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# Cetuximab and bevacizumab: preclinical data and phase II trial in recurrent or metastatic squamous cell carcinoma of the head and neck

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**Background:** We evaluated combined targeting with cetuximab, an anti-epidermal growth factor receptor (EGFR) monoclonal antibody, and bevacizumab, an anti-vascular endothelial growth factor (VEGF) monoclonal antibody, in squamous cell carcinoma of the head and neck (SCCHN).

**Patients and methods:** The combination was studied in human endothelial cells and head and neck and lung cancer xenograft model systems. Patients with recurrent or metastatic SCCHN were treated with weekly cetuximab and bevacizumab, 15 mg/kg on day 1 given intravenously every 21 days, until disease progression. Analysis of tumor biomarkers and related serum cytokines was performed.

**Results:** Cetuximab plus bevacizumab enhanced growth inhibition both *in vitro* and *in vivo*, and resulted in potent reduction in tumor vascularization. In the clinical trial, 46 eligible patients were enrolled. The objective response rate was 16% and the disease control rate 73%. The median progression-free survival and overall survival were 2.8 and 7.5 months, respectively. Grade 3–4 adverse events were expected and occurred in less than 10% of patients. transforming growth factor alpha, placenta-derived growth factor, EGFR, VEGFR2 increased and VEGF decreased after treatment but did not correlate with treatment efficacy.

**Conclusions:** Cetuximab and bevacizumab are supported by preclinical observations and are well tolerated and active in previously treated patients with SCCHN.

**Key words:** bevacizumab, cetuximab, EGFR, head and neck cancer, VEGF

## introduction

Squamous cell carcinoma of the head and neck (SCCHN) is an aggressive cancer which can be potentially cured when not associated with distant metastasis. Recurrent or metastatic (R/M) SCCHN develops in about 50% of patients and has a poor prognosis [1–3]. Systemic therapy may prolong survival and alleviate disease-related symptoms in this setting. Platinum-based combinations are commonly used for the treatment of patients with R/M SCCHN, and yield higher response rates compared with single-agent treatment but

without associated survival benefit [3–5]. A phase III randomized showed that the addition of cetuximab, an anti-epidermal growth factor receptor (EGFR) monoclonal antibody, to platinum and 5-fluorouracil (5-FU) improved the overall response rate (ORR), progression-free survival (PFS), and overall survival (OS) in patients with R/M SCCHN [6]. Also, cetuximab has modest single-agent activity in platinum-refractory SCCHN with an ORR of 13%, disease control rate (DCR) of 46%, and median OS of 5.9 months [7]. A worthwhile therapeutic aim in this disease is to improve cetuximab efficacy without enhancing toxic effects.

EGFR and vascular endothelial growth factor (VEGF) and their signaling pathways are important for solid tumor growth and dissemination, and have emerged as critical targets for novel therapeutics. Cetuximab binds to the extracellular domain of EGFR, promotes degradation of receptor and thus,

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hampers its phosphorylation and activation [8]. Bevacizumab is a humanized monoclonal antibody that binds VEGF and inhibits its function [9]. These agents have demonstrated activity in several solid tumors and were added to the armamentarium against colon [10], lung [11], and renal cancers [12]. Promising results were recently reported with the combination of bevacizumab and pemetrexed, an anti-folate agent, in R/M SCCHN [13]. It has been demonstrated that VEGF signaling is upregulated by EGFR expression [14], whereas VEGF upregulation has been associated with resistance to cetuximab [15]. Furthermore, it has been suggested that EGFR inhibition has an anti-angiogenic effect and downregulates VEGF [16]. Therefore, co-targeting of EGFR and VEGF seems attractive and may overcome resistance to EGFR inhibition. Preclinical data suggest increased inhibition of tumor growth with combined anti-EGFR and anti-angiogenesis inhibition in various tumor models [17–20]. In patients with R/M SCCHN, bevacizumab in combination with erlotinib, an anti-EGFR tyrosine kinase inhibitor resulted in acceptable toxicity and promising efficacy [21].

