

Targeting epidermal growth factor receptor in head and neck cancer: lessons learned from cetuximab

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Abstract

As early detection strategies have not been successful, most patients with head and neck cancer (HNC) present with advanced (stages III and IV) disease. Oral cavity tumors are treated primarily with surgical resection and advanced tumors of the pharynx and larynx are generally treated with combined modality therapy (chemoradiation). The major advances in the management of HNC have evolved from the integration of targeted therapeutics into treatment regimens. Presently, the most important target for new therapeutic strategies in HNC is the epidermal growth factor receptor (EGFR) and so far only cetuximab, a monoclonal antibody targeting EGFR, has been approved by the United States Food and Drug Administration in the HNC population as a radiation-sensitizing agent for patients undergoing primary radiation-based treatment and for patients with recurrent or metastatic disease. Other receptor and non-receptor kinase targeting strategies are under active clinical investigation as well. The increasing number of molecular targeting strategies in clinical development underscores the need to identify which HNC patients will respond to specific therapies. This article focuses on the current preclinical and clinical evidence of monoclonal antibodies targeting EGFR in HNC. We will first review the mechanisms of action of cetuximab, its clinical trials and side-effect profiles, and its present clinical application. Then, the current development status of other molecular antibodies and two molecular inhibitors, gefitinib and erlotinib, will be examined. Finally, by focusing on cetuximab, the current issues in EGFR targeting will be reviewed and we propose future directions of EGFR targeting. We hope that this review will provide further insight into the future directions of targeted therapy in the management of advanced HNC.

Keywords: EGFR targeting, cetuximab, head and neck cancer, chemoradiation, stage III/IV disease, gefitinib, erlotinib

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Introduction

Head and neck cancers, 90% of which are squamous cell carcinomas (SCCHN), include cancers of the oral cavity, oropharynx, hypopharynx, larynx and, to a lesser degree, the nose and paranasal sinuses. The treatment for SCCHN has mainly consisted of a combination of surgery and radiation, or combined radiation and chemotherapy depending on the site and stage of the disease and patient's health status. In most stage I and II cancers, the first treatment choice is surgery or radiation therapy. In cases of locally advanced stage III and IV cancers, systemic chemotherapy was introduced as a combined modality with surgery or radiation in the mid-1970s to overcome the poor result of

the single modality therapy of surgery and radiation therapy alone.¹ The introduction of new chemotherapeutic agents, altered fractionation radiation and their combination (chemoradiotherapy [CRT]), has further declined mortality rates.^{2,3} Despite the moderate success, the combination of conventional therapeutic modalities still demonstrates the high incidence of locoregional failure and a poor overall five-year survival rate of around 50%. Furthermore, once the tumor has become refractory to chemotherapy, the median life-expectancy is three months and the tumor response rate to second- or third-line chemotherapeutic agents is approximately 15%.⁴ The success for conventional treatment modalities is limited, in part, by the remarkable

resistance of tumors to chemotherapy. Attempts to overcome resistance with higher doses of chemotherapeutics inevitably result in an unacceptable degree of toxicity and bystander damage to normal tissues. Thus, there is a need for new therapies with novel mechanisms of action that are well tolerated and effective. The identification of these molecular alterations has allowed the development of new therapeutic approaches targeting specific differences between normal and malignant cells.

So far, a number of specific genetic events have been identified in the malignant progression of SCCHN. These genetic events include amplification and/or overexpression of oncogenes and mutations and deletions leading to the inactivation of tumor suppressor genes.⁵ Genetic alterations leading to the inactivation of tumor suppressor genes, such as p16 and p53, are frequently observed, and chromosomes 3p, 9p and 17p experience the highest loss of heterozygosity region in SCCHN and are associated with the development and progression of the disease.⁶

The identification of these molecular alterations has allowed the development of new therapeutic approaches targeting specific differences between normal and malignant cells. Presently, based on numerous preclinical and clinical studies, the most important target for new therapeutic strategies in SCCHN is the epidermal growth factor receptor (EGFR). A number of agents that are designed to target EGFR includes monoclonal antibodies, tyrosine kinase-specific inhibitors, ligand-linked immunotoxins and antisense oligonucleotides, and are currently being studied in the clinic; in fact, several of them have been successful enough to be approved by the Food and Drug Administration (FDA) as secondary drugs for different types of cancers. So far, cetuximab, an anti-EGFR monoclonal antibody, has shown the most promising result in several clinical trials and presently only cetuximab has been FDA-approved for two indications in the head and neck cancer population: (1) as a radiation-sensitizing agent for patients undergoing primary radiation based treatment and (2) for patients with recurrent or metastatic disease. However, cetuximab's indications and a thorough understanding of its side-effect profiles suggest that careful approaches in its use are warranted.

This article will focus on EGFR-targeting agents for the treatment of SCCHN. We will first review the mechanisms of action of cetuximab and provide an overview of its clinical trials, side-effect profiles and its present clinical application. Then, the current development status of other molecular antibodies and two molecular inhibitors, gefitinib and erlotinib, will be examined. Finally, by focusing on cetuximab, the current issues and future directions of EGFR targeting strategy will be discussed.

Molecular biology of EGFR in SCCHN

The EGFR (or erbB1) is a membrane receptor and a member of the tyrosine kinase family. Binding of the ligands (EGF and transforming growth factor- α , TGF- α) to the receptor's extracellular domain induces EGFR dimerization, resulting in the activation of receptor tyrosine kinase activity and

EGFR autophosphorylation on specific tyrosine residues.⁷ These phosphorylated tyrosines recruit signal transducers or adaptors that initiate various intracellular signaling pathways such as Ras/Raf/MEK/MAPK that control cellular proliferation, migration and differentiation.⁷

The alterations in the EGFR signaling pathway can cause malignant transformation through different mechanisms, including receptor overexpression, alteration in dimerization and deficiency of specific phosphatases.⁸ The overexpression of EGFR has been reported in 80–90% of SCCHN and shows a significant association with EGFR gene amplification, but not with mutations.⁹ The overexpression of EGFR has been found in dysplastic lesions and histologically normal mucosa from SCCHN patients, indicating that EGFR upregulation represents an early event in carcinogenesis.¹⁰ Furthermore, EGFR overexpression has been found to correlate with more aggressive disease, resistance to both chemotherapy and radiotherapy and a poor prognosis.^{11,12}

Epidermal growth factor receptor antagonists

A number of agents have been designed to target EGFR with different mechanisms, including monoclonal antibodies, tyrosine kinase-specific inhibitors, ligand-linked immunotoxins and antisense oligonucleotides, and are currently being studied in the clinical setting. The furthest in development are cetuximab (Erbix, IMC-C225), panitumumab, gefitinib (Iressa, ZD1839) and erlotinib (Tarceva, OSI-774) (Figure 1). Gefitinib and erlotinib are orally active EGFR inhibitors, while cetuximab and panitumumab are anti-EGFR monoclonal antibodies.

Cetuximab: monoclonal antibody to EGFR

Cetuximab is an immunoglobulin G₁ (IgG₁) monoclonal antibody that targets the extracellular ligand-binding domain of EGFR with high affinity. It is a recombinant, human/mouse chimeric monoclonal antibody that binds specifically to the extracellular domain of human EGFR (Figure 1). Cetuximab is composed of the Fv regions of a murine anti-EGFR antibody with human IgG₁ heavy- and kappa light-chain constant regions and has an approximate molecular weight of 152 kDa. Cetuximab is produced in mammalian (murine myeloma) cell culture. It competitively binds to the receptor and induces the downregulation of EGFR, preventing further receptor binding and activation of the ligands. Ultimately, this antibody-mediated blockade of EGFR results in the inhibition of cell proliferation. Also in response to EGFR blockade, tumor cells produce markedly reduced angiogenesis factors, and blood vessel formation in human tumor xenografts was inhibited.¹³

Preclinical studies show that cetuximab inhibits the proliferation of cell lines expressing EGFR, including SCCHN cell lines, and increases the cytotoxic effects of chemotherapy and radiation.^{14,15} Based upon the observations of a synergistic effect of cetuximab in xenograft models, cetuximab has been administered in combination with

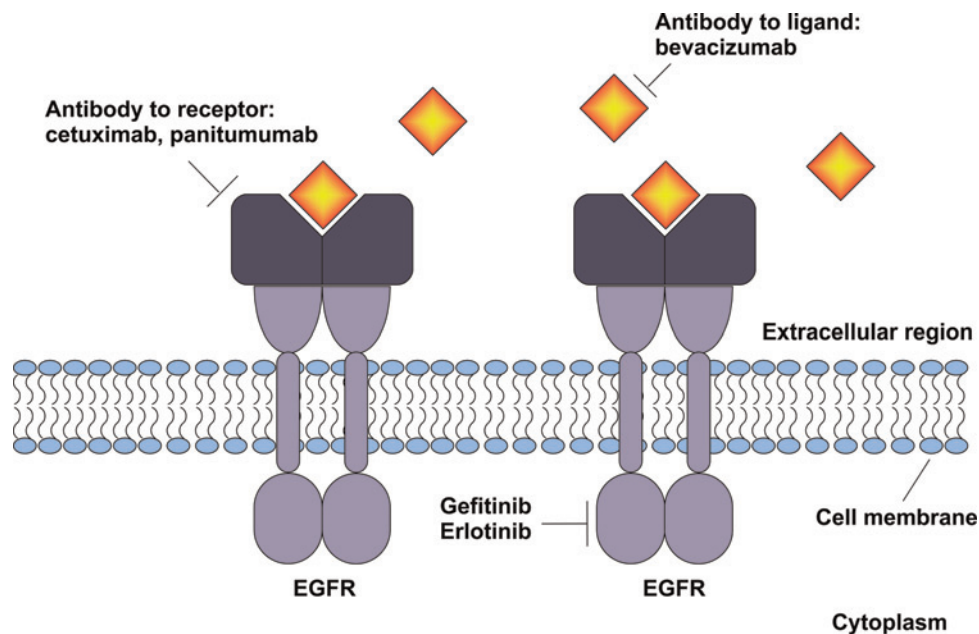


Figure 1 Diagram of epidermal growth factor receptor (EGFR) and sites of action in EGFR targeting for cetuximab, gefitinib and erlotinib (A color version of this figure is available in the online journal)

chemotherapy and/or radiation therapy, enhancing their antitumor activities in a number of clinical trials.

