vol. 24, supplement 18, 2006.

[16] M. J. Moore, D. Goldstein, J. Hamm, et al., "Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group," *Journal of Clinical Oncology*, vol. 25, no. 15, pp. 1960–1966, 2007.

[17] M. Fukuoka, S. Yano, G. Giaccone, et al., "Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer," *Journal of Clinical Oncology*, vol. 21, no. 12, pp. 2237–2246, 2003.

[18] M. G. Kris, R. B. Natale, R. S. Herbst, et al., "Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial," *Journal of the American Medical Association*, vol. 290, no. 16, pp. 2149–2158, 2003.

[19] N. Thatcher, A. Chang, P. Parikh, et al., "Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer)," *The Lancet*, vol. 366, no. 9496, pp. 1527–1537, 2005.

[20] G. Giaccone, R. S. Herbst, C. Manegold, et al., "Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 1," *Journal of Clinical Oncology*, vol. 22, no. 5, pp. 777–784, 2004.

[21] R. S. Herbst, G. Giaccone, J. H. Schiller, et al., "Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 2," *Journal of Clinical Oncology*, vol. 22, no. 5, pp. 785–794, 2004.

[22] K. Kelly, L. Gaspar, K. Chansky, K. S. Albain, J. Crowley, and D. R. Gandara, "Low incidence of pneumonitis on SWOG 0023: a preliminary analysis of an ongoing phase III trial of concurrent chemoradiotherapy followed by consolidation docetaxel and Iressa/placebo maintenance in patients with inoperable stage III non-small cell lung cancer," in *Proceedings of the Annual Meeting of the American Society of Clinical Oncology*, Orlando, Fla, USA, May 2005, abstract no. 7058.

[23] T. S. Mok, S. Leong, X. Liu, et al., "Gefitinib vs carboplatin/paclitaxel in clinically selected chemonaive patients with advanced non-small cell lung cancer in Asia (ipass): randomized, open-label, phase III study," in *Proceedings of*

the 33rd European Society for Medical Oncology (ESMO '08), Stockholm, Sweden, September 2008.

[24] F. Ciardiello and G. Tortora, "EGFR antagonists in cancer treatment," *The New England Journal of Medicine*, vol. 358, no. 11, pp. 1160–1174, 2008.

[25] D. J. Jonker, C. J. O'Callaghan, C. S. Karapetis, et al., "Cetuximab for the treatment of colorectal cancer," *The New England Journal of Medicine*, vol. 357, no. 20, pp. 2040–2048, 2007.

[26] D. Cunningham, Y. Humblet, S. Siena, et al., "Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer," *The New England Journal of Medicine*, vol. 351, no. 4, pp. 337–345, 2004.

[27] A. F. Sobrero, J. Maurel, L. Fehrenbacher, et al., "EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 26, no. 14, pp. 2311–2319, 2008.

[28] E. Van Cutsem, M. Nowacki, I. Lang, et al., "Randomized phase III study of irinotecan and 5-FU/FA with or without cetuximab in the first-line treatment of patients with metastatic colorectal cancer (mCRC): the CRYSTAL trial," *Journal of Clinical Oncology*, vol. 25, supplement 18, 2007, abstract no. 4000.

[29] C. Bokemeyer, I. Bondarenko, J. T. Hartmann, et al., "KRAS status and efficacy of first-line treatment of patients with metastatic colorectal cancer (mCRC) with FOLFOX with or without cetuximab: the OPUS experience," in *Proceedings of the 44th Annual Meeting of the American Society of Clinical Oncology*, Chicago, Ill, USA, May-June 2008, abstract no. 4000.

[30] E. Van Cutsem, I. Lang, G. D'haens, et al., "KRAS status and efficacy in the first-line treatment of patients with metastatic colorectal cancer (mCRC) treated with FOLFIRI with or without cetuximab: the CRYSTAL experience," in *Proceedings of the 44th Annual Meeting of the American Society of Clinical Oncology*, Chicago, Ill, USA, May-June 2008, abstract no. 2.

[31] R. G. Amado, M. Wolf, M. Peeters, et al., "Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 26, no. 10, pp. 1626–1634, 2008.

[32] D. Soulieres, N. N. Senzer, E. E. Vokes, M. Hidalgo, S. S. Agarvala, and L. L. Siu, "Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck," *Journal of Clinical Oncology*, vol. 22, no. 1, pp. 77–85, 2004.

[33] J. B. Vermorken, R. Mesia, F. Rivera, et al., "Platinum-based chemotherapy plus cetuximab in head and neck cancer," *The New England Journal of Medicine*, vol. 359, no. 11, pp. 1116–1127, 2008.

[34] B. Burtness, M. A. Goldwasser, W. Flood, B. Mattar, and A. A. Forastiere, "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," *Journal of Clinical Oncology*, vol. 23, no. 34, pp. 8646–8654, 2005.

[35] J. A. Bonner, P. M. Harari, J. Giralt, et al., "Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck," *The New England Journal of Medicine*, vol. 354, no. 6, pp. 567–578, 2006.

[36] M. Bloomston, A. Bhardwaj, E. C. Ellison, and W. L. Frankel, "Epidermal growth factor receptor expression in pancreatic carcinoma using tissue microarray technique," *Digestive Surgery*, vol. 23, no. 1-2, pp. 74–79, 2006.

[37] E. Tsiambas, A. Karameris, A. C. Lazaris, et al., "EGFR alterations in pancreatic ductal adenocarcinoma: a chromogenic in situ hybridization analysis based on tissue microarrays," *Hepato-Gastroenterology*, vol. 53, no. 69, pp. 452–457, 2006.

[38] P. A. Philip, J. Benedetti, C. Fenoglio-Preiser, et al., "Phase III study of gemcitabine plus cetuximab versus gemcitabine in patients with locally advanced or metastatic pancreatic adenocarcinoma: SWOG S0205 study," *Journal of Clinical Oncology*, vol. 25, p. 199s, 2007.

[39] R. Pirker, A. Szczesna, J. von Pawel, et al., "FLEX: a randomized, multicenter, phase III study of cetuximab in combination with cisplatin/vinorelbine (CV) versus CV alone in the first-line treatment of patients with advanced non-small cell lung cancer (NSCLC)," *Journal of Clinical Oncology*, vol. 26, supplement 15, 2008, abstract no. 3.

[40] F. R. Hirsch, R. S. Herbst, C. Olsen, et al., "Increased *EGFR* gene copy number detected by fluorescent in situ hybridization predicts outcome in non-small-cell lung cancer patients treated with cetuximab and chemotherapy," *Journal of Clinical Oncology*, vol. 26, no. 20, pp. 3351–3357, 2008.

[41] R. M. Giusti, K. A. Shastri, M. H. Cohen, P. Keegan, and R. Pazdur, "FDA drug approval summary: panitumumab (Vectibix™)," *The Oncologist*, vol. 12, no. 5, pp. 577–583, 2007.

[42] J. R. Hecht, A. Patnaik, J. Berlin, et al., "Panitumumab monotherapy in patients with previously treated metastatic colorectal cancer," *Cancer*, vol. 110, no. 5, pp. 980–988, 2007.

[43] E. Van Cutsem, M. Peeters, S. Siena, et al., "Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 25, no. 13, pp. 1658–1664, 2007.

[44] E. Van Cutsem, S. Siena, Y. Humblet, et al., "An open-label, single-arm study assessing safety and efficacy of panitumumab in patients with metastatic colorectal cancer refractory to standard chemotherapy," *Annals of Oncology*, vol. 19, no. 1, pp. 92–98, 2008.

[45] J. R. Hecht, E. Mitchell, T. Chidiac, et al., "An updated analysis of safety and efficacy of oxaliplatin (Ox)/bevacizumab (bev) +/− panitumumab (pmab) for first-line treatment (tx) of metastatic colorectal cancer (mCRC) from a randomized, controlled trial (PACCE)," in *Proceedings of the Gastrointestinal Cancers Symposium*, Orlando, Fla, USA, January 2008, abstract no. 273.

[46] C. Langner, M. Ratschek, P. Rehak, L. Schips, and R. Zigeuner, "Are heterogenous results of EGFR immunoreactivity in renal cell carcinoma related to non-standardised criteria for staining evaluation?" *Journal of Clinical Pathology*, vol. 57, no. 7, pp. 773–775, 2004.

[47] B. Escudier, T. Eisen, W. M. Stadler, et al., "Sorafenib in advanced renal cell carcinoma," *The New England Journal of Medicine*, vol. 356, no. 2, pp. 125–134, 2007.

[48] J. M. Llovet, S. Ricci, V. Mazzaferro, et al., "Sorafenib in advanced hepatocellular carcinoma," *The New England Journal of Medicine*, vol. 359, no. 4, pp. 378–390, 2008.

[49] A. Cheng, Y. Kang, Z. Chen, et al., "Randomized phase III trial of sorafenib versus placebo in Asian patients with advanced hepatocellular carcinoma," *Journal of Clinical Oncology*, vol. 26, supplement 15, 2008, abstract no. 4509.

[50] G. Abou-Alfa, P. Johnson, J. Knox, et al., "Preliminary results from a phase II, randomized, double-blind study of sorafenib plus doxorubicin versus placebo plus doxorubicin in patients with advanced hepatocellular carcinoma," *European Journal of*

Cancer Supplements, vol. 5, no. 4, p. 259, 2007, abstract no. 3500.

[51] V. Gupta-Abramson, A. B. Troxel, A. Nellore, et al., "Phase II trial of sorafenib in advanced thyroid cancer," *Journal of Clinical Oncology*, vol. 26, no. 29, pp. 4714–4719, 2008.

[52] H. Zhong and J. P. Bowen, "Molecular design and clinical development of VEGFR kinase inhibitors," *Current Topics in Medicinal Chemistry*, vol. 7, no. 14, pp. 1379–1393, 2007.

[53] I. Smith, M. Procter, R. D. Gelber, et al., "2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial," *The Lancet*, vol. 369, no. 9555, pp. 29–36, 2007.

[54] R. J. Motzer, M. D. Michaelson, B. G. Redman, et al., "Activity of SU11248,a multitargetted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma," *Journal of Clinical Oncology*, vol. 24, no. 1, pp. 16–24, 2006.

[55] R. J. Motzer, B. I. Rini, R. M. Bukowski, et al., "Sunitinib in patients with metastatic renal cell carcinoma," *Journal of the American Medical Association*, vol. 295, no. 21, pp. 2516–2524, 2006.

[56] R. J. Motzer, T. E. Hutson, P. Tomczak, et al., "Phase III randomized trial of sunitinib malate (SU11248) versus interferon alfa (IFN-a)as a first line systemic therapy for patients with metastatic renal cell carcinoma (mRCC)," *The New England Journal of Medicine*, vol. 356, no. 2, pp. 115–124, 2007.

[57] D. Cella, J. Z. Li, J. C. Cappelleri, et al., "Quality of life in patients with metastatic renal cell carcinoma treated with sunitinib or interferon alfa: results from a phase III randomized trial," *Journal of Clinical Oncology*, vol. 26, no. 22, pp. 3763–3769, 2008.

[58] G. D. Demetri, A. T. van Oosterom, C. R. Garrett, et al., "Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial," *The Lancet*, vol. 368, no. 9544, pp. 1329–1338, 2006.

[59] N. U. Lin, V. Dieras, D. Paul, et al., "EGF105084, a phase II study of lapatinib for brain metastases in patients (pts) with HER2+ breast cancer following trastuzumab (H) based systemic therapy and cranial radiotherapy (RT)," *Journal of Clinical Oncology*, vol. 25, supplement 18, 2007, abstract no. 1012.

[60] M. Ekenel, A. M. Hormigo, S. Peak, L. M. DeAngelis, and L. E. Abrey, "Capecitabine therapy of central nervous system metastases from breast cancer," *Journal of Neuro-Oncology*, vol. 85, no. 2, pp. 223–227, 2007.

[61] C. E. Geyer Jr., A. Martin, B. Newstat, et al., "Lapatinib (L) plus capecitabine (C) in HER2+ advanced breast cancer (ABC): genomic and updated efficacy data," *Journal of Clinical Oncology*, vol. 25, supplement 18, 2007, abstract no.1035.

[62] M. A. Cobleigh, C. L. Vogel, D. Tripathy, et al., "Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease," *Journal of Clinical Oncology*, vol. 17, no. 9, pp. 2639–2648, 1999.

[63] C. L. Vogel, M. A. Cobleigh, D. Tripathy, et al., "Efficacy and safety of trastuzumab as a single agent in first-line treatment of *HER2*-overexpressing metastatic breast cancer," *Journal of Clinical Oncology*, vol. 20, no. 3, pp. 719–726, 2002.

[64] D. J. Slamon, B. Leyland-Jones, S. Shak, et al., "Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2," *The New England Journal of Medicine*, vol. 344, no. 11, pp. 783–792, 2001.

[65] E. H. Romond, E. A. Perez, J. Bryant, et al., "Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer," *The New England Journal of Medicine*, vol. 353, no. 16, pp. 1673–1684, 2005.

[66] Y. Lu, X. Zi, Y. Zhao, D. Mascarenhas, and M. Pollak, "Insulin-like growth factor-I receptor signaling and resistance to transtuzumab (Herceptin)," *Journal of the National Cancer Institute*, vol. 93, no. 24, pp. 1852–1857, 2001.

[67] C. E. Geyer, J. Forster, D. Lindquist, et al., "Lapatinib plus capecitabine for HER2-positive advanced breast cancer," *The New England Journal of Medicine*, vol. 355, no. 26, pp. 2733–2743, 2006.

[68] A. Di Leo, H. L. Gomez, Z. Aziz, et al., "Phase III, double-blind, randomized study comparing lapatinib plus paclitaxel with placebo plus paclitaxel as first-line treatment for metastatic breast cancer," *Journal of Clinical Oncology*, vol. 26, no. 34, pp. 5544–5552, 2008.

[69] E. O'Keefe, T. Battin, and R. Payne Jr., "Epidermal growth factor receptor in human epidermal cells: direct demonstration in cultured cells," *Journal of Investigative Dermatology*, vol. 78, no. 6, pp. 482–487, 1982.

[70] L. B. Nanney, J. A. McKanna, C. M. Stoscheck, G. Carpenter, and L. E. King Jr., "Visualization of epidermal growth factor receptors in human epidermis," *Journal of Investigative Dermatology*, vol. 82, no. 2, pp. 165–169, 1984.

[71] M. R. Green, D. Phil, and J. R. Couchman, "Differences in human skin between the epidermal growth factor receptor distribution detected by EGF binding and monoclonal antibody recognition," *Journal of Investigative Dermatology*, vol. 85, no. 3, pp. 239–245, 1985.

[72] C. Piérard-Franchimont, A. Colige, J. Arrese Estrada, C. M. Lapière, and G. E. Piérard, "Immunohistochemical expression of epidermal growth factor receptors in nuclei of a subpopulation of keratinocytes and sweat gland cells," *Dermatologica*, vol. 183, no. 1, pp. 7–9, 1991.

[73] L. B. Nanney, C. M. Stoscheck, L. E. King Jr., R. A. Underwood, and K. A. Holbrook, "Immunolocalization of epidermal growth factor receptors in normal developing human skin," *Journal of Investigative Dermatology*, vol. 94, no. 6, pp. 742–748, 1990.

[74] W. Jacot, D. Bessis, E. Jorda, et al., "Acneiform eruption induced by epidermal growth factor receptor inhibitors in patients with solid tumors," *British Journal of Dermatology*, vol. 151, no. 1, pp. 238–241, 2004.

[75] L. B. Saltz, N. J. Meropol, P. J. Loehrer Sr., M. N. Needle, J. Kopit, and R. J. Mayer, "Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the pidermal growth factor receptor," *Journal of Clinical Oncology*, vol. 22, no. 7, pp. 1201–1208, 2004.

[76] H. Q. Xiong, A. Rosenberg, A. LoBuglio, et al., "Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with gemcitabine for advanced pancreatic cancer: a multicenter phase II trial," *Journal of Clinical Oncology*, vol. 22, no. 13, pp. 2610–2616, 2004.

[77] R. Pérez-Soler, J. P. Delord, A. Halpern, et al., "HER1/EGFR inhibitor-associated rash: future directions for management and investigation outcomes from the HER1/EGFR inhibitor rash management forum," *The Oncologist*, vol. 10, no. 5, pp. 345–356, 2005.

[78] A. F. S. Galimont-Collen, L. E. Vos, A. P. M. Lavrijsen, J. Ouwerkerk, and H. Gelderblom, "Classification and management of skin, hair, nail and mucosal side-effects of epidermal

growth factor receptor (EGFR) inhibitors," *European Journal of Cancer*, vol. 43, no. 5, pp. 845–851, 2007.

[79] B. Escudier, C. Szczylik, T. Eisen, et al., "Randomized phase III trial of the RAF kinase and VEGFR inhibitor sorafenib (BAY-43-9006) in patients with advanced renal cell cancer (RCC)," in *Proceedings of the Annual Meeting of the American Society of Clinical Oncology*, Orlando, Fla, USA, May 2005, abstract no. 4510.

[80] R. J. Motzer, B. I. Rini, R. M. Bukowski, et al., "Sunitinib in patients with metastatic renal cell carcinoma," *The Journal of the American Medical Association*, vol. 295, no. 21, pp. 2516–2524, 2006.

[81] K. K. L. Mak and S. Y. Chan, "Epidermal growth factor as a biologic switch in hair growth cycle," *The Journal of Biological Chemistry*, vol. 278, no. 28, pp. 26120–26126, 2003.

[82] R. Murillas, F. Larcher, C. J. Conti, M. Santos, A. Ullrich, and J. L. Jorcano, "Expression of a dominant negative mutant of epidermal growth factor receptor in the epidermis of transgenic mice elicits striking alterations in hair follicle development and skin structure," *EMBO Journal*, vol. 14, no. 21, pp. 5216–5223, 1995.

[83] YM BioSciences Inc., January 2009, http://www.nimotuzumab.com.

[84] J. M. Uribe, C. M. Gelbmann, A. E. Traynor-Kaplan, and K. E. Barrett, "Epidermal growth factor inhibits Ca^{2+}-dependent Cl^{-} transport in T84 human colonic epithelial cells," *American Journal of Physiology*, vol. 271, no. 3, pp. C914–C922, 1996.

[85] M. H. Cohen, G. A. Williams, R. Sridhara, G. Chen, and R. Pazdur, "FDA drug approval summary: gefitinib (ZD1839) (Iressa®) tablets," *The Oncologist*, vol. 8, no. 4, pp. 303–306, 2003.

[86] Iressa [package insert], AstraZeneca, London, UK, 2003.

[87] T. Takano, Y. Ohe, M. Kusumoto, et al., "Risk factors for interstitial lung disease and predictive factors for tumor response in patients with advanced non-small cell lung cancer treated with gefitinib," *Lung Cancer*, vol. 45, no. 1, pp. 93–104, 2004.

[88] D. Makris, A. Scherpereel, M. C. Copin, et al., "Fatal interstitial lung disease associated with oral erlotinib therapy for lung cancer," *BMC Cancer*, vol. 7, article 150, pp. 1–4, 2007.

[89] J. S. Lind, E. F. Smit, K. Grünberg, S. Senan, and F. J. Lagerwaard, "Fatal interstitial lung disease after erlotinib for non-small cell lung cancer," *Journal of Thoracic Oncology*, vol. 3, no. 9, pp. 1050–1053, 2008.

[90] D. R. Spigel, M. Lin, V. O'Neill, and J. D. Hainsworth, "Final survival and safety results from a multicenter, open-label, phase 3b trial of erlotinib in patients with advanced nonsmall cell lung cancer," *Cancer*, vol. 112, no. 12, pp. 2749–2755, 2008.

[91] Pfizer, Sutent (sunitinib) Prescribing Information, 2007.

[92] E. A. Perez, J. A. Byrne, W. Isaac, et al., "Cardiac safety experience in 3127 patients treated with lapatinib," *Annals of Oncology*, vol. 17, supplement 9, 2006.

[93] H.-J. Lenz, "Management and preparedness for infusion and hypersensitivity reactions," *The Oncologist*, vol. 12, no. 5, pp. 601–609, 2007.

[94] B. Melichar, J. Cerman Jr., and E. Malířová, "Successful management of infusion reaction accompanying the start of cetuximab therapy," *Supportive Care in Cancer*, vol. 15, no. 4, pp. 445–449, 2007.

[95] M. N. Needle, "Safety experience with IMC-C225, an anti-epidermal growth factor receptor antibody," *Seminars in Oncology*, vol. 29, no. 5, supplement 14, pp. 55–60, 2002.

[96] C. H. Chung, B. Mirakhur, E. Chan, et al., "Cetuximab-induced anaphylaxis and IgE specific for galactose-α-1,3-galactose," *The New England Journal of Medicine*, vol. 358, no. 11, pp. 1109–1117, 2008.

[97] M. W. Saif and M. Cohenuram, "Role of panitumumab in the management of metastatic colorectal cancer of metastatic colorectal cancer," *Clinical Colorectal Cancer*, vol. 6, no. 2, pp. 118–124, 2006.

[98] M. W. Saif, J. Peccerillo, and V. Potter, "Successful re-challenge with panitumumab in patients who developed hypersensitivity reactions to cetuximab: report of three cases and review of literature," *Cancer Chemotherapy and Pharmacology*, vol. 63, no. 6, pp. 1017–1022, 2009.

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Review Article

Update in Antiepidermal Growth Factor Receptor Therapy in the Management of Metastatic Colorectal Cancer

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The approval of anti-epidermal growth factor receptor (EGFR) monoclonal antibodies in the treatment of metastatic colorectal cancer (CRC) has expanded the armamentarium against this disease. This paper will review the historical progress and recent clinical developments of anti-EGFR therapies in the treatment of metastatic CRC. Novel strategies of targeting the EGFR pathway to improve efficacy as well as ongoing research in identifying specific molecular predictors of response will be discussed.

1. Introduction

A decade ago, the systemic treatment of colorectal cancer (CRC) consisted only of fluoropyrimidine-based chemotherapy administered alone or in combination with either oxaliplatin or irinotecan in an empirical fashion, guided by serial measurements of radiological response. Owing to the remarkable advances in our understanding of the molecular mechanisms of carcinogenesis, target-based therapies are now commonly used as in the treatment of many types of cancer, including CRC. Cetuximab and panitumumab are monoclonal antibody against the epidermal growth factor receptor (EGFR) that has been approved for the treatment of patients with metastatic CRC [1, 2]. The optimal clinical application of anti-EGFR agents in the management of CRC patients and the identification of predictive markers are the main focus of research in recent years. This article will concentrate on the developments and controversies of anti-EGFR therapy in the management of CRC.

2. EGFR as a Target in Colorectal Cancer

The concept of manipulation of EGFR in the treatment of epithelial malignancies such as colorectal and lung cancers has actually been envisaged since the mid 1960s [1, 2]. It was during that period that the EGFR protein was first isolated, characterized, and recognized as a potential therapeutic target. Throughout the last 40 years, advances made in basic and clinical research have enhanced our understanding of this target, and many different classes of EGFR inhibitors are now at various stages of clinical development.

EGFR is a 170 kD member of the ErbB receptor tyrosine kinase family of signaling proteins, and its ligands include epidermal growth factor (EGF), transforming growth factor-α (TGF-α), heparin-binding EGF (HB-EGF), and amphiregulin (AR). Ligand binding, dimerization, and phosphorylation of EGFR lead to activation of downstream proteins, leading to a cascade mediating cell growth and survival [3]. Two different anti-EGFR strategies are currently available in the therapeutic armamentarium: (1) monoclonal antibodies that prevent EGFR ligand binding and (2) tyrosine-kinase inhibitors (TKIs) that block phosphorylation of the intracellular tyrosine kinase component of the EGFR. Both of these strategies dampen signal transduction through some of the downstream pathways such as RAS/RAF/mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)-AKT cascades, thus limiting cell growth, proliferation, invasion, angiogenesis, and metastasis [3, 4].

Table 1: Comparison between cetuximab and panitumumab.

	Cetuximab	Panitumumab
Structure	Chimeric IgG-1, 30% murine	Fully humanized: IgG-2
Hypersensitivity reaction	3%	1%
Half life	5 days	7.5 days
Treatment schedule	1-2 weekly	2-weekly
Antibody-dependent cell mediated cytotoxicity (ADCC)	Yes Fc Domain of IgG-1	No ADCC reported

3. Anti-EGFR Monoclonal Antibodies

Cetuximab (chimeric IgG1 monoclonal antibody) and panitumumab (fully humanized IgG2 monoclonal antibody) are two anti-EGFR monoclonal antibodies currently approved in the treatment of metastatic CRC. The structural difference between the IgG-1-based cetuximab and IgG-2-based panitumumab is believed to have implications on their mechanisms of action. Preclinical studies have suggested that the cetuximab molecule is able to induce antibody-dependent cell-mediated cytotoxicity (ADCC) [4, 5], where natural killer cells, monocytes, and eosinophils are recruited to lyse the targeted cells (i.e., tumor cells). Being a chimeric antibody, cetuximab is also associated with a slightly higher incidence of hypersensitivity and infusional reactions when compared with fully humanized panitumumab (see Table 1).

3.1. Cetuximab. Pharmacokinetics studies have shown that cetuximab's binding affinity for EGFR was shown to be one log higher than its natural ligand and its mechanism of action is thought to be competitive inhibition of ligand binding to EGFR. Cetuximab alone resulted in in vitro and in vivo growth inhibition in multiple tumor types, including CRCs [4, 5]. Cetuximab was able to enhance the antitumor effect of irinotecan, as evident from an experiment on the HT-29 CRC xenograft model, where cetuximab and irinotecan given in combination resulted in a greater degree of tumor growth delay than when either agent was given alone [5]. Furthermore, cetuximab has been shown to overcome acquired resistance against irinotecan in vivo. This was shown as an experiment where the addition of cetuximab resulted in shrinkage of tumor xenografts which were otherwise progressing after previous treatment with irinotecan.

The landmark BOND trial [2] randomized 329 patients with metastatic CRC which were EGFR-positive and refractory to irinotecan, to either cetuximab alone or in combination with irinotecan in a 2 : 1 ratio. EGFR positivity was defined as 1+ staining by immunohistochemistry, and "irinotecan-refractory" status was defined as disease progression on or within 3 months of irinotecan-based therapy. Cross-over was allowed from the monotherapy arm to the combination arm upon disease progression. Objective response rate (ORR) was significantly in favor of the combination arm (22.9% versus 10.8%, $P = .007$). Fifty-six patients who were randomized to cetuximab alone eventually crossed over to the combination arm, while 3.6% and 35.7% of these patients achieved partial response and stable disease, respectively. This study led to the US Food Drug Administration (FDA) approval of cetuximab in patients with irinotecan-refractory meta static CRC. Subsequently, the NCIC-CO.17 study randomized patients who had failed at least 2 lines of prior therapies, to either supportive care or cetuximab alone [6]. In this study where no cross-over was allowed, there was a statistically significant advantage in median overall survival (OS) favoring the cetuximab arm (6.1 months) compared with supportive care (4.6 months). Partial responses occurred in 23 patients (8.0%) in the cetuximab group but none in the group assigned to supportive care alone ($P < .001$).

Cetuximab has also been investigated in the first-line setting. The "CRYSTAL" [7] study is a multicentre phase III trial which randomized more than 1000 patients with metastatic CRC, to either the "FOLFIRI" regimen alone (Irinotecan, infusional 5-fluorouracil and leucovorin in a 2-weekly schedule), or in combination with cetuximab at a weekly schedule. The primary endpoint (progression-free survival (PFS)) was met in the study, where patients randomized to the combination arm had a significantly longer progression-free survival (8.9 months versus 8.0 months; $P = .036$) than the chemotherapy alone arm, but there was no difference in overall survival in the initial intention-to-treat analysis. Response rate was also significantly better in the combination arm (46.9% versus 38.7%; $P = .005$), resulting in a larger number of patients being down staged enough to undergo resection of liver metastases (9.8% versus 4.5%). The "OPUS" study [8] is another first-line randomized phase II study, which randomized 337 chemotherapy-nave patients with metastatic CRC, to either the FOLFOX-4 regimen or in combination with cetuximab in a 1:1 fashion. The overall RR was 45.6% in the combination arm versus 35.7% in FOLFOX-4 alone arm. In the "ACROBAT" study, Tabernero et al. reported on 42 patients who were treated with FOLFOX-4 plus cetuximab, showing a confirmed ORR of 81% [9]. Encouragingly, 10 patients (23%) underwent resection of previously unresectable metastases, 8 of them had liver metastases. The resection with curative intent rate of 23% achieved in this study is therefore comparable with the highest reported for unselected patients.