Cytokines released by tumor cells have been implicated in the tumor growth and resistance to treatment. Hepatocyte growth factor (HGF)-mediated mesenchymal–epithelial transition activation has emerged as a novel mechanism of cetuximab resistance in colorectal cancer (CRC) [22] and gefitinib resistance in non-small-cell lung cancer cells with EGFR-activating mutations [23]. Placenta-derived growth factor (PIGF) seems to play a key role in the angiogenic rescue as it is upregulated in cancer patients treated with anti-VEGF agents [24, 25]. A significant difference in the expression of PIGF, IP-10, interleukin (IL)-8, and IL-1 alpha between the sensitive and the resistant to bevacizumab cell lines has been observed (S.K., unpublished data). Fibroblast growth factor (FGF) has been long recognized as an inducer of tumor angiogenesis [26, 27] and it has been also implicated in development of resistance to VEGFR inhibition [28].

We initially investigated the combination of cetuximab and bevacizumab *in vitro* and in human tumor xenografts in the laboratory (T.H., P.M.H.) and subsequently designed and conducted a phase II study to evaluate the efficacy of this regimen in patients with R/M SCCHN. Also, we studied baseline tumor biomarkers as well as the effect of treatment on serum cytokines that relate to EGFR and angiogenesis.

## materials and methods

### preclinical studies

We employed an *in vitro* human umbilical vein endothelial cell (HUVEC) growth inhibition assay; methods have been reported previously [29]. HUVEC (Cambrex, East Rutherford, NJ) were treated in culture with bevacizumab 0.1  $\mu\text{M}$   $\pm$  cetuximab 0.1  $\mu\text{M}$  for 96 h. VEGF was added into plates (25 ng/ml). Viable cells were quantified with crystal violet assay.

To evaluate growth inhibition *in vivo*, athymic mice bearing human lung (H226; generously provided by Drs John Minna and Adi Gazdar of the University of Texas Southwestern Medical School, Dallas) or head and neck (SCC1; generously provided by Dr Thomas E. Carey of the University of Michigan, Ann Arbor) tumor xenografts were randomized into four treatment groups: IgG (control), bevacizumab 0.75 mg/kg, cetuximab 1.25

mg/kg, or combined bevacizumab and cetuximab. At day 25 after implanting, drug injections were administered intraperitoneally twice weekly for three doses (D25, D29, and D32) for H226 bearing xenografts and five doses (D25–D39) for SCC-1 bearing xenografts. Tumor specimens were obtained from mice following treatment and tumor volumes were measured. The proliferation of tumor cells was examined by immunohistochemistry (IHC) using the proliferative marker proliferating cell nuclear antigen (PCNA). We studied the effect on angiogenesis using an *in vivo* angiogenic Matrigel plug assay following tumor inoculation in athymic mice as described previously [29]. Briefly, mice with H226 tumors implanted into dorsal Matrigel plugs were treated with bevacizumab  $\pm$  cetuximab. The Matrigel plug was formed following injection of extracellular matrix solution. H226 cells were soaked with a sponge and inserted into the Matrigel plug. Twenty-four hours later, mice were treated with bevacizumab (0.75 mg/kg), cetuximab (1.25 mg/kg), or in combination for 1 week. Before sacrifice, FITC (fluorescein isothiocyanate)-dextran was injected via the tail vein and the distribution of blood vessels in the plugs was visualized under fluorescence microscopy.

### patient selection

Key eligibility criteria included age 18 or older with histologically or cytologically documented R/M SCCHN, not eligible for curative intent surgical or radiation therapy, with measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) definitions [30] and Eastern Cooperative Oncology Group (EGOG) performance status 0–2. No more than one prior adjuvant/neoadjuvant chemotherapy and/or concomitant chemoradiotherapy regimen that may have included biologic/targeted agent and no more than 1 prior treatment (chemotherapy or biologic/targeted) for R/M SCCHN was allowed. No prior treatment with EGFR or angiogenesis inhibitors was permitted. Patients were required to have absolute neutrophil count  $\geq 1000$  per  $\mu\text{l}$  and platelet count  $\geq 75\,000$  per  $\mu\text{l}$ , total bilirubin within normal range, AST and ALT  $\leq 5$  times the institutional upper limit of normal, creatinine clearance  $>60$  ml/min, and urine protein to urine creatinine ratio of  $<1$ . Patients with tumors that invaded major vessels, history of abdominal fistula, gastrointestinal perforation, intra-abdominal abscess within 28 days prior to registration, serious non-healing wound, ulcer, or bone fracture, history of thrombosis, or on therapeutic anticoagulation were excluded. Ineligible were also patients with uncontrolled hypertension, or serious comorbidities. Institutional Review Board was obtained in each participating institution. All patients provided written informed consent.