Phase I/II clinical trial

In three successive phase I trials, 52 patients, including 26 SCCHN patients, were treated with cetuximab in a single dose, weekly multiple doses and weekly multiple doses with cisplatin, for the establishment of a safe profile and a pharmacological profile (Table 1).¹⁶ Cetuximab infusions were well tolerated at all doses tested, and the most commonly reported adverse events were fever and chill, asthenia, transaminidase elevation, nausea and skin toxicities. A dose of complete EGFR saturation was achieved before the maximum-tolerated dose (MTD). Although these studies were not designed to analyze clinical response, tumor regression was demonstrated in two patients with SCCHN treated with cetuximab in combination with cisplatin.

Shin *et al.*²⁶ have reported results of a phase IB trial of cetuximab in combination with cisplatin in patients with recurrent SCCHN. After cetuximab infusion, EGFR tyrosine kinase activity was significantly reduced and EGFR/cetuximab complexes were detected. The loading dose of 400 mg/m² with a maintenance dose of 250 mg/m² achieved a high percentage of saturation of EGFR in tumor tissue, and the combination of cetuximab with cisplatin achieved a high percentage of tumor response. Cetuximab also showed a synergistic effect in combination with cisplatin; six of nine evaluable patients achieved major responses, including two complete responses.

A dose-escalation study in combination with radiation therapy tested 15 treatment naïve patients with advanced SCCHN with a loading dose of 100–500 mg/m², followed by weekly infusions of 100–250 mg/m² for seven to eight weeks (Table 1).¹⁷ All patients achieved an objective

response; 13 patients had complete remissions, while two patients experienced a partial response. The most commonly reported adverse events were fever, asthenia, transaminase elevation, nausea and skin toxicities that were grades 1 and 2 in most patients. Based on the pharmacological parameters and adverse events, a loading dose of 400–500 mg/m² and a maintenance weekly dose of 250 mg/m² were recommended. A definite conclusion on whether cetuximab increases the local toxicity of radiation cannot be reached because of the small number of patients in this study.

Two ongoing Eastern Cooperative Oncology Group (ECOG) trials are testing cetuximab in different settings. ECOG 2303 is a phase II trial examining cetuximab as a neoadjuvant therapy with induction chemotherapy and chemoradiation.²⁷ Sixty-seven patients with stage III/IV SCCHN were treated with cetuximab, paclitaxel and carboplatin. After eight weeks, the complete response rate at the primary tumor site was determined by biopsy. In 26 (65%), no residual tumor was detected after treatment. ECOG 3303 is a preliminary trial of concurrent radiation, cisplatin and cetuximab in unresectable, locally advanced SCCHN.²⁸ Thus far, 51 patients have been evaluated. Twenty-three percent were found to have a complete response, 25% partial response and 31% stable disease. Three patients (5%) had progressive disease. As with the other trials, the most common adverse effects were neutropenia, fatigue and acneiform rash.

Phase III clinical trial for cetuximab in combination with radiotherapy

A multinational landmark phase III trial has recently provided the results of cetuximab in combination with radiotherapy (Table 1).²⁴ Over 400 patients with locoregionally advanced SCCHN received high-dose radiation therapy

Table 1 Clinical trial results of cetuximab in head and neck cancer

Tumor type	Phase	Vector name	Delivery mode	No. of patients evaluable	No. and type of responses	Adverse reactions	Reference
Bladder, breast, head and neck, kidney, lung, ovary, prostate	I	Cetuximab	IV	28	18 stable disease; 10 progressive	Asthenia, chills, fever, nausea, skin toxicities, transaminase elevation	16
Head and neck	I	Cetuximab	IV plus radiation therapy	15	13 complete response; 2 partial response	Asthenia, fever, nausea, skin toxicities, transaminase elevation	17
Head and neck	II	Cetuximab	IV plus cisplatin	114	2 complete response; 15 partial response; 66 stable disease; 31 progressive	Anemia, nausea, rash	18
Head and neck	II	Cetuximab	IV plus cisplatin/carboplatin	78	10 partial response; 41 stable disease; 27 progressive	Anemia, asthenia, rash	19
Head and neck	II	Cetuximab	IV plus radiation therapy	16	2 complete response; 13 partial response; 1 progressive	Fever, nausea, rash	20
Head and neck	II	Cetuximab	IV	85	13 partial response; 34 stable disease; 38 progressive	Asthenia, rash	21
Head and neck	III	Cetuximab	IV plus cisplatin	44	1 complete response; 5 partial response; 32 stable disease; 6 progressive	Hypersensitivity, neutropenia, rash	22
Head and neck	III	Cetuximab	IV plus cisplatin	117	Overall response: 26% (cetuximab) versus 10% (placebo)	Fatigue, nausea, neutropenia, rash	23
Head and neck	III	Cetuximab	IV plus radiation therapy	424	Overall response: 74% (+cetuximab) versus 64% (–cetuximab)	Rash	24
Head and neck	III	Cetuximab	IV plus platinum-based chemotherapy	442	Overall response: 36% (+cetuximab) versus 20% (–cetuximab)	Rash	25

either alone or together with cetuximab (initial dose 400 mg/m², followed by subsequent weekly doses of 250 mg/m²), with the initial dose administered one week prior to radiotherapy. The study met both its primary endpoint of locoregional control (LRC) and its secondary endpoint of overall survival, both of which were statistically significant. The median duration of LRC was significantly improved among cetuximab plus radiotherapy group compared with radiotherapy-alone group (24.4 versus 14.9 months, $P = 0.005$). Cetuximab plus radiotherapy also demonstrated a significant improvement in the median overall survival. With a median follow-up of 54 months, the median survival with cetuximab plus radiotherapy was 49 months compared with 29.3 months for patients receiving radiotherapy alone ($P = 0.03$).²⁴ It is notable that the increase in survival is achieved without worsening of radiation-induced adverse effects. However, it has the limitation that the trial does not compare the cetuximab combination with a platinum-based CRT treatment, as is currently the standard-of-care for patients with SCCHN. Also, the trial does not administer the same radiation regimens to all patients, which complicates how the results should be interpreted.

So far, based on the response rate and improvement in survival, the FDA has approved use of cetuximab as a radiation-sensitizing agent for head and neck cancer

patients undergoing primary radiation-based treatment. Based on this approval, it is expected that cetuximab will be part of the standard-of-care in the management of locally advanced head and neck cancer.

Phase II/III clinical trial for cetuximab in combination with chemotherapy

The fact that patients with recurrent SCCHN who failed to respond to platinum-based therapy have less than 5% chance of response to second-line treatment and less than two months of median progression-free survival (PFS), has led to a multi-institutional trial of cetuximab in combination with cisplatin in patients with recurrent SCCHN refractory to cisplatin containing chemotherapy (Table 1).²² The final report of the efficacy and safety of cetuximab in combination with cisplatin in patients with recurrent SCCHN refractory to cisplatin containing chemotherapy was presented at the 2002 American Society of Clinical Oncology meeting.²⁹ Patients with metastatic or recurrent SCCHN were given a standard cytotoxic regimen of either cisplatin/paclitaxel or cisplatin/5-fluorouracil (5-FU). After two treatment cycles, responding patients continued the initial treatment regimen while patients with either stable disease (45 patients) or progressive disease (27 patients) then proceeded to the experimental regimen consisting of

cetuximab/cisplatin. The cetuximab/cisplatin regimen was generally well tolerated and cetuximab appears to convert a percentage of cisplatin non-responders to responders; the unverified response rate in stable disease group and disease progression group were 20% and 21%, respectively, which is higher than expected with standard cytotoxic therapy alone.

In a phase III randomized trial conducted by the ECOG to determine the effect of the addition of cetuximab on the recurrent and/or metastatic SCCHN, patients were assigned to receive cisplatin every four weeks, with weekly cetuximab or placebo (Table 1).²³ Among 117 evaluable patients treated, the addition of cetuximab to cisplatin significantly improved the response rate compared with placebo (26% versus 10%; $P = 0.03$). However, PFS and overall survival were not significantly improved by the addition of cetuximab (Figure 2). The development of skin toxicity was significantly associated with a reduction in the risk of death: the hazard ratio for survival by skin toxicity in cetuximab-treated patients was 0.42. The development of a dose-related acne-like rash which is a sterile, non-suppurative folliculitis is the most common adverse event occurring in nearly all patients treated with cetuximab. It generally appears during the first three weeks of treatment, with complete resolution within one to three months after discontinuation. Interestingly, the analysis in four phase II studies of patients with colorectal cancer, SCCHN, and pancreatic cancer demonstrated the relationship between the presence and

the severity of the rash and survival. Patients who developed the acne-like rash survived longer than those who did not develop a rash, and those with a more intense rash survived longer still, suggesting that skin rash may be an important clinical surrogate of efficacy.³⁰ The presence and intensity of the cetuximab-induced acne-like rash predict increased survival in studies across multiple malignancies.

