

## Research Article

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## Chemoprevention of Head and Neck Cancer with Celecoxib and Erlotinib: Results of a Phase Ib and Pharmacokinetic Study

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### Abstract

Epidermal growth factor receptor (EGFR) and COX-2 inhibitors synergistically inhibit head and neck squamous cell carcinoma tumorigenesis in preclinical studies. We conducted a phase I and pharmacokinetic study with the erlotinib and celecoxib combination in patients with advanced premalignant lesions. Thirty-six subjects with oral leukoplakia, mild, moderate, or severe dysplasia, or carcinoma *in situ* were screened for study participation; 12 consented and received therapy for a median of 5.38 months. Erlotinib was escalated following a standard 3+3 design at 50, 75, and 100 mg orally daily and celecoxib was fixed at 400 mg twice daily for 6 months. Biopsy of lesions and cytobrush of normal mucosa were performed at baseline, 3, 6, and 12 months. Erlotinib pharmacokinetics were analyzed in 10 subjects. The maximum tolerated dose of erlotinib with celecoxib 400 mg BID was 50 mg per day with skin rash being the main observed toxicity. Overall histologic response rate was 63% (complete response, 43%; partial response, 14%; stable disease, 29%; and disease progression, 14%). With median follow-up of 36 months, mean time to progression to higher-grade dysplasia or carcinoma was 25.4 months. Downregulation of EGFR and p-ERK in follow-up biopsies correlated with response to treatment. Larger average erlotinib V/F (approximately 308 L) and CL/F (8.3 L/h) compared with previous studies may be related to relatively large average bodyweights. Average erlotinib  $t_{1/2}$  was 25.6 hours. Encouraging responses to the celecoxib and erlotinib combination correlated with EGFR pathway inhibition. Although erlotinib-related rash was the main limitation to dose escalation, the intervention was well tolerated. *Cancer Prev Res*; 7(3); 283–91. ©2013 AACR.

### Introduction

The epithelial growth factor receptor (EGFR) is expressed in a wide variety of malignant tumors, including head and neck, colon, pancreatic, non-small cell lung, breast, kidney,

ovarian, bladder carcinomas and gliomas (1–3). The incidence of EGFR expression in head and neck squamous cell carcinoma (HNSCC) is over 90%, suggesting that EGFR inhibition may be effective in HNSCC (4–6). One approach to block EGFR activity involves the use of small molecule tyrosine kinase inhibitors (TKI) that target the intracellular domain of EGFR. This ability to specifically inhibit intracellular tyrosine kinase activity has been observed over precise dose ranges (7, 8).

A broad range of laboratory investigations, animal models, and epidemiologic studies provide evidence that inhibition of COX-2 pathways may contribute to cancer treatment in general (9–11) and HNSCC in particular (12, 13). In HNSCC, COX-2 is expressed in both tumor tissue and adjacent epithelium, with increased expression in invasive carcinoma compared with normal epithelium. COX-2 inhibition has been shown to result in cell growth inhibition in HNSCC cell lines (14). Furthermore, we have previously elucidated the differential expression pattern of COX-2 in stages of head and neck premalignant lesions and invasive carcinoma (15). Our findings indicated that COX-2 is involved in early and intermediate stages of carcinogenesis

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in HNSCC. COX-2 levels increased progressively throughout all stages of carcinogenesis. This may reflect a role for COX-2 in this process, further supporting the rationale for COX-2 inhibition as a valid strategy for cancer chemoprevention. Based on this evidence, a number of chemoprevention and therapeutic trials in HNSCC using COX-2 inhibitors (COX-2I) are under way (16). Evidence suggests that COX inhibitors, including nonsteroidal anti-inflammatory drugs (NSAID), protect against a variety of tumors (9, 17, 18). In patients with familial adenomatous polyposis, celecoxib, a selective COX-2I, caused a 28% reduction in the number of colorectal polyps compared with 4.5% reduction for placebo (19). In HNSCC, Western blot analysis showed expression of COX-2 in mucosa of subjects at different stages of carcinogenesis and not in normal mucosa, suggesting a possible role for COX-2 inhibition in HNSCC chemoprevention (20). Previous studies suggested that NSAIDs might have a similar effect in delaying the growth of head and neck tumor cell lines (21–23). Furthermore, prior studies using the COX-2I as a single agent in oral premalignant lesions revealed evidence of improvement in the degree of dysplasia (24).