3.2. Panitumumab. The US FDA approval of panitumumab was based on a pivotal multinational phase III study that involved over 400 patients [10]. This study compared panitumumab versus best supportive care (BSC), allowing cross-over from the BSC arm to panitumumab upon disease progression. The median PFS time was 8 weeks for panitumumab and 7.3 weeks for BSC. After a 12-month followup period, response rates were 10% for panitumumab and 0% for BSC ($P < .0001$). The lack of difference in OS (hazard ratio HR 1.00; 95% confidence interval, CI 0.82 to 1.22) maybe attributed to the cross-over design, where 76% of patients in the BSC arm subsequently received panitumumab. As expected with anti-EGFR therapies, skin-related toxicities occurred in 90% of patients in the panitumumab group but no patients had grade 3 or 4 infusional reactions.

4. EGFR Tyrosine Kinase Inhibitors

Although not approved for the treatment of CRC, small molecule inhibitors of the EGFR tyrosine kinase (TKI) have been shown to have meaningful activity in different tumor types such as lung and pancreatic cancer. In contrast to EGFR monoclonal antibodies, the site of action of these drugs is intracellular at the ATP-binding site of the EGFR TK domain. Compared with monoclonal antibodies, TKIs may potentially inhibit multiple targets and tend not to induce receptor downregulation.

4.1. Gefitinib. Gefitinib is a low-molecular-weight competitive inhibitor of ATP-binding pocket of the EGFR TK domain [11], which is approved for the treatment of nonsmall cell lung cancer. Clinical trials of gefitinib as a single agent in CRC reported no objective tumor responses [12–14] however, a sizable proportion of patients did have disease stabilization. A phase II trial which compared the 250 mg versus a 500 mg daily dosing of gefitinib [15], reported 1 partial response in a patient who received the 500 mg dose. Paired biopsies pre- and posttreatment biopsies performed in 28 patients showed that 84% had no change or increase in the expression level of phosphorylated EGFR, MAP kinase, and Ki67 after treatment. Gefitinib and chemotherapy in combination have also been investigated in several trials, where the response rates seemed to be superior to the historically reported rates of chemotherapy alone [16–18]. However, there were significantly more toxicities particularly with respect to neutropenia and diarrhea with the combination. Studies combining gefitinib with irinotecan also resulted in greater toxicity, with some trials requiring early termination.

4.2. Erlotinib. Erlotinib has also been studied in advanced CRC. There have been mixed reports of some clinical activity when used as a single agent. As with gefitinib, erlotinib produced a higher response rate when combined with chemotherapy. High incidence of toxicity was noted when given in combination with systemic chemotherapy, especially in two trials where erlotinib was given in combination with FOLFOX and bevacizumab [19, 20], with the latter trial having prematurely closed due to toxicity.

5. Incidence and Implications of Skin Rash

Anti-EGFR monoclonal antibodies and EGFR TKIs are associated with a distinctive skin rash. The rash is characterized histologically as a neutrophilic infiltrate in perifollicular areas within the basal layer of the skin, which is different from that seen in typical acne. Skin toxicity is generally observed within 2 to 3 weeks after the start of treatment and gradually resolves in most patients, even when anti-EGFR treatment is continued. In the BOND study, the most frequently observed adverse event to cetuximab was the skin rash, and in the panitumumab study, Hecht et al. [21] reported a 95% incidence of acneiform skin rash of any grade. Grade 3 rash occurred in 3% of patients and none experienced grade 4 skin toxicities. An association between the severity of acneiform rash and efficacy to cetuximab has been well described. Retrospective analysis of the BOND data showed a clear association between higher grades of skin reaction with RR and time to progression (TTP) [2]. This association is also seen with panitumumab [10] and seems to hold true in the treatment of tumors of other sites with this class of agents.

The "EVEREST" study [22] was a phase I/II dose-escalation study, where patients who were receiving cetuximab were randomized at 22 days, to either standard dose of weekly (250 mg/m2/week) cetuximab or an escalating dose of cetuximab until the development of grade 3 toxicity, with a maximum ceiling dose of 500 mg/m2/week. Skin and tumor biopsies were obtained. Preliminary report suggested that the PFS in standard-dose arm was 3.9 months and 4.8 months in the dose-escalated arm. Dose-related increases in pharmacokinetic parameters (e.g., Cmax, AUC) were observed in the escalated arm. The authors concluded that cetuximab dose escalation up to 500 mg/m2/w may improve ORR in patients who experienced no or slight skin reactions on standard-dose cetuximab.

6. Combining Targeted Therapies in Colorectal Cancer

Combinations of multiple targeted agents with or without the additional of chemotherapy have also been investigated. The BOND-2 trial [23] randomized irinotecan-refractory patients to either 2 drugs (bevacizumab and cetuximab) or 3 drugs (bevacizumab, cetuximab and irinotecan). The results were encouraging, with the 3-drug arm resulting in a better TTP and ORR than the 2-drug arm. This study also showed for the first time that monoclonal antibodies in combination could induce an ORR of 20% in the absence of chemotherapy. Nonstatistical comparison with result of the BOND-1 study suggested that bevacizumab may enhance the effects of irinotecan-cetuximab combination, with an ORR of 37% as compared to 23% reported for the cetuximab/irinotecan arm [2, 23] (see Table 2). Subsequent to the BOND-2 study, 3 other randomized trials have been reported on the feasibility of combining monoclonal antibodies in the treatment of CRC. The PACCE study [24] randomized 824 treatment-nave patients with metastatic CRC to oxaliplatin-based chemotherapy plus bevacizumab, with or without panitumumab, and 230 patients to irinotecan-based chemotherapy plus bevacizumab with or without panitumumab. The study was terminated at a preplanned interim analysis after 231 events were reported in patients who received oxaliplatin-based therapy, where a statistically significant increase in PFS was reported in the arm without panitumumab. In contrast, the Dutch CAIRO-2 trial [25] has a similar study design to the PACCE trial and involved over 700 patients. Patients received capecitabine, oxaliplatin, and bevacizumab with or without additional cetuximab. As expected, preliminary result reported a significantly higher incidence of grade 3-4 skin rash in the cetuximab-containing arm, without a statistically significant difference in mortality in either arm. The CALGB/SWOG 80405 intergroup trial was a 3-arm study, which randomized patients with metastatic CRC to chemotherapy and cetuximab, chemotherapy and

Table 2: Nonstatistical comparison of the results of the BOND-2 [23] and BOND-1 study [1].

BOND2	BOND2		BOND1	BOND1
Cetuximab, irinotecan, and bevacizumab	Cetuximab and bevacizumab		Cetuximab and irinotecan	Cetuximab alone
No. of Patients	43	40	218	111
Prior treatment with oxaliplatin (%)	87	89	62	64
Response rates (%)	37	20	23	11
Time to progression (months)	7.3	4.9	4.1	1.5
Median overall survival (months)	14.5	11.4	8.6	6.9

bevacizumab, or chemotherapy plus cetuximab and bevacizumab. This study has been suspended following a decision by the CALGB Data and Safety Monitoring Board as from June 2008, pending a protocol revision in view of the data on KRAS mutation (see below) [26]. The reason why the PACCE study failed to demonstrate a survival advantage in the panitumumab-bevacizumab arm remains unclear. It is possible that the dose of the antibodies was inappropriate leading to excessive toxicity and reduced efficacy. Patient selection has also proven critical in the optimal use of anti-EGFR antibodies as the use of EGFR staining to predict response and outcome has been severely challenged. Thus, a combination approach of targeted agents in metastatic CRC remains controversial and it is the consensus among gastrointestinal oncologists that such an approach remains experimental.

7. EGFR Expression in Colorectal Cancer

EGFR as determined by immunohistochemical (IHC) methods was the first biomarker investigated as a potential predictor of response. It is overexpressed in over 80% of colorectal cancers [27]. However, EGFR expression as measured by immunohistochemistry does not predict clinical benefit [28, 29]. Initial observations in a small retrospective series by Lenz et al. [30] noted that more than 20% of EGFR-negative patients developed major objective responses. An extensive retrospective analysis was reported in 2005 by Chung et al. [29]. They reported a response rate of 25% in EGFR-negative patients (4 out of 16) given cetuximab and irinotecan. This was comparable and indistinguishable from the response rate of 23% seen in two separate clinical trials with EGFR-positive patients [1, 31]. Given this data, immunohistochemical (IHC) demonstration of EGFR expression is no longer required before starting cetuximab therapy in practice. Similarly, in the phase II trial, the response to treatment with panitumumab in patients with metastatic CRC was similar, irrespective of the level of EGFR protein expression assessed by IHC analysis [32].

8. Potential Predictors of Response to Anti-EGFR Therapy

8.1. Activating EGFR Gene Mutations. There has been much interest in determining whether EGFR gene mutations may play a role in affecting response to cetuximab or panitumumab. Previous research in nonsmall cell lung cancer [33] has shown that EGFR TK mutations predict benefit from EGFR TKIs. However, several retrospective studies on tumor biopsies and cell lines found that EGFR gene mutations in CRC are extremely rare [34, 35]. No significantly different gene mutations were found between responders and nonresponders to treatment.

8.2. EGFR Gene Amplification. It has been hypothesized that DNA is a more stable molecule than a protein and thus EGFR gene copy number maybe more accurate reflection of the EGFR status than IHC expression [36]. In a cohort of patients treated with cetuximab or panitumumab, EGFR gene amplification with increased copy number has been shown to significantly correlate with objective response to treatment [36]. This raises the possibility that patients with high EGFR copy number may be more likely to respond to treatment with anti-EGFR therapies.

8.3. KRAS Gene Mutation. KRAS is a guanosine triphosphate (GTP)-binding protein that acts as a critical on-off switch in cellular growth and survival pathways. It plays a key role in the RAS/MAPK signally pathway located downstream of many growth factor receptors, including EGFR, and involved in carcinogenesis. Mutations of KRAS that result in the constitutive activation of MAPK pathway downstream occurs in about 40% of colorectal cancers [37]. A retrospective analysis reported by Lièvre et al. [38] analyzed tumor samples from 30 patients treated with cetuximab. A KRAS mutation was found in 13 tumors (43%) and was significantly associated with absence of response to cetuximab. None of the patients with response to cetuximab harbored a KRAS mutation. The overall survival of patients without KRAS mutation was significantly higher compared with those patients with a mutated tumor ($P = .016$; median survival 16.3 versus 6.9 months). An increase in EGFR copy number was also significantly associated with objective tumor response.

It has been hypothesized that irrespective of the level of EGFR expression, the presence of a KRAS mutation is associated with a constitutive activation of the RAS/MAPK pathway, leading to cell proliferation which cannot be significantly inhibited by cetuximab. KRAS mutations have also been implicated in resistance against EGFR TKIs in lung adenocarcinomas [39].

Large scale retrospective reviews retrieved archived tumor tissue from prior cetuximab and panitumumab trials. KRAS mutation analysis was performed tested in tumor samples collected from over 1000 participants of the "CRYSTAL"

[8], "OPUS" [40], NCIC-CO.17 trial [41] and panitumumab trials [42]. Beneficial effects of anti-EGFR antibodies were limited to a subgroup of patients with wild-type KRAS tumors. This has led to the recommendation that all patients with advanced CRC who are being considered for cetuximab or panitumumab should undergo KRAS testing, and if the cancer bears a mutated KRAS gene, they should not receive an antibody that targets EGFR.

9. Conclusion

Throughout the last decade, significant advancement in our understanding of the molecular mechanisms of metastatic CRC has been made. Anti-EGFR monoclonal antibodies approved for use in the metastatic setting have broadened the therapeutic armamentarium in the treatment of metastatic CRC. The most effective sequence and combinations of anti-EGFR therapy with chemotherapy and, or bevacizumab in order to achieve cytotoxic potentiation with limited toxicity need to be addressed. Advances made in the identification of predictive biomarkers such as KRAS mutations allow us to select distinct groups of patients who are most likely to benefit from cetuximab therapy.

References

[1] H. Hurwitz, L. Fehrenbacher, W. Novotny, et al., "Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer," *The New England Journal of Medicine*, vol. 350, no. 23, pp. 2335–2342, 2004.

[2] D. Cunningham, Y. Humblet, S. Siena, et al., "Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer," *The New England Journal of Medicine*, vol. 351, no. 4, pp. 337–345, 2004.

[3] N. Normanno, A. De Luca, C. Bianco, et al., "Epidermal growth factor receptor (EGFR) signaling in cancer," *Gene*, vol. 366, no. 1, pp. 2–16, 2006.

[4] J. Mendelsohn and J. Baselga, "Epidermal growth factor receptor targeting in cancer," *Seminars in Oncology*, vol. 33, no. 4, pp. 369–385, 2006.

[5] N. E. Hynes and H. A. Lane, "ERBB receptors and cancer: the complexity of targeted inhibitors," *Nature Reviews Cancer*, vol. 5, no. 5, pp. 341–354, 2005.

[6] D. J. Jonker, C. J. O'Callaghan, C. S. Karapetis, et al., "Cetuximab for the treatment of colorectal cancer," *The New England Journal of Medicine*, vol. 357, no. 20, pp. 2040–2048, 2007.

[7] E. Van Cutsem, I. Lang, G. D'Haens, et al., "*KRAS* status and efficacy in the first-line treatment of patients with metastatic colorectal cancer (mCRC) treated with FOLFIRI with or without cetuximab: the CRYSTAL experience," *Journal of Clinical Oncology*, vol. 26, no. 20S, 2008, abstract 2.

[8] C. Bokemeyer, I. Bondarenko, A. Makhson, et al., "Cetuximab plus 5-FU/FA/oxaliplatin (FOLFOX-4) versus FOLFOX-4 in the first-line treatment of metastatic colorectal cancer (mCRC): OPUS, a randomized phase II study," *Journal of Clinical Oncology*, vol. 25, no. 18S, 2007, abstract 4035.

[9] J. Tabernero, E. Van Cutsem, E. Díaz-Rubio, et al., "Phase II trial of cetuximab in combination with fluorouracil, leucovorin, and oxaliplatin in the first-line treatment of metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 25, no. 33, pp. 5225–5232, 2007.

[10] E. Van Cutsem, M. Peeters, S. Siena, et al., "Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 25, no. 13, pp. 1658–1664, 2007.

[11] A. E. Wakeling, S. P. Guy, J. R. Woodburn, et al., "ZD1839 (Iressa): an orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy," *Cancer Research*, vol. 62, no. 20, pp. 5749–5754, 2002.

[12] J. Baselga, D. Rischin, M. Ranson, et al., "Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types," *Journal of Clinical Oncology*, vol. 20, no. 21, pp. 4292–4302, 2002.

[13] M. Ranson, L. A. Hammond, D. Ferry, et al., "ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial," *Journal of Clinical Oncology*, vol. 20, no. 9, pp. 2240–2250, 2002.

[14] R. S. Herbst, A.-M. Maddox, M. L. Rothenberg, et al., "Selective oral epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 is generally well-tolerated and has activity in non-small-cell lung cancer and other solid tumors: results of a phase I trial," *Journal of Clinical Oncology*, vol. 20, no. 18, pp. 3815–3825, 2002.

[15] M. L. Rothenberg, B. LaFleur, D. E. Levy, et al., "Randomized phase II trial of the clinical and biological effects of two dose levels of gefitinib in patients with recurrent colorectal adenocarcinoma," *Journal of Clinical Oncology*, vol. 23, no. 36, pp. 9265–9274, 2005.

[16] J. T. Hartmann, J. P. Pintoffl, H. Kröning, C. Bokemeyer, M. Holtmann, and T. Höhler, "Gefitinib in combination with oxaliplatin and 5-fluorouracil in irinotecan-refractory patients with colorectal cancer: a phase I study of the arbeits gemeinschaft internistische onkologie (AIO)," *Onkologie*, vol. 31, no. 5, pp. 237–241, 2008.

[17] T. Kuo, C. D. Cho, J. Halsey, et al., "Phase II study of gefitinib, fluorouracil, leucovorin, and oxaliplatin therapy in previously treated patients with metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 23, no. 24, pp. 5613–5619, 2005.

[18] G. A. Fisher, T. Kuo, M. Ramsey, et al., "A phase II study of gefitinib, 5-fluorouracil, leucovorin, and oxaliplatin in previously untreated patients with metastatic colorectal cancer," *Clinical Cancer Research*, vol. 14, no. 21, pp. 7074–7079, 2008.

[19] J. A. Meyerhardt, K. Stuart, C. S. Fuchs, et al., "Phase II study of FOLFOX, bevacizumab and erlotinib as first-line therapy for patients with metastastic colorectal cancer," *Annals of Oncology*, vol. 18, no. 7, pp. 1185–1189, 2007.

[20] D. R. Spigel, J. D. Hainsworth, H. Burris, et al., "Phase II study of FOLFOX4, bevacizumab, and erlotinib as first-line therapy in patients with advanced colorectal cancer," in *Proceedings of ASCO Gastrointestinal Cancers Annual Symposium*, San Francisco, Calif, USA, January 2006, abstract 238.

[21] J. Hecht, E. Mitchell, J. Baranda, et al., "Panitumumab antitumor activity in patients (pts) with metastatic colorectal cancer (mCRC) expressing low (1–9%) or negative (<1%) levels of epidermal growth factor receptor (EGFr)," in *Proceedings of ASCO Gastrointestinal Cancer Annual Symposium*, San Francisco, Calif, USA, January 2006, abstract 350.

[22] S. Tejpar, M. Peeters, Y. Humblet, et al., "Phase I/II study of cetuximab dose-escalation in patients with metastatic colorectal cancer (mCRC) with no or slight skin reactions on

cetuximab standard dose treatment (EVEREST): pharmacokinetic (PK), pharmacodynamic (PD) and efficacy data," *Journal of Clinical Oncology*, vol. 25, no. 18S, 2007, abstract 4037.

[23] L. B. Saltz, H.-J. Lenz, H. L. Kindler, et al., "Randomized phase II trial of cetuximab, bevacizumab, and irinotecan compared with cetuximab and bevacizumab alone in irinotecan-refractory colorectal cancer: the BOND-2 study," *Journal of Clinical Oncology*, vol. 25, no. 29, pp. 4557–4561, 2007.

[24] J. Hecht, T. Chidiac, E. Mitchell, et al., "An interim analysis of efficacy and safety from a randomized controlled trial of panitumumab with chemotherapy pluse bevacizumab in metastastic colorectal cancer," in *Proceedings of the 9th World Congress on Gastrointestinal Cancer*, Barcelona, Spain, June 2007, abstract 33.

[25] J. Tol, M. Koopman, C. J. Rodenburg, et al., "A randomised phase III study on capecitabine, oxaliplatin and bevacizumab with or without cetuximab in first-line advanced colorectal cancer, the CAIRO2 study of the Dutch Colorectal Cancer Group (DCCG). An interim analysis of toxicity," *Annals of Oncology*, vol. 19, no. 4, pp. 734–738, 2008.

[26] A. P. Venook, C. D. Blanke, D. Niedzwiecki, et al., "Revisiting the cancer and leukemia group B/southwest oncology group 80405 trial: a phase III trial of chemotherapy and biologic agents for patients with untreated advanced colorectal adenocarcinoma," *Clinical Colorectal Cancer*, vol. 6, no. 7, pp. 536–538, 2007.

[27] J.-P. Spano, C. Lagorce, D. Atlan, et al., "Impact of EGFR expression on colorectal cancer patient prognosis and survival," *Annals of Oncology*, vol. 16, no. 1, pp. 102–108, 2005.

[28] R. Adams and T. Maughan, "Predicting response to epidermal growth factor receptor-targeted therapy in colorectal cancer," *Expert Review of Anticancer Therapy*, vol. 7, no. 4, pp. 503–518, 2007.

[29] K. Y. Chung, J. Shia, N. E. Kemeny, et al., "Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry," *Journal of Clinical Oncology*, vol. 23, no. 9, pp. 1803–1810, 2005.

[30] H.-J. Lenz, E. Van Cutsem, S. Khambata-Ford, et al., "Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan, oxaliplatin, and fluoropyrimidines," *Journal of Clinical Oncology*, vol. 24, no. 30, pp. 4914–4921, 2006.

[31] L. B. Saltz, N. J. Meropol, P. J. Loehrer Sr., M. N. Needle, J. Kopit, and R. J. Mayer, "Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor," *Journal of Clinical Oncology*, vol. 22, no. 7, pp. 1201–1208, 2004.

[32] D. J. Freeman, T. Juan, M. Reiner, et al., "Association of K-ras mutational status and clinical outcomes in patients with metastatic colorectal cancer receiving panitumumab alone," *Clinical Colorectal Cancer*, vol. 7, no. 3, pp. 184–190, 2008.

[33] T. J. Lynch, D. W. Bell, R. Sordella, et al., "Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib," *The New England Journal of Medicine*, vol. 350, no. 21, pp. 2129–2139, 2004.

[34] T. D. Barber, B. Vogelstein, K. W. Kinzler, and V. E. Velculescu, "Somatic mutations of *EGFR* in colorectal cancers and glioblastomas," *The New England Journal of Medicine*, vol. 351, no. 27, pp. 2883–2885, 2004.

[35] H. Nagahara, K. Mimori, M. Ohta, et al., "Somatic mutations of epidermal growth factor receptor in colorectal carcinoma," *Clinical Cancer Research*, vol. 11, no. 4, pp. 1368–1371, 2005.

[36] M. Moroni, S. Veronese, S. Benvenuti, et al., "Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study," *The Lancet Oncology*, vol. 6, no. 5, pp. 279–286, 2005.

[37] H. J. N. Andreyev, A. R. Norman, D. Cunningham, J. R. Oates, and P. A. Clarke, "Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study," *Journal of the National Cancer Institute*, vol. 90, no. 9, pp. 675–684, 1998.

[38] A. Lièvre, J.-B. Bachet, D. Le Corre, et al., "*KRAS* mutation status is predictive of response to cetuximab therapy in colorectal cancer," *Cancer Research*, vol. 66, no. 8, pp. 3992–3995, 2006.

[39] W. Pao, T. Y. Wang, G. J. Riely, et al., "*KRAS* mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib," *PLoS Medicine*, vol. 2, no. 1, p. e17, 2005.

[40] C. Bokemeyer, I. Bondarenko, J. Hartmann, et al., "*KRAS* status and efficacy of first-line treatment of patients with metastatic colorectal cancer (mCRC) with FOLFOX with or without cetuximab: the OPUS experience," *Journal of Clinical Oncology*, vol. 26, no. 15S, 2008, abstract 4000.

[41] C. S. Karapetis, S. Khambata-Ford, D. J. Jonker, et al., "K-ras mutations and benefit from cetuximab in advanced colorectal cancer," *The New England Journal of Medicine*, vol. 359, no. 17, pp. 1757–1765, 2008.

[42] R. G. Amado, M. Wolf, M. Peeters, et al., "Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 26, no. 10, pp. 1626–1634, 2008.

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Review Article

Anti-EGFR Therapy: Mechanism and Advances in Clinical Efficacy in Breast Cancer

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This review will focus on recent advances in the application of antiepidermal growth factor receptor (anti-EGFR) for the treatment of breast cancer. The choice of EGFR, a member of the ErbB tyrosine kinase receptor family, stems from evidence pinpointing its role in various anti-EGFR therapies. Therefore, an increase in our understanding of EGFR mechanism and signaling might reveal novel targets amenable to intervention in the clinic. This knowledge base might also improve existing medical treatment options and identify research gaps in the design of new therapeutic agents. While the approved use of drugs like the dual kinase inhibitor Lapatinib represents significant advances in the clinical management of breast cancer, confirmatory studies must be considered to foster the use of anti-EGFR therapies including safety, pharmacokinetics, and clinical efficacy.

1. Introduction

Despite the availability of a new array of biomarkers and a widely adapted clinically relevant/treatment-oriented approach of classifying breast cancer cases over the last decade, categorization of breast cancer is an ongoing challenge which is being revisited more frequently by the scientific community. The goal is to fine-tune the diagnostic assignment of breast cancer cases with the hope that this will adequately address and improve the effectiveness of selecting treatment modalities, particularly in regard to the choice of use of monoclonal antibodies (MoAbs) and small molecule tyrosine kinase inhibitors (smTKIs) against EGFR, a clinical strategy collectively referred to as anti-EGFR therapy. EGFR is a member of the ErbB/HER family of tyrosine kinase receptors, which also includes its well-documented family member ErbB2, clinically referred to as HER-2/neu. Anti-EGFR therapy has found application for cases from all three major breast cancer subclasses, respectively, the hormone-sensitive/insensitive group, the ER+/− and HER-2/neu+/− groups, and the basal-like/triple negative (−) group. Of note, HER-2/neu may also be a genetic biomarker since it has a more significant correlation with a selective HER-2 (+ve) population of breast cancer cases than EGFR. Preliminary studies show that anti-EGFR therapy has moderate clinical efficacy not only on EGFR-expressing cells, but on HER-2-expressing and -overexpressing cells as well, suggesting that the treatment outcome may depend on the expression and responsiveness of the heterodimerization of HER-2 with EGFR. Although both EGFR and HER-2 (+ve) are favored biomarkers of efficacy in many ongoing anti-EGFR clinical studies, their expression is not sufficiently robust as a prognosticator for clinical outcomes and should not be singularly used as a criterion for evaluating the responsiveness of breast cancer cases to anti-EGFR treatment regimens [1]. Tumor targets for anti-EGFR therapy include early and advanced stage, and metastatic breast cancer as well as an array of other solid tumors that are not part of this review; data from recent studies suggest that various anti-EGFR/TKI combinations may not only treat but also lower progression rates of these forms of cancer.

The primary focus of this article is to review and summarize recent advances in anti-EGFR therapies in order to generate a clinically relevant profiling system; a complementary objective is to relate the structure of EGFR with its downstream signaling mechanisms particularly in the context of inhibition by administered anti-EGFR therapies. Database search engines like MEDLINE, PubMed, Scopus, and ENTREZ were used, and the articles were selected according to the criteria: (i) anti-EGFR therapy

and clinical efficacy in breast cancer, (ii) publications from 1998–2008, and (iii) using reviews/conferences/special reports/randomized clinical trials/phase II and III trials/general research articles. It is hoped that reviews like this can help to elucidate the mechanisms involved in anti-EGFR therapy as well as define relationships between the overexpression of EGFR and other biomarkers of breast cancer. Recent data regarding responsiveness to combination and multiregiment chemotherapies may also provide insight on the mechanism and activity of anti-EGFR therapies, specifically that of the dual kinase inhibitor, Lapatinib (GW572016), which is capable of targeting both the EGFR and HER-2/neu tyrosine kinases that are often overexpressed in breast cancer cells [4].

2. EGFR and Its Role in Breast Cancer

EGFR is a member of the EGFR/ErbB/HER family of Type I transmembrane tyrosine kinase receptors, which includes ErbB1/HER-1 (EGFR itself), ErbB2/HER-2/neu, ErbB3/HER-3, and ErbB4/HER-4. The ErbB receptors play an essential role in organ development and growth by regulating both the differentiation and morphology of cells and tissues. However, specific members, most notably EGFR, are frequently overexpressed, and this aberrant expression and the signaling event it elicits induce erroneous development and unrestricted proliferation in a number of human malignancies including breast cancer [5]. Members of the ErbB gene family, respectively, ErbB1, ErbB3, and ErbB4 can be activated by various growth factor ligands, for example, the epidermal growth factor (EGF). In contrast, no known ligand has been demonstrated for ErbB2/HER-2/neu, despite that it still plays an integral role in several signaling pathways as well as tumorigenesis. Activation of EGFR inevitably involves homo- or heterodimerization of EGFR with another EGFR molecule, or a different member of the ErbB family (e.g., HER-2), which in turn induces the amplified signaling cascade (Figure 1). Increased activation of EGFR and/or HER-2 will eventually result in uncontrolled proliferation, a hallmark of cancer cells. Additionally, the cells harboring overexpressed EGFR or improper regulation of EGFR activation may decrease apoptosis, increase metastasis and even angiogenesis. Dysfunctional EGFR-signaling networks are reportedly present in a cohort of breast carcinomas with poor prognosis [5, 6].

