The Potential Predictive Value of Cyclooxygenase-2 Expression and Increased Risk of Gastrointestinal Hemorrhage in Advanced Non–Small Cell Lung Cancer Patients Treated with Erlotinib and Celecoxib

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Abstract

Purpose: Celecoxib, a cyclooxygenase-2 (COX-2) inhibitor, potentiates antitumor effects of erlotinib in preclinical studies, and COX-2 is frequently expressed in non–small cell lung cancer (NSCLC). With these observations, we designed a phase II trial to evaluate the efficacy and safety of erlotinib plus celecoxib in advanced NSCLC.

Experimental Design: Previously treated stage IIIB/IV NSCLC patients were given celecoxib at 400 mg orally twice daily and erlotinib at 150 mg orally daily until disease progression. Planned accrual was 40 patients. Tissue was collected for epidermal growth factor receptor (EGFR) analysis and COX-2 immunohistochemistry.

Results: Twenty-six patients were enrolled (17 men, 9 women; median age, 66 years). Eighteen and 21 patients had tissue available for EGFR analysis and COX-2 immunohistochemistry, respectively. The median progression-free survival (PFS) and overall survival were 2.0 and 9.2 months, respectively. Eleven of 21 patients tested had increased tumor COX-2 expression, which was strongly associated with prolonged PFS ($P = 0.048$). Four patients on anticoagulation or with a history of peptic ulcer disease had grade 3/grade 4 upper gastrointestinal bleeding (GIB), prompting early study closure. Three patients with GIB had endoscopy that found peptic ulcers.

Conclusions: The combination of erlotinib and celecoxib does not seem superior to erlotinib alone in unselected patients. However, longer PFS with high-tumor COX-2 expression suggests that trials of EGFR and COX-2 inhibitors may be warranted in this patient subset. GIB observed in our trial supports excluding patients with a history of peptic ulcer disease or those requiring therapeutic anticoagulation from future EGFR and COX-2 inhibitor studies.

Advanced non-small cell lung cancer (NSCLC) treatment has reached a therapeutic plateau with conventional cytotoxic agents (1). The epidermal growth factor receptor (EGFR) was identified as a possible novel treatment target in NSCLC, and phase II studies of the EGFR tyrosine kinase inhibitors (TKI) erlotinib and gefitinib yielded response rates of 10% to 18% in previously treated advanced NSCLC (2–5). A subsequent phase III trial in advanced NSCLC that compared erlotinib versus placebo revealed a significant survival improvement with erlotinib, and in a similarly designed phase III study with gefitinib, there was a trend for longer survival (6, 7). Erlotinib is now a standard, Food and Drug Administration–approved second-line or third-line treatment for advanced NSCLC. Based on practical and theoretical considerations, combining EGFR TKI and conventional cytotoxic agents was an attractive treatment strategy. However, four large randomized studies in unselected patients showed no survival advantage in patients treated with chemotherapy and an EGFR TKI versus patients given chemotherapy alone (8–11).

The disappointing results with cytotoxics and EGFR TKI have led to the exploration of other approaches to enhance the effectiveness of EGFR TKI. One approach involves targeting cyclooxygenase 2 (COX-2). COX-2, an eicosanoid enzyme induced by stress and cytokines, is overexpressed in many human malignancies and is thought to have a role in the pathogenesis of NSCLC (12). Preclinical data showed that increased COX-2 expression via suppression of E-cadherin may modulate epithelial-mesenchymal transition, which is associated with increased cancer invasiveness and metastatic potential (13). Preclinical observations in colon cancer cell lines suggest that increased COX-2 expression is associated with induction of the EGFR and growth stimulation (14). With its relative frequent expression in NSCLC, COX-2 was identified as a

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potentially important therapeutic target in conjunction with EGFR, and COX-2 inhibition potentiated the effect of EGFR TKI in preclinical studies (15, 16).

Based on these considerations, celecoxib, a COX-2 inhibitor shown to decrease adenomatous polyp formation in patients with familial adenomatous polyposis, was chosen to combine with erlotinib for a phase II trial of erlotinib and celecoxib in stage IV NSCLC patients who had failed one previous chemotherapy regimen. The dose of celecoxib for this trial was selected based on the dose found to produce regression of colonic polyps in familial adenomatous polyposis (17). The primary objective of the study was to determine the efficacy of the regimen and see if there was enough activity to warrant further evaluation in larger phase III trials. Results of this multicenter, phase II trial of erlotinib combined with celecoxib, stopped early due to apparent increased gastrointestinal toxicity, are reported here.