Similar positive results were observed in a phase III trial of cetuximab in combination with platinum-based chemotherapy as a first-line treatment for SCCHN (EXTREME).²⁵ Of the 442 patients, 220 with recurrent or metastatic SCCHN were treated with either cisplatin or carboplatin, fluorouracil plus cetuximab. The addition of cetuximab significantly prolonged the median overall survival from 7.4 months in the chemotherapy-alone group to 10.1 months in the group that received chemotherapy plus cetuximab ($P = 0.04$). Longer PFS was also observed in the group that received chemotherapy and cetuximab (5.6 months) when compared with the group that received only chemotherapy (3.3 months) ($P < 0.001$) and increased the response rate from 20% to 36% ($P < 0.001$). The most common grade 3 or 4 adverse events in both groups were anemia, neutropenia and thrombocytopenia.

While EXTREME is the first study in over 25 y in which any treatment for SCCHN has shown a survival advantage in the recurrent setting, what was not addressed was the complex question of sequence, cross-resistance and synergism. The EXTREME trial did not show whether the survival advantage of the addition of cetuximab would be retained if patients were first treated with the chemotherapy regimen and received cetuximab upon progression. If cetuximab were as effective when given after progression on platinum and 5-FU, then combined toxicity and costs could be minimized for patients and payors. As with other targeted therapy combinations, the costs incurred are substantial; drug charges and extra weekly visits are estimated to cost at least three times more when cetuximab is added to cisplatin and 5-FU for the management of SCCHN.

So far, based on the response rate, the FDA has approved use of cetuximab for patients with recurrent or metastatic head and neck cancer. Based on this approval, it is expected that cetuximab will be one of the treatment options for recurrent disease as either a single agent or used in combination with chemotherapy. However, due to the lack of clear evidence for the improvement in survival, its use will be limited.

Side-effect profiles of cetuximab therapy

As cetuximab is becoming more and more popular in the management of head and neck cancer, a thorough understanding of its side-effect profile is important, especially for the community oncologist as some effects such as hypersensitivity and cardiopulmonary toxicity can be fatal. Therefore, in making decisions for the use of cetuximab, a careful comparison between conventional CRT and cetuximab-based therapy needs to consider each patient's

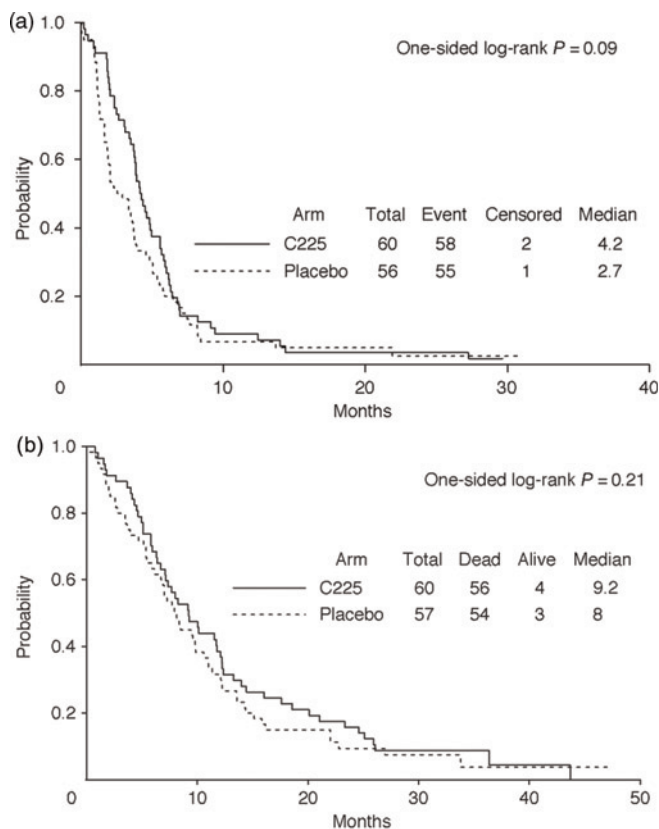


Figure 2 Kaplan-Meier estimates of progression-free survival (a) and overall survival (b) among all patients in phase III trial who were randomly assigned to cisplatin plus cetuximab or cisplatin alone²³

clinical status and risk profile. The information regarding the side-effect profiles have been accumulated since the phase I trial studied in multiple different cancers¹⁶ and the several clinical trials using cetuximab either as a single agent or combined with chemotherapy have provided valuable information in specific clinical settings.^{16,22,23,26,29,30} Additionally, the phase III study reported by Bonner *et al.*²⁴ provides a systemic analysis for general side-effect profiles and several specific and important side-effects of cetuximab when it is combined with radiotherapy. In the following section, we have summarized the overall side-effects profile, with a focus on the data from Bonner *et al.*²⁴ (Table 2).

General side-effect profiles

The most common adverse events seen in patients receiving cetuximab as a single agent ($n = 103$) were acneiform rash (76%), asthenia (45%), pain (28%), fever (27%) and weight loss (27%).^{16,26} The most common adverse events seen in patients receiving cetuximab with irinotecan ($n = 354$) or cetuximab as a single agent ($n = 420$) were acneiform rash (88%/90%), asthenia/malaise (73%/48%), diarrhea (72%/25%), nausea (55%/29%), abdominal pain (45%/26%), vomiting (41%/25%), fever (34%/27%), constipation (30%/26%) and headache (14%/26%)¹⁵ (Table 3).

The most common adverse events seen in cetuximab combination with radiation therapy ($n = 208$) versus radiation alone ($n = 212$) were mucositis-stomatitis (93%/94%), acneiform rash (87%/10%), radiation dermatitis (86%/90%), weight loss (84%/72%), xerostomia (72%/71%), dysphagia (65%/63%), asthenia (56%/49%), nausea (49%/37%), constipation (35%/30%) and vomiting (29%/23%).²⁴ Additional serious adverse events associated with cetuximab in combination with radiation therapy in patients with head and neck cancer were mucositis (6%), radiation dermatitis (3%), confusion (2%) and diarrhea (2%).³¹

Table 3 Incidence of selected adverse events ($\geq 10\%$) of cetuximab in patients with locoregionally advanced SCCHN as modified from the reference (The Internet Drug Index)

Body system preferred term	Cetuximab plus radiation ($n = 208$)		Radiation therapy alone ($n = 212$)	
	Grades 1–4	Grades 3 and 4	Grades 1–4	Grades 3 and 4
% of Patients				
Body as a whole				
Asthenia	56	4	49	5
Fever*	29	1	13	1
Headache	19	<1	8	<1
Infusion reaction†	15	3	2	0
Infection	13	1	9	1
Chills*	16	0	5	0
Digestive				
Nausea	49	2	37	2
Emesis	29	2	23	4
Diarrhea	19	2	13	1
Dyspepsia	14	0	9	1
Metabolic/nutritional				
Weight loss	84	11	72	7
Dehydration	25	6	19	8
Respiratory				
Pharyngitis	26	3	19	4
Skin/appendages				
Acneiform rash‡	87	17	10	1
Radiation dermatitis	86	23	90	18
Application site reaction	18	0	12	1
Pruritus	16	0	4	0

*Includes cases also reported as infusion reaction

†Infusion reaction is defined as any event described at any time during the clinical study as 'allergic reaction' or 'anaphylactoid reaction', or any event occurring on the first day of dosing described as 'allergic reaction', 'anaphylactoid reaction', 'fever', 'chills', 'chills and fever', or 'dyspnea'

‡Acneiform rash is defined as any event described as 'acne', 'rash', 'maculopapular rash', 'pustular rash', 'dry skin' or 'exfoliative dermatitis'

Table 2 Antitumor efficacy of phase III cetuximab trial combined with radiotherapy (modified from Bonner *et al.*²⁴)

Variable	Radiotherapy alone (n = 213)	Radiotherapy plus cetuximab (n = 211)	Hazard ratio (95% CI)*	P value†
Locoregional control				
Median duration (months)	14.9	24.4	0.68 (0.52–0.89)	0.01
Rate at 2 y (%)	41	50		
Progression-free survival				
Median duration (months)	12.4	17.1	0.70 (0.54–0.90)	0.01
Rate at 2 y (%)	37	46		
Overall survival‡				
Median duration (months)	29.3	49	0.74 (0.57–0.97)	0.03
Rate at 3 y (%)	45	55		
Median duration according to radiotherapy regimen (months)				
Once daily	15.3	18.9	1.01	
Twice daily	53.3	58.9	0.74	
Concomitant boost	31	>66.0	0.64	
Cumulative incidence of distant metastasis				
Rate at 1 y (%)	10	8		
Rate at 2 y (%)	17	16		

*The hazard ratio is for the outcome in the group assigned to radiotherapy plus cetuximab as compared with the group assigned to radiotherapy alone. Outcomes were as follows: progression of locoregional disease or death (in the analysis of locoregional control), progression of disease or death (in the analysis of progression-free survival) and death (in the analysis of overall survival). CI denotes confidence interval

†P values were calculated by the log-rank test

‡Median follow-up was 54.0 months in both groups

Hypersensitivity reaction

Grade 3/4 infusion-related hypersensitivity reactions, rarely with fatal outcome (<1 in 1000), occurred in approximately 3% (46/1485) of patients receiving cetuximab therapy, characterized by rapid onset of airway obstruction (bronchospasm, stridor, hoarseness), urticaria, hypotension and/or cardiac arrest.^{15–17,22–24,26,29} Severe infusion reactions require immediate and permanent discontinuation of cetuximab therapy. Most reactions (90%) were associated with the first infusion of cetuximab despite the use of prophylactic antihistamines.²⁴ Caution must be exercised with every cetuximab infusion as there were patients who experienced their first severe infusion reaction during later infusions. A one-hour observation period is recommended following the cetuximab infusion. Longer observation periods may be required in patients who experience infusion reactions.