To better understand the role of EGFR in breast carcinogenesis, the aforementioned relationship will be analyzed in several parts. First, it is important to thoroughly investigate thesignaling pathway and mechanism of EGFR to properly examine the correlation that exists between breast cancer and anomalous EGFR expression; in this case, scrutiny of the structure of EGFR and the role it plays in cell signaling is imperative. Secondly, anti-EGFR therapies for breast cancer either in ongoing clinical phase testing or already FDA-approved are reviewed or summarized in the tables, with focus directed to specific developments and progress in clinical efficacy in recent years. Examples discussed in detail in Section 3 include Cetuximab, a monoclonal antibody against EGFR and the most widely used anti-EGFR therapy in solid tumor treatment regimens; Lapatinib, an innovative small molecule tyrosine kinase inhibitor of EGFR with a unique dual-TKI inhibitory activity against both EGFR and HER-2/neu, which shows improved clinical efficacy and has heightened the expectation for new breast cancer therapies. Although many recent studies have demonstrated a beneficial role of Lapatinib used in combination with anti-EGFR therapy, only selected examples will be reviewed to illustrate how Lapatinib may be strategically explored to improve our understanding of the synergy resulting from its use associated with anti-EGFR therapy.

2.1. EGFR Structure and Signaling Pathway Mechanism. The EGFR and the activated signaling cascades it elicits play an integral role in the mechanism and efficacy of anti-EGFR therapies (Figure 1). Examination of the EGFR structure (Figure 2) provides a contextual framework for the inception and development of two major strategies of anti-EGFR therapy, respectively, anti-EGFR monoclonal antibodies and small molecule tyrosine kinase inhibitors (smTKIs). Examples of monoclonal antibodies for EGFR are found in Cetuximab and Trastuzumab. Anti-EGFR drugs belonging to smTKIs and in clinical trials include Erlotinib, Lapatinib, and Gefitinib.

2.1.1. Molecular Analysis of EGFR Structure

(1) Ectodomain: Ligand-Binding Domain. Domains I–IV make up the EGFR ectodomain, a 621-kDa structure responsible for ligand binding and dimerization, both of which are considered molecular antecedents for the induced conformational changes required for the activation of the internal tyrosine kinase. Although the EGFR ectodomain was first crystallized in 1998, the detailed structure of the EGFR ectodomain dimer bound to ligand EGF was not resolved until 2002 and provided information on a completely novel and unexpected mode of activation for the EGFR signaling pathway involving the dimerization process [2, 7–9].

Domain I or L1 (where L: leucine-rich domain) shares sequence and structural homology with the Domain III or L2, both of which are involved in ligand binding based on site directed mutagenesis and deletion mutation studies [10]. Domains II and IV, also referred to CR1 and CR2 reflecting their high content of cysteine residues and the potential for forming intradomain disulfide bonds, are important in facilitating the overall conformational change induced by binding of the ligand to EGFR. The CR1 also contains a "protruding loop" capable of bending in a relatively straight, ligand-binding site of EGFR (Figure 2(b)). This flexible molecular feature presumably enables binding of the ligand between the two L domains and at the same time permits contacts to be made with the "protruding loops" in CR1.

(2) Transmembrane and Juxtamembrane Domains. The transmembrane domain consists of 23 amino acids and plays an important role in anchoring the receptor to the lipid bilayer of the cell. More than 50% of the

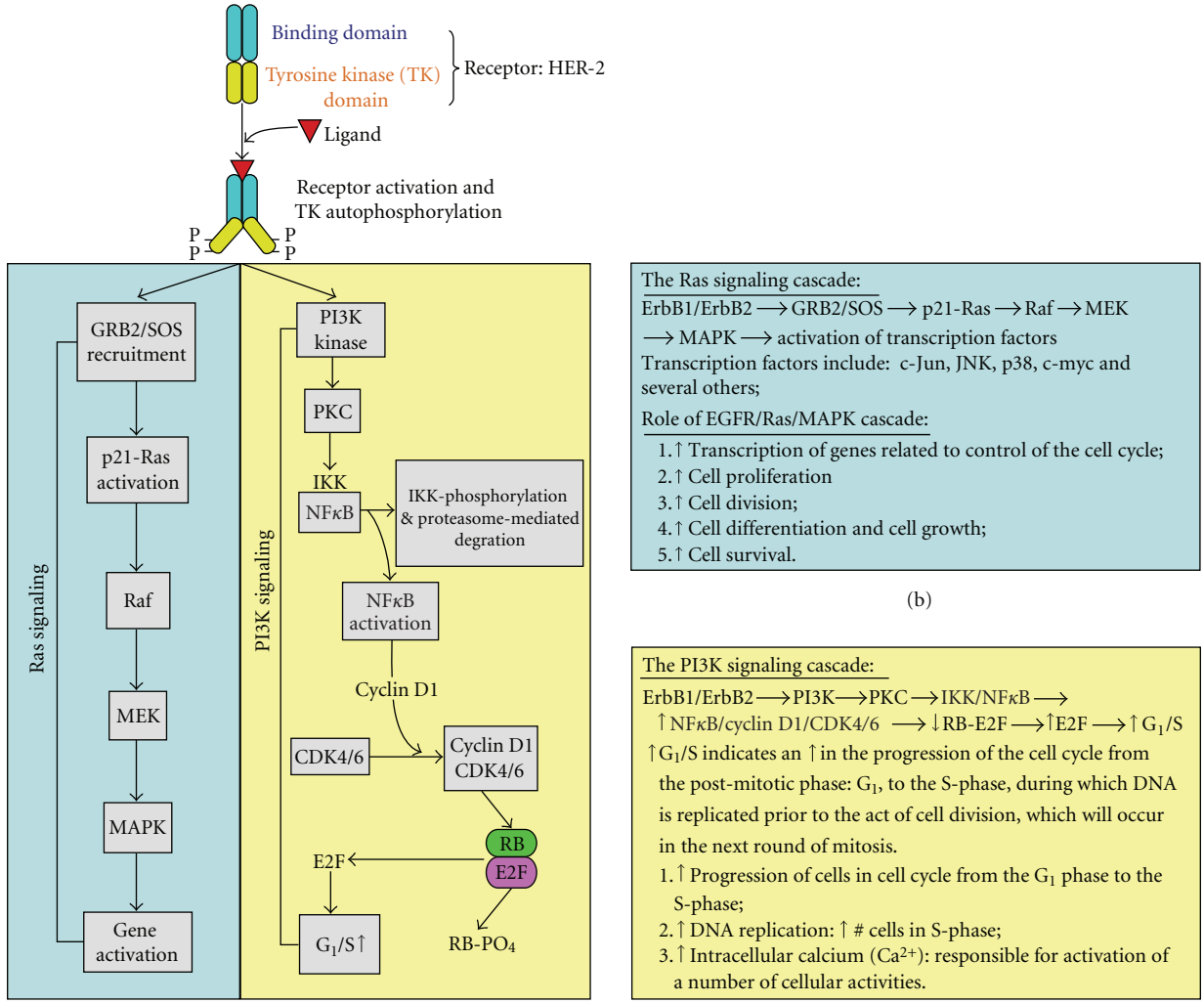


Figure 1: The EGFR Signaling Pathway. (a) Upon EGF-ligand binding to the EGFR there is subsequent dimerization (homo- or hetero-) and tyrosine kinase residue auto-/transphosphorylation of dimer partners, which in turn initiates the actual downstream signaling pathways. (b) Ras signaling cascade in tabulated form. (c) PI3K signaling cascade in tabulated form.

transmembrane domain is localized in caveolae or lipid rafts [10], through posttranslational modifications, such as N-linked glycosylation, resulting in enrichment of EGFR in defined locality of the membrane and hence faster receptor dimerization following binding of the ligand [11]. Adjacent to the transmembrane domain facing intracellularly is the juxtamembrane domain which is believed to regulate various functional aspects of EGFR including control of the tyrosine kinase activity, downregulation of the EGFR, ligand internalization, and receptor sorting. Of note, this domain also has binding motifs that allow it to interact with second messengers like calmodulin [10].

(3) Tyrosine Kinase Domain. The tyrosine kinase domain (TKD) is essential for the functional activation of the receptor and consequently the induction of the EGFR signaling pathways for the control of cell division and proliferation. The TKD has a bilobate arrangement marked by an N-lobe, an activation loop, and a C-lobe [3]. This molecular configuration accommodates binding of the substrate and ATP at the active site, enabling substrate phosphorylation to occur in concomitance with the hydrolysis of ATP (Figure 2(c)). The TKD contains important tyrosine (Y) residues that can assume various states of phosphorylation/dephosphorylation. Knockdown or deletion studies of the ectodomain suggest that it regulates the dimerization as well as prevent constitutive activation of the tyrosine kinase. Binding of the ligand to the ectodomain relieves some of the steric hindrances normally imposed on the tyrosine kinase activity, resulting in activation. Site-directed mutagenesis or deletion analysis in the TKD shows that it is involved in EGFR dimerization, auto- and transphosphorylation and also activation of the signaling cascades, all of which have

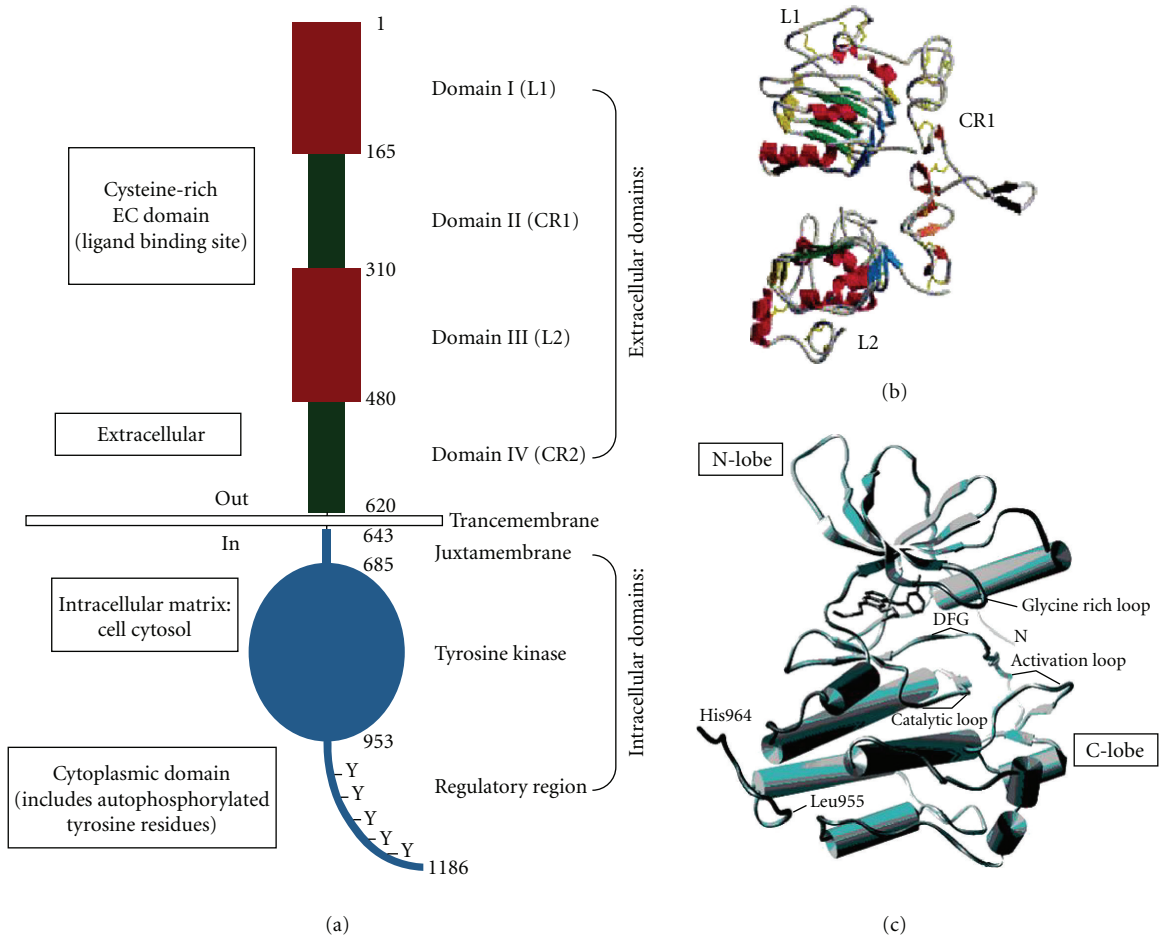


Figure 2: (a) Basic Structure of EGFR demonstrating relevant domains. *(I) The extracellular domains*: (1) domain I: L1; (2) domain II: CR1; domain III: L2; domain IV: CR2. *(II) Transmembrane domains*. *(III) The* intracellular *domains* (1) juxtamembrane domain; (2) tyrosine kinase domain; (3) regulatory region domain. The phosphorylation of several substrates by the tyrosine kinase domain of the EGFR receptor is responsible for activating the various signaling cascades seen in Figure 1. (b) *Structure of domains I–IV of EGFR* (no ligand bound). Note the "protruding loop" in domain II (CR1) directed away from the *C-shaped region* of the ligand-binding zone formed by domains I, II, and III. (c) The tyrosine kinase domain of EGFR showing the N-lobe and C-lobe flanking the activation loop and active site cleft [2, 3].

been exploited in the development of small molecule tyrosine kinase inhibitors (smTKIs) for targeting EGFR in various cancer types including breast cancer.

(4) The Activation Loop, Active Site, and C-Terminal Tail Regions of EGFR. The activation loop of the tyrosine kinase of EGFR is quite distinct from other receptors harboring tyrosine kinases. Namely, whereas most receptor TKDs require phosphorylation for tyrosine kinase activation, this does not appear to be the case in EGFR. For example, the phosphorylation of Tyr^{845} has little affect on the EGFR kinase activity [3], possibly due to a conformational arrangement that directs the activation loop away from the active site rendering it refractory to the state of phosphorylation of the receptor tyrosine kinase. Activation of the EGFR tyrosine kinase phosphorylates numerous targets, including itself (autophosphorylation), a different EGFR (homodimerization), HER-2/neu of the ErbB gene family (heterodimerization), and nonreceptor substrates such as Grb2/SOS, STATs, PLC, and/or PI3K, which in turn initiate the signaling cascades of MAPK/ERK, STAT, PIP_2, and AKT, respectively. Not surprisingly, therefore, mutations in this region can cause a substantial decrease in kinase activity, an outcome considered desirable in cancer therapy and may underlie the therapeutic efficacy of smTKIs. By binding to the TKD of EGFR, smTKIs may act by sterically interfering with the binding of both the substrate and ATP necessary for phosphorylation, resulting in an overall decreased signaling activity of the EGFR.

Lastly, it is important to mention that the tyrosine kinase activity of EGFR is tightly regulated via its own internal regulatory region located at the C-terminal tail of the structure, which involves the tyrosine residue cluster with the potential of being transphosphorylated during EGFR-dimerization. It is noteworthy that EGFR dimerization induces phosphorylation of several tyrosine residues including Tyr^{1069} Tyr^{1092} Tyr^{1110} Tyr^{1116} Tyr^{1172} Tyr^{1197}, creating

docking sites for the recruitment of other adaptor molecules and signaling proteins. These attributes suggest that the tyrosine-rich C-terminal tail is a phosphorylable, mobile structure connected to a relatively stationary TKD.

In summary, the EGFR may be divided into two functional substructures. The first one consists of the extracellular ectodomain responsible for ligand binding, dimerization, and the initiation of signal transduction. The ectodomain has been the thematic target of anti-EGFR therapy, vis-à-vis, development of monoclonal antibody directed at the ligand binding region, which inactivates EGFR through competitive inhibition of ligand binding, as well as by inducing overall downregulation of EGFR through increased receptor internalization. Examples include the monoclonal antibodies like Cetuximab and Trastuzumab, which play an extremely critical role in anti-EGFR therapy. Currently, both these two drugs and Panitumumab are the only anti-EGFR monoclonal antibodies approved by the FDA for use in the clinic. The second major functional substructure of EGFR is the tyrosine kinase domain located on the intracellular side of the plasma membrane. This domain plays a key role in the activation of signaling cascades involved in cell proliferation, division, and differentiation; therefore, inhibition of the tyrosine kinase enzymatic activity of EGFR using small molecule TKIs is a clinically relevant treatment option for breast cancer patients.

2.2. Breast Cancer and the Signaling Mechanism of EGFR. As a member of the ErbB receptor family, the EGFR plays important roles in cell signaling, proliferation, differentiation, and apoptosis. Signaling is initiated by binding of ligands to the extracellular domain of the EGFR. Six well-characterized ligands of EGFR have been identified, respectively, EGF, transforming growth factor-α (TGFα), amphiregulin, heparin binding EGF-like growth factor, betacellulin, and epiregulin. Ligand binding induces conformational change resulting in heterodimerization and the activation of the major signaling pathways seen in Figure 1.

2.2.1. Statistics and Etiology of Breast Carcinogenesis. Breast cancer is the most common cancer and a major cause of morbidity and premature loss of life in women worldwide, accounting for approximately 7% of all cancer-related deaths [12]. The highest rates of breast cancer in the world are seen in the United States, where approximately 1 out of every 8 women will develop invasive breast cancer, which is responsible for almost 3% of all deaths in American women [13]. Given the grim statistics, the need for more sensitive and reliable detection methods is obvious. Equally urgent are treatment modalities that are modest in cost, easily compliant, effective, have low to no toxicities, and capable of targeting the multifaceted and heterogeneous nature of breast carcinogenesis.

Currently, there is still lack of understanding of the natural history of breast cancer. It had been hypothesized that lobular carcinoma in situ (LCIS) represented a precursor lesion of invasive cancer, and, based on this, mastectomy was initially recommended [14]. Later studies have shown that the risk of subsequent breast cancer is bilateral. Moreover, it became evident that LCIS is not a premalignant lesion, but rather a marker that identifies women at an increased risk for subsequent development of invasive breast cancer, with the risk remaining elevated even beyond two decades. In a large prospective study from the National Surgical Adjuvant Breast and Bowel Project involving a 5-year follow-up of 182 women with LCIS managed with excisional biopsy alone, eight women developed ipsilateral breast tumors (four with invasive tumors), and three women developed contralateral breast tumors (two with invasive tumors) [15]. Therefore, it remains unclear whether or not LCIS progresses to ductal carcinoma in situ (DCIS) during breast carcinogenesis. On the other hand, DCIS is a bona fide precursor for invasive ductal carcinoma and lacks estrogen receptor (ER) expression. Furthermore, DCIS frequently overexpresses mutated p53, HER-2/neu, and EGFR, all of which show some clinical correlation with resistance to hormone therapy and increased risk for the development of invasive, metastatic breast cancer. Patients with ER(+ve)/PR(+ve) disease usually respond more favorably to hormonal therapy (as compared to individuals with ER(−ve)/PR(−ve) status), presumably in part due to the overexpression of HER-2 and EGFR in ER(−ve) cells that provide "acquired growth stimulation autonomy." These findings suggest that strategies cotargeting HER-2 and EGFR expression or their functions might have therapeutic and preventive potentials particularly in ER(−ve) breast carcinoma cases.

2.2.2. Expression/Function of HER-2/EGFR and Signaling in Breast Carcinogenesis. The EGFR gene is frequently altered by gene amplification or overexpression at the mRNA and protein levels in sporadic breast cancer cases. Numerous polypeptide ligands sharing an EGF-like motif have been identified and shown to be capable of inducing EGFR dimerization with different kinetics, eliciting signals of variable durations, and coupled signal transduction to specific sets of cytoplasmic proteins. In principle, therefore, this "ligand-initiated receptor-mediated signaling-executed" molecular relay system might generate a large combinatorial set of biological readouts with enormous potential for diversification, fine tuning, and stringent control of cellular functions and responses. Of note, the HER-2/EGFR has been proposed to act as a master regulator of a signaling network that drives breast carcinoma epithelial cell proliferation; HER-2 gene amplification was observed in 92% of breast cancer specimens and overexpression of HER-2 at the mRNA, and protein levels have been correlated with cancer virulence, resistance to therapy, and poor prognosis. As discussed, each member of the EGFR gene family has a multifunction structural organization comprised of an extracellular ligand-binding/interacting domain connected by a transmembrane span to an intracellular kinase domain. In an uninduced state, the EGFR is organized such that the autoinhibitory loops flanking the kinase active site sterically inhibit it from binding substrates. Binding to ligands induces EGFR homo- or heterodimerization concomitant with its autoactivation by a transphosphorylation mechanism involving specific

tyrosine residues located in the intracellular domain. In turn, phosphorylated EGFR undergoes conformational changes that create additional docking sites for adaptor proteins, kinases, and intracellular messengers. Therefore, a tightly regulated, dynamic equilibrium presumably exists between inhibited, activated, monomeric, and dimeric EGFR in order for proper cell signaling to ensue. If any one of these mechanisms goes awry, the results can be fatal to the cell and often can be fatal to the organism. The same considerations may well contribute to the observed clinical efficacy or lack thereof in EGFR-targeted therapies.

The realization that HER-2 is a master regulator of a signaling network that drives epithelial cell proliferation identifies this protein as a target for cancer therapy. When overexpressed, the HER-2 protein may be constitutively active, that is, signaling from the receptor occurs by a ligand-independent manner. Under these conditions, growth-promoting signals may be continuously transmitted into the cells in the absence of ligand. As a result, multiple intracellular signal transduction pathways become activated, resulting in unregulated cell growth and, in some instances, oncogenic transformation. Figure 1 depicts some of the signaling pathways elicited in response to ligand binding to EGFR and either EGFR/EGFR homodimerization or EGFR/HER-2 heterodimerization reactions. It also demonstrates the cascade of events resulting in the transmission of signals into the nucleus and subsequent cell proliferation and gene activation. The intracellular signaling pathways of EGFR and HER-2 are thought to involve Ras-MAPK, and PI3K-, PKC-, NFκB-mediated pathways. Many clinical trials have observed a poor clinical outcome and shortened survival time for women whose breast tumors have HER-2 amplification. An inverse correlation of ER and HER-2 levels between ER(+ve) and ER(−ve) breast cancer cells has been demonstrated, which probably accounts for the development of tamoxifen resistance in breast cancer cells.

2.2.3. Signaling Cross-Talk and Acquisition of Endocrine Resistance. Multiple lines of evidence implicate breast cancer development and progression as under the control of steroid hormones, in particular estrogens, via their interaction with estrogen receptors (ERs) and cross-talk of ER with receptors including EGFR [16–18]. The classical mechanism of ER signaling involves binding of estrogens to intracellular ER, triggering a multitude of events that culminate in altered transcription of estrogen-responsive genes. In sequence, protein synthesis occurs resulting in cell proliferation, angiogenesis, breast cancer growth, progression, and metastasis [19–21]. The ER-induced signaling mechanism coupled with the fact that well over two thirds of breast cancers exhibit high expression of ER, have provided the rationale for preventing and treating breast cancer by estrogen antagonism, highlighted by the discovery of tamoxifen. By selectively modulating the ER, tamoxifen is considered the mainstay of estrogen antagonist therapy and among the most effective systemic treatmentfor women with ER-positive breast cancer at all stages today [21]. A serious obstacle, however, is intrinsicor acquired resistance to endocrine agents. Manypatients present with primary (de novo) resistance to endocrinetherapy, despite high tumor levels of ER, and all patients withadvanced disease eventually acquire resistance [22].

What underlies the refractoriness to endocrine therapies? A number of possible explanations may be considered. For example, in addition to the aforementioned activation of intracellular ER for transcription, estrogens have also been shown to bind membrane-associated ER [20]. Evidence also exists on ER activation by a ligand-independent but growth factor-dependent kinase-mediated mechanism [16]. Important contributing factors for resistance to endocrine therapy include the levels of both ER and ER coregulatory proteins, amplified extra- and intracellular signaling from growth factor-mediatedpathways, as well as cross-talk between the ER pathwayand other growth factor and kinase networks [16–18]. Other mechanisms may involve amplification and/or mutations of key proteins involved in cross-talk, as well induction of promiscuity and/or antagonism to therapeutic agents through mutational and posttranslational modification events [21]. It is possible that aberrations and dysfunctions in these and other mechanisms may occur with increasing frequency during the development of the endocrine-resistant phenotype. Delineation of the interplay between the estrogens, ER, and ER cross-talk with receptors like EGFR will be an important diagnostic and prognostic objective in anti-EGFR therapy. Similarly, discovery and development of novel agents that can reverse resistance by targeting the ER and its downstream signaling events, or by selective modulation of the ER:EGFR cross-talk might improve therapeutic response rates.

In summary, identifying the factors and pathways responsible for endocrine resistance and defining ways to overcome it are research gaps in need of further study and will remain important diagnostic and therapeutic challenges in the continuing war to better manage and treat breast cancer.

2.2.4. Breast Cancer Treatment Using Multitarget Strategy Related to HER-2 Signaling. The amplification of the HER-2 gene and overexpression of the HER-2 protein is frequently observed (10–40%) in human breast cancer patients [23] and has been suggested to associate with tumor aggressiveness, prognosis, and responsiveness to hormonal and cytotoxic agents. These observations suggest that HER-2 is an appropriate target for tumor-specific therapies, some of which are listed as follows.

(1) A humanized monoclonal antibody against HER-2, rhuMAbHER-2 (Trastuzumab), is already approved for clinical use in the treatment of patients with metastatic breast cancer. Some forms of HER-2 overexpressing breast tumors can be successfully treated using antireceptor monoclonal antibodies, *for example*, Herceptin. However, because multiple proteins are involved in growth-signaling pathways, development of a uniformly active therapy may be strategically challenging. Herceptin inhibited the

Table 1: Response criteria and evaluation ratings used in the classification of clinical efficacy and safety/toxicity scoring of anti-EGFR therapies for solid tumors. General classification schemes used in review of clinical efficacy and safety, WHO criteria [24].

General classification schemes used in review of clinical efficacy and safety:
Objective response and tumor response were evaluated by the WHO Criteria [24].
Adverse events (AEs) were assessed at each cycle using the common toxicity criteria (CTC).
Cardiac failure/cardiac toxicity was graded based on the NYHA classification system.
The Cardiac Review and Evaluation Committee (CREC) evaluates cardiac dysfunction.
Factors for clinical efficacy of treatment:
In an intent-to-treat (ITT) population, in order to evaluate the overall response rate of the individual "patient-drug interaction."
Overall response (OR): complete response (CR) + partial response (PR)
Clinical benefit (CB): complete response (CR) + partial response (PR) + stable disease (SD) ≥ 6 months
Time-to-disease-progression (TTP): the time from randomization (randomized initiation of drug/therapy.
Treatment regimen to be tested) to "disease-progression" or death (whichever event occurs first).

P13K-dependent pathway, and not the MAPK pathway. Also, blockade of HER-2 function alone without the interception of the committed, associated downstream events may restrict the effectiveness of therapeutic interventions.

(2) Tyrosine kinase inhibitors, such as emodin, which block HER-2 phosphorylation and its intracellular signaling.

(3) Heat shock protein Hsp90-associated signal inhibitors, which induce degradation of tyrosine kinase receptors, such as HER-2.

3. Classes of Anti-EGFR Therapy

3.1. Advances in the Clinical Efficacy of Anti-EGFR Therapies for Breast Cancer Treatment. The race for a successful breast cancer treatment intensified during the late 1990s and 2000s, resulting in the development of innovative anti-EGFR therapies in the last few years including both monoclonal antibodies (MoAbs) and small molecule tyrosine kinase inhibitors. To systematically analyze and summarize the clinical outcome of these anti-EGFR therapies, it is useful to identify and define key terms used in clinical trials. The relevant key terms can be found in various tables presented below, as appropriate (*see Table* 1).