Preclinical and clinical studies have described the interaction between EGFR and COX-2 and shown that targeting these two pathways can synergistically or additively block progression of HNSCC growth *in vitro* and *in vivo* (14, 25, 26), providing a scientific basis for using the combination of EGFR-TKI and COX-2I as a chemopreventive approach in HNSCC. We report here the results of a phase I clinical trial and pharmacokinetic studies of this combination in subjects with premalignant lesions (i.e., leukoplakia, erythroplakia, and/or erythroleukoplakia) of the oral cavity oropharynx or larynx. The correlation of response with biomarker modulation was reported in a separate publication (27).

## Patients and Methods

### Patient accrual

Between October 24, 2006, and June 28, 2012, 36 subjects with documented premalignant lesions, including mild (mild-D), moderate, or severe oral leukoplakia, and carcinoma *in situ* (CIS) were screened. Lesion sites included oral cavity oropharynx, and the larynx. Participants were eligible regardless of their smoking status. Eligibility requirements included Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, age  $\geq 18$  years, adequate bone marrow, liver and renal function, signed written informed consent, negative serum pregnancy test ( $\beta$ -HCG) within 72 hours of receiving treatment, willingness to use appropriate contraception during study participation for women of child-bearing potential, adequate pulmonary function (FEV<sub>1</sub> and Forced vital capacity  $\geq 60\%$  by spirometry), adequate cardiac function (echocardiogram with normal Left Ventricular ejection fraction).

Subjects were excluded from participation if they had documented hyperplasia only, acute intercurrent illness or recent major surgery, history of previous malignancies

excluding stage I or II cancers rendered disease-free more than 1 year from time of consent, documented pregnancy, were breast feeding, had active cardiovascular events, including angina, unstable angina, palpitation, tachycardia, arrhythmia, a recent cerebrovascular accident or myocardial infarction within 6 months from enrollment, documented history of coagulopathy and/or were taking warfarin or warfarin-derivative anticoagulants, hypertension not adequately controlled by medication, history of congestive heart failure greater than New York Heart Association (NYHA) grade 2, confusion, disorientation, or a history of major psychiatric illness impairing their understanding of the informed consent, history of intake of COX-2I or EGFR-TKI within 3 months of study entry, documented history of interstitial lung disease or known connective tissue disease, history of NSAID-induced ulcers or participation in a clinical trial using an investigational drug within 12 months of enrollment.

Study participants were required to have a complete inspection of the oral cavity oropharynx and larynx. A baseline biopsy for initial diagnosis and grading was mandatory. All evaluable patients were required to have repeated biopsies at 3, 6, and 12 months from initiation of therapy. Biopsies of suspicious lesions in the oral cavity or laryngeal lesions were performed using standard "punch biopsy" procedure with approximately a 3-mm punch. If a follow-up biopsy was deemed difficult to obtain at the suspicious site, a brushing or wash was performed instead. Histologic assessments were performed by a head and neck pathologist. Buccal scrapings (cytobrush) for target lesions and normal buccal mucosa were performed at baseline, 3, 6, and 12 months as surrogates for biomarker modulation.

### Drug treatment

Participants received a fixed dose of celecoxib 400 mg orally BID continuously for 6 months. Erlotinib was dose escalated at 3 dose levels of 50, 75, and 100 mg orally every day for 6 months. Dose escalation followed a standard 3+3 escalation design.

### Definition of response

Response evaluation was based on pathologic examination of the degree of dysplasia observed and recorded by an expert head and neck pathologist. Pathologic complete response was defined as complete disappearance of dysplasia from the epithelium. Pathologic partial response was defined as improvement of dysplasia by at least one degree (i.e., severe dysplasia becomes moderate dysplasia). Pathologic minor response or stable disease was defined as minor focal improvement without change of degree of dysplasia (i.e., focal improvement from moderate to mild dysplasia with still moderate dysplasia overall) or no pathologic changes after treatment. Pathologic progressive disease was defined as worsening by at least one degree of dysplasia (i.e., mild to moderate dysplasia) or development of invasive cancer on or following treatment.

**Table 1.** Evaluable patient characteristics, baseline pathology, pathologic response, and time to progression

Patient	Age (y)	Gender	Site	Lesion	Pathologic response	Progression/discontinuation	Time to progression or last evaluation (months)/cause of withdrawal
1	50	F	Tongue	MD	CR	Y	4
2	42	M	Tongue	MD	PR	Y	26
3	41	F	Tongue	SD	SDi	Y	26
4	65	F	Tongue	SD	PD	Y	6
5	62	M	Buccal mucosa	SD	CR	Y	55
6	64	M	Larynx	Mild-D	SDi	Y	6
7	40	M	Larynx	MD	CR	N	36
8	69	F	Tongue	HG	NE	D (elevated creatinine)	Renal insufficiency
9	74	F	Tongue	MD	NE	D (grade 3 rash)	Rash
10	87	F	BOT	SD	NE	D (withdrew)	Patient chose to withdraw
11	46	F	Tongue	HG	NE	D (withdrew)	Withdrew after signing consent
12	57	F	Tongue	HG	N/A	N/A	Excluded from efficacy analysis

Abbreviations: BOT, base of tongue; D, discontinued therapy before the first evaluation of response; F, female; HG, high-grade dysplasia; M, male; MD, moderate dysplasia; N, no progression as of the last follow-up; NE, nonevaluable; SD, severe dysplasia; SDi, stable disease; Y, documented progression.