Cardiopulmonary toxicity

Cardiopulmonary arrest and/or sudden death occurred in 2% (4/208) of patients with SCCHN treated with radiation therapy and cetuximab as compared with none of 212 patients treated with radiotherapy alone.²⁴ Cetuximab in combination with radiation therapy should be used with caution in patients with known coronary artery disease, congestive heart failure and arrhythmias. Close monitoring of serum electrolytes, including serum magnesium, potassium and calcium, during and after cetuximab therapy is recommended.

Skin rash

In clinical studies of cetuximab, dermatological toxicities, including acneiform rash, skin drying and fissuring, and inflammatory and infectious sequelae (e.g. blepharitis, cheilitis, cellulites and cyst) were reported. Severe (grade 3/4) acneiform rash was reported in 17% of 208 patients with head and neck cancer treated with cetuximab plus radiation and in 1% of 103 patients treated with cetuximab as a single agent.²⁴

The biology underlying the observed association between EGFR inhibition and rash is not clearly understood. EGFR polymorphisms could possibly account for both therapeutic efficacy and cutaneous toxicity. One study by Amador *et al.*³² found that a lower number of CA single-sequence repeats in intron 1 of the EGFR gene correlates with both response to EGFR inhibition and development of skin toxicity. Alternatively, the association of treatment efficacy with the development of rash may simply be a reflection of adequate drug exposure.

An additional concern is that early development of rash may not be predictive of outcome. The study by Vermorken *et al.*²⁵ demonstrates that although development of skin reactions and acne-like rash appeared to trend toward improved response and disease control, early development of these toxicities (within the first three weeks of therapy) was not associated with improved response, time to progression or survival. It is notable that most cutaneous

toxicities that developed in patients enrolled in this study were mild.

Two possible mechanisms of EGFR inhibitor-related cutaneous toxicity exist, each with implications for mitigating rash severity. The most simple and likely mechanism is direct inhibition of EGFR in the skin itself. Keratinocytes principally express HER1/EGFR as the primary HER family member.³³ The administration of a single dose of cetuximab results in a dose-dependent decrease in EGFR protein expression levels in skin biopsies over time.³⁴ As a result, topical therapies for rash that block skin EGFR inhibition are in clinical development.^{35,36} A second proposed mechanism for the observed rash is the result of a systemic immunological reaction.³⁷ EGFR-associated rash has been observed to be self-limited in a small number of patients, improves over time during continuous therapy and can be managed effectively with steroids and immunosuppressive agents. Because EGFR inhibitor-associated rash is related to clinical anticancer activity, improved treatment of this toxicity may permit more patients to benefit from treatment with EGFR inhibitors.

Hypomagnesemia

The incidence of hypomagnesaemia (both overall and severe [grades 3 and 4]) was increased in patients receiving cetuximab alone or in combination with chemotherapy as compared with those receiving best supportive care or chemotherapy alone based on ongoing, controlled clinical trials in 244 patients. Approximately one-half of these patients receiving cetuximab experienced hypomagnesaemia and 10–15% experienced severe hypomagnesaemia. Electrolyte repletion was necessary in some patients and in severe cases, intravenous replacement was required.

Other monoclonal antibodies targeting EGFR

hR3

hR3 is a humanized murine monoclonal antibody to EGFR. hR3 was tested for safety and efficacy in combination with radiation therapy in patients with stage III/IV SCCHN not amenable to surgery in a phase Ib/IIa trial. hR3 was generally well tolerated without significant hypersensitivity. The most common side-effects were nausea/vomiting, headache and fatigue. Seven complete responses and one progressive disease were observed in eight evaluable patients at week 12 or 24 assessments.³⁸

Matuzumab

Matuzumab is another humanized monoclonal antibody of the IgG₁ subclass that binds selectively to the EGFR, competing with both EGF and TGF- α for binding. In contrast to the chimeric antibody cetuximab, the humanized antibody has a prolonged half-life, approximately 6–7 d, allowing for a less frequent administration schedule.³⁹ Currently, matuzumab is in clinical studies for the treatment

of various solid tumors known to overexpress the EGFR including SCCHN.^{40,41}

Panitumumab

Panitumumab is a fully human monoclonal antibody that binds to the extracellular ligand-binding domain of the EGFR. By blocking binding of natural ligands to the EGFR, panitumumab inhibits the function of the receptor. This results in the blockade of EGFR-mediated signaling pathways, causing G1/G0 cell-cycle arrest, growth inhibition and apoptosis.⁴² It has been approved by the US FDA as a single agent for the treatment of metastatic colorectal cancer. Interestingly, in metastatic colorectal cancer, panitumumab activity was found to be limited to patients whose tumors expressed wild-type *KRAS*; the response rate was 17% versus 0% in patients with tumors with *KRAS* mutations.^{43,44} The PFS was longer for patients with wild-type *KRAS* tumors treated with panitumumab. Whether the presence of wild-type *KRAS* is also associated with response to panitumumab in patients with SCCHN tumors is unknown. The overexpression of *HRAS* appears to be fairly common in SCCHN tumors, although *KRAS* mutations are infrequent.⁴⁵

Phase I clinical trials

Panitumumab has shown efficacy in clinical trials for patients with advanced SCCHN. An initial phase I study of this agent was conducted in 19 treatment-naïve patients with stage III/IV disease.⁴⁶ Panitumumab (2.5 mg/kg) was administered with weekly carboplatin plus radiotherapy. Of the 15 patients evaluable for response, 13 (87%) had a complete response. The most common adverse events included dysphagia, mucositis and acneiform rash. These preliminary results suggest that panitumumab, in combination with carboplatin and radiation therapy, may potentially be effective for the treatment of patients with advanced SCCHN.

Phase II and III clinical trials

Currently, there are a number of phase II trials of panitumumab in SCCHN. PRISM is a non-randomized, open-label, multicenter phase II study designed to evaluate single-agent panitumumab as second-line therapy in patients with platinum-refractory recurrent or metastatic SCCHN. In this trial, all patients will receive panitumumab and treatment will be continued until disease progression, as measured by tumor assessment, or unacceptable toxicity, study withdrawal, death or end of study.

Another phase II study (PARTNER) is a randomized, multicenter, open-label trial designed to evaluate the addition of panitumumab to a combination of docetaxel and cisplatin for first-line treatment of metastatic or recurrent SCCHN. The study allows for the cross-over to panitumumab monotherapy for patients with disease progression on docetaxel (Taxotere)/cisplatin alone. Patients eligible for the PARTNER trial must have histologically or cytologically confirmed metastatic and/or recurrent SCCHN not curable

by surgery and/or radiation therapy. The primary study endpoint for this trial is PFS. Secondary endpoints include overall response rate, time to response, duration of response, rate of disease control and safety. Currently, however, this study has been suspended for safety analysis.

There are also two phase II trials studying panitumumab in the treatment of locally advanced SCCHN. One trial examines the difference in LRC rate at two years in subjects receiving CRT or panitumumab plus radiotherapy as first-line treatment for locally advanced SCCHN. The second trial seeks to evaluate the PFS of locoregionally advanced (stages III/IV) SCCHN patients undergoing postoperative CRT with panitumumab. Notably, this study will also attempt to correlate efficacy parameters with a number of biomarkers, including EGFR and downstream pathway activation.

Currently, there is one phase III trial examining panitumumab in SCCHN. This study seeks to compare the PFS of patients with locally advanced SCCHN treated with radiotherapy and high-dose cisplatin versus radiotherapy and panitumumab.

Gefitinib: molecular inhibitor targeting EGFR

Gefitinib is an oral EGFR-specific anilinoquinazoline that reversibly inhibits autophosphorylation of EGFR (Figure 1). Preclinical studies show that it inhibits proliferation of cell lines expressing even low levels of EGFR and inhibited the growth of tumor xenografts in nude mice. In addition, it is the first drug of its class to be approved by the FDA as third-line treatment for patients with advanced NSCLC. However, it should be noted that its indication has recently been limited to those patients who have benefited previously or are benefiting from gefitinib treatment.

Phase I clinical trial

In several phase I studies investigating the tolerability and efficacy in patients with a variety of solid tumors, including SCCHN, gefitinib was generally well tolerated; the most common side-effects were mild/moderate (grade 1/2) reversible rash and diarrhea, whose incidence and severity increased with increasing dose.^{47,48} The MTD was over 700 mg/d. Partial responses and disease stabilization were observed at doses over 150 mg/d with no suggestion that higher doses provided greater antitumor activity. Similarly, pre- and post-treatment skin biopsy results from cancer patients revealed that EGFR signaling was inhibited at doses over 150 mg/d, with no clear dose dependence above this level. Based on these results, two doses below the MTD were chosen for evaluation in Phase II studies: 250 mg/d, at which dose responses had been seen with minimum toxicity, and 500 mg/d, the highest dose that has been tolerated on prolonged treatment.^{47,48} Two phase II studies have been designed to determine whether 250 or 500 mg/d of gefitinib has optimal activity as a monotherapy in recurrent SCCHN.

