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Molecular and Clinical Responses in a Pilot Study of Gefitinib with Paclitaxel and Radiation in Locally Advanced Head and Neck Cancer

Carter Van Waes, M.D., Ph.D.^{1,2}, Clint T. Allen, M.D.¹, Deborah Citrin, M.D.², David Gius, M.D., Ph.D.², A. Dimetrios Colevas, M.D.³, Nancy A. Harold, R.N.⁴, Susan Rudy, R.N., C.R.N.P.¹, Liesl Nottingham, M.D.¹, Christine Muir, R.N.⁴, Zhong Chen, Ph.D.¹, Anurag K. Singh, M.D.², Janet Dancey, M.D.³, and John C. Morris, M.D.⁴

¹Head and Neck Surgery Branch, National Institute of Deafness and Communication Disorders, National Institutes of Health, Bethesda, MD 20892, USA.

²Radiation Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892, USA.

³Investigational Drug Branch, Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD 20892, USA.

⁴Metabolism Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892, USA.

Abstract

Purpose—Epidermal growth factor receptor (EGFR) overexpression in head and neck squamous cell carcinoma (HNSCC) stimulates tumor cell proliferation, inhibits apoptosis, and increases chemotherapy and radiation resistance. We examined the toxicity, safety and the effects on EGFR signaling in tumor biopsies from patients with locally advanced HNSCC treated with the EGFR signaling inhibitor gefitinib (GEF) combined with weekly intravenous paclitaxel (PAC) and radiation therapy (RT).

Methods and Materials—A pilot phase I dose-escalation study. Eligibility included stage III-IVB HNSCC, age ≥18 years, no prior RT or chemotherapy, adequate organ function and informed consent. Endpoints included determination of maximum tolerated dose (MTD) and analysis of treatment effect on EGFR signaling, tumor cell proliferation and apoptosis in biopsies.

Results—Ten patients were treated. The MTD of this combination was GEF 250 mg/d with PAC 36 mg/m² I.V. weekly × 6 with concurrent RT. Grade 3/4 toxicities included prolonged (>8 weeks) stomatitis (7 patients), infection (1), and interstitial pneumonitis (1). There were five complete responses (CR) and two partial responses (PR). Of 7 patients undergoing serial biopsies, only one

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Corresponding author: John C. Morris, M.D. Mark O. Hatfield Clinical Research Center, Room 4-5330 10 Center Drive, Bethesda, MD 20892-1457 TEL: (301) 402-2912 FAX: (301) 402-1001 jmorris@mail.nih.gov.

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demonstrated a reduction in phosphorylated-EGFR, decreased downstream signaling and reduced cellular proliferation after initiating GEF.

Conclusions—GEF inhibition of EGFR was observed in only one of seven tumors studied. The addition of GEF to PAC and RT did not appear to improve the response of locally advanced HNSCC compared to our prior experience with PAC and RT alone. This treatment appeared to delay recovery from stomatitis.

Keywords

Epidermal growth factor receptor; head and neck cancer; gefitinib; paclitaxel; radiation

INTRODUCTION

EGFR, a member of the c-Erb B family of growth factor receptors is overexpressed in 90% of HNSCC.¹ EGFR is a 180 kDa transmembrane receptor tyrosine kinase that transduces signals for a diverse group of ligands. EGFR overexpression and mutation increase its dimerization, autophosphorylation and intracellular signaling.² Multiple downstream pathways inducible by EGFR or other receptors are activated in HNSCC. These include mitogen-activated protein kinases ERK1/2 that activate transcription factor AP-1 and promotes cell proliferation; phosphatidylinositol 3-kinase (PI3-K) that activates AKT and nuclear factor-kappa B (NF- κ B)/RELA; and JAK-STAT3, all of which promote cancer cell survival.³⁻⁶ These pathways also activate genes involved in inflammation, angiogenesis, and metastasis. EGFR expression is correlated with larger tumor size, advanced stage, greater risk of recurrence and metastases, and shortened survival.^{7,8} Moreover, EGFR expression is associated with resistance of tumor to the effects of radiation and chemotherapy.⁹⁻¹²