Materials and Methods

Study design. This was a phase II single-arm, multiinstitutional trial of stages IIIB and IV NSCLC patients whose cancer had progressed after previous chemotherapy. Previous treatment was limited to one prior chemotherapy regimen. Patients received erlotinib 150 mg daily plus celecoxib at 400 mg twice daily. Both medicines were given orally. Dose reductions were limited to persistent grade 2 toxicities with exception of dyspepsia and hypersensitivity to celecoxib for which doses were interrupted. Dosing interruptions also occurred for grade 3 and grade 4 toxicities. The study design was based on the TWOSTAGE program from the Southwest Oncology Group Statistical Center.[6] Forty patients were to be enrolled for the first stage of this trial to detect at least two responses, and if obtained, an additional 40 patients would be enrolled to give the study 95% confidence to accept the regimen based on 12 total responses. The study was stopped prematurely after four of the first 26 patients presented with grades 3 and 4 gastrointestinal bleeding (GIB).

Eligibility requirements included histiologically or cytologically proved NSCLC, measurable disease, Eastern Cooperative Group performance status (0-2), adequate hematologic function (leukocytes, >3,000/μL; absolute neutrophil count, >1,500/μL; platelets, >100,000), adequate renal function (creatinine clearance, >60 mL/min/1.73 m^2), and adequate liver function (bilirubin within institutional normal limits, transaminases <2.5× institutional upper limit of normal). In addition, patients taking a histamine 3 blocker or a proton-pump inhibitor or those previously treated with an EGFR inhibitor were not included. Low-dose aspirin and anticoagulation were allowed in this study, and the international normalized ratio, while on warfarin, was to be monitored weekly. Other exclusion criteria included hypersensitivity to celecoxib or other nonsteroidal antiinflammatory drugs, brain metastasis, or other significant comorbidity (coronary artery disease, symptomatic heart failure). Patients with active gastrointestinal ulcers were excluded from the study. The previous chemotherapy regimen did not need to contain a platinum component. Nonsmoking status was defined by lifetime consumption of <100 cigarettes. Submission of tumor tissue for analyses of EGFR gene mutations, EGFR gene copy number, and COX-2 protein expression was an eligibility requirement, but results of the assays did not exclude patients from the study.

Objective response was assessed according to RECIST criteria of measurable and nonmeasurable lesions. Progression-free survival (PFS) and overall survival (OS) were measured in months from the start of initial erlotinib treatment to the time of disease progression or death. Progressive disease was defined as progression within 70 days of treatment. Disease control rate was defined as the total percentage of patients with complete response, partial response, and stable disease.

Immunohistochemistry. Formalin-fixed paraffin blocks were cut in 5-μm sections and then deparaffinized and rehydrated by standard technique. For COX-2 staining, a microwave antigen retrieval method was carried out in citrate buffer (pH 6.0), and the tissue was stained using a Ventana ES Histo-stainer (Ventana Medical Systems) with supplied diaminobenzidine and avidin-biotin conjugate immunoperoxidase chemistry. The COX-2 (106112) monoclonal antibody (Cayman Chemical) was used for cytoplasmic staining at a dilution of 1:200. The EGFR (clone H11) mouse monoclonal antibody (Dako Corp., M3563) was used to stain the cell membrane at 1:200 dilution using the Dako Auto Stainer Plus automated staining system. The DakoCytomation Envision+ System-HRP(DAB) (K4007) staining chemistry was used along with a proteinase K (Dako, S3020) digestion step for antigen retrieval. All slides were counterstained with hematoxylin.

Tumor samples were to be scored according to the methods described previously in Buckingham et al. (18). For COX-2 expression, tumors were rescored to duplicate methods described by Edelman et al., with staining frequency and intensity of all tumor cells on each slide estimated on a scale of 0 to 3 without knowledge of clinical patient data (19, 20). As such, COX-2 intensity was measured from 0 to 3, with 0 referring to no staining uptake, 3 referring to dark brown staining (saturated), and 1 and 2 referring to faint and intermediate staining, respectively. COX-2 frequency (percentage of cells staining) was measured from 0 to 3, with 0 referring to staining less of 1%, 1 for staining between 1% and 9%, 2 for staining between 10% and 49%, and 3 for staining between 50% and 100%. All slides were scored twice, independently, by the same pathologist, without discrepancies in the final classification, as described below. The reproducibility of the scoring between different observers was not determined. The COX-2 index was calculated by multiplying the intensity score by the frequency score. An index of ≤4 was classified as low COX-2 expression, and an index of >4 was classified as high COX-2 expression. COX-2 intensity, frequency, and index, as defined here, were included in statistical analyses.