Phase II clinical trial

In one phase II study of patients with recurrent or metastatic SCCHN, patients were treated with single-agent

gefitinib 500 mg/d as second-line therapy (Table 4).⁴⁹ Among 47 patients assessable for response, one complete and four partial responses were observed, with a response rate of 10.6%, and 20 patients had stable disease with a disease control rate of 53%. The median time to disease progression (TTP) and overall survival were 3.4 and 8.1 months, respectively. The only grade 3 toxicity encountered was diarrhea in three patients. Performance status and development of skin toxicity were found to be strong predictors of response, progression and survival. While other reports have showed no statistically significant correlation between the presence of the rash and TTP or survival, this study demonstrated that development of rash was associated with statistically and clinically meaningful improvements in TTP and overall survival ($P = 0.0002$ and 0.001 , respectively).

Another phase II trial treated patients with recurrent SCCHN with gefitinib at the level of 250 mg/d. Of the 14 patients evaluable for response, four had stable disease. Toxicity was assessed in 17 patients; the most common adverse events were grade 1/2 rash in 50% of patients and grade 1/2 diarrhea in 20% of patients. Further data from this patient population will be assessed.⁵⁰

Phase III clinical trial

Recent results from a phase III trial of gefitinib and docetaxel have been published.⁶⁰ Two hundred and seventy patients with refractory or metastatic SCCHN were enrolled to receive either docetaxel and a placebo (arm A) or docetaxel and gefitinib (arm B). The primary endpoint was to demonstrate an improvement in median overall survival. The study, however, was terminated at interim analysis in November 2008 because it was highly unlikely that the primary endpoint could be met. The median overall survival was six months in arm A versus 6.8 months in arm B ($P = 0.74$). The objective response rate in 168 evaluable patients was 6% in arm A versus 14% in arm B ($P = 0.16$). The median time to progression was two months and 3.5 months ($P = 0.03$) and median PFS 2.2 months and 3.3 months ($P = 0.18$) in arms A and B, respectively. The authors concluded that while the addition of gefitinib to docetaxel was well tolerated and improves time to progression, it did not improve survival in previously treated and poor PFS patients with refractory or metastatic SCCHN.

Two phase III comparative studies of gefitinib 250 and 500 mg/d versus chemotherapy for previously treated patients with SCCHN are under development. Patients are

Table 4 Clinical trial results of gefitinib and erlotinib in head and neck cancer

Tumor type	Phase	Vector name	Delivery mode	No. of patients evaluable	No. and type of responses	Adverse reactions	Reference
Head and neck, lung, nasopharynx, salivary, thyroid	I	Gefitinib	Oral dose	16	2 partial response, 10 stable disease, 4 progressive	Diarrhea, fatigue, neutropenia, rash	51
Head and neck	I	Gefitinib	Oral dose plus radiation/chemoradiation	23	21 complete response, 2 stable disease	Diarrhea, neutropenia, rash	52
Head and neck	I/II	Gefitinib	Oral dose plus radiation	16	4 complete response, 8 partial response, 1 stable disease, 3 progressive	Hepatic toxicity, stomatitis	53
Head and neck	II	Gefitinib	Oral dose	47	1 complete response; 4 partial response; 20 stable disease; 22 progressive	Acne-like rash, diarrhea	49
Head and neck	II	Gefitinib	Oral dose plus chemotherapy/radiation	50	18 complete response, 22 partial response, 7 stable disease, 2 progressive	Diarrhea, mucositis	54
Head and neck	III	Gefitinib	Oral dose	256	Two complete response, 14 partial response, 141 stable disease, 99 progressive	Diarrhea, nausea, rash	55
Head and neck	I	Erlotinib	Oral dose	31	9 objective response, 20 stable disease, 2 progressive	Asthenia, diarrhea, rash	56
Head and neck	I/II	Erlotinib	Oral dose plus cisplatin	39	1 complete response, 8 partial response, 21 stable disease, 9 progressive	Anemia, dry skin, fatigue, hypomagnesia, lymphopenia, rash	57
Head and neck	I/II	Erlotinib	Oral dose plus bevacizumab	46	4 complete response, 3 partial response, 14 stable disease, 25 progressive	Diarrhea, rash	58
Head and neck	II	Erlotinib	Oral dose	78	10 partial response; 23 stable disease; 45 progressive	Acne-like rash, diarrhea, fatigue, headache, nausea and vomiting	59

randomized to one of three treatment arms: chemotherapy alone or chemotherapy plus gefitinib at one of two dose levels, 250 or 500 mg/m².

Erlotinib: molecular inhibitor to EGFR

Erlotinib is another anilinoquinazoline derivative and orally active, reversible EGFR inhibitor that can induce both cell-cycle arrest in G1 and apoptosis (Figure 1). It inhibits EGFR autophosphorylation and its selectivity is more than 1000 times greater than other tyrosine kinase inhibitors. It reduced EGFR-associated phosphorylation by about 70% 24 h after a single 100 mg/kg dose. In mouse xenograft models, concurrent erlotinib and cisplatin chemotherapy produced increased antitumor activity over that of cisplatin alone, without any increased toxicity.

Phase I clinical trial

In a phase I dose-escalating pharmacological study for feasibility of erlotinib in patients with advanced solid malignancies including head and neck cancer, patients were administered in one of three regimens: once daily for three days a week, every three out of four weeks; once daily for every three out of four weeks; and on a continuous, uninterrupted schedule.⁶¹ Oral administration of erlotinib was well tolerated and demonstrated antitumor activity. Diarrhea and skin rash were the principal toxicities and dose-limiting toxicities (DLT) were observed over 150 mg/d; three patients treated at the 200 mg/d dose level experienced DLT, grade 3 or 4 diarrhea that was manageable. The 150 mg/d on a continuous schedule was selected for subsequent phase II studies based on its safety and tolerability profile.^{61,59}

Phase II clinical trial

Further evaluation of erlotinib in patients with recurrent or metastatic SCCHN has been performed to determine the efficacy of erlotinib as a single agent in phase II study (Table 4).⁵⁹ Of 115 patients enrolled, 47% of patients received erlotinib at 150 mg daily throughout the entire study, 6% had dose escalations and 46% required dose reductions and/or interruptions. Five patients achieved partial responses on study, for an overall objective response rate of 4.3%. Forty-four patients achieved disease stabilization for a median duration of 16.1 weeks. The median PFS and the median overall survival were 9.6 weeks and 6.0 months, respectively. Rash and diarrhea were the most common drug-related toxicities, encountered in 79% and 37% of patients, respectively, although the severity was mild to moderate in most cases.

As reported in other EGFR targeting agents, subgroup analyses revealed a statistically significant difference in overall survival favoring patients who developed at least grade 2 skin rashes versus those who did not ($P = 0.045$). Furthermore, in an analysis of three phase II trials of single-agent erlotinib in patients with refractory non-small-cell lung carcinoma (NSCLC), SCCHN and

ovarian cancers, the incidence of rash was 75%, 79% and 82%, respectively, after the first dose of erlotinib. In all three trials, however, patients with rash had significantly longer survival than those without rash ($P = 0.0001$, 0.039, 0.009).

In addition to monotherapeutic approach, several studies are ongoing, evaluating erlotinib in combination with chemotherapeutics patients with various solid tumors including SCCHN. In these studies, there were no evidence of overlapped toxicities or interactions between erlotinib and chemotherapeutics and combined treatment achieved promising objective responses.

Lapatinib: molecular inhibitor to EGFR and HER2

Lapatinib (Tykerb) is an orally active small molecule that reversibly inhibits EGFR and HER2, leading to the inhibition of mitogen-activated protein kinase and phosphoinositide 3-kinase signaling in EGFR-expressing and HER2-overexpressing tumor cells and xenografts. Lapatinib-treated tumor cells undergo apoptosis or growth arrest, depending upon the cell type. Lapatinib has already been approved by the US FDA as a treatment for HER2-positive metastatic breast cancer.

Phase I clinical trials

In a phase I study of the safety/tolerability and efficacy of lapatinib, 67 heavily pretreated patients with EGFR-expressing and/or HER2-overexpressing metastatic cancers, including head and neck cancer, were randomly assigned to one of five dose cohorts of lapatinib administered once daily.⁶² Lapatinib was well tolerated at doses ranging from 500 to 1600 mg once daily. Fifty-nine patients were evaluable for disease assessment. Four patients had a partial response and all had metastatic breast cancer. Twenty-four patients experienced stable disease, including three with head and neck cancer. The most common drug-related toxicities were diarrhea (42%) and rash (31%). Unlike other EGFR inhibitors, the incidence and severity of the skin rash was not associated with the clinical response. This lack of correlation may be due to the unique 4-anilinoquinazoline structure of lapatinib in contrast to gefitinib and erlotinib which are quinazolines.

Phase II clinical trial

Phase II trials have begun to evaluate the efficacy of lapatinib in SCCHN. One multi-institutional trial enrolled patients with refractory or metastatic SCCHN into two cohorts: arm A consisted of 27 patients without prior exposure to an EGFR inhibitor, while arm B consisted of 15 patients with prior exposure to an EGFR inhibitor.⁶³ All subjects were treated with lapatinib 1500 mg optical density. Consistent with the phase I studies, the most frequent adverse events were diarrhea (40%), fatigue (21%), rash (21%) and nausea (14%). No objective response was observed in either arm. In an intent-to-treat analysis,

stable disease was the best response observed in 37% of arm A and 20% of arm B subjects. The median PFS was 1.6 months in arm A and 1.7 months in arm B. While lapatinib was well tolerated in both arms, it appears to have little activity in either EGFR inhibitor-naïve or refractory patients.

The promising results of lapatinib in combination with chemoradiation have led to a phase III trial (MAINTYNANCE) of lapatinib in high-risk SCCHN patients following surgery. A total of 680 patients with locally advanced SCCHN that have undergone surgery will be treated with radiotherapy and cisplatin plus lapatinib.