A number of new agents targeting EGFR inhibit its signaling¹³ including EGFR-selective tyrosine kinase inhibitors (TKI). GEF was the first of these agents to be widely studied. GEF suppressed the growth of EGFR expressing tumor xenografts. In addition, EGFR inhibition was demonstrated to improve responses to both radiation and cytotoxic agents in preclinical models.¹⁴⁻¹⁷ Despite initial optimism, a phase II study of single agent GEF in 52 patients with metastatic HNSCC demonstrated a disappointing response rate of 11%.¹⁸ More recently a phase I study demonstrated the feasibility of combining GEF with RT, or with weekly cisplatin and RT in HNSCC patients.¹⁹ Local-regional control at 3 years was 85% and disease-free survival was 61%. Alternative regimens of GEF combined with taxanes or other agents and RT are of interest.

Biomarkers predicting responsiveness of HNSCC to GEF remain to be defined. Correlative studies have emphasized quantitation of pre-treatment EGFR expression; however, this has not been useful in predicting responses.^{1,8} Responses in lung cancer are associated with mutations affecting the receptor ATPase site that render tumors sensitive or resistant.^{20,21} Similar mutations have not been widely detected in HNSCC.²² Evidence suggests EGFR makes a variable contribution relative to other oncogenic alterations in HNSCC that activate pathways promoting cell proliferation, survival and angiogenesis. In some cell lines and tumors, EGFR signaling plays a dominant role, while in others, expression of cytokines such as IL-1, TNF α and IL-6 can activate the AKT-NF- κ B and JAK-STAT3 pathways.^{5,6,23}

Platinum and taxane-based chemotherapy combined with RT has demonstrated radiosensitization in HNSCC.²⁴⁻²⁷ We report the results of a pilot phase I dose-escalation study to determine the safety and tolerability of GEF administered with weekly doses of PAC and concurrent RT in locally advanced HNSCC. Tumor biopsies were obtained prior to treatment to determine EGFR activation and its signaling pathways, and after 7 days of GEF prior to

beginning PAC and RT. The effects on EGFR phosphorylation, downstream AKT, ERK1/2, STAT3, and NF- κ B p65 signaling, cell proliferation, and apoptosis were examined.

METHODS AND MATERIALS

Eligibility

This study was IRB-approved and carried out in accordance with the Helsinki Declaration of 1975, as revised in 2000. Patient eligibility included untreated stage III, IVA or IVB HNSCC, or patients with a stage equivalent recurrence after surgery. Patients with potentially resectable tumors were informed of surgical options and must have indicated a preference for non-surgical treatment. Other requirements included measurable tumor by RECIST²⁷, age ≥ 18 years; hemoglobin ≥ 10 g/dL; granulocytes $\geq 1,500/\mu\text{L}$; platelets $\geq 100,000/\mu\text{L}$; serum creatinine $\leq 1.5\times$ upper limit of normal (ULN); total bilirubin $\leq 2\times$ ULN, and hepatic transaminases $\leq 2.5\times$ ULN. Patients were required to provide informed consent. Separate consents were obtained for all biopsies. Exclusions included previous radiation, chemotherapy, or EGFR-inhibitor therapy; other invasive malignancy within 5-years; pregnancy, or serious unrelated illness.

Treatment

The primary objective of this pilot phase I trial was to determine the dose-limiting toxicity (DLT), toxicity profile and MTD of daily GEF in combination with six weekly doses of PAC and concurrent RT in patients with locally advanced HNSCC. Alternating dose-escalation cohorts of GEF and PAC were planned (Table 1). Oral GEF was initiated on day 1 and continued daily for 16 weeks. Two-week drug supplies were dispensed and compliance was monitored by pill counts. PAC was initiated day 8 as a weekly 1-hour I.V. infusion for 6 doses. Three-dimensional conformal RT 180 cGy per day to a maximum tumor dose of 6,600 to 7,600 cGy was initiated after the PAC dose on day 8. Uninvolved regional lymph nodes received 4500 to 5400 cGy in 180 cGy daily fractions. Feeding gastrostomy tubes were placed when it was felt treatment would impact patient nutritional status. Use of hematopoietic growth factors and radioprotective agents were not permitted.