### treatment plan

Patients received cetuximab [provided by the National Cancer Institute (NCI) under an agreement between ImClone Systems Incorporated and the NCI Division of Cancer Treatment and Diagnosis (DCTD)] 250 mg/m<sup>2</sup>, intravenously (IV), over 60 min, on days 1, 8, and 15 (400 mg/m<sup>2</sup> IV over 120 min on cycle 1 day 1, loading dose), followed by bevacizumab (supplied by the NCI under an agreement between Genentech, Inc. and the NCI DCTD) 15 mg/kg IV, over 90 min, on day 1 of a 21-day cycle, until progression of disease or intolerable toxicity.

### patient evaluation

At baseline, patients were evaluated with history and physical examination, complete blood counts, serum chemistry, coagulation test, urine protein and creatinine and electrocardiogram. Clinical and laboratory evaluation was repeated in the beginning of each cycle. Computed tomography and/or magnetic resonance imaging was performed for baseline tumor assessment and after every two cycles thereafter. RECIST was used to evaluate response

[30]. Toxicity was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0.

### serum cytokine and tumor biomarker analysis

Thirteen angiogenesis-related cytokines were measured in patient sera using ELISA (R&D systems Inc., Minneapolis, MN) according to the manufacturer's instructions. Baseline and post 21 days of treatment serum samples from 20 patients were run in duplicates and the mean value was used for analysis. The cytokines that we examined were as follows: epidermal growth factor (EGF), VEGF, PIGF, FGF a and b (FGFa, FGFb), HGF, granulocyte-colony stimulating factor (G-CSF), transforming growth factor alpha (TGFa), IL-6, IL-8, IP-10, soluble EGFR, and soluble VEGFR (sVEGFR-2). They were analyzed by using individual ELISA kits (catalog numbers DEG0, DVE0, DPG0, DFA00, DFB5, DHG0, DCS5, DTGA0, D6050, D8000, DIP10, DEGR, and DVR20, respectively). Immunohistochemical staining of tumor tissues was performed as previously described [31]. The following antibodies were utilized; pEGFR (Cell Signaling, Danvers, MA, catalogue #4407), EGFR (Sigma, St Louis, MO, catalogue #E3138), pSTAT3 (catalogue #9145, Cell Signaling), STAT3 (Cell Signaling, catalogue #9132), pVEGFR (Calbiochem, San Diego, CA, catalogue #PC460), VEGFR (Epitomics, Burlingame, CA, catalogue #1303-1), CD31 (Dako Cytomation, Carpinteria, CA, catalogue #M0823), Ki67 (Dako North America, catalogue #M7240). Scoring of the staining intensity was performed as previously described [32].

### statistical methods

The primary end point was ORR with a the null hypothesis of 13% and assuming 90% power to detect an improvement to 30% due to the addition of bevacizumab. Using a one-sided exact binomial test at  $\alpha = 0.10$ , 45 patients were needed (one stage design). If 11 or more objective responses were observed among 45 patients, the null hypothesis would be rejected at  $\alpha = 0.05$ . To account for a 5% ineligibility rate, we planned to accrue a total of 48 patients. PFS and OS were reported on all eligible patients. A correlation of the levels of the measured cytokines before and after treatment and the ORR was performed using a two-tailed Wilcoxon test.

## results

### preclinical studies (*in vitro* and *in vivo*) combining cetuximab and bevacizumab

Combining the EGFR inhibitor cetuximab with the VEGF inhibitor bevacizumab substantially enhanced the inhibition of HUVEC growth in cell culture (supplementary Figure S1, available at *Annals of Oncology* online). Furthermore, combination therapy augmented antitumor activity over that achieved with single antibody therapy in head and neck (SCC1) and lung (H226) tumor xenograft model systems (supplementary Figure S2, available at *Annals of Oncology* online). At the end of the experiment, SCC1 xenografts treated with combined antibody therapy had markedly smaller tumor volumes ( $119 \text{ mm}^3$ ) compared with those treated with cetuximab ( $176 \text{ mm}^3$ ,  $P = 0.026$ ) or bevacizumab alone ( $541 \text{ mm}^3$ ,  $P = 0.0006$ ). Similarly, in the H226 group, the mean tumor volumes in the three treatment arms were  $541$ ,  $749 \text{ mm}^3$  ( $P = 0.252$ ), and  $1005 \text{ mm}^3$  ( $P = 0.007$ ), respectively. There was marked reduction in tumor proliferative activity with the combination regimen versus single antibody alone or control as shown by PCNA IHC (supplementary Figure S3, available at *Annals of Oncology* online). Finally, the Matrigel plug assay