Conclusion and future challenges

In the United States, each year, more than 55,000 new cases of head and neck cancer are diagnosed, and 13,000 die from the disease. Radiation, surgery and chemotherapy have been the mainstays of head and neck cancer treatment. Currently, in an effort to enhance efficacy, reduce toxicity and prevent resistance to each of these therapies, combinations of conventional therapies and molecular targeted has been the main stream of clinical research. For example, over the past decade, the important role of different growth factors and their receptors and signal transduction pathways in the genesis and progression of tumors has been well recognized and their mechanism of action and interactions is gradually being unraveled. EGFR overexpression is present in the vast majority of squamous cell head and neck cancers and carries a worse prognosis. EGFR is the target of multiple specifically targeted monoclonal antibodies and tyrosine kinases, which are in various stages of clinical development in SCCHN. The search for EGFR mutations is an area of active investigation. The incidence and impact of EGFR mutations in SCCHN have yet to be determined.

So far, out of several targeted therapeutics tested in clinical trial, incorporations of cetuximab into conventional regimens have shown a benefit to SCCHN patient patients. In fact, so far, only cetuximab has been FDA-approved for two indications in the head and neck cancer population: (1) as a radiation-sensitizing agent for patients undergoing primary radiation based treatment and (2) for patients with recurrent or metastatic disease. Cetuximab is an active agent in SCCHN, particularly when given in combination with radiation therapy and chemotherapy. However, some caution must be exercised by community oncologists when considering the addition of cetuximab to treatment. On the one hand, concomitant use of cetuximab and radiotherapy has led to a gain in survival and an improvement in the quality of life for locally advanced SCCHN. On the other hand, the addition of cetuximab has not demonstrated a clear-cut benefit; while cetuximab has been approved for the treatment of metastatic disease, the benefit is minimal. Furthermore, there is no study so far which has shown equivalent survival of cetuximab and radiation versus cisplatin and radiation. In addition, the side-effects of cetuximab, most notably skin rash and hypersensitivity, may cause physicians and patients to think twice before incorporating it into the therapeutic regimen.

Presently, out of many side-effect profiles, the hypersensitivity reaction is the most challenging problem. As this side-effect can be potentially fatal, certain measures need to be taken to give enough comfort for community oncologists who are willing to use cetuximab, but still are hesitant due to the rare but devastating side-effect. A recent study of the mechanism for the hypersensitivity reaction assessed by Chung *et al.*²⁰ at Vanderbilt University may provide certain insight for future guidelines for cetuximab therapy. In this study, multiple patient serums were obtained from two cohorts: 71 pretreatment samples from patients who subsequently received cetuximab and 69 healthy controls. Samples were analyzed for total cetuximab-specific and mouse-specific IgE. Of the 71 patients who received cetuximab, 24 had a hypersensitivity reaction, 21 of which were severe reaction. Fifteen patients had cetuximab-specific IgE, and all 15 experienced a severe hypersensitivity reaction and thus were not re-challenged. Six patients experienced a hypersensitivity reaction, but were cetuximab-specific IgE negative. Four of these patients were re-challenged and tolerated further infusion without any incidence. The serum samples from healthy volunteers were 21.7% (15/69) positive for cetuximab-specific IgE. These data suggest that in the southeastern United States, approximately 20% of patients have a pre-existing cetuximab-specific IgE and that the presence of cetuximab-specific IgE is predictive for a severe allergic reaction. Although this study needs to be repeated with a larger-scale study design, future treatment based on cetuximab may need to include the cetuximab IgE profiling as pretreatment testing, possibly by using the newly developed ImmunoCAP assay in affected areas.

Although cetuximab combined with radiotherapy showed a very exciting finding, there are still two challenging questions that need to be answered in future clinical trials. The first questions arose at the 2006 Annual Meeting of the American Society of Clinical Oncology, where the study for an induction chemotherapy with cisplatin, 5-FU and docetaxel (Taxotere) followed by CRT and surgical resection in patients with locally advanced SCCHN and an update of the EORTC (European Organization for Research and Treatment of Cancer trial 24971) which randomized SCCHN patients to receive cisplatin/5-FU or cisplatin/5-FU/docetaxel in the induction setting were reported. Both studies showed impressive, statistically significant gains in PFS and overall survival. These results have raised an important question for the treatment of SCCHN patients with good performance status. The question is how to incorporate these clinical advances with those described by Bonner *et al.* – should a patient get neoadjuvant cisplatin/5-FU/docetaxel chemotherapy followed by combined cetuximab/radiation therapy? While there are no clinical data for such a regimen in terms of either efficacy or safety, it is possible to see the use of a combination of both regimens based on the outcomes of the two recently published randomized clinical trials. In summary, integration of this therapy with the results of neoadjuvant cisplatin, 5-FU and docetaxel remains a question and ongoing clinical trials will provide a proper sequence and strategies that incorporate cetuximab in the treatment of SCCHN.

A second question based on prior clinical trial results is whether cetuximab with CRT can result in a better outcome as compared with cetuximab combined with radiation therapy. A pilot phase II demonstrated the preliminary efficacy of combining cetuximab with CRT in locally advanced SCCHN.⁶⁴ Twenty-two patients received concomitant boost radiotherapy, cisplatin (100 mg/m² intravenously weeks 1 and 4) and cetuximab (400 mg/m² intravenously week 1, followed by 250 mg/m² weeks 2–10). Although this study shows promising result, the study was closed for significant adverse events, including grade 3/4 adverse events (myocardial infarction, bacteremia and atrial fibrillation) and two deaths (one from pneumonia and one of unknown cause). Currently, a randomized phase III study is being conducted to further investigate the use of cisplatin and accelerated fractionated radiotherapy with or without cetuximab in locally advanced SCCHN.

In addition to cetuximab, lists of new targeting strategies in head and neck cancer have grown very rapidly recently, although most of them are still in the early stages of development. For example, EGFR downstream signaling pathways are the target of farnesyltransferase inhibitors and mammalian target of rapamycin inhibitors.⁶⁵ Cyclooxygenase-2 (COX-2) is overexpressed in premalignant lesions (oral leukoplakia) and in SCCHN. EGFR and COX-2 signaling pathways form a positive feedback loop. As their toxicity profiles are non-overlapping, a combination of COX-2 inhibitors and EGFR-targeting therapies looks attractive.⁶⁶ Another strategy uses inhibitors of the vascular endothelial growth factor (VEGF), although the majority of the studies examining the prognostic significance of VEGF expression did observe a worse outcome in patients with SCCHN-expressing VEGF and VEGF receptor 2 (VEGFR-2).⁶⁷ Anti-VEGF strategies include neutralizing antibodies to VEGF or VEGFR and VEGFR tyrosine kinase inhibitors.⁶⁷ Other kinase inhibitors like aurora kinase inhibitors, insulin-like growth factor inhibitors and histone acetylase inhibitors have recently gained interest as potential new promising ways of tackling tumors including SCCHN.⁶⁸

Furthermore, it is becoming increasingly clear that more subset analyses of clinical trials need to be performed. For example, in NSCLC, EGFR gene mutations and copy number can predict gefitinib sensitivity.⁶⁹ Response rates were found to be higher in patients with EGFR gene mutations or increased copy numbers. Cell line data suggest a similar case in SCCHN for both gefitinib and cetuximab.⁷⁰ These results also suggest that future therapies will be individualized to the biology of each patient's tumor, not just to the tumor type itself.

In summary, the results from current clinical trials suggest that inhibition of one aberrant pathway is not sufficient to control tumors and improve survival, due to multiple genetic mutations and multilevel stimulation of pathways. A greater understanding of an individual's cancer biology, adequate design of clinical evaluations and optimal application of drugs, as well as the development of new strategies to combine new drugs with conventional therapy and selecting the best tumor types to be targeted, are required to overcome the current limitations and to

benefit head and neck cancer patients with improved quality-of-life and extended survival.