Toxicity—Common Toxicity Criteria Adverse Event (CTCAE) v3.0 and RTOG/EROTC Toxicity Criteria were used for classification and grading of systemic and in field toxicity, respectively.²⁸ Toxicities $\geq$ grade 3 with the exception of the following were considered DLT: Patients with grade 3/4 hematological toxicity on the day of PAC administration, or who developed RTOG grade 4 stomatitis/esophagitis, or other grade 4 in-field toxicity at anytime were permitted a single 7 day treatment delay without being defined as DLT. If one of three patients in a dose cohort experienced DLT, three additional patients were entered. If an additional patient experienced DLT, it was then determined that MTD had been exceeded.

Response—Response was determined by RECIST²⁹ using physical and direct fiberoptic examination, computerized tomography or magnetic resonance imaging, and confirmed by biopsy.

Tumor biopsies—Biopsies were performed prior to GEF, after 7 days of GEF prior to PAC and RT, and again three months after completion of radiation therapy. Pre-treatment, the edge of viable tumor was biopsied. Day 8 biopsies were taken adjacent to the original biopsy. Specimens were embedded in optimum cutting temperature (OCT) media, frozen and stored at -80°C until processing.

Immunohistochemistry—Frozen tissues were sectioned at a thickness of 10 μm and placed on silanated glass slides. Immunohistochemistry (IHC) was performed using avidin-biotin immunoperoxidase. Depending on optimal conditions for the antibody, serial sections were

fixed with either cold acetone for 5 min (total and p-EGFR) or with 4% paraformaldehyde/phosphate buffered saline (PBS). For all stains except total and p-EGFR, membranes were permeabilized with 0.2% Triton X-100/PBS. Nonspecific binding was blocked using 5% serum from the secondary antibody species and endogenous tissue peroxidase was quenched with 0.6% H₂O₂. Total and p-EGFR antibodies were diluted in 1% serum/PBS, and all other antibodies were diluted in 3% BSA/Tris buffered saline (TBS), and incubated at 4°C overnight (see Supplemental Table S1 for antibodies used). Sections were incubated with a biotinylated secondary antibody followed by HRP avidin-biotin complex (Vector Labs Vectastain Elite ABC Kits, Burlingame, CA). Slides were developed with 3, 3'-diaminobenzidine (DAB) at room temperature for 1-5 minutes. Sections were counterstained with Gill's formula hematoxylin (Vector Labs), dehydrated through graded alcohol, cleared with xylenes, and mounted using Permount (Fisher Scientific; Pittsburgh, PA). Negative controls were performed for each assay replacing the primary antibody with an isotype and concentration matched control antibody. Immunostaining with a pan-cytokeratin primary antibody was used as a positive control to identify squamous tissue. Apoptosis was assessed using the ApoTag TUNEL detection kit (Chemicon Temecula, CA) following the manufacturer's protocol. Negative controls were performed by deleting terminal deoxynucleotidyl transferase (TdT) from the assay.

To score expression of each antigen, three representative 400X fields of at least 100 tumor cells were evaluated. Slides were examined on an Olympus BX51 microscope and the fields photographed with an Olympus DP70 camera. Images were archived and representative figures created using Adobe Photoshop Elements 3.0 (Adobe Systems, Inc. San Jose, CA). Images were not modified in any way from their original format.

Total and p-EGFR was scored using a four-tiered semi-quantitative method. Three 400X magnification fields for each pre- and post-treatment section were scored as 0+ for no membrane staining, 1+ for weak staining, 2+ for moderate staining, or 3+ for strong staining. Scores for each high power field were averaged and reported as plasma membrane (PM) staining.