that examines the anti-angiogenic impact of therapy confirmed the most potent reduction in tumor vascularization when cetuximab was combined with bevacizumab corresponding with tumor growth inhibition (supplementary Figure S4, available at *Annals of Oncology* online).

### patient characteristics

Table 1 shows baseline patient characteristics. From November 2006 to November 2010, a total of 48 patients were enrolled in the study from 4 participating centers. We report results in 46 eligible patients. Two patients were deemed ineligible, one because subsequent biopsy showed that measurable disease was actually osteoradionecrosis and not recurrent laryngeal cancer. The other patient required surgery for cholecystitis before starting treatment, and eventually found to have resolution of presumed target lesions that were actually infectious; this patient never initiated protocol treatment.

Of 46 eligible patients, 42 (91%) had previously received chemotherapy or chemoradiotherapy at any time: 25 as part of prior potentially curative treatment only, 12 as part of palliative treatment of recurrent disease only, and 5 as part of both potentially curative and subsequent palliative treatment. Nineteen patients had completed chemotherapy as part of a potentially curative regimen within the previous 6 months; all but one had previously received cisplatin or carboplatin. No patient had previously received targeted agent therapy. The median number of cycles delivered was 4 (range 1–21).

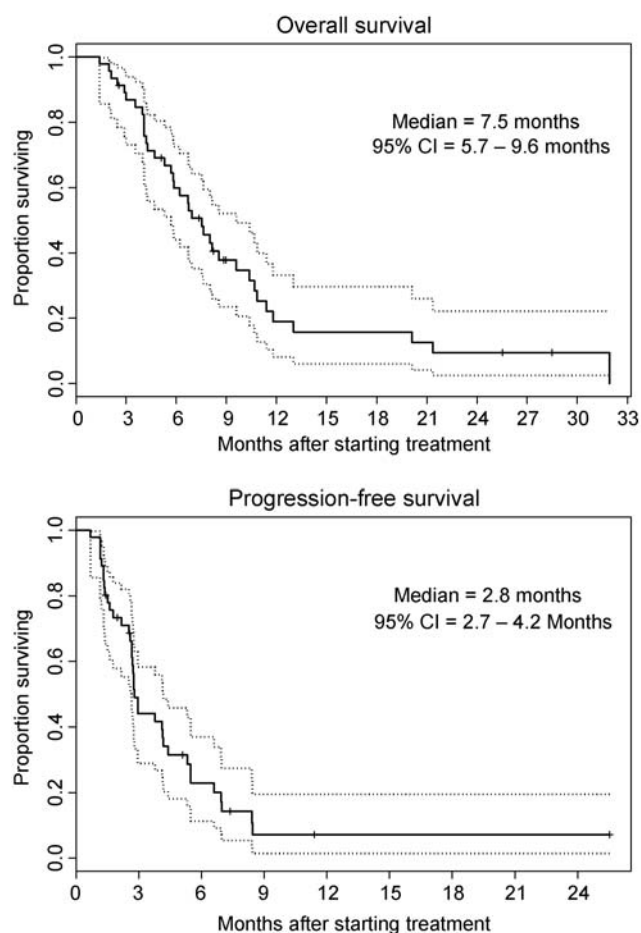
### efficacy

Of 46 eligible patients, 45 were evaluable for response (one patient was not evaluable because he died of aspiration pneumonia before post-treatment scans were performed): 7 patients achieved a partial response (PR) and an additional 26 patients had stable disease (SD) as best response. The ORR