In situ *hybridization.* Fluorescence *in situ* hybridization methods to enumerate copies of chromosome 7 and *EGFR* have recently been described in detail in Buckingham et al. and are similar to those described by Capuzzo et al. (18, 21). *EGFR* and centromere 7 probes were used to examine *EGFR*/cell, centromere 7/cell, and *EGFR*/centromere 7. Specimen slides were hybridized with two-color fluorescence *in situ* hybridization probe solutions (Vysis SpectrumOrange LSI and EGFR/SpectrumGreen CEP 7; Abbott Molecular, Inc.). Paraffin pretreatments II and III were done according to the kit package inserts. Fluorescence *in situ* hybridization slides were evaluated under a Zeiss Axioscope microscope (Carl Zeiss) with a 4′,6-diamidino-2-phenylindole single–band-pass filter to visualize nuclei, an orange single–band-pass filter set to visualize *EGFR* probe, and a green single–band-pass filter set to visualize CEP 7 (all filter sets from Abbott Molecular, Inc.). Only nuclei with morphology characteristic of malignant cells were counted.

EGFR gene mutation sequencing. DNA was extracted from paraffin-embedded specimens by manual microdissection and proteinase K digestion. *EGFR* gene mutation status was assessed using single-strand conformation polymorphism, sequence specific PCR, and direct sequencing. For sequences of primers used to amplify and sequence exons 18, 19, and 21, please refer to Buckingham et al. (18).

Statistical analysis. The associations between response to erlotinib (yes/no) and categorical covariates are tabulated, and Fisher's exact test was used to measure their significance. The Kaplan-Meier method was used to estimate the probability of OS and PFS as a function of time. Survival differences among comparator groups were analyzed

[6] www.swogstat.org/stat/public/cgi-bin/wstage3.exe

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Table 1. Patient characteristics

Patient characteristics	
Variable	***n* (%)**
Gender	
Female	9
Male	17
Age	
Median	66 y
Range	46-81 y
Stage	
IIIB	3
IV	23
Histology	
Adenocarcinoma (%)	18 (69)
Squamous cell	6 (26)
Subtype not specified	2 (15)
ECOG performance status	
0	4
1	21
2	1
Smoking	
Yes	22
No	4
Median (pack-years)	35

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

by the log-rank test. A parallel analysis using the exact rank test method was used to obtain exact permutation-based *P* values of the log rank test. Logistic regression and Cox proportional hazards models were used to select and model the effect of molecular markers and other predictors on objective response and PFS, respectively. All analyses were done using Version 9.1.3 of the SAS software (SAS Institute) and the statistical software R. All reported *P* values are two sided.

Results

Patient characteristics. Twenty-six patients were enrolled in the study. The study was stopped before its planned accrual because of serious GIB. The median age was 66 years, and 65% were male. The frequency of stage IV and stage IIIB disease was 88% and 12%, respectively. All patients received one previous regimen for advanced disease at study entry. Sixty-nine percent of patients had histology consistent with adenocarcinoma and 23% with squamous cell; 8% had unspecifiable subtypes. The majority of patients (85%) had a history of smoking, with a median of 35 pack-years. There were four patients enrolled who had never smoked. Ninety-six percent of patients (25 of 26) had Eastern Cooperative Oncology Group performance status of 1 or better (see Table 1).

Efficacy. Two patients had a partial response (8%), and eight patients had stable disease (30%) resulting in a disease control rate of 38%. There were no complete responses observed in the study. There was no significant difference in disease control rate, PFS, or OS by gender, histology, stage, or prior chemotherapy regimen, although there was a trend toward improved disease control rate in nonsmokers (75% versus 32%, $P = 0.26$). The median PFS was 2.0 months, and the median OS was 9.2 months (see Fig. 1). The 1-year survival was 31%.