Scoring of phosphorylated nuclear AKT, RELA, ERK1/2 and STAT3 was performed using the method reported by Nenutil, *et al.*³⁰ Briefly, cells with nuclear staining were counted and the percent of positive cells in each high power field was determined. Fields were then assigned a staining intensity of 1+ for weak nuclear staining, 2+ for moderate, or 3+ for strong staining. The product of the percent positive cells and staining intensity was then derived to create a histoscore range of 0-300. Scores are reported as average histoscore per high power field for each pre- and post-treatment section. Tumor proliferation and apoptosis were assessed via semi-quantitative analysis of Ki67 and TUNEL stains. Positive cells defined as nuclei staining for Ki67 or cellular staining for TUNEL, were counted in each 400X magnification field. Scores for each tumor are reported as an average of the percent positive cells per high power field to derive the proliferation and apoptotic indices, respectively.

Statistical considerations—For pre-treatment and post-treatment biopsy IHC scores for each antigen, p-values were determined (student's t-test) based on the comparisons of triplicate high power fields. A value of $p \leq 0.05$ for differences between pre- and post-treatment measures was considered significant.

RESULTS

Patients

Ten eligible patients were enrolled. Their demographics are shown in Table 2, and baseline laboratory values in Supplemental Table S2. The median age was 57.5 years (range, 41-83

years). Primary tumor sites included the oropharynx in six, oral cavity in two, the hypopharynx in one and the subglottic larynx in one patient. Five patients had stage IVB disease, two had stage IVA, and two had stage III disease. One patient with a stage II lesion underwent prior resection, but recurred with the equivalent of IVA disease.

Toxicity

The number of events of worst grade toxicity per patient is shown in Fig. 1. DLT was observed in two of four patients treated in cohort I receiving GEF 250 mg daily for 16 weeks, PAC 45 mg/m²/week × 6 with concurrent RT. Patient 1 developed leukopenia and pneumonia requiring hospitalization. Patient 4 developed interstitial lung disease secondary to GEF requiring discontinuation of GEF. Based on these toxicities it was determined that the MTD was exceeded. Three of four patients also exhibited grade 3 or higher stomatitis that ranged from 36 to 136 days duration. Subsequent patients were entered at dose level 0 consisting of GEF 250 mg daily concurrent with RT and PAC 36 mg/m²/week × 6. One of six patients treated at this dose developed pneumonia and a cardiac arrhythmia, establishing this as the MTD. RTOG grade 3 or higher stomatitis of median duration of 58 days (range, 30-70 days) was observed in four of six patients treated at this dose. Overall seven of the 10 patients experienced prolonged mucosal toxicity defined as radiation therapy. The median duration of ≥ grade 3 stomatitis or esophagitis persisting for >28 days after completion of radiation therapy. The median duration of ≥ grade 3 stomatitis for the entire group was 43 days (range, 0-136 days); however, in the group from both dose cohorts that developed ≥ grade 3 stomatitis, the median duration was 66 days (range, 30-136 days). In addition, a persistent “burning” oral mucosal dysesthesia was reported by seven patients. Other adverse events included cellulitis, diarrhea, dysphagia, esophagitis, fatigue, hypoalbuminemia, hyponatremia, hypokalemia, leukopenia, lymphocytopenia, pleural effusion, and radiation skin reaction.

Response

Five patients achieved a pathological complete response (CRp) and two had partial responses (PR) (Table 3). Three patients experienced tumor progression. Two patients that achieved a PR underwent surgical resection and were rendered disease free. The median response duration for the entire group was 109.3 weeks (range, 57.4-178.7 weeks). At a median follow-up of 109.3 weeks (range, 33.3-193.6 weeks), 4 patients have died, 3 from tumor and one from unrelated disease. Of the patients achieving a CR, all but one are alive with a tumor-free median survival of 109.3 weeks (range 79.7-178.7 weeks). One CR died of heart disease 24 months after treatment without a recurrence of tumor. Patients 2 and 6 achieved a local CR and PR, respectively, but subsequently developed metastatic disease.