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REFERENCES

- 1 Al-Sarraf M. Treatment of locally advanced head and neck cancer: historical and critical review. *Cancer Control* 2002;**9**:387–99
- 2 Jemal A, Clegg LX, Ward E, Ries LA, Wu X, Jamison PM, Wingo PA, Howe HL, Anderson RN, Edwards BK. Annual report to the nation on the status of cancer, 1975–2001, with a special feature regarding survival. *Cancer* 2004;**101**:3–27
- 3 Pignon JP, le Maitre A, Bourhis J. Meta-analyses of chemotherapy in head and neck cancer (MACH-NC): an update. *Int J Radiat Oncol Biol Phys* 2007;**69**:S112–4
- 4 Lamont JP, Kuhn JA, Nemunaitis JJ, McCarty TM. Gene therapy for head and neck cancers. *Oncology (Williston Park)* 2001;**15**:303–308; discussion 311–304
- 5 Hardisson D. Molecular pathogenesis of head and neck squamous cell carcinoma. *Eur Arch Otorhinolaryngol* 2003;**260**:502–8
- 6 Ah-See KW, Cooke TG, Pickford IR, Soutar D, Balmain A. An allelotyping of squamous carcinoma of the head and neck using microsatellite markers. *Cancer Res* 1994;**54**:1617–21
- 7 Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2000;**103**:211–25
- 8 Grunwald V, Hidalgo M. Developing inhibitors of the epidermal growth factor receptor for cancer treatment. *J Natl Cancer Inst* 2003;**95**:851–67
- 9 Hanawa M, Suzuki S, Dobashi Y, Yamane T, Kono K, Enomoto N, Ooi A. EGFR protein overexpression and gene amplification in squamous cell carcinomas of the esophagus. *Int J Cancer* 2006;**118**:1173–80
- 10 Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. *N Engl J Med* 2001;**345**:1890–900
- 11 Ang KK, Berkey BA, Tu X, Zhang HZ, Katz R, Hammond EH, Fu KK, Milas L. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. *Cancer Res* 2002;**62**:7350–6
- 12 Kong A, LeBoucher P, Leek R, Calleja V, Winter S, Harris A, Parker PJ, Larijani B. Prognostic value of an activation state marker for epidermal growth factor receptor in tissue microarrays of head and neck cancer. *Cancer Res* 2006;**66**:2834–43
- 13 Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin Cancer Res* 1995;**1**:1311–8
- 14 Herbst RS, Hong WK. IMC-C225, an anti-epidermal growth factor receptor monoclonal antibody for treatment of head and neck cancer. *Semin Oncol* 2002;**29**:18–30
- 15 Needle MN. Safety experience with IMC-C225, an anti-epidermal growth factor receptor antibody. *Semin Oncol* 2002;**29**:55–60
- 16 Baselga J, Pfister D, Cooper MR, Cohen R, Burtneiss B, Bos M, D'Andrea G, Seidman A, Norton L, Gunnett K, Falcey J, Anderson V, Waksal H, Mendelsohn J. Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. *J Clin Oncol* 2000;**18**:904–14
- 17 Robert F, Ezekiel MP, Spencer SA, Meredith RF, Bonner JA, Khazaeli MB, Saleh MN, Carey D, LoBuglio AF, Wheeler RH, Cooper MR, Waksal HW. Phase I study of anti-epidermal growth factor receptor antibody cetuximab in combination with radiation therapy in patients with advanced head and neck cancer. *J Clin Oncol* 2001;**19**:3234–43
- 18 Herbst RS, Arquette M, Shin DM, Dicke K, Vokes EE, Azarnia N, Hong WK, Kies MS. Phase II multicenter study of the epidermal growth factor receptor antibody cetuximab and cisplatin for recurrent and refractory squamous cell carcinoma of the head and neck. *J Clin Oncol* 2005;**23**:5578–87

- 19 Baselga J, Trigo JM, Bourhis J, Tortochaux J, Cortes-Funes H, Hitt R, Gascon P, Amellal N, Harstrick A, Eckardt A. Phase II multicenter study of the antiepidermal growth factor receptor monoclonal antibody cetuximab in combination with platinum-based chemotherapy in patients with platinum-refractory metastatic and/or recurrent squamous cell carcinoma of the head and neck. *J Clin Oncol* 2005;23:5568–77
- 20 Chung CH, Chan E, Berlin J, Gilbert J, Yarbrough W, Satinover S, Murphy B, Slebos RJ, Hatley T, Platts-Mills T. Cetuximab-related hypersensitivity reactions associated with pre-existing cetuximab-specific IgE antibody. *J Clin Oncol* 2007;25:9097
- 21 Vermoken JB, Trigo J, Hitt R, Koralewski P, Diaz-Rubio E, Rolland F, Knecht R, Amellal N, Schueler M, Baselga J. Open-label, uncontrolled, multicenter phase II study to evaluate the efficacy and toxicity of cetuximab as a single agent in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck who failed to response to platinum-based therapy. *J Clin Oncol* 2007;25:2171–7
- 22 Kies MS, Arquette MA, Nabell L, Quinn D, Shin DM, Needle MN, Waksal H, Hong WK, Herbst RS. Final report of the efficacy and safety of the anti-epidermal growth factor antibody Erbitux (IMC-C225), in combination with cisplatin in patients with recurrent squamous cell carcinoma of the head and neck (SCCHN) refractory to cisplatin containing chemotherapy. *Proc Am Soc Clin Oncol* 2002;21:925
- 23 Burtneess B, Goldwasser MA, Flood W, Mattar B, Forastiere AA. Phase III randomized trial of cisplatin plus placebo compared with cisplatin plus cetuximab in metastatic/recurrent head and neck cancer: an Eastern Cooperative Oncology Group study. *J Clin Oncol* 2005;23:8646–54
- 24 Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, Jones CU, Sur R, Raben D, Jassem J, Ove R, Kies MS, Baselga J, Youssoufian H, Amellal N, Rowinsky EK, Ang KK. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2006;354:567–78
- 25 Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, Erfan J, Zabolotnyy D, Kienzer HR, Cupissol D, Peyrade F, Benasso M, Vynnychenko I, De Raucourt D, Bokemeyer C, Schueler A, Amellal N, Hitt R. Platinum-based chemotherapy plus cetuximab in head and neck cancer. *N Engl J Med* 2008;359:1116–27
- 26 Shin DM, Donato NJ, Perez-Soler R, Shin HJ, Wu JY, Zhang P, Lawhorn K, Khuri FR, Glisson BS, Myers J, Clayman G, Pfister D, Falcey J, Waksal H, Mendelsohn J, Hong WK. Epidermal growth factor receptor-targeted therapy with C225 and cisplatin in patients with head and neck cancer. *Clin Cancer Res* 2001;7:1204–13
- 27 Wanebo HJ, Ghebremichael M, Burtneess B, Spencer S, Ridge J, Forastiere A, Ghebremichael M. Phase II evaluation of cetuximab (C225) combined with induction paclitaxel and carboplatin followed by C225, paclitaxel, carboplatin, and radiation for stage III/IV operable squamous cancer of the head and neck (ECOG, E2303). *J Clin Oncol* 2007;25:6015
- 28 Langer CJ, Lee JW, Patel UA, Shin DM, Argiris AE, Quon H, Ridge JA, Forastiere AA. Preliminary analysis of ECOG 3303: concurrent radiation (RT), cisplatin (DDP) and cetuximab (C) in unresectable, locally advanced (LA) squamous cell carcinoma of the head and neck (SCCHN). *J Clin Oncol* 2008;26:6006
- 29 Baselga J, Trigo JM, Bourhis J, Tortochaux J, Cortes-Funes H, Hitt R, Gascon P, Muesser M, Harstrick A, Eckardt A. Cetuximab (C225) plus cisplatin/carboplatin is active in patients (pts) with recurrent/metastatic squamous cell carcinoma of the head and neck (SCCHN) progressing on a same dose and schedule platinum-based regimen. *Proc Am Soc Clin Oncol* 2002;21:900
- 30 Saltz L, Kies MS, Abbruzzese JL, Azarnia N, Needle MN. The presence and intensity of the cetuximab-induced acne-like rash predicts increased survival in studies across multiple malignancies. *Proc Am Soc Clin Oncol* 2003;22:817
- 31 Chambers MS, Garden AS, Kies MS, Martin JW. Radiation-induced xerostomia in patients with head and neck cancer: pathogenesis, impact on quality of life, and management. *Head Neck* 2004;26:796–807
- 32 Amador ML, Oppenheimer D, Perea S, Maitra A, Cusatis G, Iacobuzio-Donahue C, Baker SD, Ashfaq R, Takimoto C, Forastiere A, Hidalgo M. An epidermal growth factor receptor intron 1 polymorphism mediates response to epidermal growth factor receptor inhibitors. *Cancer Res* 2004;64:9139–43
- 33 Lacouture ME. Mechanisms of cutaneous toxicities to EGFR inhibitors. *Nat Rev Cancer* 2006;6:803–12
- 34 Fracasso PM, Burris H 3rd, Arquette MA, Govindan R, Gao F, Wright LP, Goodner SA, Greco FA, Jones SF, Willcut N, Chodkiewicz C, Pathak A, Springett GM, Simon GR, Sullivan DM, Marcello R, Mayfield SD, Mauro D, Garrett CR. A phase 1 escalating single-dose and weekly fixed-dose study of cetuximab: pharmacokinetic and pharmacodynamic rationale for dosing. *Clin Cancer Res* 2007;13:986–93
- 35 Perez-Soler R, Zou Y, Li T, Ling Y. Steroids and immunosuppressive agents potentiate the cytotoxicity of the EGFR inhibitor erlotinib (E) in human skin keratinocytes whereas Vit K3 exerts a protective effect: implications for the management of the skin rash. *Proc Am Soc Clin Oncol* 2007;25:9124
- 36 Perez-Soler R, Zou Y, Li T, Tornos C, Ling Y. Topical vitamin K3 (Vit K3, Menadione) prevents erlotinib and cetuximab-induced EGFR inhibition in the skin. *Proc Am Soc Clin Oncol* 2006;24:3036
- 37 Perez-Soler R, Saltz L. Cutaneous adverse effects with HER1/EGFR-targeted agents: is there a silver lining? *J Clin Oncol* 2005;23:5235–46
- 38 Crombet T, Osorio M, Cruz T, Figueredo R, Koropatnick J, Reginfo E, Torres O, Perez R, Lage A. Use of the anti-EGFR antibody h-R3 in combination with radiotherapy in the treatment of advanced head and neck cancer. *Proc Am Soc Clin Oncol* 2002;21:53
- 39 Tewes M, Schleucher N, Dirsch O, Schmid KW, Rosen O, Arens HJ, Kovar A, Seeber S, Harstrick A, Vanhoefer U. Results of a phase I trial of the humanized anti epidermal growth factor receptor (EGFR) monoclonal antibody EMD 72000 in patients with EGFR expressing solid tumors. *Proc Am Soc Clin Oncol* 2002;21:378
- 40 Bier H, Hoffmann T, Haas Ivan Lierop A. Anti-(epidermal growth factor) receptor monoclonal antibodies for the induction of antibody-dependent cell-mediated cytotoxicity against squamous cell carcinoma lines of the head and neck. *Cancer Immunol Immunother* 1998;46:167–73
- 41 Kollmannsberger C, Schittenhelm M, Honecker F, Tillner J, Weber D, Oechsle K, Kanz L, Bokemeyer C. A phase I study of the humanized monoclonal anti-epidermal growth factor receptor (EGFR) antibody EMD 72000 (matuzumab) in combination with paclitaxel in patients with EGFR-positive advanced non-small-cell lung cancer (NSCLC). *Ann Oncol* 2006;17:1007–13
- 42 Carteni G, Fiorentino R, Vecchione L, Chiurazzi B, Battista C. Panitumumab a novel drug in cancer treatment. *Ann Oncol* 2007;18(Suppl. 6):vi16–21
- 43 Van Cutsem E, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, Canon JL, Van Laethem JL, Maurel J, Richardson G, Wolf M, Amado RG. 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. *J Clin Oncol* 2007;25:1658–64
- 44 Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, Juan T, Sikorski R, Suggs S, Radinsky R, Patterson SD, Chang DD. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;26:1626–34
- 45 Kiaris H, Spandidos DA, Jones AS, Vaughan ED, Field JK. Mutations, expression and genomic instability of the H-ras proto-oncogene in squamous cell carcinomas of the head and neck. *Br J Cancer* 1995;72:123–8
- 46 Wirth LJ, Posner M, Tishler RB, Haddad RI, Clark JR, Goguen L, Norris CM, Annino D, Costello R, Allen AM. Phase I study of panitumumab + chemoradiotherapy (CRT) for head and neck cancer (HNC). *J Clin Oncol* 2008;26:6007
- 47 Herbst RS, Maddox AM, Rothenberg ML, Small EJ, Rubin EH, Baselga J, Rojo F, Hong WK, Swaisland H, Averbuch SD, Ochs JL, Russo PM. 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. *J Clin Oncol* 2002;20:3815–25
- 48 Ranson M, Hammond LA, Ferry D, Kris M, Tullo A, Murray PI, Miller V, Averbuch S, Ochs J, Morris C, Feyereislova A, Swaisland H, Rowinsky EK. 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. *J Clin Oncol* 2002;20:2240–50
- 49 Cohen EE, Rosen F, Stadler WM, Recant W, Stenson K, Huo D, Vokes EE. Phase II trial of ZD1839 in recurrent or metastatic squamous cell carcinoma of the head and neck. *J Clin Oncol* 2003;21:1980–7
- 50 Cohen EE, Stenson K, Gustin DM, Lamont E, Mauer AM, Blair E, Stadler WM, Dekker A, Mallon W, Vokes EE. A phase II study of 250-mg gefitinib (ZD1839) monotherapy in recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN). *Proc Am Soc Clin Oncol* 2003;22:2021