3.2. Monoclonal Antibodies

3.2.1. Cetuximab. Cetuximab is the most commonly used anti-EGFR therapeutic agent for the treatment of solid tumors. Originally developed for treating colorectal cancer (primary: CRC & metastatic: mCRC) and squamous cell carcinoma of the head and neck and not yet approved as therapy for breast cancer, Cetuximab does provide an excellent model for the development of new MoAbs that may one day be used in breast cancer therapy. As a humanized mouse MoAb similar to others currently in development: for example, EMD72000 (Matuzumab) and hR3 (Nimotuzumab), Cetuximab differs from fully humanized MoAbs like Panitumumab, which have a lower incidence of adverse events (AEs) (e.g., rash, diarrhea) [25]. In addition, although Panitumumab blocks ligand-binding to EGFR and causes receptor internalization like humanized Cetuximab, it does not induce degradation of the receptors [25].

3.2.2. Trastuzumab. Trastuzumab is an anti-HER-2 receptor humanized MoAb that has shown significant clinical benefits for the treatment of HER-2/neu(+ve) metastatic breast cancer as a single agent [26]. Phase II study investigated the clinical efficacy and safety of Trastuzumab monotherapy given as first-line treatment once every 3 weeks in woman with HER-2(+ve) metastatic breast cancer (MBC). In 105 patients receiving five cycles of therapy, the overall response rate was 19% and the clinical benefit rate was 33%. Median time-to-progression was 3.4 months (range, 0.6 to 23.6 months).In general, the monotherapy was well tolerated and no significant AEs were reported. The most common treatment-related AEs were only mild-to-moderate rigors pyrexia, headaches, nausea, and fatigue. Tables 2(a) and 2(b) show the clinical efficacy and common AEs of Trastuzumab monotherapy [27].

Trastuzumab has also been shown to improve survival rates after chemotherapy, specifically in the Herceptin Adjuvant (HERA) study [6]. HERA is an international multicenter-randomized trial comparing 1 or 2 years of Trastuzumab treatment with observation alone after standard neoadjuvant or adjuvant chemotherapy in women with HER-2(+ve) node positive or high-risk node negative breast cancer. In an intention-to-treat analysis of a total of 5102 patients, the unadjusted hazard ratio for the risk of death with Trastuzumab compared with observation alone was 0.66 (95% CI 0.47–0.91; $P = .0115$). Overall, the hazard rates were lower for Trastuzumab treatment group after 1 year compared to the observation group [29]. After 1 year of Trastuzumab treatment, there were 218 disease-free survival events and 59 deaths, whereas 321 disease-free survival events and 90 deaths occurred in the control group. Patients with one or more grade 3 or 4 AEs were 11%, however, there were

Table 2: *Part A*. Response to first-line 3-weekly Trastuzumab monotherapy from Baselga et al. [27]. CR: Complete response; PR: Partial response; SD: Stable disease; CBR: Clinical benefit rate; PD: Progressive disease; ORR: Overall response rate; ITT: Intent-to-treat population. *Part B*. Most common treatment-related adverse events ($n = 105$), adapted from Baselga et al. [27].

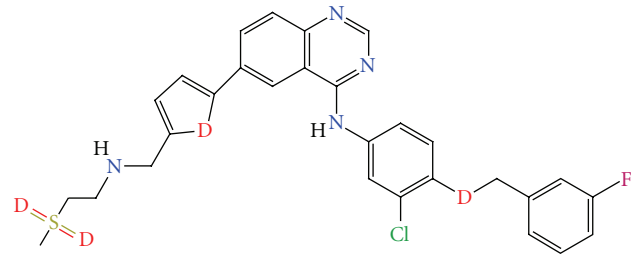
(a)

	No. of patients ($n = 105$)*	
Response	No.	%
CR	2	2
PR	18	17
SD	53	51†
CRB (CR + PR + SD > 6 months)	35	33
PD	30	29
ORR	20	19†

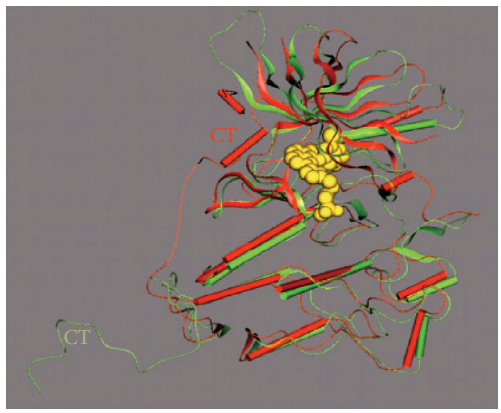
*Data missing for 2 patients.
†One patient with best response of SD in the main study period achieved CR in the 12-month follow-up period. Therefore, in the follow-up analysis, ORR was 20%.

(b)

	No. of patients	
Adverse event	No.	%
Rigors	19	18
Pyrexia	16	15
Headache	11	10
Nausea	10	10
Fatigue	10	10



(a)



(b)

Figure 3: Molecular and crystal structures of EGFR inhibitor Lapatinib and Lapatinib bound and complexed to EGFR ATP-binding pocket, respectively. (a) Molecular structure of Lapatinib (CID208908), an EGFR-ErbB2 inhibitor. (b) Overlay of EGFR in the Lapatinib and Erlotinib complexes. EGFR in the Lapatinib and Erlotinib structures is shown as red and green ribbons, respectively. Lapatinib is shown as a yellow space-filling model. The two proteins were overlaid based on residues in the COOH-terminal domain of the kinase. The COOH-terminal in both structures is CT. Disordered residues in the COOH-terminal tail of EGFR are indicated by a dashed line. The figure was prepared using QUANTA (Accelrys), adapted from Wood et al. [36].

minimal cardiac AEs in the 1 year Trastuzumab group with no reported deaths related to cardiac failures. Therefore, one-year treatment of Trastuzumab after adjuvant chemotherapy has significant overall survival rates and minimal AEs [29].

Trastuzumab in combination with chemotherapeutic agents, specifically paclitaxel and anthracycline (Doxorubicin), has shown significant increases in response rates and disease-free progression (*See Table 3*) [27–29]. The combination prolonged the median time to disease progression from 4.6 to 7.4 months, increased the overall response rate from 32 to 50%, extended duration of response from 6.1 to 9.1 months, and improved 1-year survival times from 68 to 79% compared with chemotherapy alone. The clinical efficacy of Trastuzumab alone and in combination with other chemotherapy options is shown in Table 3. The probability of survival was shown to increase by 25% for 25.4 months with Trastuzumab-*plus*-chemotherapy compared to just 20.3 months for chemotherapy alone. Therefore, it appears that Trastuzumab may sensitize cancer cells to other forms of chemotherapy. AEs were expectedly seen as in other chemotherapy-treated patients with MBC. Thus, the combination of Trastuzumab with chemotherapy (anthracycline or paclitaxel) was active for the treatment of patients with HER-2(+ve) MBC who had not been previously treated for metastatic disease. As expected, the clinical benefits from the treatment with Trastuzumab only apply to HER-2/neu(+ve) breast cancer patients, future studies should be designed using new targeted patient population.

3.3. Tyrosine Kinase Inhibitors

3.3.1. Lapatinib. Lapatinib is a member of the orally active small molecules that reversibly inhibit both ErbB1 and ErbB2 tyrosine kinases, which consequently leads to the down-regulation of both the MAPK and PI3K signaling cascades responsible for cell proliferation and survival, respectively. As a dual kinase inhibitor, Lapatinib has shown activity in a number of different metastatic and advanced tumor cell lines as well as xenografts and has recently shown positive results in clinical testing as well (*see Figure 3 and Tables* 4(a) *and* 4(b)). Inhibition of ErbB1/EGFR alone using Gefitinib and Erlotinib, examples of anti-ErbB1 smTKIs, has shown mixed clinical efficacy results for MBC [31]. Recent studies have demonstrated that it may be advantageous to inhibit ErbB1

Table 3: Efficacy of Trastuzumab when given in combination with chemotherapy in metastatic breast cancer from Slamon et al. [28].

	Tratuzumab + AC ($n = 143$)	AC alone ($n = 138$)	Trastuzumab + paclitaxel ($n = 92$)	Paclitaxel alone ($n = 96$)	Trastuzumab + chemotherapy ($n = 235$)	Chemotherapy alone ($n = 234$)
Median TTP (months)	7.8	6.1	6.9	3	7.4	4.6
	($P = .0004$)		($P = .0001$)		($P = .0001$)	
Response rate (%)	56	42	41	17	50	32
	($P = .0197$)		($P = .0002$)		($P < .0001$)	
Median duration of response (months)	9.1	6.7	10.5	4.5	9.1	6.1
	($P = .0047$)		($P = .00124$)		($P = .0002$)	
Median TTF (months)	7.2	5.6	5.8	2.9	6.9	4.5
	($P = .0014$)		($P = .0001$)		($P = .0001$)	
1-year survival (%)	83	72	72	60	79	68
	($P = .0415$)		($P = .0975$)		($P = .008$)	
Median survival (months)	26.8	22.8	22.8	18.4	25.4	20.3
					($P = .025$)	

AC: Anthracycline; TTP: Time to disease progression; TTF: Time-to-treatment failure.

and ErbB2 simultaneously in those patients overexpressing the ErbB2/HER-2/neu gene, which constitutes approximately 25% of all cases of primary breast cancer [32–35]. Interestingly, although both Lapatinib and Erlotinib bind the ATP-binding site of EGFR, only Lapatinib displays the unique dual kinase inhibitory activity. The molecular underpinning for the observed differences awaits further research in the future [36].

Clinical efficacy and safety of Lapatinib as a monotherapy has been recently tested for HER-2-amplified locally advanced cases or MBC [30]. In a total of 138 patients treated with Lapatinib for a median of 17.6 weeks, the overall response rate was 24% and the clinical benefit was 31%. The median time to response was 7.9 weeks, and the progression-free survival rates at 4 to 6 months were 63% and 43%, respectively. Response rates and common AEs are reported in Table 4. The most common AEs were diarrhea, rash, pruritus, and nausea, which were primarily grade 1 or 2 toxicities. This study supports further use of Lapatinib in first-line and early-stage HER-2-overexpressing breast cancer patients [30].

Combination therapy involving Lapatinib has had mixed results as of 2008. For example, despite the fact that Lapatinib combination therapy with Capecitabine has shown success in treatment for HER-2(+ve) advanced breast cancer treatment, a subpopulation of patients often reported occurrences of grade 4 diarrhea, as well as fatigue, headache, and dizziness [40]. The same study eventually reported a discontinuation of treatment (of combined Lapatinib-*plus*-Capecitabine) due to increased occurrence of AEs in 22 women in the combination-therapy group (13%) [40]. However, it was also reported that 18 women in the monotherapy group (12%), also experienced this high frequency of AEs, which appears to be inconsistent with the safety reports for Lapatinib in a number of other sources, which report no reports of any drug-related grade 4 AEs Table 5 [37, 38, 40].

Furthermore, regarding Phase I safety reports for Lapatinib, there were also no reports of drug-related interstitial pneumonitis or cardiac dysfunction that was normally found to be associated with other forms of ErbB-targeted therapies [37, 38]. There was still a need for further investigations regarding the clinical efficacy, safety, and pharmacokinetics of Lapatinib, and thus these were goals for the Phase II/III clinical trials for Lapatinib. The most commonly reported AEs were diarrhea (42%) and rash (31%); diarrhea incidence increased with increasing dose, whereas rash incidence had no correlation with dose regimen [37]. Lapatinib is well tolerated in doses from 500–1600 mg once daily [37].

Another study from 2006 investigated the dual kinase inhibitor activity of Lapatinib in HER-2-overexpressing breast cancer cells as well as responses of a panel of 31 characterized human breast cancer cell lines to treatment by Trastuzumab, including the use of Trastuzumab-conditioned HER-2(+ve) cell lines [4]. These studies demonstrated four key observations associated with Lapatinib treatment in breast cancer. First, they documented that the anti-proliferative effects of Lapatinib were in fact concentration dependent and were seen in all breast cancer cell lines. Second, they also reported a range of half-maximal inhibitory concentrations for Lapatinib (IC_{50}), however, the study demonstrated a significant amount of variation among these values: $IC_{50} = 0.010–18.6\,\mu$mol/L. Third, these preliminary data were also representative of long-termin vivo Lapatinib treatment regiments for breast cancer; this was ascertained

Table 4: *Part A*. Patient response rate: Lapatinib dose cohort comparison adapted from Gomez et al. [30]. Stable disease patients who had a best response of stable disease (*i.e., stable disease documented for a minimum of 7 weeks*). Clinical benefit response rates include only patients with a best response of CR, PR, or stable disease for at least 24 weeks. Disease status was assessed by an independent panel using response evaluation. Criteria in solid tumors (*see Section* 3.3). CR: Complete response; PR: Partial response. *Part B*. Patients with drug-related adverse events that occurred in >10% of patients receiving Lapatinib, Adapted from Gomez et al. [30].

(a)

	Dosing regimen					
	1500 mg once daily ($n = 69$)		500 mg twice daily ($n = 69$)		All patients ($N = 138$)	
Patient response	No.	%	No.	%	No.	%
Best response						
CR	0	0	0	0	0	0
PR	15	22	18	26	33	24
Stable disease	40	58	31	45	71	51
Progressive disease	8	12	16	23	24	17
Unknown	6	9	4	6	10	7
Response rate: CR or PR, %	21.7		26.1		23.9	
95% CI	12.7 to 33.3		16.3 to 38.1		17.1 to 31.9	
Odds ratio					0.8	
95% CI					0.3 to 1.9	
P					.691	

(b)

	Dosing regimen					
	1500 mg once daily ($n = 69$)		500 mg twice daily ($n = 69$)		All patients ($N = 138$)	
Adverse event*	No.	%	No.	%	No.	%
Diarrhea	24	35	25	36	49	36
Grade 1-2	23	33	22	32	45	33
Grade 3	1	1	3	4	4	3
Rash	19	29	18	26	37	27
Grade 1-2	19	29	17	25	36	26
Grade 3	0	0	1	1	1	1
Pruritus	14	20	11	16	25	18
Grade 1-2	14	20	11	16	25	18
Grade 3	0	0	0	0	0	0
Nausea	9	13	5	7	14	10
Grade 1-2	9	13	4	6	13	9
Grade 3	0	0	1	1	1	1

*No grade 4 adverse events occurred for these conditions.

using a 77 consecutive-day Lapatinib treatment schedule in which there was a significant reduction in the volume of human breast cancer xenografts in athymic mice compared with untreated controls [4]. The reduction in tumor volume demonstrated in the aforementioned clinical study with respect to Lapatinib is consistent with the results obtained in the laboratory setting as well (*see Figure* 4) [37, 38]. Lastly, they examined the synergistic effects of a combinatorial therapy of Lapatinib-*plus*-Trastuzumabfor which their results have indeed provided the preliminary data necessary to support the rationale for continuing research regarding the potential of Lapatinib as a combination anti-EGFR therapy with Trastuzumab in HER-2-overexpressing breast cancer and in patients with clinical resistance to Trastuzumab [4]. Thus, this review, in agreement with several earlier reports, supports further investigation of the benefits of Lapatinib as a first-line treatment regimen for early-stage HER-2-overexpressing breast cancer cell lines as well as its use in the treatment of both metastatic and locally advanced breast cancer cases [40].

3.3.2. Erlotinib. Erlotinib treatment is most commonly found in combination with other chemotherapeutic agents, including Capecitabine and Docetaxel. A study researched

Table 5: Clinical efficacy of Trastuzumab and Lapatinib as monotherapy agents for metastatic breast cancer [26, 27, 30, 39].

Study	No. of patients	Initial and following dose	OR (%)	Median TOP and range (months)
Trastuzumab				
Baselga et al. [27]	105	8 mg/kg, 6 mg/kg triweekly	19	3.4 (range 0.6–23.6)
Cobleigh et al. [26]	222	4 mg/kg, 2 mg/kg weekly	15	3.1 (range 0–≥28)
Vogel et al. [39]	114	4 mg/kg, 2 mg/kg weekly Or 8 mg/kg, 4 mg/kg weekly	26	3.8 (range 3.3–5.3)
Lapatinib				
Gomez et al. [30]	69	1500 mg once daily Or 500 mg twice daily	24	4.4 (range 0.5–23)

OR: Overall response rate; TOP: Time to progression.
To date, most lapatinib therapies are still in progress and currently being evaluated.

Table 6: Overall response for Trastuzumab, Lapatinib, Erlotinib, and Gefitinib combination therapies with chemotherapeutic agents [34, 40–43].

Study	No. of patients	Chemotherapy	Dose	OR (%)
Trastuzumab				
Slamon et al. [34]	143	Doxorubicin	Trastuzumab (4 mg/kg initial dose, 2 mg/kg weekly) Doxorubicin (60 mg/m^2)	56
	92	Paclitaxel	Trastuzumab (4 mg/kg initial dose, 2 mg/kg weekly) Paclitaxel (175 mg/m^2)	41
Marty et al. [43]	186	Docetaxel	Trastuzumab (4 mg/kg initial dose, 2 mg/kg weekly) Docetaxel (100 mg/m^2 triweekly)	34
Lapatinib				
Geyer et al. [40]	163	Capecitabine	Lapatinib (1250 mg/day) Capecitabine (2000 mg/m^2)	22*
Erlotinib				
Twelves et al. [41]	24	Capecitabine, docetaxel	Erlotinib (100 mg/day) Capecitabine (825 mg/m^2) Docetaxel (75 mg/m^2)	68
Gefitinib				
Ciardiello et al. [42]	41	Docetaxel	Geftinib (250 mg/day) Docetaxel (75 mg/m^2 or 100 mg/m^2)	54

OR: Overall response rate.
*Study was performed in women with HER2-positive metastatic breast cancer that has progressed after trastuzumab-based therapy.

the additive efficacy of Erlotinib with Capecitabine and Docetaxel [41]. The combined treatment was administered every 3 weeks, with a total of 24 women with MBC; the overall response rate was 67%. The most common treatment-related AEs were skin toxicities and diarrhea. The severe AEs were relatively low, but as the Capecitabine/Docetaxel doses were increased, the rate of grade 3 events also increased. The tolerability of the regimen has been measured and the group reported an established dosage of Erlotinib (100 mg/day), Capecitabine (825 mg/m^2), and Docetaxel (75 mg/m^2) in patients with MBC [41].

3.3.3. Gefitinib. Gefitinib has oncebeen approved by FDA as monotherapy for patients with locally advanced or metastatic nonsmall-cell lung cancer (NSCLC) [45]. However, more recently, Gefitinib has been used for the treatment of MBC, including a Phase II study of Gefitinib in combination with Docetaxel as first-line therapy in MBC [42]. In 41 patients, a response rate of 54% (95% CI 45–75%), a stable disease response of 14%, and a progressive disease response of 32% were reported. Grade 3 or 4 toxicities that were observed included neutropenia (49%), diarrhea (10%), acne-like rash (5%), and anemia (2%). Overall, the Gefitinib and Docetaxel

Table 7: Efficacy end points in intent-to-treat population, adapted from Geyer et al. [40].

End point	Lapatinib plus capecitabine ($N = 163$)	Capecitabine alone ($N = 161$)	Hazard ratio (95% CI)	P-value
Median time to progression—mo	8.4	4.4	0.49 (0.34–0.71)	<.001†
Median progression-free survival—mo	8.4	4.1	0.47 (0.33–0.67)	<.001†
Overall response—% (95% CI)	22 (16–29)	14 (9–21)		.09‡
Complete response—no. (%)	1 (<1)	0 (0)		
Partial response—no. (%)	35 (21)	23 (14)		
Clinical benefit—no. (%)	44 (27)	29 (18)		
Death—no. (%)	36 (22)	35 (22)		

End Points are based on evaluation by the independent review committee under blinded conditions.
†The P-value was calculated with the log-rank test.
‡The P-value was calculated with Fisher's exact test.

combination demonstrated an active and generally well-tolerated regimen in women with MBC who have not been previously treated with metastatic disease [42]. Gefitinib seemed very promising in early clinical phase testing for the treatment of a number of solid tumors, including NSCLC. However, the FDA recently withdrew Gefitinib from its list of clinically effective therapies for NSCLC, but is still currently under critical review in Phase II/III clinical studies for breast cancer: primary, metastatic, and advanced forms.

With respect to ER-HER2/neu cross-talk in ER/HER2/neu(+ve) breast cancer, Gefitinib has demonstrated promising responses [31]. In a tamoxifen-resistant, HER2-overexpressing MCF-7 breast cancer cell line, designated MCF-7/HER2-18, Gefitinib pretreatment was shown to block ER : EGFR receptor cross-talk, reestablish corepressor complexes with tamoxifen-bound ER on target gene promoters, eliminate tamoxifen agonistic effects, and restore tamoxifen antitumor activity both in vitro and in vivo [23].

3.4. Combinational Therapies

3.4.1. Lapatinib and Anti-ErbB2 Inhibitors: Trastuzumab. The results of Lapatinib and Trastuzumab monotherapy and combined therapy approaches for treating breast cancer are summarized in Tables 6 and 7. A particular combination therapy study involved the comparison of Lapatinib-*plus*-pAb (where pAb is a rabbit polyclonal antisera generated by vaccination with a human ErbB2 fusion protein) and Lapatinib-*plus*-Trastuzumab [46]. This study showed that Lapatinib-*plus*-Trastuzumab combination therapy had enhanced clinical efficacy compared to both Lapatinib and Trastuzumab monotherapies but had similar efficacy as the secondary combination cocktail of Lapatinib-*plus*-pAb [46]. The Lapatinib-*plus*-Trastuzumab combination therapy also showed both a significant downregulation of survivin, an important prosurvival/antiapoptosis protein, as well as enhanced apoptosis [46]. These conclusions along with the information regarding clinical efficacy and AEs in Table 7 provide sufficient preliminary evidence supporting this synergistic cooperation seen in this combination therapy. It appears that Lapatinib may in fact sensitize the cells to further treatment with Trastuzumab thereby enhancing the individual activity of both drugs.

3.4.2. Lapatinib and Capecitabine. Lapatinib combination therapy with Capecitabine has also shown success in treatment for HER-2(+ve) advanced breast cancer treatment (*see Tables 6 and 7*) [40]. Patients with HER-2(+ve) MBC who had progressed after treatment with regimens that included an anthracycline, a taxene, and Trastuzumab were randomly assigned to receive either Capecitabine alone versus Capecitabine in combination with Lapatinib. The hazard ratio for the independently assessed time to progression was 0.49 (95% CI 0.34 to 0.71; $P < .001$); the median time to progression was 8.4 months in the combination therapy group, whereas 4.4 months in the monotherapy group. The most common AEs were diarrhea, the hand-foot syndrome, nausea, vomiting, fatigue, and rash, varying from grades 1 to 3. In grade 4, diarrhea, fatigue, headache, and dizziness were reported. Discontinuation of treatment due to AEs occurred in 22 women in the combination-therapy group (13%) and in 18 women in the monotherapy group (12%) [40]. However, as previously indicated, these reports are refuted by other sources, which claim no grade 4 AEs and only minor toxicity reports for Lapatinib monotherapy, but there are no other reviews specifically regarding further investigation into the relationship between combinational therapies of Lapatinib-*plus*-Capecitabine, if any does in fact exist, positively or negatively correlated with improved outcome.

Therefore, in concurrence with combinational therapies, a major need clearly exists for further research to be done in this specific area of anti-EGFR therapy. There is a significant deficit in the hypotheses and models that can critically evaluate the data reported thus far on most combinational therapies.

4. Conclusion

Breast cancer is a disease that is responsible for approximately 1% of the mortality rate worldwide. The importance of developing new and improved therapies for its treatment

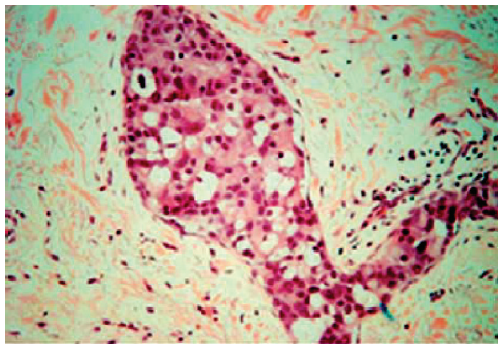
Table 8: Summary of Anti-EGFR therapy agents. The *5* anti-EGFR therapy drugs discussed in this review: these 5 drugs are currently being used or in clinical phase testing for anti-EGFR therapy of breast cancer. All of these agents are either already being used in the clinical setting or are in Phase III clinical development.

Drug name	Other names for the drug	Classification of drug	Target receptor(s) of drug	Special cancer types and efficacy	Important comments	Drug manufacturer
Cetuximab	*Erbitux* (humanized form of the murine MoAb: C225)	MoAb (chimeric IgG_1)	Blocks EGFR;	A large variety of solid tumors: -CRC/mCRC; -SCCHN;	Most widely used anti-EGFR monoclonal antibody used in solid tumor therapy (07/2007) [44];	ImClone Systems, Inc., Princeton, NJ. & NY, NY.
Trastuzumab	*Herceptin*	MoAb (human IgG_1)	Blocks HER2/neu;	Mostly widely used MoAb in treating HER2+ -overexpressing cases of BC;	Extremely important drug in breast cancer;	F. Hoffmann-La Roche Ltd, Basel, Switzerland.
Erlotinib	*Tarceva* (*OSI-774*)	smTKI	Inhibition of EGFR;	Solid tumor therapy: -Pancreatic cancer; -NSCLC (recent);	Nothing unique;	Genetech, Inc., South San Fransisco, CA.
Gefitinib	*Iressa* (*ZD1839*)	smTKI - anilinoquinazoline	Inhibition of EGFR;	Previously used for NSCLC-currently w/d by FDA; In clinical phase testing for BC as well as other metastatic & advanced cancers;	Recently withdrawn by FDA for treatment of NSCLC;	AstraZeneca Pharmaceuticals, Wilmington, DE.
Lapatinib	*Tykerb* (*GW572016*)	smTKI	Both EGFR and HER2/neu; (Dual-TKI Action)	Solid tumor therapy: -BC.	Extremely important smTKI in current BC treatment.	Glaxo-Smith-Kline, Philadelphia, PA.

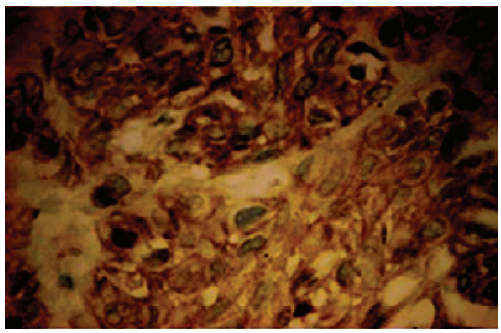
MoAb: Monoclonal antibody; EGFR: Epidermal growth factor receptor (ErbB1); HER-2/neu: Human epidermal growth factor receptor 2; smTKI: Small molecule tyrosine kinase inhibitor; w/d: Withdrawn; NSCLC: Nonsmall cell lung cancer; BC: Breast cancer; CRC: Colorectal cancer; mCRC: Metastatic colorectal cancer; SCCHN: Squamous cell carcinoma of the head and neck.

is therefore undisputable. Recently, advances in anti-EGFR therapy have given hope to the development of new breast cancer therapies with improved specificity, activity, and safety. Increasingly, there is recognition and acceptance of the unique role anti-EGFR therapy plays in the armamentarium of treatment options available to breast cancer patients. Recently, novel members of this group, such as Lapatinib, have been brought to the forefront of this research as it not only is an extremely effective drug in the clinical setting, but it also serves as an excellent model for the development of future EGFR and/or HER-2 inhibitors. The novel dual kinase inhibitor activity of Lapatinib, which displays tyrosine kinase receptor inhibitory activity against both EGFR and HER-2, is both exciting and intriguing. The unique activity of Lapatinib to inhibit both mechanisms of signaling cascades should be studied extensively in order to improve upon the current model of tyrosine kinase inhibition and its role in anti-EGFR therapy. Other drugs with similar activities to Lapatinib, such as CI-1033, a pan-ErbB tyrosine kinase inhibitor, should also be studied thoroughly in order to identify any important similarities between them and to determine how these crucial factors can perhaps be modified to enhance their activity in future anti-EGFR drug prototypes. Other areas of anti-EGFR therapy that should be investigated include the ability of the various anti-EGFR therapeutic modalities to sensitize cancer cells to other forms of chemotherapy originally considered refractory for an individual patient. This is another extremely important avenue that should be investigated exhaustively.