Effect of GEF on tumor EGFR signaling, proliferation and apoptotic responses

Paired tumor biopsies were obtained from 7 subjects prior to treatment and after 7 days of GEF. IHC staining for total and p-EGFR, p-ERK1/2, p-AKT, p-NF-κB, p-STAT3, Ki-67 and TUNEL are summarized in Table 4. Representative staining of tumor from two patients, a GEF molecular and clinical responder, and a non-responder, are shown in Fig. 2A and B, respectively. At baseline, 6 of 7 biopsies demonstrated total and p-EGFR scores >1.0 (Pts. 1-4, 7 and 9), while one showed only weak staining with both EGFR antibodies (Pt. 6), consistent with increased expression and phosphorylation of EGFR in the majority of subjects. After 7 days of GEF, 1 of 7 patients with EGFR and p-EGFR staining at baseline, showed significant redistribution of p-EGFR staining from a nuclear to a cytoplasmic pattern, and inhibition of multiple signaling phosphoproteins together with decreased proliferation by Ki-67 and increased apoptosis by TUNEL staining (Pt. 3; post-treatment, p<0.05). Isolated decreases in nuclear EGFR and p-AKT (Pt. 2), nuclear p-EGFR alone (Pt. 4), EGFR and/or p-EGFR with

decreased or increased p-ERK (Pts. 7 and 9) were observed, but these and two other subjects (Pts. 1 and 6) demonstrated no significant effects on Ki-67 or TUNEL staining.

Fig. 2B shows the pattern of staining observed in pre- and on-treatment specimens from a GEF non-responder (Pt. 1), demonstrating baseline total and p-EGFR staining >1, but no significant effect in any markers while on GEF. In this subject, the pre-treatment biopsy exhibited a predominantly cytoplasmic staining pattern with weak membrane staining for total EGFR compared to that observed in the responder (Fig. 2A), and little p-EGFR staining compared to the isotype control. Furthermore, no redistribution in total or p-EGFR was observed following GEF. Along with low p-EGFR, there was also weaker staining for p-STAT3 and p-RELA, but p-ERK, and p-AKT stained strongly in both pre- and on-treatment biopsies. Patient 6, who showed low EGFR and p-EGFR, also demonstrated relatively lower staining for most of the other phosphoproteins except p-AKT.

The single molecular responder (Pt. 3) also demonstrated a CR. Patients 1, 7, and 9 who also demonstrated no molecular response to GEF, also had a CR following GEF+PAC+RT. Among patients with a PR or PD, for whom data was available (Pts. 2, 4, and 6) exhibited nuclear staining for p-ERK, p-STAT3, p-AKT and/or p-NF- κ B that was not inhibited with GEF. This included patient 6 (Fig. 2B) with the lowest EGFR, but strong p-AKT staining and no significant GEF effect and had a PR before developing metastatic disease.

DISCUSSION

EGFR-targeted therapy provides an attractive approach for the treatment of advanced HNSCC in which EGFR activation may be a dominant contributor. As limited responses have been observed with EGFR antagonists alone, combinations of EGFR inhibitors with chemotherapy and RT are under investigation. In this pilot phase I study, we examined the EGFR inhibitor GEF in combination with PAC and RT, and found that this regimen demonstrated prolonged mucosal toxicity. Although similar grades of stomatitis were seen in our previous study of infusional PAC and RT, we did not observe protracted severe stomatitis. In addition, a “burning” quality oral mucosal dysesthesia was reported by seven patients that persisted after healing.³¹ This toxicity limits the clinical acceptability of this combination since the 50% CR rate observed in this pilot study does not represent an improvement compared to other combined chemotherapy and radiation regimens for HNSCC.^{19, 24}

Biomarker studies demonstrated phosphorylation of EGFR in 6 of 7 pre-treatment biopsies; however, limited effects on EGFR activation, molecular pathways and cellular responses were observed in tumor specimens after 7 days of GEF 250 mg/day. GEF significantly inhibited pEGFR together with p-ERK, p-AKT, p-NF- κ B and p-STAT3, with corresponding inhibition of Ki-67 and increased TUNEL in a single patient's biopsies, indicating EGFR activation may play a dominant role in only a subset of HNSCC patients.