- 51 Fury MG, Solit DB, Su YB, Rosen N, Sirotinak FM, Smith RP, Azzoli CG, Gomez JE, Miller VA, Kris MG, Pizzo BA, Henry R, Pfister DG, Rizvi NA. A phase I trial of intermittent high-dose gefitinib and fixed-dose docetaxel in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 2007;**59**:467–75
- 52 Chen C, Kane M, Song J, Campana J, Raben A, Hu K, Harrison L, Quon H, Dancey J, Baron A, Said S, Eckhardt SG, Raben D. Phase I trial of Gefitinib in combination with radiation or chemoradiation for patients with locally advanced squamous cell head and neck cancer. *J Clin Oncol* 2007;**25**:4880–6
- 53 Caponigro F, Romano C, Milano A, Solla R, Franchin G, Adamo V, Mari E, Morrica B, Pepe S. A phase I/II trial of gefitinib and radiotherapy in patients with locally advanced inoperable squamous cell carcinoma of the head and neck. *Anticancer Drugs* 2008;**19**:739–44
- 54 Hainsworth JD, Spigel DR, Burris HA 3rd, Markus TM, Shipley D, Kuzur M, Lunin S, Greco FA. Neoadjuvant chemotherapy/gefitinib followed by concurrent chemotherapy/radiation therapy/gefitinib for patients with locally advanced squamous carcinoma of the head and neck. *Cancer* 2009;**115**:2138–46
- 55 Stewart JS, Cohen EE, Licitra L, Van Herpen CM, Khorprasert C, Soulieres D, Vodvarka P, Rischin D, Garin AM, Hirsch FR, Varella-Garcia M, Ghiorgiu S, Hargreaves L, Armour A, Swaisland A, Vokes EE. Phase III study of gefitinib 250 compared with intravenous methotrexate for recurrent squamous cell carcinoma of the head and neck. *J Clin Oncol* 2009;**27**:1864–71
- 56 Thomas F, Rochemaix P, Benlyazid A, Sarini J, Rives M, Lefebvre JL, Allal BC, Courbon F, Chatelut E, Delord J-P. Pilot study of neoadjuvant treatment with Erlotinib in nonmetastatic head and neck squamous cell carcinoma. *Clin Cancer Res* 2007;**13**:7086–92
- 57 Siu LL, Soulieres D, Chen EX, Pond GR, Chin SF, Francis P, Harvey L, Klein M, Zhang W, Dancey J, Eisenhauer EA, Winquist E. Princess Margaret Hospital Phase II Consortium; National Cancer Institute of Canada Clinical Trials Group Study. Phase I/II trial of erlotinib and cisplatin in patients with recurrent or metastatic squamous cell carcinoma of the head and neck: a Princess Margaret Hospital phase II consortium and National Cancer Institute of Canada Clinical Trials Group Study. *J Clin Oncol* 2007;**25**:2178–83
- 58 Cohen EE, Davis DW, Karrison TG, Seiwert TY, Wong SJ, Nattam S, Kozloff MF, Clark JI, Yan DH, Liu W, Pierce C, Dancey JE, Stenson K, Blair E, Dekker A, Vokes EE. Erlotinib and bevacizumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck: a phase I/II study. *Lancet Oncol* 2009;**10**:246–57
- 59 Soulieres D, Senzer NN, Vokes EE, Hidalgo M, Agarwala SS, Siu LL. 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. *J Clin Oncol* 2004;**22**:77–85
- 60 Argiris AE, Ghebremichael M, Gilbert J, Burtneess B, Forastiere A. A phase III randomized, placebo-controlled trial of docetaxel (D) with or without gefitinib (G) in recurrent or metastatic (R/M) squamous cell carcinoma of the head and neck (SCCHN): a trial of the Eastern Cooperative Oncology Group (ECOG). *J Clin Oncol* 2009;**27**:6011
- 61 Hidalgo M, Siu LL, Nemunaitis J, Rizzo J, Hammond LA, Takimoto C, Eckhardt SG, Tolcher A, Britten CD, Denis L, Ferrante K, Von Hoff DD, Silberman S, Rowinsky EK. Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. *J Clin Oncol* 2001;**19**:3267–79
- 62 Burris HA 3rd, Hurwitz H, Dees EC, Dowlati A, Blackwell KL, O'Neil B, Marcom PK, Ellis MJ, Overmoyer B, Jones SF, Harris JL, Smith DA, Koch KM, Stead A, Mangum S, Spector NL. 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. *J Clin Oncol* 2005;**23**:5305–13
- 63 Abidoye OO, Cohen EE, Wong SJ, Kozloff MF, Nattam SR, Stenson K, Blair E, Day S, Dancey JE, Vokes EE. A phase II study of lapatinib (GW572016) in recurrent/metastatic (R/M) squamous cell carcinoma of the head and neck (SCCHN). *J Clin Oncol* 2006;**24**:5568
- 64 Pfister DG, Su YB, Kraus DH, Wolden SL, Lis E, Aliff TB, Zahalsky AJ, Lake S, Needle MN, Shaha AR, Shah JP, Zelefsky MJ. Concurrent cetuximab, cisplatin, and concomitant boost radiotherapy for locoregionally advanced, squamous cell head and neck cancer: a pilot phase II study of a new combined-modality paradigm. *J Clin Oncol* 2006;**24**:1072–8
- 65 Sos ML, Zander T, Thomas RK, Staratschek-Jox A, Claasen J, Wolf J. Expression of signaling mediators downstream of EGF-receptor predict sensitivity to small molecule inhibitors directed against the EGF-receptor pathway. *J Thorac Oncol* 2008;**3**:170–3
- 66 Chen Z, Zhang X, Li M, Wang Z, Wieand HS, Grandis JR, Shin DM. Simultaneously targeting epidermal growth factor receptor tyrosine kinase and cyclooxygenase-2, an efficient approach to inhibition of squamous cell carcinoma of the head and neck. *Clin Cancer Res* 2004;**10**:5930–9
- 67 Rapidis AD, Vermorken JB, Bourhis J. Targeted therapies in head and neck cancer: past, present and future. *Rev Recent Clin Trials* 2008;**3**:156–66
- 68 Mariani SM. Targeting cancer cells – more pathways, more inhibitors, more trails. Highlights of 9th annual Drug Discovery Technology World Congress; August 8–13, 2004; Boston, Massachusetts. *Med Gen Med* 2004;**6**:22
- 69 Takano T, Ohe Y, Sakamoto H, Tsuta K, Matsuno Y, Tateishi U, Yamamoto S, Nokihara H, Yamamoto N, Sekine I, Kunitoh H, Shibata T, Sakiyama T, Yoshida T, Tamura T. Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. *J Clin Oncol* 2005;**23**:6829–37
- 70 Erjala K, Sundvall M, Junttila TT, Zhang N, Savisalo M, Mali P, Kulmala J, Pulkkinen J, Grenman R, Elenius K. Signaling via ErbB2 and ErbB3 associates with resistance and epidermal growth factor receptor (EGFR) amplification with sensitivity to EGFR inhibitor gefitinib in head and neck squamous cell carcinoma cells. *Clin Cancer Res* 2006;**12**:4103–11

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