### Pharmacokinetic studies

Blood sampling for measurement of erlotinib pharmacokinetics was performed before drug administration ( $t = 0$ ), and 0.5, 1, 3, 6, 8, 24, and 48 hours after administration. Blood was centrifuged using a refrigerated centrifuge and plasma collected and frozen before being assayed for erlotinib.

### Assays of erlotinib and active metabolites

Erlotinib was extracted from plasma samples and plasma concentrations were measured by liquid chromatography/mass spectrometry (LC/MS), as detailed in Supplementary Materials. A standard curve was constructed using weighted ( $1/X^2$ ) linear regressions of the peak area ( $Y$ ) of erlotinib against the corresponding nominal concentrations of erlotinib ( $X$ , ng/mL) in blank plasma. The liquid chromatography/tandem mass spectrometry (LC/MS-MS) assay was validated with specificity, precision (coefficients of variation <15%), accuracy (>85%), matrix effects, and linearity (0.1 to 500 ng/mL;  $r > 0.99$ ). The limits of detection and quantitation for erlotinib were 0.5 and 2 pg on the column. Recoveries of erlotinib from human plasma were 80% to 82%.

### Pharmacokinetic data analysis and modeling

Plasma concentrations of erlotinib were used for pharmacokinetic modeling. Assay results (confirmed by patient drug diaries) indicated variations with respect to timings of erlotinib administration, and certain individuals were missing blood samples at critical times, making the data unsuitable for compartmental modeling on a per individual basis. However, modeling was feasible using population pharmacokinetic methods with the NONMEM modeling program

(28). The population pharmacokinetic of erlotinib was previously modeled in patients with cancer enrolled in phase II studies, using an open one-compartmental model with first-order absorption into plasma (29–31). Here, a similar modeling approach was used to study the pharmacokinetics of erlotinib in comparatively healthy subjects with premalignant lesions, using the NONMEM software (ver 7.1; ICON Development Solutions; ref. 32) run with PLT tools (version 2.6; PLTsoft). Goodness of fit was analyzed using commonly used graphical methods for population pharmacokinetic, and the influence of body weight on V/F and CL/F was explored using previously reported covariate model structures (29–31). Methods are detailed in Supplementary Materials.

## Results

### Patient characteristics

A total of 36 subjects with pathologic dysplasia (mild-D, moderate, and severe) or CIS were screened. Nineteen screened subjects were not enrolled due to social or personal reasons mostly related to reasons of convenience such as transportation or other personal commitments, in addition to concerns about toxicity, comorbidities, or ineligibility. One patient with mild-D was enrolled after the protocol was amended to include mild-D. Seventeen subjects were enrolled on the study, 3 of whom withdrew consent (one male of 69 years of age, and two females of 43 and 42 years of age). Two patients who signed informed consent were deemed to be screen failures, one secondary to prior history of oral squamous cell carcinoma and the other secondary to a history of cardiac arrhythmias. A total of 12 subjects received therapy. None of the 12 patients who received therapy had a history of

prior treated malignancy. Their characteristics with baseline pathology, response, and duration of response are described in Table 1. Two subjects were taken off treatment before response evaluation due to the following reasons: grade 3 rash, elevated serum creatinine, or urosepsis. Two subjects chose to withdraw from therapy before first response evaluation. One patient was excluded from the efficacy analysis as she was found to have microinvasive carcinoma on her resected pretherapy tissue biopsy. A total of 7 subjects were evaluable for response.