Limited responsiveness to GEF may result from heterogeneous EGFR mutations that determine sensitivity, or from other EGFR-independent pathways that promote the cancer and cause resistance to chemotherapy and radiation. EGFR mutations associated with sensitivity or resistance to GEF have been identified in NSCLC, but the same mutations have not been linked with GEF sensitivity in HNSCC, nor has the role of these other mutations been well defined.^{1, 20} Our data indicate that while co-activation of EGFR and multiple downstream phosphoproteins is common, their activation is variably associated with one another, and to responses to GEF suggesting heterogeneity in the contribution of EGFR. Ki-67 and TUNEL staining demonstrated no evidence of anti-proliferative or cytotoxic effects following 7 days of GEF suggesting EGFR-independent activation. Tumors from other patients demonstrated

co-activation and lack of responsiveness of p-EGFR and multiple phosphoprotein components of the downstream pathways consistent with insensitivity to this dose of GEF.

The basis for the responsiveness of a limited subset of HNSCC to GEF may be diverse and remains to be fully elucidated. We previously showed that EGFR may make limited and variable contributions to NF- κ B and AKT activation in a panel of HNSCC cell lines^{1, 2}, and evidence indicates that other factors such as IL-1, TNF α , and CCR-7 also contribute to ERK-AP-1, PI3K-AKT and IKK-NF- κ B signaling.³²⁻³⁵ EGFR-independent STAT3 activation via either JAK or MEK-ERK has been shown to result from NF- κ B-induced IL-6 expression by tumor and infiltrating inflammatory cells.^{5, 35, 36}

We independently confirmed heterogeneity in EGFR-dependent and independent activation and GEF sensitivity of these pathways in a panel of HNSCC cell lines by immunoblot and quantitative ELISA in the same tumor specimens using quantitative reverse phase protein microarray (RPMA).³⁷ In cell lines and tumors, absent or only partial inhibition of AKT, ERK, NF- κ B, and/or STAT3 activation was observed consistent with GEF resistance or EGFR independent activation. The drug sensitivity in these cell lines was negatively correlated with increased levels of basal and EGF-induced p-AKT, and positively correlated with EGF-induced pSTAT3. In cell lines sensitive to GEF, ERK1/2, AKT, NF- κ B and STAT3 were inhibited, as in the responding patient in this study. The independent comparison of the present study in the same tumor specimens and cell lines using RPMA supports the notion of heterogenous EGFR activation in TKI resistance. These studies also provide evidence that using IHC or RPMA for detection of inhibition of pEGFR together with pERK, pAKT, pNF- κ B and pSTAT3, with corresponding inhibition of Ki-67 and increased TUNEL staining as evidence of cytotoxicity, may be useful as early markers of responsiveness to EGFR antagonists and for selection of personalized antitumor therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CR	complete response
DLT	Dose-limiting toxicity
EGFR	epidermal growth factor receptor
GEF	gefitinib
HNSCC	head and neck squamous cell carcinoma
IHC	immunohistochemistry
MTD	maximum tolerated dose
PAC	paclitaxel
PBS	phosphate buffered saline
PR	partial response

RT	radiation therapy
TKI	tyrosine kinase inhibitor

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1.

Figure 1.

Number of worst grade adverse events observed per patient (n = 10 subjects). The Common Toxicity Criteria Adverse Event (CTCAE) v3.0 and RTOG/EROTC Toxicity Criteria were used for classification and grading of systemic and in field adverse events, respectively.

Figure 2.