Molecular correlates. Eleven of 21 patients had high expression of COX-2 (COX-2 index, >4). COX-2 index of >4 was associated with prolonged PFS compared with COX-2 index of ≤4 (2.0 months versus 5.5 months, respectively; $P_{\text{log-rank}} < 0.048$; see Figs. 2 and 3). Comparing samples with >50% of cells staining for COX-2 to cells with lesser COX-2 staining revealed a difference in median PFS (6.0 months versus 2.0 months, respectively; $P = 0.02$). Alternatively, using the COX-2 index of ≥4 for high expression and <4 for decreased expression, as in Edelman et al., high COX-2 expression only trended toward prolonged PFS (2.0 months versus 3.7 months, respectively; $P = 0.11$; refs. 19, 20).

High chromosome 7 polysomy (more than four copies per cell) was found in 50% of patients. There was no significant difference in response rate among patients with and without chromosome 7 polysomy. No patient had *EGFR* gains (more than two EGFR/chromosome 7). Two patients had an *EGFR* gene mutation detected. One male patient with an exon 19

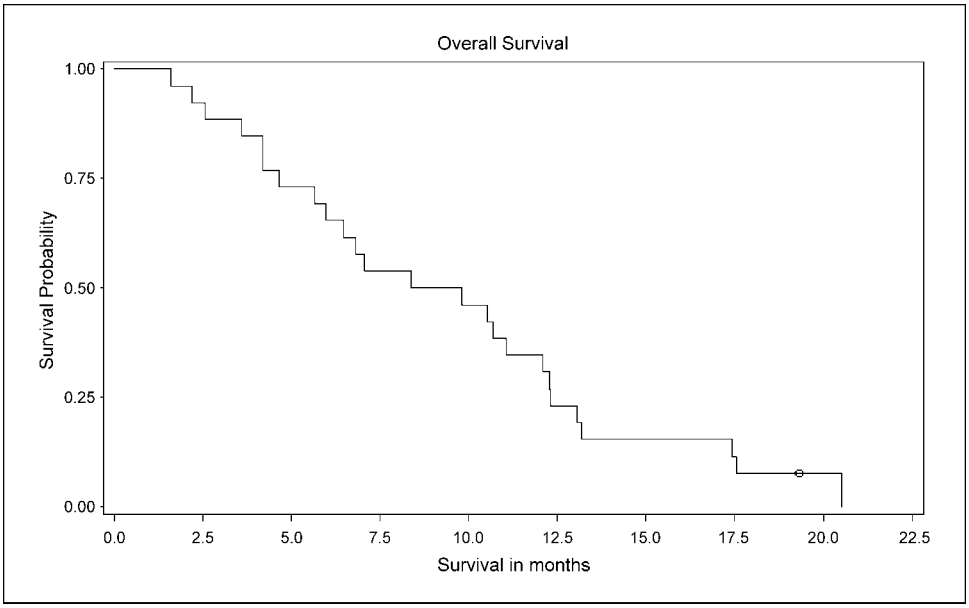

Fig. 1. Kaplan-Meier overall survival for 26 enrolled patients. The median survival was 9.2 months with a 95% confidence interval of 6.0 to 12.2 mo.

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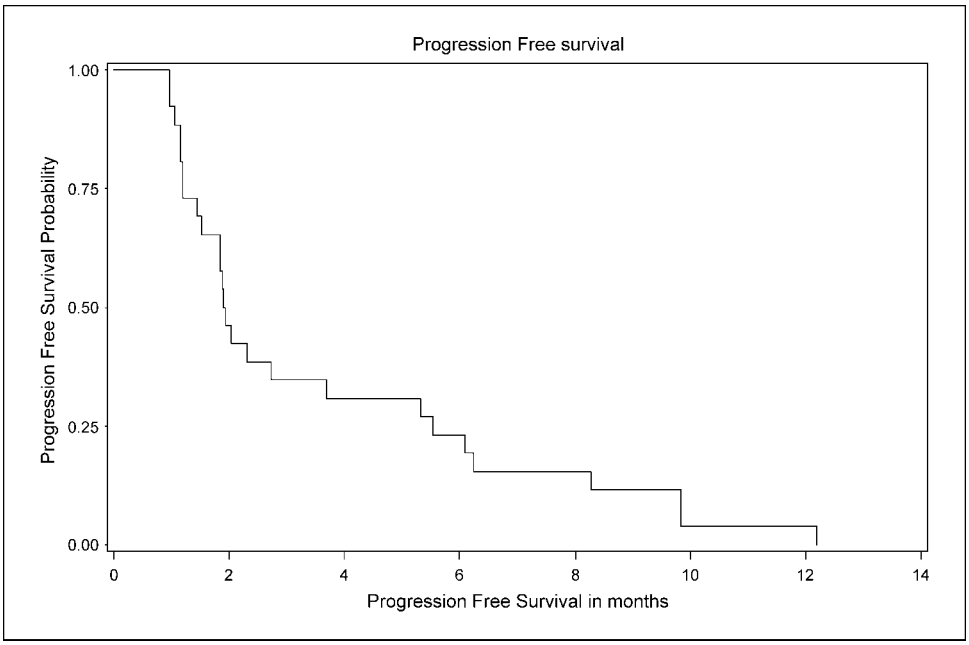