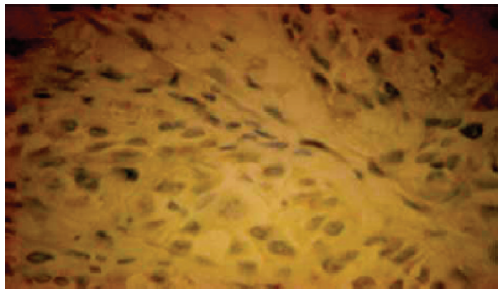
Although there is much improvement to be done, the wealth of knowledge surrounding these therapies continues to grow (*see Table 8*). This observation, along with recent advances in crystallography and docking techniques, the development of improved high-throughput analyses for identifying novel anti-EGFR activity, as well as advances in DNA/RNA-microarray technology used for classification purposes and extremely useful in the clinical setting, all continue to contribute to the overall understanding and development of these new treatment regiments as well as treating breast cancer as a whole. The design rationale of new



(a)



(b)



(c)

Figure 4: Immunohistochemical staining demonstrating the clinical efficacy of Lapatinib. Figure 4 identifies inhibition of activated, phosphorylated ErbB2/HER-2/neu (p-ErbB2) in a breast cancer patient responding to Lapatinib treatment. (a) Shows a dermal-lymphatic invasion (magenta) that is consistent with recurrent inflammatory breast cancer. (b) and (c) Show further immunohistochemical staining for p-ErbB2 performed on tumor biopsy samples obtained from patient X on days 0 (4B) and 21 (4C) of Lapatinib therapy; note the change in positive staining (brownish-yellow). There is a significant decrease in the activation of p-ErbB2 in response to Lapatinib [37, 38].

anti-EGFR therapies lies in the intimate relationship between the mechanisms of action of current forms of treatment and the structure of the EGFR. We believe that meticulous inspection of the unique intermolecular interactions of these drugs with this receptor and its family members will not only lead to future accomplishments in anti-EGFR therapy but will also increase insight into chemotherapy as a whole for breast cancer.

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References

[1] B. Moy and P. E. Goss, "Lapatinib: current status and future directions in breast cancer," *Oncologist*, vol. 11, no. 10, pp. 1047–1057, 2006.

[2] M. C. Boyd, "Epidermal growth factor receptor: structural basis for functional properties," A Literature Review Submitted to the Program for Biochemistry and Molecular Biology at New York Medical College in Partial Fulfillment of the Requirements for the Degree of Master of Science, Laboratory of Dr. Joseph Wu, New York Medical College, Valhalla, NY, USA, 2005.

[3] N. Gotoh, A. Tojo, M. Hino, Y. Yazaki, and M. Shibuya, "A highly conserved tyrosine residue at codon 845 within the kinase domain is not required for the transforming activity of human epidermal growth factor receptor," *Biochemical and Biophysical Research Communications*, vol. 186, no. 2, pp. 768–774, 1992.

[4] G. E. Konecny, M. D. Pegram, N. Venkatesan, et al., "Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells," *Cancer Research*, vol. 66, no. 3, pp. 1630–1639, 2006.

[5] A. Agrawal, E. Gutteridge, J. M. W. Gee, R. I. Nicholson, and J. F. R. Robertson, "Overview of tyrosine kinase inhibitors in clinical breast cancer," *Endocrine-Related Cancer*, vol. 12, supplement 1, pp. S135–S144, 2005.

[6] C. L. Arteaga, S. L. Moulder, and F. M. Yakes, "HER (erbB) tyrosine kinase inhibitors in the treatment of breast cancer," *Seminars in Oncology*, vol. 29, no. 3, supplement 11, pp. 4–10, 2002.

[7] T. P. J. Garrett, N. M. McKern, M. Lou, et al., "Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor," *Nature*, vol. 394, no. 6691, pp. 395–399, 1998.

[8] T. P. J. Garrett, N. M. McKern, M. Lou, et al., "Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor α," *Cell*, vol. 110, no. 6, pp. 763–773, 2002.

[9] H. Ogiso, R. Ishitani, O. Nureki, et al., "Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains," *Cell*, vol. 110, no. 6, pp. 775–787, 2002.

[10] S. Aifa, J. Aydin, G. Nordvall, I. Lundström, S. P. S. Svensson, and O. Hermanson, "A basic peptide within the juxtamembrane region is required for EGF receptor dimerization," *Experimental Cell Research*, vol. 302, no. 1, pp. 108–114, 2005.

[11] C. Mineo, G. N. Gill, and R. G. W. Anderson, "Regulated migration of epidermal growth factor receptor from caveolae," *The Journal of Biological Chemistry*, vol. 274, no. 43, pp. 30636–30643, 1999.

[12] "World Health Organization: Agency for Research on Cancer: Fact Sheet No. 297," July 2008, http://www.who.int/mediacentre/factsheets/fs297/en/index.html.

[13] American Cancer Society, "What Are the Key Statistics fo Breast Cancer?" September 2008, http://www.cancer.org/2008CAFFfinalsecured.pdf.

[14] F. W. Foote and F. W. Stewart, "Lobular carcinoma in situ: a rare form of mammary cancer," *American Journal of Pathology*, vol. 17, pp. 491–496, 1941.

[15] National Cancer Institute: Physician Data Query, "PDQ Adult Treatment Editorial Board," http://www.cancer.gov/cancertopics/pdq/treatment/breast/HealthProfessional.

[16] A. Migliaccio, G. Castoria, M. Di Domenico, et al., "Crosstalk between EGFR and extranuclear steroid receptors," *Annals of the New York Academy of Sciences*, vol. 1089, pp. 194–200, 2006.

[17] D. J. Britton, I. R. Hutcheson, J. M. Knowlden, et al., "Bidirectional cross talk between ERα and EGFR signalling pathways regulates tamoxifen-resistant growth," *Breast Cancer Research and Treatment*, vol. 96, no. 2, pp. 131–146, 2006.

[18] E. R. Levin, "Bidirectional signaling between the estrogen receptor and the epidermal growth factor receptor," *Molecular Endocrinology*, vol. 17, no. 3, pp. 309–317, 2003.

[19] R. B. Lichtner, "Estrogen/EGF receptor interactions in breast cancer: rationale for new therapeutic combination strategies," *Biomedicine and Pharmacotherapy*, vol. 57, no. 10, pp. 447–451, 2003.

[20] E. R. Levin, "Cellular functions of plasma membrane estrogen receptors," *Steroids*, vol. 67, no. 6, pp. 471–475, 2002.

[21] V. C. Jordan and B. W. O'Malley, "Selective estrogen-receptor modulators and antihormonal resistance in breast cancer," *Journal of Clinical Oncology*, vol. 25, no. 36, pp. 5815–5824, 2007.

[22] S. R. D. Johnston, L.-A. Martin, J. Head, I. Smith, and M. Dowsett, "Aromatase inhibitors: combinations with fulvestrant or signal transduction inhibitors as a strategy to overcome endocrine resistance," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 95, no. 1–5, pp. 173–181, 2005.

[23] J. Shou, S. Massarweh, C. K. Osborne, et al., "Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer," *Journal of the National Cancer Institute*, vol. 96, no. 12, pp. 926–935, 2004.

[24] World Health Organization, *WHO Handbook for Reporting Results of Cancer Treatment*, World Health Organization, Geneva, Switzerland, 1979.

[25] P. R. Dutta and A. Maity, "Cellular responses to EGFR inhibitors and their relevance to cancer therapy," *Cancer Letters*, vol. 254, no. 2, pp. 165–177, 2007.

[26] M. A. Cobleigh, C. L. Vogel, D. Tripathy, et al., "Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease," *Journal of Clinical Oncology*, vol. 17, no. 9, pp. 2639–2648, 1999.

[27] J. Baselga, X. Carbonell, N.-J. Castañeda-Soto, et al., "Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule," *Journal of Clinical Oncology*, vol. 23, no. 10, pp. 2162–2171, 2005.

[28] D. Slamon, B. Leyland-Jones, S. Shak, et al., "Addition of Herceptin™ (humanized anti-HER2 antibody) to first line chemotherapy for HER2 overexpressing metastatic breast cancer (HER2+/MBC) markedly increases anticancer activity: a randomized, multinational controlled phase III trial," *Proceedings of the American Society of Clinical Oncology*, vol. 17, no. 98a, abstract 377, 1998.

[29] I. Smith, M. Procter, R. D. Gelber, et al., "2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial," *The Lancet*, vol. 369, no. 9555, pp. 29–36, 2007.

[30] H. L. Gomez, D. C. Doval, M. A. Chavez, et al., "Efficacy and safety of lapatinib as first-line therapy for ErbB2-amplified locally advanced or metastatic breast cancer," *Journal of Clinical Oncology*, vol. 26, no. 18, pp. 2999–3005, 2008.

[31] J. Baselga, J. Albanell, A. Ruiz, et al., "Phase II and tumor pharmacodynamic study of gefitinib in patients with advanced breast cancer," *Journal of Clinical Oncology*, vol. 23, no. 23, pp. 5323–5333, 2005.

[32] W. J. Gullick, S. B. Love, C. Wright, et al., "c-erbB-2 protein overexpression in breast cancer is a risk factor in patients with involved and uninvolved lymph nodes," *British Journal of Cancer*, vol. 63, no. 3, pp. 434–438, 1991.

[33] S. Nicholson, C. Wright, J. R. C. Sainsbury, et al., "Epidermal growth factor receptor (EGFr) as a marker for poor prognosis in node-negative breast cancer patients: neu and tamoxifen failure," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 37, no. 6, pp. 811–814, 1990.

[34] D. J. Slamon, G. M. Clark, S. G. Wong, W. J. Levin, A. Ullrich, and W. L. McGuire, "Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene," *Science*, vol. 235, no. 4785, pp. 177–182, 1987.

[35] S. Tsutsui, S. Ohno, S. Murakami, Y. Hachitanda, and S. Oda, "Prognostic value of epidermal growth factor receptor (EGFR) and its relationship to the estrogen receptor status in 1029 patients with breast cancer," *Breast Cancer Research and Treatment*, vol. 71, no. 1, pp. 67–75, 2002.

[36] E. R. Wood, A. T. Truesdale, O. B. McDonald, et al., "A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells," *Cancer Research*, vol. 64, no. 18, pp. 6652–6659, 2004.

[37] H. A. Burris III, H. I. Hurwitz, E. C. Dees, et al., "Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas," *Journal of Clinical Oncology*, vol. 23, no. 23, pp. 5305–5313, 2005.

[38] N. L. Spector, W. Xia, H. Burris III, et al., "Study of the biologic effects of lapatinib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies," *Journal of Clinical Oncology*, vol. 23, no. 11, pp. 2502–2512, 2005.

[39] C. L. Vogel, M. A. Cobleigh, D. Tripathy, et al., "Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer," *Journal of Clinical Oncology*, vol. 20, no. 3, pp. 719–726, 2002.

[40] C. E. Geyer, J. Forster, D. Lindquist, et al., "Lapatinib plus capecitabine for HER2-positive advanced breast cancer," *The New England Journal of Medicine*, vol. 355, no. 26, pp. 2733–2743, 2006.

[41] C. Twelves, J. M. Trigo, R. Jones, et al., "Erlotinib in combination with capecitabine and docetaxel in patients with metastatic breast cancer: a dose-escalation study," *European Journal of Cancer*, vol. 44, no. 3, pp. 419–426, 2008.

[42] F. Ciardiello, T. Troiani, F. Caputo, et al., "Phase II study of gefitinib in combination with docetaxel as first-line therapy in metastatic breast cancer," *British Journal of Cancer*, vol. 94, no. 11, pp. 1604–1609, 2006.

[43] M. Marty, F. Cognetti, D. Maraninchi, et al., "Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group," *Journal of Clinical Oncology*, vol. 23, no. 19, pp. 4265–4274, 2005.

[44] C. M. Rocha-Lima, H. P. Soares, L. E. Raez, and R. Singal, "EGFR targeting of solid tumors," *Cancer Control*, vol. 14, no. 3, pp. 295–304, 2007.

[45] Federal Drug Administration (FDA Website), "Approved labeling for Gefitinib," http:www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm.

[46] W. Xia, C. M. Gerard, L. Liu, N. M. Baudson, T. L. Ory, and N. L. Spector, "Combining lapatinib (GW572016), a small molecule inhibitor of ErbB1 and ErbB2 tyrosine kinases, with therapeutic anti-ErbB2 antibodies enhances apoptosis of ErbB2-overexpressing breast cancer cells," *Oncogene*, vol. 24, no. 41, pp. 6213–6221, 2005.

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Review Article

Anti-EGFR-Targeted Therapy for Esophageal and Gastric Cancers: An Evolving Concept

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Cancers of the esophagus and stomach present a major health burden worldwide. In the past 30 years we have witnessed some interesting shifts in terms of epidemiology of esophago gastric cancers. Regardless of a world region, the majority of patients diagnosed with esophageal or gastric cancers die from progression or recurrence of their disease. While there are many active cytotoxic agents for esophageal and stomach cancers, their impact on the disease course has been modest at best. Median survival for patients with advanced gastroesophageal cancer is still less than a year. Therefore, novel strategies, based on our understanding of biology and genetics, are desperately needed. Epidermal growth factor receptor (EGFR) pathway has been implicated in pathophysiology of many epithelial malignancies, including esophageal and stomach cancers. EGFR inhibitors, small molecule tyrosine kinase inhibitors and monoclonal antibodies, have been explored in patients with esophageal and gastric cancers. It appears that tumors of the distal esophagus and gastroesophageal junction (GEJ) may be more sensitive to EGFR blockade than distal gastric adenocarcinomas. Investigations looking into potential molecular predictors of sensitivity to EGFR inhibitors for patients with esophageal and GEJ cancers are ongoing. While we are still searching for those predictors, it is clear that they will be different from ones identified in lung and colorectal cancers. Further development of EGFR inhibitors for esophageal and GEJ cancers should be driven by better understanding of EGFR pathway disregulation that drives cancer progression in a sensitive patient population.

1. Introduction

The estimated incidence of esophageal and gastric cancer in the United States is 16 470 and 21 500 in the United States, respectively, in 2008 [1]. Worldwide figures indicate nearly 1 300 000 new cases and an overall mortality of approximately 1 100 000 patients between esophageal and gastric cancers [2], which underscores the global challenge in dealing with these diseases. East Asia makes up for a significant proportion of new cases, with very high rates of gastric and esophageal cancer in China and Japan [3]. Some of the risk factors for the development of esophageal or gastric cancer overlap, including nutritional factors such as smoking and alcohol use. There is however a tremendous heterogeneity in terms of epidemiology of esophageal and gastric cancer. While in developing countries proximal squamous cell esophageal cancers and gastric cancers with intestinal or diffuse type histology still predominate, we have witnessed an epidemiological shift in developed countries, including the United States [4]. This relates not only to tumor histology, with esophageal adenocarcinoma now surpassing squamous carcinoma in incidence, but also to changes in primary tumor location. Adenocarcinomas of the distal esophagus and gastroesophageal junction are becoming increasingly more common than distal gastric cancers in the US and Western world. Interestingly, we are beginning to see the beginning of this trend in some countries in Latin America and Asia in the last decade. The causes of this epidemiological shift are still unclear although there is a suggestion that this phenomenon may be, at least in part, related to eradication of *Helicobacter pylori* infection in developed countries and increased incidence of

gastroesophageal reflux disease in Western world. Significant and recurrent gastroesophageal reflux disease (GERD) is associated with an eightfold increased risk of developing adenocarcinoma of the esophagus [5]. Approximately 5 to 8 percent of patients with GERD develop Barrett's esophagus, a disease characterized by dysplasia of the normal epithelium [6]. Patients with Barrett's are at a high risk of development of adenocarcinoma of the esophagus along with the gastroesophageal junction (GEJ). *Helicobacter pylori (H. pylori)* infection, on the other hand, has been shown to be a significant risk factor for the development of distal gastric cancer [7].

The development of targeted therapies for the treatment of cancers has really taken off recently, with 17 targeted therapies approved by the Food and Drug Administration (FDA) since 2000 [12]. The novel targeted therapies include monoclonal antibodies or small molecule inhibitors targeting either growth factors or growth factor receptor kinases. Of these agents, epidermal growth factor receptor (EGFR) inhibitors have played a visible role in the management of solid malignancies including colorectal cancer, metastatic non small-cell lung cancer (NSCLC), pancreatic cancer, and squamous-cell carcinoma of the head and neck (HNSCC). Currently, there are four EGFR inhibitors approved by the FDA including two small molecule tyrosine kinase inhibitors (erlotinib and gefitinib) and two monoclonal antibodies (cetuximab and panitumumab) [13–17]. The clinical use of EGFR inhibitors will likely continue to increase in the future for two main reasons. First, there are many EGFR inhibitors in the later stages of development [18]. Second, new indications for the current and novel agents are being actively pursued. This review article focuses on current experience in using therapeutic EGFR inhibitors as a therapy for patients with esophageal and gastric cancers.

2. EGFR Pathway and Implications for Therapy of Gastroesophageal Cancers

EGFR, or ErbB1, is a transmembrane receptor and a member of four structurally related tyrosine kinases. EGFR is composed of an extracellular binding domain, a transmembrane portion, and an intracellular cytoplasmic domain with tyrosine kinase functionality. In the event of ligand binding, either homodimerization or heterodimerization can occur. This process leads to tyrosine kinase autophosphorylation and activation [18]. Downstream of EGFR dimerization and activation are multiple processes that can result in cancer cell proliferation, prevention of apoptosis, tumor-induced angiogenesis, and activation of invasion and metastatic growth [19, 20].

The available therapeutic EGFR inhibitors include two classes of agents, monoclonal antibodies and small molecule tyrosine kinase inhibitors. There are significant pharmacological and therapeutic differences between the two classes of agents, which are clinically important. Small molecule tyrosine kinase inhibitors can bind intracellularly at the tyrosine kinase binding domain through competition with ATP. In contrast, monoclonal antibodies bind extracellularly, blocking ligand binding and dimerization of the receptor. Some monoclonal antibodies may have an additional mechanism of action through immune system activation. Immune system activation can result in antibody-dependent cellular cytotoxicity (ADCC) and activation of the complement system [25]. Another significant difference between monoclonal antibodies and small molecule inhibitors is the specificity of the agent. Monoclonal antibodies are very selective in nature, while small molecule TKIs can inhibit additional kinase receptors. This can theoretically increase the efficacy, but may have deleterious effect on the side effect profile [17]. Additionally, there are noteworthy pharmacokinetic differences between the two classes. Small molecule tyrosine kinase inhibitors such as erlotinib and gefitinib are dosed orally on a continuous daily basis due to short half lives. In addition, oral administration may not be practical or effective for some patients with gastrointestinal malignancies due to the lack of anatomic integrity or decreased absorption caused by primary malignancy. Monoclonal antibodies such as cetuximab and panitumumab can only be given intravenously, but have an extended half life of approximately seven days [26]. This does offer increased adaptability of the antibody dosing in regard to a specific regimen (weekly or biweekly), and future studies are exploring feasibility of further prolonging dosing intervals [18, 27]. The disposition of monoclonal antibodies is also more straightforward, as they are cleared and recycled by reticuloendothelial cells, mostly in the liver. This is in contrast to small molecule tyrosine kinase inhibitors metabolized by CYP450 system, which does create a possibility for potentially adverse interactions with other drugs and food ingredients.

An increasingly explored method of predicting the efficacy of EGFR inhibitors is through assessing the cutaneous adverse effects as a correlate of response. Rash is a common adverse effect of EGFR inhibitors and occurs in approximately 45%–100% of patients. Mechanistically, the rash is likely due to the expression of EGFR in the epidermal layers of the skin and is dose dependent [28]. Many studies have shown a consistent relationship between rash and both response to therapy and survival. The first study to report this finding was in patients with colorectal cancer [29] but has been also shown in patients with NSCLC [30], HNSCC [31], and ovarian carcinoma [32]. The observation has been seen with both small molecule TKIs and monoclonal antibodies targeting EGFR. As the rash is both dose dependent and correlates with survival, there is interest in increasing the dose in patients that do not develop a significant rash. In the EVEREST study, a phase I/II study of cetuximab in patients with metastatic colorectal cancer, patients were dose escalated until a greater than grade 2 adverse effect occurred or until a maximum dose of 500 mg/m^2 [33]. Over half of the patients were able to achieve the maximum dose while on treatment. While the primary endpoint was not efficacy and the sample size was small, the single agent response rate was 30% in the escalating dose arm versus 13% in the standard dose arm. While the quality of life and discontinuation rates need to be considered when using this strategy, these results are promising and should be considered in future studies with EGFR inhibitors.

3. Standard Chemotherapy for Esophageal and Gastric Adenocarcinomas

The establishment of standard chemotherapy for esophageal and gastric cancer still remains a moving target, despite of decades of intense clinical investigations [3]. While both esophageal and gastric cancers respond to many different cytotoxic agents, responses are usually short lasting and systemic chemotherapy so far have shown only a modest success in prolonging survival of patients with advanced or metastatic disease [34]. Five-year survival rate for esophageal cancer, all stages included, is only about 15%–25%. This underscores late diagnosis and limited efficacy of potentially curative modalities such as surgery and chemoradiation. For patients with unresectable or metastatic disease, which account for more of the 50% of new cases, prognosis is dismal, with a median survival of less than one year. The role of systemic therapy is palliation. Commonly used chemotherapy regimens for metastatic disease usually include a combination of fluoropyrimidine (5-fluorouracil or capecitabine) and a platinum drug (cisplatin, oxaliplatin or carboplatin) [35, 36]. Taxanes such as docetaxel and paclitaxel have activity, either alone or in combination with a fluoropyrimidine or platinum [37, 38]. Another promising, and also well-tolerated combination is a combination of irinotecan and cisplatin [39]. Older agents, such as anthracyclines (doxorubicin, epirubicin), and topoisomerase II inhibitors (etoposide), or vinca (navelbine), have also been used with a modest success [40]. While there is evidence of some incremental improvement with regard to efficacy and tolerability of chemotherapy combinations, their impact on the natural history of esophageal cancer has been disappointing thus far [41].

For gastric adenocarcinomas, a commonly used regimen is a combination of 5-fluorouracil and cisplatin (CF). More recently, combination regimens such as ECF (epirubicin, cisplatin and fluorouracil) and DCF (docetaxel, cisplatin and fluorouracil) have demonstrated increased efficacy compared to CF but at the expense of additional toxicity [42–44]. Substitution of 5-fluorouracil with capecitabine and of cisplatin with oxaliplatin has resulted in encouraging activity and good tolerability (REAL trial) [45]. Continuous infusion is better tolerated than bolus 5-fluorouracil, especially when it is combined with other drugs, such as irinotecan or oxaliplatin in patients with gastroesophageal carcinomas [46, 47]. S-1 (TS-1) is another oral fluoropyrimidine that has been approved for the therapy of gastric cancer in Japan; confirmatory trials are in progress in Europe and the US [48]. Despite addition of several novel cytotoxic drugs, the median survival for patients with locally advanced unresectable or metastatic gastric cancer still falls short of reaching 12 months. Thus, there is a real need to expand therapeutic options for this group of patients. Lately the focus has been on targeted therapeutics. Newer and better tolerated combination regimens also provide a superior platform for adding and testing novel targeted agents.

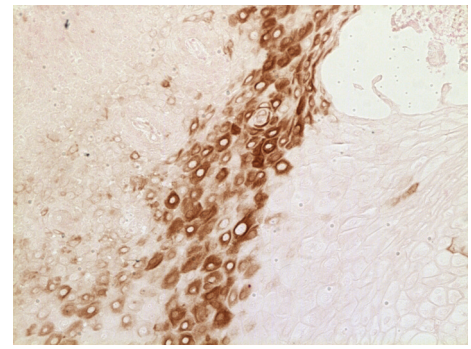

Figure 1: A section of human gastroesophageal adenocarcinoma stained by an anti-EGFR monoclonal antibody and biotin streptavidin 3,3′-diaminobenzidine method (Courtesy of Dr. Amanda Baker, University of Arizona).

4. Evidence for EGFR Pathway Disregulation in Gastric and Esophageal Malignancies

The importance of the EGFR receptor lies in the downstream effects of activation. The primary intracellular pathways implicated following phosphorylation of EGFR are the phosphoinositol-3-kinase (PI3K)/Akt and RAS/mitogen-activated protein kinase (MAPK) pathways [49, 50]. The PI3K pathway is involved in apoptotic and survival signaling, and downstream of this pathway is the mammalian target of rapamycin (MTOR). The RAS/MAPK pathway is involved in cancer cell proliferation, which is responsible for progression from the G1 to S phase, and gene transcription [51].

In esophageal cancer, overexpression of EGFR by immunohistochemistry (IHC) is very common, occurring in approximately 80% of patients with adenocarcinoma and squamous cell carcinoma [52]. Additionally, amplification of the EGFR gene has been detected in some esophageal adenocarcinomas. Fluorescence in situ hybridization (FISH) analysis shows amplification in about 8%–30% of cases [53–55]. Multiple studies have shown that increased EGFR expression is associated with an overall decrease in survival in patients with esophageal cancer [56]. In contrast, overexpression of EGFR by IHC occurs less frequently in gastric cancer, at a rate of less than 40%. Additionally, in a large study of 511 patients only 2.3% of patients had gene amplification measured by FISH [57]. In this study overexpression of EGFR resulted in a statistically significant decrease in survival. Based on these findings, multiple phase I/II studies of small molecule tyrosine kinase inhibitors and monoclonal antibodies have been initiated for patients with esophageal and gastric cancers.

5. Clinical Trials of EGFR Inhibitors in Esophageal and Gastric Cancer

5.1. Tyrosine Kinase Inhibitors. Some of the first clinical trials of EGFR inhibitors in esophageal and gastric cancers were those involving small molecule tyrosine kinase inhibitors. Gefitinib (Iressa) was the first in the new class of small

Table 1: Trials of oral EGFR tyrosine kinase inhibitors.

	Phase	Number of patients	Anatomic site	Histology	Treatment regimen	Outcomes	Comments
Ferry et al. [8]	II	27	Esophagus	27/27 adenocarcinoma	Gefitinib 500 mg PO daily	mOS 4.5 months mPFS 1.9 months 3/27 PR (11%) 7/27 SD (26%)	Prior chemotherapy: 18/27 (67%)
Janmaat et al. [9]	II	36	Esophagus	26/36 adenocarcinoma (72%) 9/36 squamous cell (25%) 1/36 adenosquamous (3%)	Gefitinib 500 mg PO daily	mOS 5.5 months mPFS 2 months 1/36 PR (3%) 10/36 SD (28%)	Second-line treatment. 8/36 not assessable for response
Dragovich et al. [10]	II	70	26/70 Gastric (37%) 44/70 GEJ (63%)	70/70 adenocarcinoma	Erlotinib 150 mg PO daily	mOS GEJ 6.7 months mOS Gastric 3.5 months mTTF GEJ 2 months mTTF Gastric 1.6 months GEJ: 1/43 CR (2%), 3/43 PR (7%), 5/43 SD (12%)	All responses in esophageal GEJ cohort. No responses seen in the gastric cohort
Hecht et al. [11]	II	25	13/25 GEJ (52%) 12/25 Esophagus (48%)	25/25 adenocarcinoma	Lapatinib 1000–1500 mg PO daily	No responses seen 2/25 SD (8%)	Elevated TGF-alpha expression correlated with shorter TTP

molecule inhibitors to be tested clinically. At doses of 250–500 mg/day gefitinib had demonstrated clinical activity, especially in chemotherapy refractory patients with non small cell cancer. Ferry et al. conducted a phase II trial in patients with advanced esophageal carcinoma [8]. Twenty seven patients with unresectable or metastatic adenocarcinoma of the esophagus or gastroesophageal junction, and no more then one prior chemotherapy regimen were treated with 500 mg/d of gefitinib. Overall the therapy was well tolerated with diarrhea and skin rash being the most common adverse events, as expected. The median overall survival was 4.5 months and progression free survival was 1.9 months. There were three (11%) partial responders and 26% of patients had stable disease as their best response. Two of the seven patients tested had EGFR mutations but were not predictive of response. The other markers of EGFR pathway activation were analyzed in paired biopsies but did not correlate with response. Again, due to small number of tissue samples analyzed correlative analyses were of the limited scope. In another study with gefitinib for esophageal cancer, authors tested pre- and post treatment tumor samples in 24 patients. However, no correlation of change in expression of EGFR, pAKT, and pERK was demonstrated [9]. Rojo et al. reported on a pharmacodynamic investigations of tumor biopsies obtained from patients with gastric (77%) and gastroesophageal junction (21%) carcinomas treated with two different doses of gefitinib 250 and 500 mg/d [58]. Authors were able to obtain 46 (out of 70 subjects) paired pre and post-treatment biopsies. Sample analysis was stratified as Japanese and non-Japanese patients and as lower and higher dose of gefitinib. Gefitinib therapy was associated with significant downregulation of phosphorylated EGFR, but not of pMAPK and pAKT. Interestingly, increase in apoptosis was associated with increased exposure (dose) to gefitinib. Although there was some evidence of biological effect on EGFR pathway, it did not translate in clinical benefit in this study. Of note, compared to the other two trials the majority of patients in this trial had distal gastric tumors (see Table 1).