**Table 1.** Patient characteristics ( $n = 46$ )

Median age (range)	61.5 (33–92)
Sex, no. (%)	
Male	34 (74)
Female	12 (26)
ECOG performance status, no. (%)	
0	11 (24)
1	31 (67)
2	4 (9)
Primary site, no. (%)	
Oropharynx	17 (37)
Oral cavity	14 (30)
Larynx	7 (15)
Others	8 (17)
Recurrent disease, no. (%)	44 (96)
Recurrence $\leq$ 6 months of prior curative therapy, no. (%)	32 (73)
Prior radiation, no. (%)	44 (96)
Prior surgery, no. (%)	30 (65)
Prior chemotherapy, no. (%)	42 (91)
1 prior palliative regimen, no. (%)	17 (37)

ECOG, Eastern Cooperative Oncology Group.



**Figure 1.** Kaplan-Meier estimates of (A) overall survival and (B) progression-free survival with 95% confidence intervals (dotted lines).

was 16% (95% CI 7% to 24%) and the DCR was 73%. One patient achieved a PR after two cycles but progressed on subsequent assessment after four cycles and was classified as having SD as best response. Among nine surviving patients, the median follow-up was 9.7 months (range 2.6–39.4 months). The median OS was 7.5 months (95% CI, 5.7–9.6 months; Figure 1) and the median PFS 2.8 months (95% CI 2.7–4.2 months; Figure 1).

### safety

The most common drug-related adverse event was rash. Twelve patients developed grade 1 and 25 patients developed grade 2 rash. Grade 3 or 4 toxic effects were observed in less than 10% of patients (Table 2). In addition to two cases of grade 3 bleeding (one of which from the rectum and the other from a benign skin ulcer that occurred after three and four cycles of treatment, respectively), four patients had grade 2 hemorrhage and six patients grade 1 hemorrhage. Three patients developed grade 2 hypertension. One patient required tracheostomy for airway obstruction during treatment on study; this was considered an unrelated event. Two patients died of aspiration pneumonia in the context of which one developed hypoxemia complicated by cardiac ischemia and the other acute renal

**Table 2.** Grade 3/4 toxic effects (n = 46)

Toxicity	Grade 3, no. (%)	Grade 4, no. (%)
Proteinuria	–	1 (2%)
Cardiac ischemia	–	1 (2%)
Hyponatremia	3 (7%)	–
Dysphagia	4 (9%)	–
Rash	4 (9%)	–
Fatigue	3 (7%)	–
Pain	4 (9%)	–
Hypertension	3 (7%)	–
Infection	3 (7%)	–
Hemorrhage	2 (4%)	–

**Table 3.** Changes in serum cytokines after cetuximab and bevacizumab

Analyte	Median baseline (pg/ml)	Median post-treatment (pg/ml)	Median ratio	Raw signed rank P-value	Adjusted P-value
TGF $\alpha$	34.95	114.2	3.3711	0.0001	0.0013
PIGF	24.5	47	1.9453	0.0001	0.0013
VEGF	547.7	59.45	0.1203	0.0001	0.0013
EGFR	16.4	29.15	1.5875	0.0001	0.0013
IP.10	356	471	1.25	0.0025	0.0625
VEGFR.2	4459	5023	1.1032	0.0227	0.0493
FGFb	15.5	14.95	0.7346	0.1168	0.2170
FGFa	0	0	0.0000	0.1684	0.2736
IL.6	9.65	14.3	1.3831	0.2788	0.3625
HGF	4754	4549	0.9622	0.2958	0.3625
GCSF	27.95	42.05	1.2652	0.2958	0.3625
EGF	17.8	22.75	0.7878	0.4326	0.4326

failure. Both events were considered related to complications of pneumonia and unrelated to study drugs.

### biomarkers and clinical outcome

Baseline and post-treatment serum samples from 20 patients were run in duplicates for 13 different cytokines. There were no significant difference in characteristics or outcomes among the patients with available serum samples and the ones who did not have samples collected and analyzed. The following serum cytokines were shown to substantially change after 1 cycle of treatment with cetuximab and bevacizumab, even after adjustment for false discovery: TGF $\alpha$ , PIGF, EGFR, VEGFR2, and VEGF (Table 3). The concentrations of these cytokines increased in post-treatment serum except VEGF that decreased (supplementary Figure S5, available at *Annals of Oncology* online). However, neither baseline levels of cytokines, their change after treatment, nor baseline tumor biomarkers correlated with efficacy parameters (data not shown).