Fig. 2. Kaplan-Meier progression-free survival for 26 enrolled patients. The median progression-free survival was 2.0 months with a 95% confidence interval of 1.5 to 3.7 months.

mutation had a partial response to treatment. He had squamous histology and a history of having never smoked. The other patient had an exon 21 mutation detected and had progressive disease on treatment. The patient was male, had adenocarcinoma histology, and had a heavy smoking history (see Table 2).

Toxicity. Four patients had grades 3 to 4 upper GIB prompting study closure. Three of the patients consented to endoscopy and had documented bleeding peptic ulcers. One patient who bled was on therapeutic low molecular weight heparin, and two patients were supratherapeutic on warfarin therapy (both with international normalized ratio of >10). The remaining patient was not taking anticoagulation but had a remote history of peptic ulcer disease. One other patient was on anticoagulation but did not bleed, and three patients were taking low-dose aspirin without bleeding complications. All patients had normal platelets at the time of GIB.

Grades 1 to 2 rash was observed in 85% of patients, and 65% of patients had grades 1 to 2 diarrhea. Grades 1 to 2 fatigue was reported in 30% of the patients. There were three patients with grade 3 toxicities that were not related to GIB. One patient had grade 3 fatigue, one patient had grade 3 pneumonitis, and one patient had grade 3 esophageal stricture (see Table 3). Four patients had celecoxib dose reductions, and two patients had erlotinib dose reductions. Five patients were taken off the study due to the toxicity of the regimen, four because of GIB, and one because of rash and fatigue.

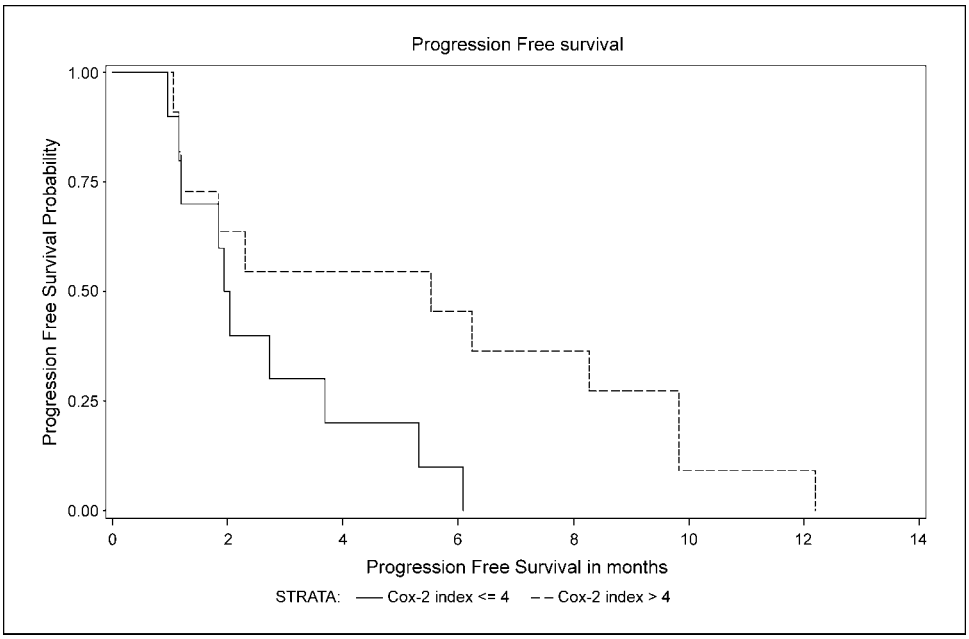

Fig. 3. Kaplan-Meier progression-free survival separated by COX-2 expression. Eleven patients had COX-2 index of ≤4 with a median progression-free survival of 2.0 months versus 10 patients that had COX-2 index >4 with a median progression-free survival of 5.6 months, $P < 0.048$.