The largest trial in this population was Southwest Oncology Group Trial 0127, which included 70 patients [10]. The patients were stratified on the basis of tumor location on (1) distal esophageal and gastroesophageal junction adenocarcinoma and (2) distal gastric adenocarcinomas. The gastric strata was closed after the first phase due to lack of activity ($n = 26$) while esophageal/GEJ strata completed full accrual ($n = 46$). Interestingly, all of the objective responses (1 complete and 4 partial) were observed in esophageal/GEJ arm (overall response rate 9%, CI 3–22%). Diagnostic archived biopsies were obtained on 54 patients and analyzed for EGFR, pAKT, and TGF-alpha by immunohistochemistry. There was no correlation with anti-tumor activity. Investigators also analyzed tumor biopsies for EGFR gene amplification and for mutations involving exons 18, 19, and 21. There was no evidence of EGFR gene

Table 2: Trials of anti EGFR monoclonal antibodies.

	Phase	Number of patients	Anatomic site	Histology	Treatment regimen	Outcomes	Comments
Gold et al. [21]	II	55	Esophagus	55/55 adenocarcinoma	Cetuximab 400 mg/m^{2}IV × 1, then 250 mg/m^2 IV weekly	mOS 4 months mPFS 1.8 months	2nd line treatment
Ku et al. [22]	II	8	Esophagus/GEJ	7/8 adenocarcinoma (87%) 1/8 squamous cell (13%)	CPT 11 65 mg/m^2 + Cisplatin 30 mg/m^2 weekly 2/3 weeks Cetuximab 400 mg/m^2 × 1, then 250 mg/m^2 IV weekly	mTTP 4.4 months 1 PR, 2 SD	All patients received prior CPT 11/cisplatin. Accrual ongoing
Pinto et al. [23]	II	38	34/38 Gastric 4/38 GEJ	38/38 adenocarcinoma	CPT 11 180 mg/m^2 IV D1 5-FU 400 mg/m^2 IV bolus D1, 5-FU 600 mg/m^2 CIVI D1-2, Leucovorin 100 mg/m^2 IV D1 every 2 weeks × 24 weeks (FOLFIRI) Cetuximab 400 mg/m^2 × 1, then 250 mg/m^2 IV weekly	mTTP 8 months median expected survival 16 months 4/34 CR (12%), 11/34 PR (32%), 16/34 SD (47%)	Untreated advanced/ metastatic disease
Han et al. [24]	II	38	38/38 gastric	38/38 adenocarcinoma	Oxaliplatin 100 mg/m^2 IV D1 Leucovorin 100 mg/m^2 IV D1, 5-FU 1200 mg/m^2/d CIVI × 46 hours (mFOLFOX6) Cetuximab 400 mg/m^2 × 1, then 250 mg/m^{2}IV weekly	mTTP 5.5 months mOS 9.9 months 19/38 PR (50%), 16/38 SD (42%)	EGF and TGF-alpha levels inversely correlated with response

mOS: median overall survival; mPFS: median progression free survival; PR: partial response; SD: stable disease; GEJ: gastroesophageal junction; TTF: time to failure; TTP: time to progression; CPT 11: irinotecan; EGF: epidermal growth factor; TGF-alpha: transforming growth factor-alpha.

amplification or any of selected mutations in 54 tested tissue specimens. In a separate study [59] authors investigated the stability of pAKT in specimens obtained by en-block resection versus those obtained by needle or endoscopic biopsies. There was great variability between two approaches, raising the concern about stability of phosphorylated kinases when tumor samples are obtained by different procedures and from different resources, in a setting of a multicenter trial.

Lapatinib, an oral inhibitor of EGFR and HER 2 was also tested in patients with upper gastrointestinal malignancies [11]. No objective responses were observed and only two of twenty five treated patients achieved disease stabilization.

5.2. Therapeutic Monoclonal Antibodies. Experience with anti-EGFR monoclonal antibodies is less extensive. Investigators from SWOG reported results of a phase II study of cetuximab (Erbitux) in 55 patients with metastaic esophageal adenocarcinoma [21] (see Table 2). The patients were allowed to have one prior chemotherapy regimen for advanced disease. The median overall survival was 4 months and there were three unconfirmed partial responses. A group from Memorial Sloan Kettering reported on their study of a combination of cetuximab plus irinotecan and cisplatin in irinotecan/cisplatin refractory patients with esophageal cancer [22]. Only one partial response was seen out of eight patients that were evaluable for response.

Two trials have been published on the use of cetuximab combination therapy for advanced gastric cancer patients. In a cetuximab + FOLFIRI trial involving 38 patients, 34 had untreated gastric adenocarcinoma [23]. Combination therapy results were promising with a median time-to-progression of 8 months. Correlative analysis of this study showed no association between either EGFR expression or rash and response to cetuximab. The combination of FOLFOX6 and cetuximab was also studied in 38 gastric

cancer patients [24]. Response rates were similar to the previous trial at approximately 50%, but median time-to-progression was 5.5 months. Again, as in the previous trial, EGFR expression was not predictive of response to therapy or overall survival. Low levels of epidermal growth factor (EGF) and transforming growth factor alpha (TGF-a) did correlate with response, but had no statistically significant effect on overall survival.

Based on the currently available clinical data it appears that small molecule EGFR tyrosine kinase inhibitors have activity in gastroesophageal cancers. Trials with gefitinib and erlotinib have consistently demonstrated that the benefit is limited to about 10% of patients with distal esophageal and gastroesophageal junction carcinomas. Gastric adenocarcinomas appear to be resistant, at least in a mono therapy setting. This magnitude of anti tumor activity was seen with EGFR inhibitors in non small cell cancer and colorectal cancer and also with anti-HER2 therapy in patients with breast cancer. However, unlike with lung cancer (EGFR gene amplification, EGFR gene mutation, lack of KRAS mutation) and colorectal cancers (lack of KRAS mutations), molecular markers of sensitivity to EGFR blockade are currently unknown for gastroesophageal carcinomas. Despite a valiant effort to identify these markers, more robust and comprehensive tissue-based analyses are needed in order to better select patients with gastroesophageal adenocarcinomas that may derive clinical benefit from EGFR inhibitors.

6. Conclusion and Future Prospects

EGFR inhibitors have shown modest clinical activity, primarily in patients with esophageal and gastroesophageal junction adenocarcinomas. While there is an always present motivation to quickly integrate targeted therapies and combine them with cytotoxic drugs we believe that it is prudent to make some additional efforts in order to optimize efficacy of these agents before we launch in to large and expensive randomized trials. Could a subset of patients likely to benefit be prospectively identified on the basis of tumor genotype or pharmacogenomic testing? As we have seen, molecular drivers that determine sensitivity to EGFR inhibitors in esophageal and GEJ adenocarcinomas are different from those important in lung and colorectal cancers. This needs further investigation in order to be able to identify subset of patients that will benefit from EGFR blockade. Is it possible to further optimize efficacy by increasing dose of EGFR inhibitors in selected patients (i.e., treat to > grade 2 skin rash), which appears to be true for patients with colorectal cancer? As we have seen, adenocarcinomas of esophagus, gastroesophageal junction, and distal stomach are recognized as distinctive entities in terms of their pathophysiology and epidemiology. This is also likely to be true when we are considering biology of these tumors. By lumping together all these cancers in our clinical trials we are increasing the chance of diluting any significant clinical benefit and reducing our ability to make further progress in terms of drug development. Therefore, it is important that future trials in addition to histology stratify patients based on the location of their primary tumor (i.e., esophageal adenocarcinomas, gastroesophageal junction, and distal gastric tumors) and their molecular characteristics. We expect further advancement of this therapeutic concept for patients with esophago gastric cancers to be driven by development of novel and more potent EGFR inhibitors, along with the development of "omics" technology allowing for a more comprehensive pathway analysis, validation of biologic targets of interest and identification of specific biomarkers.

References

[1] A. Jemal, R. Siegel, E. Ward, et al., "Cancer statistics, 2008," *CA: Cancer Journal for Clinicians*, vol. 58, no. 2, pp. 71–96, 2008.

[2] D. M. Parkin, F. Bray, J. Ferlay, and P. Pisani, "Estimating the world cancer burden: Globocan 2000," *International Journal of Cancer*, vol. 94, no. 2, pp. 153–156, 2001.

[3] A. Ohtsu, "Chemotherapy for metastatic gastric cancer: past, present, and future," *Journal of Gastroenterology*, vol. 43, no. 4, pp. 256–264, 2008.

[4] W. J. Blot and J. K. McLaughlin, "The changing epidemiology of esophageal cancer," *Seminars in Oncology*, vol. 26, no. 5, supplement 15, pp. 2–8, 1999.

[5] J. Lagergren, R. Bergström, A. Lindgren, and O. Nyrén, "Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma," *The New England Journal of Medicine*, vol. 340, no. 11, pp. 825–831, 1999.

[6] P. C. Enzinger and R. J. Mayer, "Esophageal cancer," *The New England Journal of Medicine*, vol. 349, no. 23, pp. 2241–2252, 2003.

[7] P. Correa, J. Fox, E. Fontham, et al., "*Helicobacter pylori* and gastric carcinoma. Serum antibody prevalence in populations with contrasting cancer risks," *Cancer*, vol. 66, no. 12, pp. 2569–2574, 1990.

[8] D. R. Ferry, M. Anderson, K. Beddard, et al., "A phase II study of gefitinib monotherapy in advanced esophageal adenocarcinoma: evidence of gene expression, cellular, and clinical response," *Clinical Cancer Research*, vol. 13, no. 19, pp. 5869–5875, 2007.

[9] M. L. Janmaat, M. I. Gallegos-Ruiz, J. A. Rodriguez, et al., "Predictive factors for outcome in a phase II study of gefitinib in second-line treatment of advanced esophageal cancer patients," *Journal of Clinical Oncology*, vol. 24, no. 10, pp. 1612–1619, 2006.

[10] T. Dragovich, S. McCoy, C. M. Fenoglio-Preiser, et al., "Phase II trial of erlotinib in gastroesophageal junction and gastric adenocarcinomas: SWOG 0127," *Journal of Clinical Oncology*, vol. 24, no. 30, pp. 4922–4927, 2006.

[11] J. R. Hecht, S. G. Urba, M. Koehler, et al., "Lapatinib monotherapy in recurrent gastrointestinal malignancy: phase II efficacy and biomarker analyses," in *Proceedings of the American Society of Clinical Oncology, Gastrointestinal Cancers Symposium*, 2008, abstract no. 43.

[12] "Oncology drugs approved by the FDA," December 2008, http://www.centerwatch.com/patient/drugs/area12.html.

[13] "Cetuximab approved by FDA for treatment of head and neck squamous cell cancer," *Cancer Biology & Therapy*, vol. 5, no. 4, pp. 340–342, 2006.

[14] R. M. Giusti, K. A. Shastri, M. H. Cohen, P. Keegan, and R. Pazdur, "FDA drug approval summary: panitumumab (Vectibix™)," *Oncologist*, vol. 12, no. 5, pp. 577–583, 2007.

[15] M. H. Cohen, J. R. Johnson, Y.-F. Chen, R. Sridhara, and R. Pazdur, "FDA drug approval summary: erlotinib (Tarceva®) tablets," *Oncologist*, vol. 10, no. 7, pp. 461–466, 2005.

[16] P. Laurent-Puig and J. Taieb, "Lessons from Tarceva in pancreatic cancer: where are we now, and how should future trials be designed in pancreatic cancer?" *Current Opinion in Oncology*, vol. 20, no. 4, pp. 454–458, 2008.

[17] M. V. Karamouzis, J. R. Grandis, and A. Argiris, "Therapies directed against epidermal growth factor receptor in aerodigestive carcinomas," *Journal of the American Medical Association*, vol. 298, no. 1, pp. 70–82, 2007.

[18] F. Ciardiello and G. Tortora, "EGFR antagonists in cancer treatment," *The New England Journal of Medicine*, vol. 358, no. 11, pp. 1160–1174, 2008.

[19] N. E. Hynes and H. A. Lane, "ERBB receptors and cancer: the complexity of targeted inhibitors," *Nature Reviews Cancer*, vol. 5, no. 5, pp. 341–354, 2005.

[20] N. E. Hynes and H. A. Lane, "Erratum: ERBB receptors and cancer: the complexity of targeted inhibitors," *Nature Reviews Cancer*, vol. 5, no. 7, p. 580, 2005.

[21] P. J. Gold, B. Goldman, S. Iqbal, et al., "Cetuximab as second-line therapy in patients with metastatic esophageal cancer: a phase II Southwest Oncology Group Study," in *Proceedings of the American Society of Clinical Oncology Annual Meeting*, 2008, abstract no. 4536.

[22] G. Y. Ku, M. A. Shah, L. H. Tang, et al., "Cetuximab (C225) plus irinotecan/cisplatin (CPT/Cis) for CPT/Cis-refractory esophageal cancer," in *Proceedings of the American Society of Clinical Oncology Annual Meeting*, 2008, abstract no. 15580.

[23] C. Pinto, F. Di Fabio, S. Siena, et al., "Phase II study of cetuximab in combination with FOLFIRI in patients with untreated advanced gastric or gastroesophageal junction adenocarcinoma (FOLCETUX study)," *Annals of Oncology*, vol. 18, no. 3, pp. 510–517, 2007.

[24] S.-W. Han, D.-Y. Oh, S.-A. Im, et al., "Phase II study and biomarker analysis of cetuximab combined with modified FOLFOX6 in advanced gastric cancer," *British Journal of Cancer*, vol. 100, no. 2, pp. 298–304, 2009.

[25] K. Imai and A. Takaoka, "Comparing antibody and small-molecule therapies for cancer," *Nature Reviews Cancer*, vol. 6, no. 9, pp. 714–727, 2006.

[26] J. Baselga, D. Pfister, M. R. Cooper, et al., "Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin," *Journal of Clinical Oncology*, vol. 18, no. 4, pp. 904–914, 2000.

[27] J. Tabernero, P. Pfeiffer, and A. Cervantes, "Administration of cetuximab every 2 weeks in the treatment of metastatic colorectal cancer: an effective, more convenient alternative to weekly administration?" *The Oncologist*, vol. 13, no. 2, pp. 113–119, 2008.

[28] M. E. Lacouture, "Mechanisms of cutaneous toxicities to EGFR inhibitors," *Nature Reviews Cancer*, vol. 6, no. 10, pp. 803–812, 2006.

[29] L. Saltz, M. S. Rubin, H. Hochster, et al., "Acne-like rash predicts response in patients treated with cetuximab (IMC-C225) pluls irinotecan (CPT-11) in CPT-11 refractory colorectal cancer (CRC) that expresses epidermal growth factor receptor (EGFR)," *Clinical Cancer Research*, vol. 7, p. 3766s, 2001, abstract no. 559.

[30] B. Wacker, T. Nagrani, J. Weinberg, K. Witt, G. Clark, and P. J. Cagnoni, "Correlation between development of rash and efficacy in patients treated with the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib in two large phase III studies," *Clinical Cancer Research*, vol. 13, no. 13, pp. 3913–3921, 2007.

[31] D. Soulieres, N. N. Senzer, E. E. Vokes, M. Hidalgo, S. S. Agarvala, and L. L. Siu, "Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck," *Journal of Clinical Oncology*, vol. 22, no. 1, pp. 77–85, 2004.

[32] A. N. Gordon, N. Finkler, R. P. Edwards, et al., "Efficacy and safety of erlotinib HCl, an epidermal growth factor receptor (HER1/EGFR) tyrosine kinase inhibitor, in patients with advanced ovarian carcinoma: results from a phase II multicenter study," *International Journal of Gynecological Cancer*, vol. 15, no. 5, pp. 785–792, 2005.

[33] S. Tejpar, M. Peeters, Y. Humblet, et al., "Phase I/II study of cetuximab dose-escalation in patients with metastatic colorectal cancer (mCRC) with no or slight skin reactions on cetuximab standard dose treatment (EVEREST): pharmacokinetic (PK), pharmacodynamic (PD) and efficacy data," *Journal of Clinical Oncology*, vol. 25, no. 18, supplement, 2007, abstract no. 4037.

[34] P. C. Enzinger and R. J. Mayer, "Esophageal cancer," *The New England Journal of Medicine*, vol. 349, no. 23, pp. 2241–2252, 2003.

[35] H. Bleiberg, T. Conroy, B. Paillot, et al., "Randomised phase II study of cisplatin and 5-fluorouracil (5-FU) versus cisplatin alone in advanced squamous cell oesophageal cancer," *European Journal of Cancer*, vol. 33, no. 8, pp. 1216–1220, 1997.

[36] D. H. Ilson, J. Ajani, K. Bhalla, et al., "Phase II trial of paclitaxel, fluorouracil, and cisplatin in patients with advanced carcinoma of the esophagus," *Journal of Clinical Oncology*, vol. 16, no. 5, pp. 1826–1834, 1998.

[37] D. H. Ilson, A. Forastiere, M. Arquette, et al., "A phase II trial of paclitaxel and cisplatin in patients with advanced carcinoma of the esophagus," *Cancer Journal*, vol. 6, no. 5, pp. 316–323, 2000.

[38] S. Petrasch, A. Welt, A. Reinacher, U. Graeven, M. König, and W. Schmiegel, "Chemotherapy with cisplatin and paclitaxel in patients with locally advanced, recurrent or metastatic oesophageal cancer," *British Journal of Cancer*, vol. 78, no. 4, pp. 511–514, 1998.

[39] D. H. Ilson, L. Saltz, P. Enzinger, et al., "Phase II trial of weekly irinotecan plus cisplatin in advanced esophageal cancer," *Journal of Clinical Oncology*, vol. 17, no. 10, pp. 3270–3275, 1999.

[40] T. C. Kok, A. van der Gaast, J. Dees, et al., "Cisplatin and etoposide in oesophageal cancer: a phase II study. Rotterdam Oesophageal Tumour Study Group," *British Journal of Cancer*, vol. 74, no. 6, pp. 980–984, 1996.

[41] M. A. Shah and G. K. Schwartz, "Treatment of metastatic esophagus and gastric cancer," *Seminars in Oncology*, vol. 31, no. 4, pp. 574–587, 2004.

[42] A. Webb, D. Cunningham, J. H. Scarffe, et al., "Randomized trial comparing epirubicin, cisplatin, and fluorouracil versus fluorouracil, doxorubicin, and methotrexate in advanced esophagogastric cancer," *Journal of Clinical Oncology*, vol. 15, no. 1, pp. 261–267, 1997.

[43] E. van Cutsem, V. M. Moiseyenko, S. Tjulandin, et al., "Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V25 Study Group," *Journal of Clinical Oncology*, vol. 24, no. 31, pp. 4991–4997, 2006.

[44] J. A. Ajani, "Optimizing docetaxel chemotherapy in patients with cancer of the gastric and gastroesophageal junction: evolution of the docetaxel, cisplatin, and 5-fluorouracil regimen," *Cancer*, vol. 113, no. 5, pp. 945–955, 2008.

[45] D. Cunningham, N. Starling, S. Rao, et al., "Capecitabine and oxaliplatin for advanced esophagogastric cancer," *The New England Journal of Medicine*, vol. 358, no. 1, pp. 36–46, 2008.

[46] M.-D. Seo, K.-W. Lee, J. H. Lim, et al., "Irinotecan combined with 5-fluorouracil and leucovorin as second-line chemotherapy for metastatic or relapsed gastric cancer," *Japanese Journal of Clinical Oncology*, vol. 38, no. 9, pp. 589–595, 2008.

[47] S. Y. Oh, H.-C. Kwon, B.-G. Seo, S.-H. Kim, J.-S. Kim, and H.-J. Kim, "A phase II study of oxaliplatin with low dose leucovorin and bolus and continuous infusion 5-fluorouracil (modified FOLFOX-4) as first line therapy for patients with advanced gastric cancer," *Acta Oncologica*, vol. 46, no. 3, pp. 336–341, 2007.

[48] J. A. Ajani, F.-C. Lee, D. A. Singh, et al., "Multicenter phase II trial of S-1 plus cisplatin in patients with untreated advanced gastric or gastroesophageal junction adenocarcinoma," *Journal of Clinical Oncology*, vol. 24, no. 4, pp. 663–667, 2006.

[49] A. Toker and M. Yoeli-Lerner, "Akt signaling and cancer: surviving but not moving on," *Cancer Research*, vol. 66, no. 8, pp. 3963–3966, 2006.

[50] P. J. Roberts and C. J. Der, "Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer," *Oncogene*, vol. 26, no. 22, pp. 3291–3310, 2007.

[51] S. Meloche and J. Pouyssegur, "The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition," *Oncogene*, vol. 26, no. 22, pp. 3227–3239, 2007.

[52] M. Takaoka, H. Harada, C. D. Andl, et al., "Epidermal growth factor receptor regulates aberrant expression of insulin-like growth factor-binding protein 3," *Cancer Research*, vol. 64, no. 21, pp. 7711–7723, 2004.

[53] M. Al-Kasspooles, J. H. Moore, M. B. Orringer, and D. G. Beer, "Amplification and over-expression of the EGFR and erbB-2 genes in human esophageal adenocarcinomas," *International Journal of Cancer*, vol. 54, no. 2, pp. 213–219, 1993.

[54] A. M. Rygiel, F. Milano, F. J. Ten Kate, et al., "Gains and amplifications of c-myc, EGFR, and 20.q13 loci in the no dysplasia-dysplasia-adenocarcinoma sequence of barrett's esophagus," *Cancer Epidemiology Biomarkers & Prevention*, vol. 17, no. 6, pp. 1380–1385, 2008.

[55] C. T. Miller, J. R. Moy, L. Lin, et al., "Gene amplification in esophageal adenocarcinomas and barrett's with high-grade dysplasia," *Clinical Cancer Research*, vol. 9, no. 13, pp. 4819–4825, 2003.

[56] R. I. Nicholson, J. M. W. Gee, and M. E. Harper, "EGFR and cancer prognosis," *European Journal of Cancer*, vol. 37, supplement 4, pp. S9–S15, 2001.

[57] M. A. Kim, H. S. Lee, H. E. Lee, Y. K. Jeon, H. K. Yang, and W. H. Kim, "EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number," *Histopathology*, vol. 52, no. 6, pp. 738–746, 2008.

[58] F. Rojo, J. Tabernero, J. Albanell, et al., "Pharmacodynamic studies of gefitinib in tumor biopsy specimens from patients with advanced gastric carcinoma," *Journal of Clinical Oncology*, vol. 24, no. 26, pp. 4309–4316, 2006.

[59] A. F. Baker, T. Dragovich, N. T. Ihle, R. Williams, C. Fenoglio-Preiser, and G. Powis, "Stability of phosphoprotein as a biological marker of tumor signaling," *Clinical Cancer Research*, vol. 11, no. 12, pp. 4338–4340, 2005.

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Research Article

Response of Patient-Derived Non-Small Cell Lung Cancer Xenografts to Classical and Targeted Therapies Is Not Related to Multidrug Resistance Markers

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Tumor cells that are nonsensitive to anticancer drugs frequently have a multidrug resistant (MDR) phenotype. Many studies with cell lines and patient material have been done to investigate the impact of different resistance markers at protein and mRNA level in drug resistance but with contradictory outcome. In the present study, 26 well-characterised patient-derived non-small cell lung cancer xenografts were used. The known chemosensitivity to etoposide, carboplatin, gemcitabine, paclitaxel and erlotinib was compared to the protein and mRNA expression of BCRP, LRP, MDR1, and MRP1. Further, four of these xenografts were short-term treated to analyse possible regulation mechanisms after therapeutic interventions. We found a borderline correlation between the *bcrp* mRNA expression and the response of xenografts to etoposide. All other constitutive mRNA and protein expression levels were not correlated to any drug response and were not significantly influenced by a short term treatment. The present results indicate that the expression levels of MDR proteins and mRNA investigated do not play an important role in the chemoresistance of NSCLC in the in vivo situation.

1. Introduction

Lung cancer is still one of the most frequent cancers with about 1 million incidences worldwide each year. The 5-year survival rate is low with 10–15% compared to other cancers. For chemotherapeutic treatment the classical drugs like etoposide, gemcitabine, carbo- or cisplatin, vinorelbine, docetaxel, and paclitaxel are used. For some years also targeted therapies like tyrosine kinase inhibitors, like gefitinib, and erlotinib have been introduced into clinical trials. However, some patients seem to exhibit an intrinsic resistance or develop an acquired resistance under treatment. It was shown that active drug efflux transporters of the ATP binding cassette (ABC) were involved that actively extrudes a range of structurally and functionally diverse drugs [1, 2]. Three human ABC transporters are primarily associated with the multidrug resistance, namely, P-glycoprotein (P-gp, MDR1, ABCB1), multidrug resistance protein 1 (MRP1, ABCC1), and breast cancer resistance protein (BCRP, ABCG2). They have broad and, to a certain extent, overlapping substrate specificities and are involved in transport processes for a variety of drugs used in chemotherapy. So it was shown that etoposide can be transported by MRP1 [3] and MDR1 that is also able to cause resistance to bulky amphipathic drugs, such as paclitaxel [1]. The lung cancer related protein (LRP) is associated with multidrug resistance because it was found to be overexpressed in an NSCLC cell line selected for doxorubicin resistance that did not express MDR1 [4]. Moreover, it was reported that erlotinib was a substrate for BCRP [5–7].

Most studies used only small numbers of lung cancer cell lines selected for resistance or patient material that was correlated with clinical features [8, 9]. It was turned out that MRP1 played a major role in the intrinsic resistance. Further on, an activation of MDR1 expression during chemotherapy was suggested [10]. Additionally, it was shown that the

response to Taxol-based chemotherapy was related to MDR1 but not LRP expression [11]. These partially conflicting data require further research.

Therefore we initiated a study in patient-derived NSCLC xenografts that were not selected for resistance and revealed a high coincidence with the original tumor [12]. We wanted to address the question if the level of resistance markers on mRNA or protein level is correlated with the response of xenografts to classical cytotoxic drugs (etoposide, carboplatin, gemcitabine, and paclitaxel) or targeted therapy (erlotinib).

2. Methods

2.1. Animal Experiments. 26 recently established NSCLC xenografts were used for this study (Table 1). The chemosensitivity was tested recently [12] so here described only shortly.

All animal experiments were done in accordance with the United Kingdom Co-ordinating Committee on Cancer Research regulations for the Welfare of Animals and of the German Animal Protection Law and approved by the local responsible authorities. The chemotherapeutic responsiveness of the passagable tumors was determined in male NMRI:nu/nu mice. One tumor fragment each was transplanted subcutaneously to the mice. At palpable tumor size (50–100 mm^3) mice each was randomised to treatment and control groups The following drugs and treatment modalities were used: etoposide (Vepesid, Bristol-Meyers Squibb) 10 mg/kg/d, qd 1–5, i.p.; carboplatin (Mayne Pharma Deutschland GmbH) 75 mg/kg/d, qd 1 and 8, i.p.; gemcitabine (Gemzar, Lilly Deutschland) 60–80 mg/kg/d, qd 1, 4, 7, 10, i.p.; paclitaxel (Taxol, Sigma) 12.5 mg/kg/d, qd 1–5, i.v.; erlotinib (Tarceva, Hoffmann-LaRoche) 50 mg/kg/d, qd 1–5, 8–12, orally. Doses and schedules were chosen according to previous experience in animal experiments and represent the maximum tolerated or efficient doses. The injection volume was 0.2 mL/20 g body weight.