Immunohistochemical staining of EGFR, signal transduction components, proliferation (Ki-67) and for apoptosis (TUNEL) in tumor biopsies obtained pre-treatment, and after 7 days of GEF. **A.** Molecular and histological response was observed in 1 of 7 patients with EGFR and p-EGFR staining at baseline (Pt. 3). Redistribution of pEGFR staining from a nuclear (grey bars) to a cytoplasmic pattern (black bars), and inhibition of multiple signaling phosphoproteins together with decreased proliferation by Ki-67 and increased apoptosis by TUNEL staining was detected pre- and on-treatment ($p < 0.05$). **B.** Pattern of staining observed in pre- and on-treatment specimens from a GEF non-responder, that demonstrated baseline total and p-EGFR staining > 1 , but a lack of GEF effect for any of the markers assayed. In this subject, the pre-treatment biopsy exhibited a mixed cytoplasmic (black bars) and nuclear pattern (grey bars) with relatively weak membrane staining for total EGFR compared to that observed in the responder in panel A, and little p-EGFR staining when compared to isotype control. No significant redistribution in total or pEGFR was observed following GEF. Along with low pEGFR, there was also relatively weaker staining for p-STAT3 or p-RELA, but pERK, and pAKT stained strongly in both pre-and on-treatment biopsies.

Table 1

Dose Cohorts of Gefitinib and Paclitaxel.

<u>Dose Level</u>	<u>Gefitinib (mg/day)</u>	<u>Paclitaxel (mg/m²/week ×6 weeks)</u>
0 *	250	36
1	250	45
2	250	56
3	500	45
4	500	56

* Dose-reduction cohort: GEF administered concurrent with RT. All other dose levels were to receive a total of 16 weeks GEF.

Table 2

Patient Characteristics.

Characteristic	Number (%)
Total patients	10
Median age in years [range]	57.7 [41-83]
Sex	
Male	7 (70)
Female	3 (30)
Race	
Caucasian	6 (60)
Hispanic	3 (30)
African	1 (10)
Tumor site	
Oral cavity	2 (20)
Oropharynx	6 (60)
Hypopharynx	1 (10)
Subglottic larynx	1 (10)
Tumor differentiation	
Well	1 (10)
Moderate	7 (70)
Poor	2 (20)
T classification	
< T4	8 (80)
T4	2 (20)
N classification	
< N2	4 (40)
≥ N2	6 (60)
TNM Stage at diagnosis	
II	1 (10)
III	2 (20)
IVA	2 (20)
IVB	5 (50)
Prior surgical resection	1 (10)
Unresectable disease	3 (30)
Weight loss ≥ 5% baseline	4 (40)
Median ECOG PS [range]	1 [0-2]
Hypoalbuminemia [grade 1]	5 (50)
History of heavy alcohol use *	4 (40)
Smoking status	
Ever smoker	6 (60)
Never smoker	4 (40)

ECOG PS, eastern cooperative oncology group performance status; CTC, common toxicity criteria.

* Heavy alcohol use defined as ≥ 2 alcoholic drinks daily.

Table 3

Treatment Responses.

Patient Number	Primary Tumor Site	TNM Stage	Resectable *	Treatment						Tumor Response	
				Gefitinib		Paclitaxel					RT
				Dose (mg/day)	Time (wk)	Dose (mg/m ² /wk)	Time (wk)	Dose (Gy)			
001 [†]	Oropharynx	T4N3	Yes	250	5	45	5	70	CRp		
002	Oral cavity	T1N1	Yes	250	16	45	6	72	PD		
003	Oropharynx	T2N3	Yes	250	16	45	6	70	CRp		
004 [‡]	Oropharynx	T2N2	Yes	250	4	45	4	70	PR		
005	Hypopharynx	T3N3	No	250	8	36	6	70	PD		
006	Oropharynx	T4N3	No	250	8	36	6	76	PR		
007	Oropharynx	T2N2	Yes	250	8	36	6	70	CRp		
008	Subglottic larynx	T3N0	Yes	250	8	36	6	70	PD		
009 [‡]	Oral cavity	T3N1	Yes	250	8	36	6	70	CRp		
010	Oropharynx	T2N0	No	250	8	36	6	70	CRp		

CRp, complete response pathologically confirmed; PR, partial response; PD, progressive disease; XRT, radiotherapy

*Tumor were considered unresectable if the tumor encased the carotid artery, extended to the pterygoid space or skull base, or involved the prevertebral musculature.