### Dose escalation and toxicity

Three subjects were enrolled on cohort 1 (erlotinib 50 mg daily) with no dose limiting toxicities (DLT) observed, allowing escalation to cohort 2 (erlotinib 75 mg daily). One patient had a grade 3 rash at dose level 2 and cohort 2 was expanded to 6 subjects. As a second subject had grade 3 rash, the maximum allowable dose of erlotinib was deemed to be 75 mg. One patient was enrolled at a dose level 3 (erlotinib 100 mg daily) before toxicity analysis of the patients who received 75 mg/day and received continued therapy for the entire duration of the study with no documented grade 3 toxicities. Following dose deescalation, 2 additional patients received erlotinib 50 mg/day. None of the 5 subjects treated at the 50 mg/day dose experienced a DLT. The maximum tolerated dose (MTD) of erlotinib in combination with celecoxib 400 mg BID was therefore determined to be 50 mg/day. The observed grade 2–3 toxicities were as follows for the 12 treated patients: rash 2 of 12 (17%), mucositis 1 of 12 (17%), mouth sores 3 of 12 (25%), leukopenia 1 of 12 (8%), anemia 1 of 12 (8%), hyperglycemia 2 of 12 (17%), hypoalbuminemia 1 of 12 (8%), hypoglycemia 1 of 12 (8%), throat infection 1 of 12 (8%), urosepsis 1 of 12 (8%), and elevated creatinine 1 of 12 (8%; Table 2).

### Clinical outcome

Responses were evaluated for 7 subjects using the last documented histologic response at 3, 6, or 12 months. At baseline, 3 of 7 (43%) had moderate dysplasia, 3 of 7 had severe dysplasia (43%), and 1 of 7 had mild-D (14%). Three of seven achieved a complete remission (CR, 43%), 1 of 7 partial remission (PR, 14%), 1 of 7 progressive disease (PD, 14%), and 2 of 7 had stable disease (29%) with an overall Response Rate of 57% and overall clinical benefit of 86% (histologic responses to therapy are shown in Fig. 1). The median time to achieving a documented response from the time of enrollment was 5.6 months. All 3 patients who achieved a CR also had complete disappearance of their lesions by visual inspection. At the time of the last analysis, 6 of 7 patients (85%) had documented progression: 1 to stage I invasive carcinoma 6 months after completion of therapy for moderate dysplasia of the lateral tongue, 1 to stage II oral cavity carcinoma 4 months after completion of therapy, 1 to invasive squamous cell carcinoma after having stable severe dysplasia for 26 months, 2 with recurrent moderate dysplasia and severe dysplasia at 26 and 6 months, respectively,

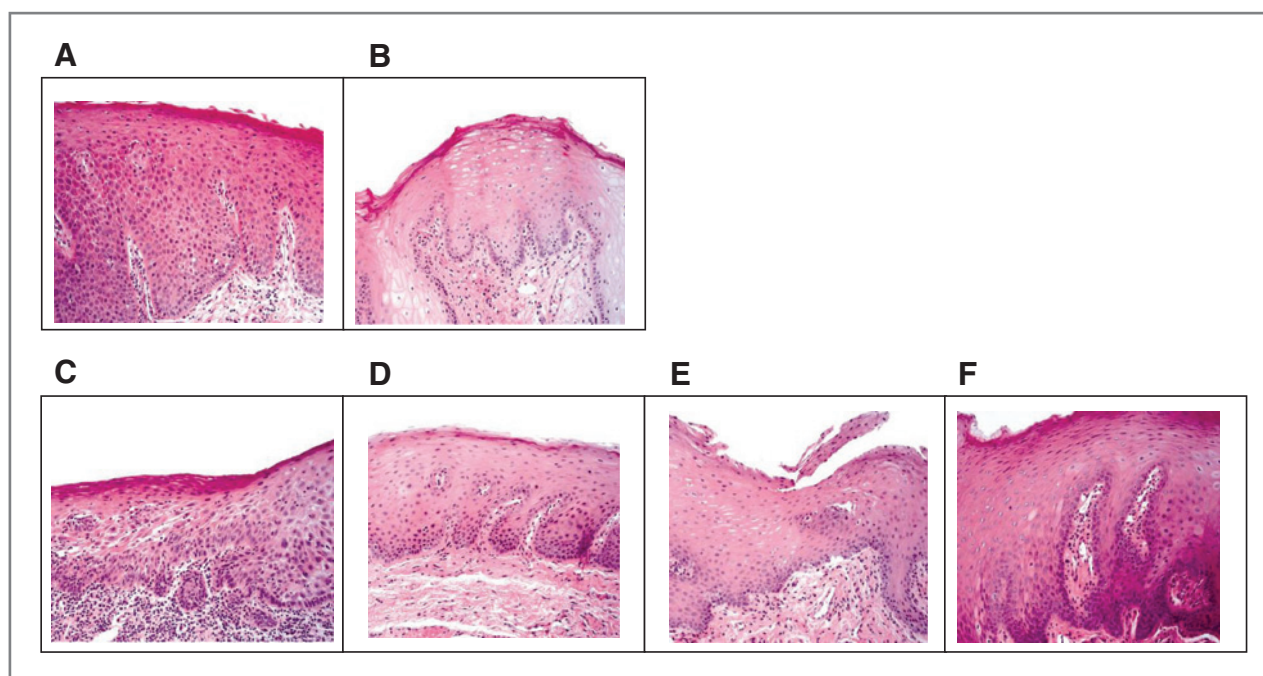
**Table 2.** Toxicities including grades 1 to 4