In this study, the four models 7406, 7433, 7700, and 7747 were selected because of their differential chemosensitivity (Table 1). 7406 was choosen because it was the only model that not responded to carboplatin and gemcitabine at once but responded to erlotinib. The other models were randomly selected but should represent the high response rates of all tumors to carboplatin and paclitaxel (models 7433, 7747) and gemcitabine and paclitaxel (7700). At the same time they should not respond to more than two drugs to keep the factors of influence low. For the short-term treated xenografts three mice each were randomised to treatment and control groups. The drug doses and application mode were the same as described above except that the treatment was carried out for three days. 24 hours after the last treatment the mice were sacrificed, tumors were snap frozen and stored at −80°C. Total RNA and protein were isolated for the analysis.

2.2. Real-Time PCR. RNA was isolated with RNA Isolation Kit (Qiagen) according to the manufacturers instructions. Total RNA was reversely transcribed using TaqMan Reverse Transcription Reagents (Applied Biosystems (AB)) and TaqMan quantitative real-time PCR performed using cDNA corresponding to 40 ng RNA per reaction. Gene and species specific primers for *bcrp, lrp, mdr1, mrp1,* and *β-actin* and TaqMan Fast Mastermix (AB) were used according to the manufacturers instructions and amplifications carried out on the StepOne Plus Real-Time PCR system (AB) with 45 cycles. Each sample was done in two replicates. Normalised ΔC_T values were obtained by subtracting the *β-actin* C_T from the gene of interest C_T. Tumor samples have been done 2-fold and as positive controls MDA-MB-231/BCRP, A549 and MT3/ADR were used.

2.3. Immunoblotting. Lysates for immunoblotting were prepared by adding lysis buffer (150 mM NaCl, 20 mM Tris, 1% Triton X-100, 0,5% sodiumdesoxycholate, 0.5% SDS, 2 mM EDTA, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphat; pH 7.7) containing protease and phosphatase inhibitors (Sigma-Aldrich) to the tumor tissue. The protein concentration was determined using BioRad Protein Assay (BioRad Laboratories GmbH). Tumor lysates (20 μg) were separated on 8% SDS-page polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were blocked and incubated with the primary antibodies (BCRP, 801-029-C125, Alexis; LRP, 610512, BD) overnight at 4°C. The secondary antibody (115-035-003, Jackson Immuno Research) was conjugated with horseradish peroxidase. Protein bands were visualized using the enhanced chemiluminescence detection system (GE Health Amersham Life Science Inc). To verify equal protein loading, the blots were stripped and reprobed for β-actin (Sigma). MDA-MB-231/BCRP and A549 were used as positive controls.

2.4. FACS Analyses. One piece of each tumour was crudely cut into smaller pieces and further separated with a cell strainer till a cell suspension was obtained. Approximately 1×10^6 cells were used for analyses. After blocking with goat serum, cells were incubated with the primary antibody (MDR1 557001, BD; MRP1, 557594, BD) and secondary Cy3-conjugated goat anti mouse antibody (115-165-146, Jackson Immuno Research). As positive control MT3/ADR breast cancer cells were used.

2.5. Statistical Analyses. Analyses of the mRNA or protein expression levels in comparison with the response to treatment have been done. The correlation according to Spearman and the *P*-values was calculated with the SPSS software. The correlation coefficient (r) could range between 0 (no correlation) and 1 (strong correlation).

3. Results

3.1. Constitutive Protein Expression in the 26 NSCLC Models. BCRP protein was detected in 18/26 xenografts with a weak to strong intensity. LRP could be found in 24 xenografts with

Table 1: Chemosensitivity testing, constitutive protein, and mRNA expression of BCRP, LRP, MDR1, and MRP1 in 26 xenografts and the positive controls. Response: − negativ: 100–50% T/C, + 35–50% T/C, ++ 21–35% T/C, +++ 6–20%T/C, ++++ 0–5% T/C, tox-toxic, n.t.—not tested; protein expression: − not detected, + weak, ++ medium, +++ strong expression; % of positive cells; mRNA expression: normalised ΔC_T values; etp—etoposide, carpl—carboplatin, gem—gemcitabine, paltx—paclitaxel, erlo—erlotinib.

	Before treatment												
	Chemosensitivity					Protein expression				mRNA expression			
LuCa	etp	Carpl	gem	paltx	erlo	BCRP	LRP	MDR1 [%]	MRP1 [%]	bcrp	lrp	mdr1	mrp1
7064	++	−	−	++	++	−	+	15.4	13.8	12.19	8.01	17.54	7.10
7126	−	−	+++	−	++	+++	+	15.3	18.2	4.17	5.04	18.18	3.45
7166	++	++	+	−	−	−	+++	13.7	13.8	15.46	6.45	0	7.16
7177	−	++	+++	−	++	−	++	14.5	29.5	10.42	5.33	16.97	2.02
7187	−	++	+++	−	−	−	++	15.4	18.3	8.13	6.90	0	7.55
7198	−	+	+	+	−	+++	++	27.2	20.9	10.13	6.18	21.24	6.25
7298	+	+	++	++	−	−	++	14.4	13.1	15.03	5.79	17.19	6.33
7336	−	(+)	++	+++	−	+++	+	13.3	13.6	8.08	6.68	0	7.02
7343	−	+++	+++	++	−	+	+	20.8	24.1	8.34	5.85	0	5.23
7387	−	−	+++	++++	−	++	++	15.4	29.9	6.15	7.64	10.90	7.57
7406	+	+	+++	+++	−	+++	+	29.4	22.4	14.87	7.61	14.97	8.10
7414	−	++	+++	+++	+	−	++	17.9	15.6	7.13	5.69	0	6.21
7433	−	+++	−	++++	−	−	+++	18.8	22.1	6.99	5.68	17.20	3.73
7462	−	+	++++	+++	++	+	−	39.8	31.3	7.65	5.24	9.36	7.78
7466	−	−	++++	++++	++	+	++	15.5	18.6	8.62	7.56	22.43	8.65
7506	−	++++	(+)	+++	−	+	+	65.8	19.0	10.96	7.07	15.94	6.98
7530	++++	−	tox	+++	−	+++	+	20.1	16.1	14.27	8.24	14.35	8.01
7558	−	++++	+++	−	−	+++	++	24.0	25.3	15.90	6.26	18.64	6.38
7612	−	++++	−	+++	−	−	+	17.7	15.5	11.09	5.89	16.98	6.04
7668	−	+++	tox	++++	−	+++	−	17.2	24.8	7.55	6.12	13.42	6.17
7700	−	−	++++	++	−	++	+++	18.1	13.9	15.81	6.01	17.32	7.96
7747	−	++	−	++	−	+	++	62.0	15.0	15.19	4.82	18.29	6.26
7766	−	+++	+	++	−	+	+	33.1	18.0	6.72	5.18	17.88	4.14
7860	+	−	++	+++	−	+	++	20.6	22.4	11.16	4.96	16.41	5.47
7913	−	+	(+)	+++	−	+	++	14.0	n.t.	13.50	5.64	18.47	6.17
7915	n.t	n.t	n.t	n.t	−	+	++	16.0	13.8	14.27	8.49	16.21	6.87
MDA-MB-231/BCRP										2.51			
A549											9.97		5.54
MT3/ADR												3.49	

different expression levels. Two NSCLC 7462 and 7668 lacked expression of LRP. MDR1 and MRP1 proteins were detected in all xenografts with an almost equal expression level (see Table 1).

3.2. Constitutive mRNA Expression in the 26 NSCLC Models. *Bcrp* was expressed in the xenografts in a ΔC_T range between 4 (7126) and 16 (7558). *Mdr1* was detected in all xenografts except in five (7166, 7187, 7336, 7343, and 7414). The highest expression with a ΔC_T value of 9 was found in xenograft 7462, the lowest level with a ΔC_T 22 in 7466. Nearly half of the other xenografts (13) had ΔC_T value in a dose range between 15 and 19. The expression of *mrp1* varied from ΔC_T values of 2 to 8. In 13 xenografts a ΔC_T value between 6 and 7 was found. The *lrp* levels ranged in all xenografts between ΔC_T 5 and 8, hence presenting a relatively homogeneous expression. *Bcrp* and *mdr1* had the most heterogeneous mRNA expression pattern, and the overall expression level of*lrp* and *mrp1* was higher than that of *bcrp* and *mdr1* in the 26 xenografts.

A borderline correlation between chemosensitivity and mRNA expression was found in the comparison of etoposide and *bcrp* ($r = 0.490$). All 6 xenografts sensitive to etoposide showed a lower *bcrp* expression ΔC_T 13,8 (±1.6) whereas the resistant tumors had a mean ΔC_T of 9,6 (±3.3). The comparison of the *lrp, mdr1,* and *mrp1* expression with the chemosensitivity towards the different drugs revealed no further correlations.

3.3. mRNA Expression in the Xenografts after Short-Term Treatment. RNA was isolated after short-term treatment of the xenografts 7406, 7433, 7700, and 7747.

In all four xenografts the mRNA of *bcrp*, *lrp, mdr1,* and *mrp1* could be detected. For one and the same xenograft

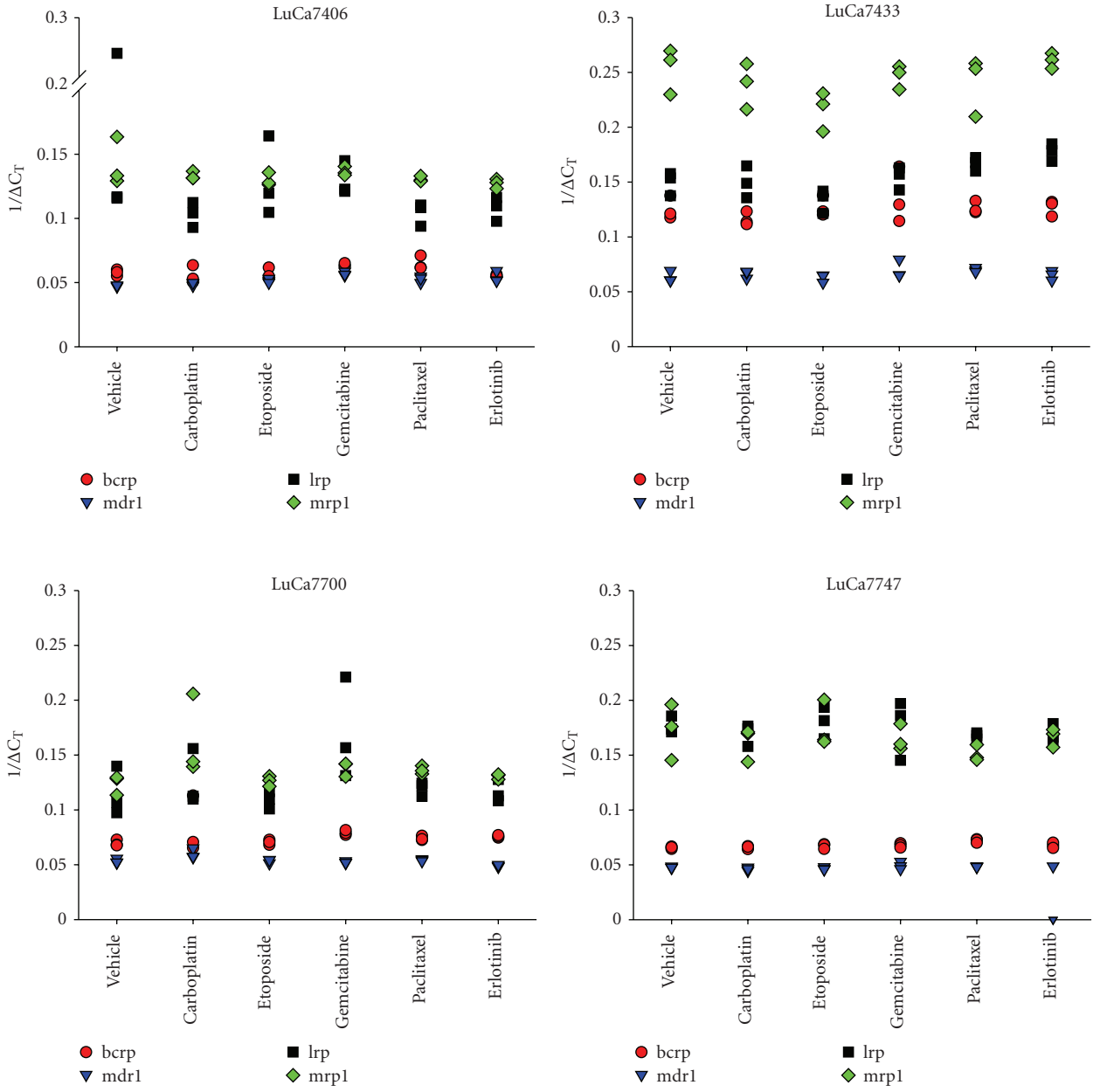

Figure 1: mRNA expression of *bcrp, lrp, mdr1,* and *mrp1* after short-term treatment in xenografts 7406, 7433, 7700, and 7747. Treatment was performed for three consecutive days. Three tumor samples per group were taken 24 hours after last treatment.

the mRNA expression was independent of the treatment (Figure 1). The ΔC_T values differed in a range of two. No significant up- or down-regulations of the mRNA after treatment with etoposide, carboplatin, gemcitabine, paclitaxel, and erlotinib could be observed.

3.4. Protein Expression in the Xenografts after Short-Term Treatment. The BCRP and MRP1 proteins were detected at a medium or weak expression level, whereas LRP had a strong expression in the all xenografts. MDR1 protein could be found in all xenografts. All groups of one model showed an equal expression level of BCRP, LRP, and MDR. There was no regulation detectable after treatment in each NSCLC xenograft (data not shown).

4. Discussion

A large number of studies dealing with questions of intrinsic or acquired drug resistance used cell line-based approaches. Hence, it was shown that amplification and overexpression of *BCRP* emerged as the dominant resistance mechanism in MDR1 and MRP1-deficient mouse fibroblast and kidney cell lines that were selected for resistance to etoposide [13]. In the present study, comparing etoposide response to *bcrp* mRNA expression the tendency was shown that all sensitive

xenografts had a lower expression level than the nonsensitive tumors. Similar correlations could not be found at the protein level. Recently, it was demonstrated that erlotinib was a substrate for BCRP and MDR1 which may explain the resistance seen in the clinics [6]. In our study, we did not observe any correlation between response and BCRP or MDR1 expression neither at protein nor at mRNA level.

Various studies showed that the expression of *LRP* closely reflected the chemoresistance profile of many tumor cell lines and clinical cancer [4, 14–17]. Elevated LRP levels were observed in cell lines resistant to cytotoxic agents like doxorubicin, etoposide, vincristine, and cisplatin [4, 18, 19]. In nonselected NSCLC cells LRP protein and mRNA expression levels correlated with resistance to cisplatin [20]. However, in the present study, no correlation was observed regarding resistance to etoposide, carboplatin or other drugs and the expression of LRP. In other studies, likewise, no correlation with relevant clinical or clinicopathological parameters could be demonstrated [21, 22]. Anyway, in non-small cell and small cell lung cancer patients, the expression was different with the highest expression found in chemoresistant NSCLC [21].

In the present study, a relation of *MRP1* expression neither to cisplatin nor to etoposide response was seen. In contrast, other authors reported that MRP1 expression was correlated with lower chemosensitivity to etoposide, but not to cisplatin in lung cancer cell lines and patients [8, 23, 24]. NSCLC patients were found to exhibit mostly low, but occasionally high MRP1 mRNA expression levels [25]. Another study indicated that either one, or both, MDR1 or MRP1 was frequently expressed in NSCLC, and expression of *mrp1* was found to be predominant over *mdr1* at the mRNA level [26]. This could be confirmed in the present study as it detected almost equal mRNA expression levels among the xenografts. In general, the *mrp1* level was higher than that of the *mdr1*.

For *MDR1* expression also contradictory literature exists. Some concluded that Taxol-based chemotherapy response of NSCLC patients was related to MDR1 but not LRP expression [11] while others suggested that MDR proteins (LRP, MDR1, and MRP1) may not play an important role in the chemoresistance and drug efflux of NSCLC cells [9]. We were not able to demonstrate any correlation between the chemosensitivity and the expression of MDR. Even after short-term treatment no remarkable changes of mRNA or protein could be observed.

One reason for the different results described in literature and found by us could be the model system used. While we used patient-derived xenografts that were not selected for any drug resistance, many other studies included cell lines passaged over years or selected for resistance under high drug concentrations. The *in vivo* situation is different because the drug availability and exact "in-tumor" concentration are not exactly known. However, the response rates of xenografts were similar to those observed in human Phase II studies with the same agents [12, 27]. Patient-derived xenografts allow the detailed investigation of therapy related markers and their dynamic regulation in a well-standardized and clinically related way.

Moreover, the multidrug resistance is regarded to be a multifactorial phenomenon in which more than the markers studied in the present study could be involved.

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References

[1] P. Borst and R. O. Elferink, "Mammalian ABC transporters in health and disease," *Annual Review of Biochemistry*, vol. 71, pp. 537–592, 2002.

[2] A. H. Schinkel and J. W. Jonker, "Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview," *Advanced Drug Delivery Reviews*, vol. 55, no. 1, pp. 3–29, 2003.

[3] É. Bakos and L. Homolya, "Portrait of multifaceted transporter, the multidrug resistance-associated protein 1 (MRP1/ABCC1)," *Pflügers Archiv European Journal of Physiology*, vol. 453, no. 5, pp. 621–641, 2007.

[4] R. J. Scheper, H. J. Broxterman, G. L. Scheffer, et al., "Overexpression of a M_r 110,000 vesicular protein in non-P-glycoprotein-mediated multidrug resistance," *Cancer Research*, vol. 53, no. 7, pp. 1475–1479, 1993.

[5] J. Li, G. Cusatis, J. Brahmer, et al., "Association of variant ABCG2 and the pharmacokinetics of epidermal growth factor receptor tyrosine kinase inhibitors in cancer patients," *Cancer Biology and Therapy*, vol. 6, no. 3, pp. 432–438, 2007.

[6] S. Marchetti, N. A. de Vries, T. Buckle, et al., "Effect of the ATP-binding cassette drug transporters ABCB1, ABCG2, and ABCC2 on erlotinib hydrochloride (Tarceva) disposition in in vitro and in vivo pharmacokinetic studies employing Bcrp1$^{-/-}$/Mdr1a/1b$^{-/-}$ (triple-knockout) and wild-type mice," *Molecular Cancer Therapeutics*, vol. 7, no. 8, pp. 2280–2287, 2008.

[7] C. Özvegy-Laczka, J. Cserepes, N. B. Elkind, and B. Sarkadi, "Tyrosine kinase inhibitor resistance in cancer: role of ABC multidrug transporters," *Drug Resistance Updates*, vol. 18, no. 1, pp. 15–26, 2005.

[8] W. Berger, L. Elbling, E. Hauptmann, and M. Micksche, "Expression of the multidrug resistance-associated protein (MRP) and chemoresistance of human non-small-cell lung cancer cells," *International Journal of Cancer*, vol. 73, no. 1, pp. 84–93, 1997.

[9] K. Ikuta, K. Takemura, K. Sasaki, et al., "Expression of multidrug resistance proteins and accumulation of cisplatin in human non-small cell lung cancer cells," *Biological & Pharmaceutical Bulletin*, vol. 28, no. 4, pp. 707–712, 2005.

[10] W. Berger, U. Setinek, P. Hollaus, et al., "Multidrug resistance markers P-glycoprotein, multidrug resistance protein 1, and lung resistance protein in non-small cell lung cancer: prognostic implications," *Journal of Cancer Research and Clinical Oncology*, vol. 131, no. 6, pp. 355–363, 2005.

[11] J.-F. Chiou, J.-A. Liang, W.-H. Hsu, J.-J. Wang, S.-T. Ho, and A. Kao, "Comparing the relationship of taxol-based chemotherapy response with P-glycoprotein and lung resistance-related

protein expression in non-small cell lung cancer," *Lung*, vol. 181, no. 5, pp. 267–273, 2003.

[12] I. Fichtner, J. Rolff, R. Soong, et al., "Establishment of patient-derived non-small cell lung cancer xenografts as models for the identification of predictive biomarkers," *Clinical Cancer Research*, vol. 14, no. 20, pp. 6456–6468, 2008.

[13] J. D. Allen, S. C. van Dort, M. Buitelaar, O. van Tellingen, and A. H. Schinkel, "Mouse breast cancer resistance protein (Bcrp1/Abcg2) mediates etoposide resistance and transport, but etoposide oral availability is limited primarily by P-glycoprotein," *Cancer Research*, vol. 63, no. 6, pp. 1339–1344, 2003.

[14] M. A. Izquierdo, G. L. Scheffer, M. J. Flens, R. H. Shoemaker, L. H. Rome, and R. J. Scheper, "Relationship of LRP-human major vault protein to in vitro and clinical resistance to anticancer drugs," *Cytotechnology*, vol. 19, no. 3, pp. 191–197, 1996.

[15] V. A. Kickhoefer, K. S. Rajavel, G. L. Scheffer, W. S. Dalton, R. J. Scheper, and L. H. Rome, "Vaults are up-regulated in multidrug-resistant cancer cell lines," *The Journal of Biological Chemistry*, vol. 273, no. 15, pp. 8971–8974, 1998.

[16] A. B. Schroeijers, A. C. Siva, G. L. Scheffer, et al., "The M_r193,000 vault protein is up-regulated in multidrug-resistant cancer cell lines," *Cancer Research*, vol. 60, no. 4, pp. 1104–1110, 2000.

[17] A. C. Siva, S. Raval-Fernandes, A. G. Stephen, et al., "Up-regulation of vaults may be necessary but not sufficient for multidrug resistance," *International Journal of Cancer*, vol. 92, no. 2, pp. 195–202, 2001.

[18] C. M. Laurençot, G. L. Scheffer, R. J. Scheper, and R. H. Shoemaker, "Increased LRP mRNA expression is associated with the MDR phenotype in intrinsically resistant human cancer cell lines," *International Journal of Cancer*, vol. 72, no. 6, pp. 1021–1026, 1997.

[19] B. Wyler, Y. Shao, E. Schneider, et al., "Intermittent exposure to doxorubicin in vitro selects for multifactorial non-P-glycoprotein-associated multidrug resistance in RPMI 8226 human myeloma cells," *British Journal of Haematology*, vol. 97, no. 1, pp. 65–75, 1997.

[20] W. Berger, L. Elbling, and M. Micksche, "Expression of the major vault protein LRP in human non-small-cell lung cancer cells: activation by short-term exposure to antineoplastic drugs," *International Journal of Cancer*, vol. 88, no. 2, pp. 293–300, 2000.

[21] A.-M. C. Dingemans, J. van Ark-Otte, P. van der Valk, et al., "Expression of the human major vault protein LRP in human lung cancer samples and normal lung tissues," *Annals of Oncology*, vol. 7, no. 6, pp. 625–630, 1996.

[22] M. Volm, J. Mattern, and R. Koomagi, "Expression of lung resistance-related protein (LRP) in non-small cell lung carcinomas of smokers and non-smokers and its predictive value for doxorubicin resistance," *Anticancer Drugs*, vol. 8, no. 10, pp. 931–936, 1997.

[23] G. Giaccone, J. van Ark-Otte, G. J. Rubio, et al., "MRP is frequently expressed in human lung-cancer cell lines, in non-small-cell lung cancer and in normal lungs," *International Journal of Cancer*, vol. 66, no. 6, pp. 760–767, 1996.

[24] M. Filipits, V. Haddad, K. Schmid, et al., "Multidrug resistance proteins do not predict benefit of adjuvant chemotherapy in patients with completely resected non-small cell lung cancer: International Adjuvant Lung Cancer Trial Biologic Program," *Clinical Cancer Research*, vol. 13, no. 13, pp. 3892–3898, 2007.

[25] K. Nooter, A. M. Westerman, M. J. Flens, et al., "Expression of the multidrug resistance-associated protein (MRP) gene in human cancers," *Clinical Cancer Research*, vol. 1, no. 11, pp. 1301–1310, 1995.

[26] S. Roy, E. Kenny, S. Kennedy, et al., "MDR1/P-glycoprotein and MRP-1 mRNA and protein expression in non-small cell lung cancer," *Anticancer Research*, vol. 27, no. 3A, pp. 1325–1330, 2007.

[27] R. Perez-Soler, B. Kemp, Q. P. Wu, et al., "Response and determinants of sensitivity to paclitaxel in human non-small cell lung cancer tumors heterotransplanted in nude mice," *Clinical Cancer Research*, vol. 6, no. 12, pp. 4932–4938, 2000.

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Review Article

TGF-β1-Induced Expression of the Poor Prognosis SERPINE1/PAI-1 Gene Requires EGFR Signaling: A New Target for Anti-EGFR Therapy

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Increased transforming growth factor-β (TGF-β) expression and epidermal growth factor receptor (EGFR) amplification accompany the emergence of highly aggressive human carcinomas. Cooperative signaling between these two growth factor/receptor systems promotes cell migration and synthesis of stromal remodeling factors (i.e., proteases, protease inhibitors) that, in turn, regulate tumor invasion, neo-angiogenesis and inflammation. ranscript profiling of transformed human cells revealed that genes encoding wound healing, matrix remodeling and cell cycle proteins (i.e., the "tissue repair" transcriptome) are significantly up-regulated early after growth factor stimulation. The major inhibitor of plasmin generation, plasminogen activator inhibitor-1 (PAI-1), is among the most highly induced transcripts during the phenotypic transition initiated by TGF-β maximal expression requires EGFR signaling. PAI-1 induction occurs early in the progression of incipient epidermal squamous cell carcinoma (SCC) and is a significant indicator of poor prognosis in epithelial malignancies. Mouse modeling and molecular genetic analysis of complex systems indicates that PAI-1 regulates the temporal/spatial control of pericellular proteolysis, promotes epithelial plasticity, inhibits capillary regression and facilitates stromal invasion. Defining TGF-β1-initiated signaling events that cooperate with an activated EGFR to impact the protease-protease inhibitor balance in the tumor microenvironment is critical to the development of novel therapies for the clinical management of human cancers.

1. Introduction

Transition of a normal epithelial cell to an early malignant phenotype often involves mutation of the p53 and p21^{ras} genes and progressive increases in autocrine TGF-β1 expression [1–10]. Elevated TGF-β1 production, in fact, typifies advanced pathologies in both mouse and human SCC [8, 10, 11]. Despite relatively high concentrations of TGF-β in the immediate tumor microenvironment, some malignant epithelial cells become refractory to TGF-β1-initiated proliferative arrest likely due to reductions in either TGF-βRII and/or SMAD4 levels as well as the now recognized p21^{ras}-dependent antagonism of TGF-β1-mediated growth inhibition/apoptosis [10–13]. In certain epithelial malignancies, moreover, resistance to TGF-β1-mediated growth suppression is often coupled with EGFR amplification or dysregulated EGFR signaling, particularly during the later stages of tumor development [14–19]. The associated reprogramming of gene expression initiates and perpetuates TGF-β1-induced cellular "plasticity" (usually referred to as epithelial-to-mesenchymal transition or EMT) which facilites tumor invasion and metastasis [8, 20–25].

Microarray of the EMT transcriptome in several clinically relevant model systems has provided insights into the specific repertoire of "plasticity" genes. Plasminogen activator inhibitor type-1 (PAI-1; SERPINE1), the major physiologic regulator of the pericellular plasmin-generating cascade, is a prominent member of the subset of TGF-β1-induced, EMT-associated genes in human malignant keratinocytes [21, 26, 27]. In epithelial cells undergoing a mesenchymal-like conversion in response to the E-cadherin transcriptional repressors Snail, Slug or E47, PAI-1 upregulation appears to be an essential characteristic of the plastic phenotype [28]. The association between PAI-1 expression and tumor

"progression" has significant clinical implications. Current data suggest that this serine protease inhibitor maintains an angiogenic "scaffold," stabilizes nascent capillary vessel structure, and facilitates tumor cell invasion through precise control of the peritumor proteolytic microenvironment [29–31]. Increased PAI-1 expression is, in fact, an early event in the progression of epidermal SCC, often localizing to tumor cells and myofibroblasts at the invasive front [24, 32–36] and, most importantly, is a biomarker with significant prognostic value [37]. Indeed, two of the best-validated prognostic indicators (level of evidence [LOE] = 1) in breast carcinoma are the serine protease urokinase plasminogen activator (uPA) and its endogenous inhibitor PAI-1 [38]. Certain PAI-1 tumor thresholds predict both poor prognosis and reduced disease-free survival in patients with breast, lung, ovarian, and oral SCC [29, 38] with the expression amplitude frequently associated with the 4G polymorphism at the PE1 E box motif in the PAI-1 promoter [37]. Identification of PAI-1 in tumor-proximal stromal myofibroblasts, furthermore, implies a more global involvement in modulating cellular invasive potential [34–36], perhaps as a matricellular effector of epithelial motility [39], invasion and the associated angiogenic response [24, 30, 31, 40, 41].