### discussion

Accumulated observations suggest that there is a cross talk between EGFR and VEGFR pathways [14–16] and that up-

regulation of VEGF and angiogenesis may be associated with resistance to EGFR inhibitors [17]. Benavente et al. [33] reported that EGFR inhibitor-resistant human SCCHN tumor cell lines had increased proliferation rate, overexpressed several proteins involved in the EGFR down-streaming pathway (AKT, MAPK) and showed cross-resistance to ionizing radiation compared with the EGFR inhibitor-sensitive parental population. In addition, EGFR inhibitor-resistant tumors exhibit extensive vascularization and growth of the vessels in the tumor site [33]. We studied the combination of bevacizumab and cetuximab in head and neck (SCC1) and lung cancer (H226) xenograft model systems and demonstrated enhanced tumor growth delay. The difference in growth delay reached statistical significance in all comparisons (combination versus each drug alone) in SCC1 cells thereby encouraging further investigation of this combination in patients with SCCHN. Further, we examined the effect of the combination on angiogenesis using the Matrigel plug assay and showed that this combination decreases tumor blood supply when compared with the effect of each drug alone. These results suggest that enhanced antitumor activity can be achieved by combining the two distinct monoclonal antibodies bevacizumab and cetuximab, and support a strategy for the simultaneous targeting of EGFR and VEGF signaling pathways in the treatment of SCCHN. These results are consistent with previous preclinical observations with endostatin and EGFR antisense [20].

Based on promising laboratory observations, we conducted a multicenter trial in patients with previously treated SCCHN. Our clinical trial showed that the combination of cetuximab and bevacizumab results in DCR and survival outcomes numerically superior to what has been reported for cetuximab alone or erlotinib, an EGFR-tyrosine kinase inhibitor [7, 34, 35]. We observed a DCR of 73% and a median OS of 7.5 months versus a DCR of 46% and median OS of 5.9 months reported by Vermorken et al. [7]. However, the primary objective of demonstrating a major improvement in response rate over what was previously reported for cetuximab alone 16% in this study versus 10–13% in cetuximab alone studies [7, 34] was not achieved in this study. Cohen et al. [21] reported an ORR of 15% and median OS of 7.1 months with erlotinib plus bevacizumab, results similar to the current study. We acknowledge several limitations when comparisons are made to historical controls. The inclusion criteria in our study were somewhat different than the criteria used by Vermorken et al. [7] and comparable with those used by Cohen et al. [21]. Seventy-six percent of our patients had performance status of 1 or 2 and 73% had an early relapse after chemoradiotherapy or were previously treated with one regimen for R/M SCCHN. In a similar patient population of previously treated R/M SCCHN, irinotecan and docetaxel had very poor activity with an ORR of 3% and median OS of 5 months [36].

In this study, we explored the modulation of several cytokines in the serum of treated patients. Although we demonstrated that several serum cytokines (TGf $\alpha$ , PIGF, EGFR, VEGFR2, VEGF) changed after combined EGFR and VEGF inhibition, even after adjusting for false discovery, cytokine levels did not correlate with efficacy. Nevertheless, the

sample size of the study was small; therefore, this study may have been underpowered to detect small differences in clinical outcomes between groups.

The combination of anti-EGFR and anti-VEGF agents has been tested in several clinical trials in solid tumors. In a randomized phase II study (BOND-2) in the second-line treatment setting in CRC, Saltz et al. [10] showed that cetuximab plus bevacizumab with or without irinotecan is feasible and active. Unlike these promising results, two subsequent phase III studies in CRC demonstrated that the addition of an anti-EGFR monoclonal antibody to the standard cytotoxic agent plus bevacizumab was associated with decreased PFS [37, 38]. Phase III clinical trial evaluation of this approach is currently ongoing in lung cancer (NCT00946712), and appears worthy of examination for selected epithelial malignancies. We have initiated a phase II randomized trial in locally advanced SCCHN that evaluates the addition of bevacizumab to a regimen with radiotherapy, cetuximab and pemetrexed (NCT00703976). Several other novel EGFR and angiogenesis targeted agents are undergoing clinical investigation. Co-targeting of these important pathways remains of major interest in cancer therapy.

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