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Abdominal cramping	2			
Alopecia	2			
Anemia	2	1		
Anxiety	2			
Decreased protein	2			
Leukopenia	1	1		
Depression	3			
Diarrhea	5			
Dry Eyes	4			
Dry Skin	6			
Elevated LDH	3			
Elevated serum creatinine	4	1		
Elevated alkaline phosphatase	3			
Elevated ALT	5			
Elevated AST	4			
Fatigue	6			
Hyperbilirubinemia	2			
Hypercholesterolemia	2			
Hyperglycemia	7	2		
Hypoalbuminemia	3	1		
Hypocalcemia	4			
Hypoglycemia	1	1		
Hypokalemia	2			
Hyponatremia	3			
Mouth sores	9	3		
Mucositis	3		1	
Nausea	4			
Neuropathy	3			
Pruritis	2			
Rash	8		2	
Shortness of breath	3			
Strep throat		1		
Urosepsis	0	0	1	
Vomiting	2			

and 1 patient with recurrent high-grade dysplasia 55 months after achieving a CR for severe dysplasia of the buccal mucosa. One patient continues to be in CR 36 months after treatment of a high-grade dysplasia of the vocal cord. The median duration of follow-up for the 7 evaluable subjects was 36 months. The mean time to progression to a higher-grade dysplasia or carcinoma was 25.4 months (Fig. 2). No patients had died by the time of data analysis.

### Correlative biomarker studies

Tissue analysis comparing baseline biopsies with follow-up samples after treatment revealed decreases in EGFR and p-ERK levels in the patients' last biopsies. These changes were correlated significantly with clinical responses to

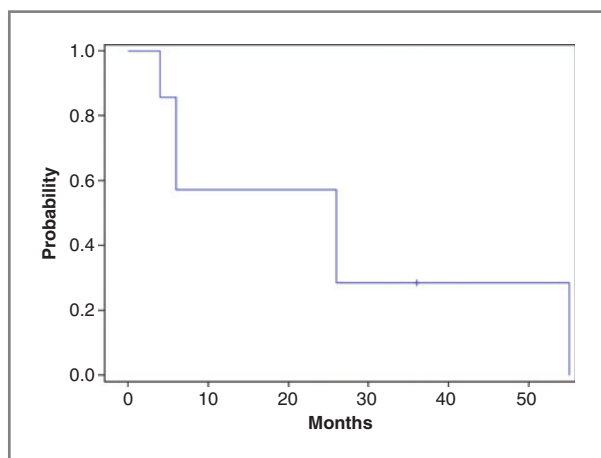


**Figure 1.** Histologic responses from biopsies of 2 patients treated with celecoxib and erlotinib. A, patient 1, baseline severe dysplasia; B, patient 1, hyperplasia and no dysplasia at 3 months; C, patient 2, baseline moderate to severe dysplasia; D, patient 2, mild dysplasia at 3 months; E, patient 2, hyperplasia and no dysplasia at 6 months; F, patient 2, hyperkeratosis at 12 months.

therapy ( $P = 0.019$  and  $P = 0.006$  for EGFR and p-ERK, respectively). The correlative biomarker studies were described in detail elsewhere (27).

#### Pharmacokinetic studies

Plasma concentrations of erlotinib and the demethyl metabolite (OSI-420) from 10 subjects administered were plotted versus time (Fig. 3A). Erlotinib concentrations only were modeled, as OSI-420 concentrations were <10% of erlotinib and curves were parallel. Four subjects administered erlotinib after the 24-hour blood draw,