[†] Patient 001 experienced Grade 4 leukopenia and Grade 3 aspiration pneumonia.

[‡] Patient 004 experienced Grade 3 interstitial pneumonitis.

[‡] Patient 009 experienced Grade 4 pneumonia and cardiac arrhythmia.

Table 4

Summary of Pre- and Post-treatment Immunohistochemistry Scores*

Patient†	Total EGFR											
	Cytoplasmic			Nuclear			p-EGFR			p-STAT3		
	PRE	POST		PRE	POST		PRE	POST		PRE	POST	
001	1.7 (0.6)	1.7 (0.6)		27.9 (3.2)	27.7 (1.8)		1.7 (0.6)	1.7 (0.6)		125.4 (57.7)	91.2 (1.2)	
002	1.0 (0)	1.7 (0.6)		37.8 (11.9)	5.8 (5.4)		1.0 (0)	1.3 (0.6)		181.6 (15.2)	170.4 (7.2)	
003	1.0 (0)	1.7 (0.6)		74.7 (8.7)	26.0 (4.1)		1.7 (0.6)	1.7 (0.6)		88.4 (2.6)	27.9 (25.7)	
004	1.5 (0.5)	0.8 (0.3)		84.8 (2.8)	81.7 (54.2)		1.7 (0.6)	1.0 (0)		125.4 (57.3)	143.8 (50.6)	
006	0.5 (0)	0.8 (0.3)		126.8 (38)	136.4 (24)		0.2 (0.2)	0.2 (0.2)		43.5 (15.2)	51.4 (15.2)	
007	1.5 (0.5)	0.8 (0.3)		150.5 (28)	39.6 (10.9)		1.2 (0.3)	0.4 (0.2)		96.6 (1.3)	87.1 (9.6)	
009	1.7 (0.6)	1.3 (0.6)		72.3 (17.1)	127.8 (20)		1.5 (0.5)	0.7 (0.3)		127.9 (55.9)	230.9 (55.6)	
										80.6 (28.8)	262.8 (46.6)	
										127.9 (55.9)	230.9 (55.6)	
										191.4 (5.0)	261.8 (55.9)	
										94.8 (1.3)	95.1 (3.4)	
										57.1 (4.9)	49.4 (4.6)	
										71.1 (11.6)	80.4 (8.3)	
										84.9 (4.5)	75.8 (11.1)	
										59.5 (18.3)	48.9 (11.4)	
										45.3 (7.6)	52.7 (9.5)	
										61.6 (18.4)	54 (28.9)	
										136.5 (42.9)	127.1 (57.1)	
										125.2 (59.5)	127.1 (57.1)	
										76 (6.3)	41.2 (9.5)	
										82.4 (6.1)	7.6 (5.4)	
										235.4(57.4)	124 (30.6)	
										170.2 (31.6)	79 (13.6)	
										295.1 (1.4)	262.2 (56.1)	
										78.8 (5.5)	85.9 (6.9)	
										66.8 (9.0)	59.7 (7.8)	
										10.7 (3.1)	30.1 (23.8)	
										5.6 (1.5)	2.5 (0.9)	
										5.1 (1.3)	3.6 (1.1)	
										1.4 (0.4)	8.1 (1.9)	

Statistically significant (<0.05, students t-test) changes in staining have bolded p-values.

* p=stain scores (EGFR, p-EGFR), histoscores (nuclear EGFR, nuclear p-EGFR, p-Erk, p-STAT3, p-Akt, p-NF-κB), proliferation indices (Ki67) and apoptotic indices (TUNEL) are given as an average (standard deviation) of three high power fields per tumor biopsy.

† Only patients listed in table had evaluable pre- and post-treatment tumor biopsies