Recent findings clearly implicate EGFR/MEK/*rho*-ROCK signaling as required for PAI-1 expression in TGF-β1-stimulated cells. E box motifs (CACGTG) in the PAI-1 PE1/PE2 promoter regions, moreover, are platforms for a MAP kinase-directed upstream stimulatory factor (USF) subtype switch (USF-1 → USF-2) in response to growth factor addition [42–44] suggesting that the EGFR/MEK/*rho*-ROCK axis impacts PAI-1 expression through USF-dependent transcriptional controls. The continued definition of TGF-β1-activated pathways that influence expression of this important target gene may lead to therapeutically useful approaches to manage human cancer. This paper, therefore, reviews data regarding the rapid transactivation of the EGFR in TGF-β1-stimulated cells suggesting cooperativity between TGF-β1 and EGFR → MAP kinase pathways in PAI-1 gene expression.

2. EGFR Signaling Is Required for TGF-β1-Induced PAI-1 Expression

TGF-β1 mobilizes both SMAD-dependent and -independent signaling [45] although the individual roles of specific cross-pathway events on PAI-1 expression are not well understood. Several recent studies demonstrated that TGF-β1-induced rapid EGFR transactivation highlighting cooperativity between TGF-β1 and EGFR signaling events in vascular, epithelial, and endothelial cells. Indeed, PAI-1 induction in response to TGF-β1 is significantly attenuated by an EGFR pharmacologic inhibitor (AG1478), by molecular targeting of EGFR activity (i.e., by adenoviral delivery of $EGFR^{Y721A}$ kinase-dead constructs) and, more importantly, by genetic ablation of the EGFR in mouse fibroblasts [43, 46, 47] with PAI-1 "rescue" evident in $EGFR^{-/-}$ cells engineered to express an EGFR construct. TGF-β1 treatment, moreover, specifically increased EGFR phosphorylation at the Y845 *src*-target residue; either mutation of this residue ($EGFR^{Y845F}$) or transfection of a DN $pp60^{c\text{-}src}$ construct completely blocked TGF-β1-dependent PAI-1 induction. Similarly, TGF-β1 failed to stimulate PAI-1 expression in cultured mouse embryonic fibroblasts (MEFs) genetically deficient in three *src* family kinases (i.e., c-*src*, c-*yes*-, c-*fyn*- null fibroblasts; $SYF^{-/-/-}$) compared to identically stimulated wild-type $SYF^{+/+/+}$ cells. PAI-1 synthesis was restored in $SYF^{-/-/-}$ MEFs engineered to re-express a wild-type $pp60^{c\text{-}src}$ [47] providing proof-of-principle for involvement of this particular *src* kinase in the inductive response. The highly specific *src* family kinase inhibitor SU6656, morevover, effectively blocked TGF-β1-initiated increases in both $pp60^{c\text{-}src}$ and EGFR phosphorylation as well as $pp60^{c\text{-}src}$ and EGFR activation (at the Y416 and Y845 residues, resp.). $pEGFR^{Y845}$ phosphorylation in response to TGF-β1 was evident, furthermore, in wild type but not $SYF^{-/-/-}$ fibroblasts. The TGF-β1-dependent formation of $EGFR/pp60^{c\text{-}src}$ complexes [46] and $EGFR^{Y845}$ phosphorylation and the inhibition of TGF-β1- (but not PDGF-) induced PAI-1 expression by the $EGFR^{Y845F}$ mutant as well as a DN-Src construct [47] collectively implicate $EGFR/pp60^{c\text{-}src}$ interactions and, in particular, the $EGFR^{Y845}$ $pp60^{c\text{-}src}$ site in the kinase domain activation loop in signal propagation [48]. The time course of TGF-β1-initiated SMAD2/3 activation, in contrast, was similar in both wild type and $SYF^{-/-/-}$ MEFs confirming that, in the context of either EGFR or *src* family kinase deficiency, SMAD2/3 activation occurs but is not sufficient for PAI-1 induction. TGF-β1 stimulated ERK1/2 phosphorylation in $EGFR^{+/+}$ but not in $EGFR^{-/-}$ cells consistent with prior observations that TGF-β1-dependent ERK1/2 activation is downstream of EGFR signaling [43, 46]. $EGFR^{-/-}$ MEFs, however, are fully capable of responding to exogenous TGF-β1 as SMAD2 was effectively activated (i.e., phosphorylated) in both wild type and $EGFR^{-/-}$ fibroblasts [47].

3. The PAI-1 Gene Is a Model of TGF-β1-Initiated Cooperative EGFR Signaling

While TGF-β1 receptors phosphorylate SMADs downstream of growth factor engagement, it appears that the Rho/ROCK pathway modulates the duration of SMAD2/3 phosphorylation [47]. How Rho/ROCK impact TGF-β1-initiated SMAD2/3 activation and subcellular localization [49, 50] is not known but this pathway may function to provide efficient SMAD2/3 activation for extended periods. Alternatively, Rho/ROCK signaling may be required to inhibit negative regulation of SMAD2/3 function by inactivation of SMAD phosphatases sustaining, thereby, SMAD2/3 transcriptional actions (e.g., [51, 52]). TGF-β1-induced SMAD2 phosphorylation is not altered by EGFR blockade either pharmacologically (with AG1478), molecularly (by expression of $EGFR^{Y721A}$ or $EGFR^{Y845F}$), or by the genetic absence of EGFR [47]. Clearly, while SMAD2/3 activation may be necessary it is not sufficient for TGF-β1-stimulated PAI-1 expression in the absence of EGFR signaling.

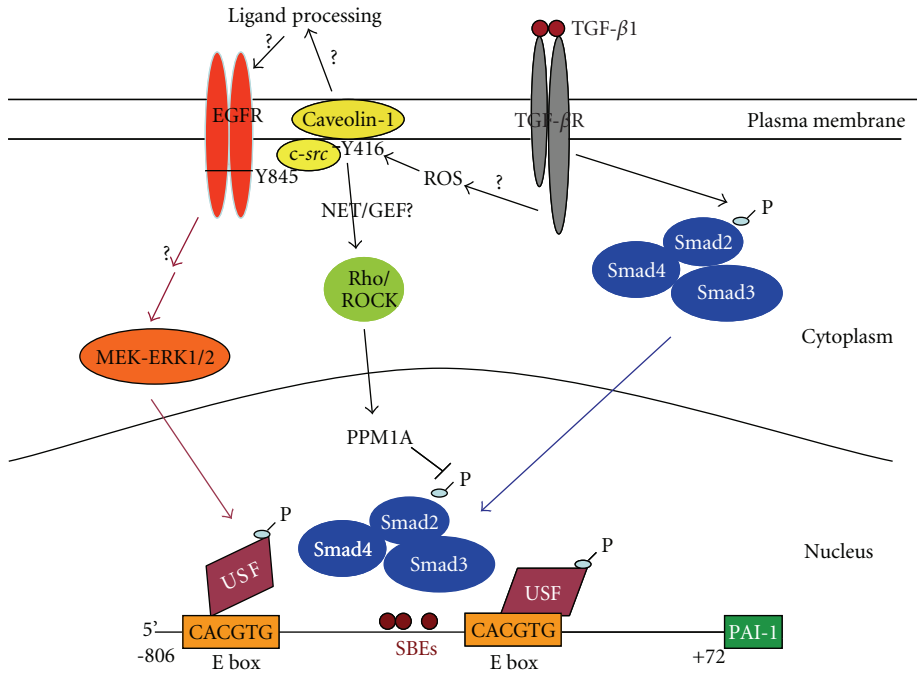

Figure 1: Model for TGF-β1-induced PAI-1 expression. TGF-β1 activates two distinct signaling pathways to initiate PAI-1 transcription. Rho/ROCK are required to maintain SMAD phosphorylation and ERK activation (through to be defined mechanisms) while the pp60$^{c\text{-}src}$-activated EGFR (at the Y845 site) signals to MEK-ERK initiating ERK/USF interactions resulting in USF phosphorylation and a subtype (USF-1 → USF-2) switch (e.g., [44]) at the PAI-1 PE1/PE2 E box sites. Collectively, these promoter-level events stimulate high level PAI-1 expression in response to TGF-βR occupancy. The actual mechanism underlying EGFR activation in response to TGF-β1 may involve direct recruitment of *src* kinases to the EGFR or the processing/release of a membrane-anchored EGFR ligand (e.g., HB-EGF). Events associated with TGF-β1 stimulation of the RhoA/ROCK pathway are similarly unclear. Rho/ROCK may regulate the activity and/or function of the SMAD phosphatase PPM1A impacting, thereby, the duration of SMAD-dependent transcription of target genes such as PAI-1. (modified from [47]).

It is apparent, therefore, that TGF-β1 stimulates PAI-1 expression through two distinct but cooperating pathways that involve EGFR/pp60$^{c\text{-}src}$ → MEK/ERK signaling and EGFR-independent, but Rho/ROCK-modulated, TGF-βR-directed SMAD and ERK activation [47]. Interference with any of the specific individual elements in this dual cascade (EGFR/pp60$^{c\text{-}src}$/MEK or Rho/p160ROCK) markedly reduced, and in some cases, completely inhibited PAI-1 expression. One model consistent with the available data [24, 40, 43, 44, 47, 53] suggests that SMADs and specific MAP kinase-targeted bHLH-LZ factors (such as USF) occupy their separate binding motifs at the critical TGF-β1-responsive PE2 region E box in the PAI-1 promoter (Figure 1). Dominant-negative interference with USF DNA-binding ability significantly reduced TGF-β1-mediated PAI-1 transcription[43, 44, 53]. Since MAP kinases regulate the DNA-binding and transcriptional activites of USF [40, 43], TGF-βR signaling through SMAD2/3 may actually cooperate with EGFR/MEK-ERK-activated USF to attain high level PAI-1 expression [40, 47]. SMADs are known to interact with E box-binding HLH-LZ factors such as TFE3 at the PE2 site in the PAI-1 geneat least in one cell type [54]. There is evidence, in fact, to suggest that such interacting complexes impact PAI-1 gene control since USF occupancy of the PAI-1 PE2 region E box site, which is juxtaposed to three SMAD-recognition elements, modulates transcription in response to TGF-β1 or serum [40, 43, 44, 53]. Current data indicate that recruitment of this multicomponent complex likely requires participation of the TGF-β1-stimulated EGFR → MEK/ERK and Rho/ROCK pathways for the optimal response of the PAI-1 gene to TGF-β1.

The mechanism of MAP kinase activation in TGF-β1-stimulated cells is just becoming clear. Upon ligand binding, the TGF-βRII undergoes autophosphorylation on three tyrosines (Y259, Y336, Y424), while Y284 is a target site for *src* kinases [55]. TGF-βRI is also subject to tyrosine phosphorylation postreceptor accupancy [56]. Such phosphorylated tyrosine residues provide docking sites for recruitment of Grb2/Shc/SOS complexes with subsequent mobilization of the *ras-raf*-MEK-ERK cascade [46, 47, 55]. Although ERKs are prominently activated in response to TGF-β1 [40, 43], perhaps the JNK and p38 MAP kinase pathways are better characterized targets of TGF-β1-initiated signaling. TGF-β1 rapidly activates JNK through MKK4 and p38 via MKK3/6 perhaps even in a cell type-specific fashion contributing to the mechanistic complexity of pathway cross-talk. Each of these kinase systems, moreover, has been implicated in a cell type-dependency of PAI-1 gene control [40, 43, 55]. Should such pathways prove uniquely or, at least, preferentially utilized in specific cellular lineages, they may provide tumor type-specific targets for intervention therapy.

4. EGFR as a Potential Therapeutic Target for Regulating PAI-1 Expression

Modulation of EGFR/HER1 signaling by specific receptor function (kinase domain) inhibitors or neutralizing antibodies against specific EGFR1 ligands (e.g., HB-EGF antibodies) can be an attractive therapeutic modality (particularly in the context of neoplastic diseases associated with elevated TGF-β1 levels). This strategy would likely impact not only PAI-1 suppression but has the potential to regulate other proinvasive target genes. There is, in fact, increasing evidence that TGF-β1-induced connective tissue growth factor and fibronectin expression similarly involve EGFR/HER1 cooperative pathways (Samarakoon and Higgins, unpublished data). Moreover, PAI-1 repression by EGFR signaling blockade may also suppress tumor angiogenesis consistent with the well-established role of PAI-1 as an inhibitor of endothelial apoptosis and neovessel regression [40]. Combinatorial targeting of PAI-1 function using established small molecule PAI-1 inhibitors and genetic-based PAI-1 expression attenuation [40] coupled with disruption of EGFR signaling (e.g., with cetuximab or erlotinib) may impact, therefore, both cancer invasion and the associated angiogenic response, particularly in the context of a TGF-β1-rich tumor microenvironment.

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References

[1] D. Breitkreutz, P. Boukamp, C. M. Ryle, H.-J. Stark, D. R. Roop, and N. E. Fusenig, "Epidermal morphogenesis and keratin expression in c-Ha-*ras*-transfected tumorigenic clones of the human HaCaT cell line," *Cancer Research*, vol. 51, no. 16, pp. 4402–4409, 1991.

[2] A. Dlugosz, G. Merlino, and S. H. Yuspa, "Progress in cutaneous cancer research," *Journal of Investigative Dermatology Symposium Proceedings*, vol. 7, no. 1, pp. 17–26, 2002.

[3] P. Boukamp, "UV-induced skin cancer: similarities—variations," *Journal der Deutschen Dermatologischen Gesellschaft*, vol. 3, no. 7, pp. 493–503, 2005.

[4] P. Boukamp, W. Peter, U. Pascheberg, et al., "Step-wise progression in human skin carcinogenesis in vitro involves mutational inactivation of p53, rasH oncogene activation and additional chromosome loss," *Oncogene*, vol. 11, no. 5, pp. 961–969, 1995.

[5] B. R. Smoller, "Squamous cell carcinoma: from precursor lesions to high-risk variants," *Modern Pathology*, vol. 19, supplement 2, pp. S88–S92, 2006.

[6] K. Y. Tsai and H. Tsao, "The genetics of skin cancer," *American Journal of Medical Genetics Part C*, vol. 131, no. 1, pp. 82–92, 2004.

[7] R. J. Akhurst and A. Balmain, "Genetic events and the role of TGFβ in epithelial tumour progression," *The Journal of Pathology*, vol. 187, no. 1, pp. 82–90, 1999.

[8] W. Cui, D. J. Fowlis, S. Bryson, et al., "TGFβ1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice," *Cell*, vol. 86, no. 4, pp. 531–542, 1996.

[9] G. Portella, S. A. Cumming, J. Liddell, et al., "Transforming growth factor β is essential for spindle cell conversion of mouse skin carcinoma in vivo: implications for tumor invasion," *Cell Growth & Differentiation*, vol. 9, no. 5, pp. 393–404, 1998.

[10] R. Derynck, R. J. Akhurst, and A. Balmain, "TGF-β signaling in tumor suppression and cancer progression," *Nature Genetics*, vol. 29, no. 2, pp. 117–129, 2001.

[11] M. Oft, R. J. Akhurst, and A. Balmain, "Metastasis is driven by sequental elevation of H-ras and Smad2 levels," *Nature Cell Biology*, vol. 4, no. 7, pp. 487–494, 2002.

[12] G. Han, S.-L. Lu, A. G. Li, et al., "Distinct mechanisms of TGF-β1-mediated epithelial-to-mesenchymal transition and metastasis during skin carcinogenesis," *The Journal of Clinical Investigation*, vol. 115, no. 7, pp. 1714–1723, 2005.

[13] M. Kretzschmar, J. Doody, I. Timokhina, and J. Massagué, "A mechanism of repression of TGFfβ/Smad signaling by oncogenic Ras," *Genes & Development*, vol. 13, no. 7, pp. 804–816, 1999.

[14] O. Rho, L. M. Beltrán, I. B. Gimenez-Conti, and J. DiGiovanni, "Altered expression of the epidermal growth factor receptor and transforming growth factor-α during multistage skin carcinogenesis in SENCAR mice," *Molecular Carcinogenesis*, vol. 11, no. 1, pp. 19–28, 1994.

[15] S. H. Yuspa, "The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis," *Journal of Dermatological Science*, vol. 17, no. 1, pp. 1–7, 1998.

[16] P. O-Charoenrat, P. Rhys-Evans, H. Modjtahedi, W. Court, G. Box, and S. Eccles, "Overexpression of epidermal growth factor receptor in human head and neck squamous carcinoma cell lines correlates with matrix metalloproteinase-9 expression and in vitro invasion," *International Journal of Cancer*, vol. 86, no. 3, pp. 307–317, 2000.

[17] P. O-Charoenrat, P. H. Rhys-Evans, D. J. Archer, and S. A. Eccles, "C-*erb*B receptors in squamous cell carcinomas of the head and neck: clinical significance and correlation with matrix metalloproteinases and vascular endothelial growth factors," *Oral Oncology*, vol. 38, no. 1, pp. 73–80, 2002.

[18] N. Moghal and P. W. Sternberg, "Multiple positive and negative regulators of signaling by the EGF-receptor," *Current Opinion in Cell Biology*, vol. 11, no. 2, pp. 190–196, 1999.

[19] T. Shimizu, H. Izumi, A. Oga, et al., "Epidermal growth factor receptor overexpression and genetic aberrations in metastatic squamous-cell carcinoma of the skin," *Dermatology*, vol. 202, no. 3, pp. 203–206, 2001.

[20] J. Zavadil and E. P. Böttinger, "TGF-β and epithelial-to-mesenchymal transitions," *Oncogene*, vol. 24, no. 37, pp. 5764–5774, 2005.

[21] J. Zavadil, M. Bitzer, D. Liang, et al., "Genetic programs of epithelial cell plasticity directed by transforming growth factor-β," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 12, pp. 6686–6691, 2001.

[22] J. P. Thiery and J. P. Sleeman, "Complex networks orchestrate epithelial-mesenchymal transitions," *Nature Reviews Molecular Cell Biology*, vol. 7, no. 2, pp. 131–142, 2006.

[23] H. Peinado, M. Quintanilla, and A. Cano, "Transforming growth factor β-1 induces snail transcription factor in epithelial cell lines. Mechanisms for epithelial mesenchymal transitions," *The Journal of Biological Chemistry*, vol. 278, no. 23, pp. 21113–21123, 2003.

[24] C. E. Wilkins-Port, C. E. Higgins, J. Freytag, S. P. Higgins, J. A. Carlson, and P. J. Higgins, "PAI-1 is a critical upstream regulator of the TGF-β1/EGF-induced invasive phenotype in mutant p53 human cutaneous squamous cell carcinoma,"

Journal of Biomedicine and Biotechnology, vol. 2007, Article ID 85208, 8 pages, 2007.

[25] C. E. Wilkins-Port and P. J. Higgins, "Regulation of extracellular matrix remodeling following transforming growth factor-β1/epidermal growth factor-stimulated epithelial-mesenchymal transition in human premalignant keratinocytes," *Cells Tissues Organs*, vol. 185, no. 1–3, pp. 116–122, 2007.

[26] L. Qi, S. P. Higgins, Q. Lu, et al., "SERPINE1 (PAI-1) is a prominent member of the early $G_0 \rightarrow G_1$ transition "wound repair" transcriptome in p53 mutant human keratinocytes," *Journal of Investigative Dermatology*, vol. 128, no. 3, pp. 749–753, 2008.

[27] S. Akiyoshi, M. Ishii, N. Nemoto, M. Kawabata, H. Aburatani, and K. Miyazono, "Targets of transcriptional regulation by transforming growth factor-β: expression profile analysis using oligonucleotide arrays," *Japanese Journal of Cancer Research*, vol. 92, no. 3, pp. 257–268, 2001.

[28] G. Moreno-Bueno, E. Cubillo, D. Sarrió, et al., "Genetic profiling of epithelial cells expressing E-cadherin repressors reveals a distinct role for snail, slug, and E47 Factors in epithelial- mesenchymal transition," *Cancer Research*, vol. 66, no. 19, pp. 9543–9556, 2006.

[29] P. A. Andreasen, L. Kjøller, L. Christensen, and M. J. Duffy, "The urokinase-type plasminogen activator system in cancer metastasis: a review," *International Journal of Cancer*, vol. 72, no. 1, pp. 1–22, 1997.

[30] K. Bajou, V. Masson, R. D. Gerard, et al., "The plasminogen activator inhibitor PAI-1 controls in vivo tumor vascularization by interaction with proteases, not vitronectin: implications for antiangiogenic strategies," *Journal of Cell Biology*, vol. 152, no. 4, pp. 777–784, 2001.

[31] K. Bajou, A. Noël, R. D. Gerard, et al., "Absence of host plasminogen activator inhibitor 1 prevents cancer invasion and vascularization," *Nature Medicine*, vol. 4, no. 8, pp. 923–928, 1998.

[32] Y.-J. Chen, S.-C. Lin, T. Kao, et al., "Genome-wide profiling of oral squamous cell carcinoma," *The Journal of Pathology*, vol. 204, no. 3, pp. 326–332, 2004.

[33] P. Lindberg, Å. Larsson, and B. S. Nielsen, "Expression of plasminogen activator inhibitor-1, urokinase receptor and laminin γ-2 chain is an early coordinated event in incipient oral squamous cell carcinoma," *International Journal of Cancer*, vol. 118, no. 12, pp. 2948–2956, 2006.

[34] M. Illemann, U. Hansen, H. J. Nielsen, et al., "Leading-edge myofibroblasts in human colon cancer express plasminogen activator inhibitor-1," *American Journal of Clinical Pathology*, vol. 122, no. 2, pp. 256–265, 2004.

[35] B. V. Offersen, B. S. Nielsen, G. Høyer-Hansen, et al., "The myofibroblast is the predominant plasminogen activator inhibitor-1-expressing cell type in human breast carcinomas," *American Journal of Pathology*, vol. 163, no. 5, pp. 1887–1899, 2003.

[36] C. Robert, I. Bolon, S. Gazzeri, S. Veyrenc, C. Brambilla, and E. Brambilla, "Expression of plasminogen activator inhibitors 1 and 2 in lung cancer and their role in tumor progression," *Clinical Cancer Research*, vol. 5, no. 8, pp. 2094–2102, 1999.

[37] E. Vairaktaris, C. Yapijakis, Z. Serefoglou, et al., "Plasminogen activator inhibitor-1 polymorphism is associated with increased risk for oral cancer," *Oral Oncology*, vol. 42, no. 9, pp. 888–892, 2006.

[38] B. Hundsdorfer, H.-F. Zeilhofer, K. P. Bock, et al., "Tumour-associated urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 in normal and neoplastic tissues of patients with squamous cell cancer of the oral cavity-clinical relevance and prognostic value," *Journal of Cranio-Maxillofacial Surgery*, vol. 33, no. 3, pp. 191–196, 2005.

[39] F. Maquerlot, S. Galiacy, M. Malo, et al., "Dual role for plasminogen activator inhibitor type 1 as soluble and as matricellular regulator of epithelial alveolar cell wound healing," *American Journal of Pathology*, vol. 169, no. 5, pp. 1624–1632, 2006.

[40] P. J. Higgins, "TGF-β1-stimulated $p21^{ras}$-ERK signaling regulates expression of the angiogenic SERPIN PAI-1," *Recent Research Developments in Biochemistry*, vol. 7, pp. 31–45, 2006.

[41] C. Maillard, M. Jost, M. U. Rømer, et al., "Host plasminogen activator inhibitor-1 promotes human skin carcinoma progression in a stage-dependent manner," *Neoplasia*, vol. 7, no. 1, pp. 57–66, 2005.

[42] M.-D. Galibert, S. Carreira, and C. R. Goding, "The Usf-1 transcription factor is a novel target for the stress-responsive p38 kinase and mediates UV-induced *Tyrosinase* expression," *The EMBO Journal*, vol. 20, no. 17, pp. 5022–5031, 2001.

[43] S. M. Kutz, C. E. Higgins, R. Samarakoon, et al., "TGF-β1-induced PAI-1 expression is e box/USF-dependent and requires EGFR signaling," *Experimental Cell Research*, vol. 312, no. 7, pp. 1093–1105, 2006.

[44] L. Qi, R. R. Allen, Q. Lu, C. E. Higgins, R. Garone, L. Staiano-Coico, and P. J. Higgins, "PAI-1 transcriptional regulation during the $G_0 \rightarrow G_1$ transition in human epidermal keratinocytes," *Journal of Cellular Biochemistry*, vol. 99, no. 2, pp. 495–507, 2006.

[45] R. Derynck and Y. E. Zhang, "Smad-dependent and Smad-independent pathways in TGF-β family signalling," *Nature*, vol. 425, no. 6958, pp. 577–584, 2003.

[46] R. Samarakoon, C. E. Higgins, S. P. Higgins, S. M. Kutz, and P. J. Higgins, "Plasminogen activator inhibitor type-1 gene expression and induced migration in TGF-β1-stimulated smooth muscle cells is $pp60^{c-src}$/MEK-dependent," *Journal of Cellular Physiology*, vol. 204, no. 1, pp. 236–246, 2005.

[47] R. Samarakoon, S. P. Higgins, C. E. Higgins, and P. J. Higgins, "TGF-β1-induced plasminogen activator inhibitor-1 expression in vascular smooth muscle cells requires $pp60^{c-src}$/$EGFR^{Y845}$ and Rho/ROCK signaling," *Journal of Molecular and Cellular Cardiology*, vol. 44, no. 3, pp. 527–538, 2008.

[48] R. Ishizawar and S. J. Parsons, "C-Src and cooperating partners in human cancer," *Cancer Cell*, vol. 6, no. 3, pp. 209–214, 2004.

[49] S. Chen, M. Crawford, R. M. Day, et al., "RhoA modulates Smad signaling during transforming growth factor-β-induced smooth muscle differentiation," *The Journal of Biological Chemistry*, vol. 281, no. 3, pp. 1765–1770, 2006.

[50] T. Kita, Y. Hata, K. Kano, et al., "Transforming growth factor-β2 and connective tissue growth factor in proliferative vitreoretinal diseases: possible involvement of hyalocytes and therapeutic potential of Rho kinase inhibitor," *Diabetes*, vol. 56, no. 1, pp. 231–238, 2007.

[51] S. Itoh and P. ten Dijke, "Negative regulation of TGF-β receptor/Smad signal transduction," *Current Opinion in Cell Biology*, vol. 19, no. 2, pp. 176–184, 2007.

[52] X. Lin, X. Duan, Y.-Y. Liang, et al., " PPM1A functions as a Smad phosphatase to terminate TGFβ signaling," *Cell*, vol. 125, no. 5, pp. 915–928, 2006.

[53] R. R. Allen, L. Qi, and P. J. Higgins, "Upstream stimulatory factor regulates E box-dependent PAI-1 transcription in human epidermal keratinocytes," *Journal of Cellular Physiology*, vol. 203, no. 1, pp. 156–165, 2005.

[54] X. Hua, Z. A. Miller, G. Wu, Y. Shi, and H. F. Lodish, "Specificity in transforming growth factor β-induced transcription of the plasminogen activator inhibitor-1 gene: interactions of promoter DNA, transcription factor μE3, and Smad proteins," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 23, pp. 13130–13135, 1999.

[55] Y. E. Zhang, "Non-Smad pathways in TGF-β signaling," *Cell Research*, vol. 19, no. 1, pp. 128–139, 2009.

[56] M. K. Lee, C. Pardoux, M. C. Hall, et al., "TGF-β activates Erk MAP kinase signalling through direct phsophorylation of ShcA," *The EMBO Journal*, vol. 26, pp. 3957–3967, 2007.