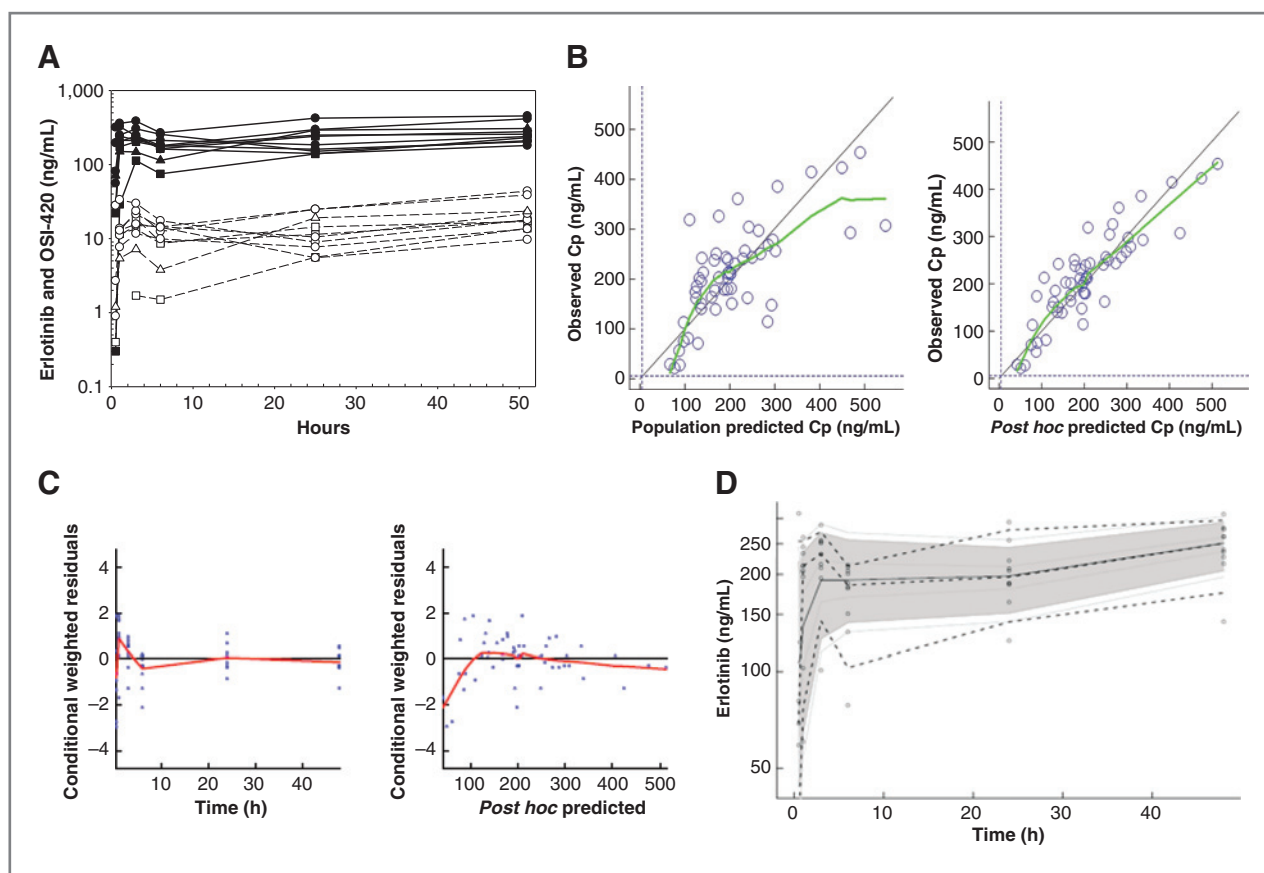


**Figure 2.** Time to progression to higher-grade dysplasia or carcinoma. Mean time to progression was 25.4 months. Median time for follow-up was 36 months.

whereas the rest did so before their 24-hour dose. Also, 6 subjects had plasma concentrations similar to  $C_{max}$  before the 48-hour sampling time. Based on the previously reported erlotinib  $T_{max}$  (2–3 hours) and long elimination half-life ( $t_{1/2} > 24$  hour), these undocumented timings were inputted as 3 hours before the 48-hour blood draw (31).

The pharmacokinetic parameters of a basic model with no covariates (model 1), and a model relating V/F as proportional with subject body weight (model 2), are summarized in Table 3. Both estimated V/F, CL/F, and absorption rate constant ( $K_a$ ) with reasonable precision. Including body weight as a covariate of V/F improved the model fit (difference in objective function = 5.09), without the need for additional fitted parameters. Unlike previous reports, inclusion of body weight as a covariate of CL/F did not improve model fit further, possibly due to the limited range of body weights in this study (29). There was >20% shrinkage for  $K_a$  in model 1 and for  $K_a$  and V/F in model 2, indicative of underestimation of interindividual parameter variability (IIV) for these parameters. Neither model allowed estimation of the intersubject variability (IIV) of CL/F. Model 2 was used for all further analyses.

There was reasonable symmetry of predicted population and *post hoc* concentrations versus observed data (Fig. 3B), with a slight tendency to overestimate certain lower concentrations. As expected, the individual *post hoc* predictions (with IIV) had greater correlations with observations ( $r^2 = 0.508$  and  $0.783$ , respectively). Conditioned weighted residuals (CWRES) versus time and *post hoc* predictions (Fig. 3C) were confirmatory of an adequately fitted model.



**Figure 3.** A, plasma concentrations of erlotinib and OSI-420 (demethyl erlotinib) versus time. Subjects self-administered 50 mg ( $\square$ ,  $n = 3$ ), 75 mg ( $O$ ,  $n = 6$ ), or 100 mg erlotinib ( $\Delta$ ,  $n = 1$ ) every day. Closed symbols and solid lines represent erlotinib concentrations, open symbols and dashed lines represent the OSI-420 metabolite. Plasma concentrations of erlotinib were used for pharmacokinetic modeling. B, observed versus final model predicted plasma concentrations of erlotinib. The left plot is based on the "typical" prediction without including model variability (IIV), whereas the right plot is based on the complete model including IIV (*post hoc* fit). The linear regression coefficients ( $r^2$ , observed vs. predicted) for the respective plots were 0.508 and 0.783, respectively. Inclusion of the error model (*post hoc* model) decreased the standard errors of prediction of the model from 22% to 15%. C, CWRES (39) were plotted versus time and *post hoc* predicted concentrations. The curvilinear plots depict local regression trend smoother fits to the data. The curvilinear plots in C and D depict local regression trend smoother (R Supersmoother) fits to the data. D, model validation by "precision corrected visual predictive check" (40), in which observations are normalized to the median (75 mg every day) dosing scheme. One thousand simulations were performed per subject and used to compute percentile ranges ( $P_5$ ,  $P_{25}$ ,  $P_{50}$ ,  $P_{75}$ , and  $P_{95}$ ) normalized to median dose and body weight. Actual observations were also normalized, and superimposed over these ranges for comparison. The shaded area indicates 90% confidence interval; solid lines indicate percentiles: 2.5, 97.5 (red); 5, 95 (blue); 25, 75 (green); 50 (black). Dashed lines indicate percentiles 5, 50, and 95 of observations.

Internal model validation using a precision corrected visual predictive check plot (Fig. 3D) for model 2 resulted in 12% and 14% of observations greater than or less than the predicted 95th and 5th percentiles, respectively, which could indicate some variance shrinkage. However, the performance may be adequate given only 51 plasma concentrations. Due to data limitations, a study of the influence of celecoxib on the pharmacokinetics of erlotinib was not feasible in this study.

## Discussion

The combination of erlotinib and celecoxib has been shown to synergistically inhibit head and neck cancer cell growth in preclinical studies performed by our group (14, 25). Prior phase I trials have examined the clinical activity of this combination with radiation therapy in patients with recurrent HNSCC and advanced lung cancer

(33). Unlike our study, the dose of erlotinib was fixed in these studies and the celecoxib dose was escalated. Grade 3 toxicities, including dermatitis and mucositis, were also reported. Our study focused on primary chemoprevention in healthy subjects with premalignancies; therefore, our threshold of defining DLTs was lower, given the chemopreventive nature of our investigation. Although both celecoxib and erlotinib have been used as single agents in chemoprevention studies for different tumors, including colorectal cancer and HNSCC (19, 34, 35), the combination has not been extensively explored in the chemoprevention setting.

The results of this phase I clinical trial support our hypothesis that EGFR-TKI and COX-2I can inhibit tumorigenesis in premalignant lesions of the head and neck. Based on our conservative approach in assessing toxicities, we determined the MTD of erlotinib in this combination to be

**Table 3.** Population pharmacokinetic parameters of erlotinib

OF (–2LL)	–45.72	–50.81
Parameters (RSE, %)	Basic model	kg on V
CL/F (L/h)	8.34 (4.99)	8.29 (5.10)
V <sub>1</sub> /F (L)	295.2 (12.69)	308.35 (10.03)
K <sub>a</sub> (h <sup>–1</sup> )	1.00 (29.55)	1.02 (31.09)
IIV <sup>a</sup> , σ <sup>2</sup> (% shrinkage)		
IIV of V/F	33.02 (14.92)	21.19 (28.01)
IIV of K <sub>a</sub>	83.87 (20.18)	66.09 (22.36)
σ <sup>2</sup> (%)	31.97 (11.63)	32.7 (9.48)
Number significant digits	3.8	4.9

NOTE: No reference intravenous dose was administered so that the fraction (F) of orally absorbed drug is unknown, as no reference intravenous dose was administered (assumed 1 for calculations). CL/F, oral clearance, V is the volume of distribution (normalized for an 81.7-kg individual in the case of the covariate model); K<sub>a</sub>, first-order oral absorption rate constant. IIV are interindividual variance estimates, σ<sup>2</sup> = residual (intraindividual) variance and OF is the value of NONMEM objective function. Relative standard errors (RSE) of parameters were calculated as % CV.

<sup>a</sup>IIV, σ<sup>2</sup>, and RSE were reported as percentage coefficient of variations (% CV), noting that the formula for % CV for a log-normal distributed parameter =  $\sqrt{(e^{\omega^2} - 1)} \times 100$ , in which ω<sup>2</sup> is the variance of a log-normally distributed parameter. The percentage of shrinkage estimates was obtained from the NONMEM output.

50 mg/day. This regimen was fairly well tolerated with erlotinib-induced rash being the most commonly observed grade 3 toxicity preventing dose escalation. We believe erlotinib at 50 mg per day would be a well-tolerated dose using this combination.

A major pitfall of our study is the small number of patients who were evaluable for response. Despite this, our correlative biomarker studies showing a downregulation in EGFR and p-ERK levels, which correlated with response to therapy (27), as well as an observed pathologic response rate of 63% with a mean duration of 20.9 months support the clinical benefit of this intervention. This will, however, require further confirmation in a larger phase II study.

There are multiple challenges and obstacles in implementing any chemopreventive intervention in a large population of healthy subjects. These include difficulty in accrual, toxicity, and quality-of-life implications in an otherwise healthy patient population, among others. As chemopreventive agents need to be delivered continuously over a rather extended period of time, it is important to select agents with a very favorable toxicity profile. Erlotinib-induced skin rash has become an accepted consequence in patients with advanced metastatic non-small cell lung cancer when compared with the dangers and side effects of cytotoxic chemo-

therapy. This is clearly not the case in a chemoprevention patient population such as ours. It could be argued that this is a rather high price to pay in the chemopreventive setting. In our experience, the toxicity of our intervention has been one major limitation to accrual and to dose escalation on the study. Other chemopreventive trials in head and neck cancer are incorporating erlotinib or celecoxib as a single agent or in combination with other agents. In a study using single-agent celecoxib in oral premalignant lesions, 12 of 18 biopsies showed improvement in the degree of dysplasia after 12 weeks (24). Whereas the majority of our treated subjects had severe or high-grade dysplasia, 50% of subjects in the Wirth study had mild dysplasia. Still these results raise the question of whether there is an advantage in combining the two agents versus using single agents. The results of trials using single-agent erlotinib are eagerly awaited. Our rationale for combining celecoxib and erlotinib stems from our preclinical data showing a clear synergism between EGFR and COX-2 blockade as far as inhibition of HNSCC progression and growth *in vitro* and *in vivo* (14, 25, 26).

The toxicity concern clearly opens the door to exploring better tolerated agents such as natural compounds as future chemopreventive agents, which is currently the focus of several groups including ours (36–38).

Our pharmacokinetic results indicated a larger average V/F (308.35 L) in our study compared with previous studies (approximately 220 L), which is roughly proportional to the average body weights in the two studies (approximately 69 and 82 kg, respectively). Furthermore, the average CL/F in the present study (8.29 L/h) was larger than that reported (approximately 3.95 L/h). Because CL/F of erlotinib increases less than proportionally with body weight (29), it is likely that factors in addition to body weight were responsible for the higher CL/F, e.g., the lower dose used in this study (50 to 100 mg every day) compared with 150 mg every day in the previous phase II trials. Furthermore, Thomas and colleagues reported that erlotinib CL/F was influenced by drug transporter polymorphisms (e.g., ABCB1, CYP3A5, and ABCG2; refs. 29, 31), which were not characterized in this study. The average t<sub>1/2</sub> of erlotinib observed in this study (ln2 × V/CL = 25.6 h) was shorter than in the study with patients with cancer (approximately 37.5 hours).

In summary, despite concerns about toxicity, this phase I study demonstrated clinical responses that correlated with downregulation of activated protein levels of the EGFR pathway. This is further supported by our preclinical findings (27). By the time of the last evaluation most patients had progressed to higher-grade dysplasia or invasive carcinoma; however, in some instances progression to dysplasia took more than 4 years from the time of documented CR and 1 patient continues to have a durable CR after 3 years. The preliminary efficacy signal is therefore encouraging and supports future evaluation of this regimen in a phase II efficacy trial. Our study remains the first to combine an EGFR-TKI and COX-2I with pharmacokinetic analysis for patients with premalignancies of the head and neck. The half-life of erlotinib in our study was

shorter than that in other studies (approximately 37.5 hours). Larger studies are necessary to determine whether coadministration of celecoxib influences the pharmacokinetics of erlotinib.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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