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Table 2. Immunohistochemical and molecular characteristics

Variable	No. patients	Objective response	Median PFS	Median OS
Chromosome 7 polysomy				
<4 copies/cell	9	0	1.9 mo	8.5 mo
≥4 copies/cell	9	1	2.1 mo	10.7 mo
		$P = 1.0$	$P_{log\text{-}rank} = 0.60$	$P_{log\text{-}rank} = 0.77$
EGFR gene mutations				
Present*	2	1	5.4 mo	8.0 mo
Absent	24	1	1.9 mo	9.2 mo
		$P = 0.15$		
COX-2 frequency				
≤2 (<50% staining)	11	0	2.0 mo	10.7 mo
>2 (≥50% staining)	10	2	6.0 mo	11.0 mo
		$P = 0.21$	$P_{log\text{-}rank} = 0.02$	$P_{log\text{-}rank} = 0.98$
COX-2 index †				
≤4	10	0	2.0 mo	9.6 mo
>4	11	2	5.6 mo	11.2 mo
		$P = 0.48$	$P_{log\text{-}rank} = 0.048$	$P_{log\text{-}rank} = 0.82$
COX-2 index ‡				
<4	6	0	2.0 mo	8.8 mo
≥4	15	2	3.73 mo	11.2 mo
		$P = 1.0$	$P_{log\text{-}rank} = 0.11$	$P_{log\text{-}rank} = 0.31$

NOTE: Pathology was not sufficient for all patients.
*One mutation was detected in exon 19 and one in exon 21.
† COX-2 index intensity multiplied by frequency, with intensity scored from 0 to 3 and frequency scored as 0 for <1%, 1 for 1% to 9%, 2 for 10% to 49%, 3 for 50% to 100% of the tumor cells staining positive for the COX-2 antibody. COX-2 intensity was scored from 0 to 3 based on no, faint, intermediate, or dark staining.
‡ COX-2 intensity as per Edleman et al. (19, 20).

Discussion

The response rate, median PFS, and median OS observed in our study are similar to results reported for single-agent erlotinib (5, 6). Other investigators who have studied combinations of an EGFR TKI and a COX-2 inhibitor in unselected NSCLC patients reported outcomes with their two-drug combinations, which seem to be similar to single agent erlotinib results (refs. 22–25; Table 4). Results from a single-arm phase II NSCLC trial evaluating second line docetaxel plus celecoxib and from a randomized phase II study comparing second line chemotherapy doublets with or without celecoxib in unselected patients suggested that adding celecoxib did not enhance the efficacy of chemotherapy (26, 27). In addition, the rate of pathologic complete remission in early-stage NSCLC patients who received neoadjuvant treatment consisting of paclitaxel-carboplatin-celecoxib did not seem to be higher than the pathologic complete remission rate reported for the same chemotherapy doublet alone (28, 29). In contrast, Reckamp et al., at phase I trial, noted a response rate of 33% with erlotinib and celecoxib in stage IV NSCLC patients. However, their relatively high response rate may have been due to patient selection, as *EGFR* activating mutations were found in the tumors from five of the seven responders (25).

High COX-2 expression has been reported to be a poor prognostic factor in NSCLC. Completely resected adenocarcinomas with high COX-2 expression were associated with shortened patient survival in the series by Achiwa et al. (30) In a recent metaanalysis, Mascaux et al. found that high COX-2 expression was associated with a trend for shorter survival in NSCLC patients, particularly with early-stage disease (31, 32). In preclinical models, high COX-2 expression is antiapoptotic, stimulates epithelial cell growth and invasion, and leads to EGFR TKI resistance through activation of the extracellular signal-regulated kinase/mitogen-activated protein kinase pathway (15, 33–36). The anticarcinogenic mechanism of celecoxib likely involves inhibition of prostaglandin E receptor 2 production through inhibition of other pathways, including protein kinase B and ornithine decarboxylase. Calcium ATPase and carbonic anhydrases might also mediate the antineoplastic effects of COX-2 inhibitors (37).

We observed a significant improvement in PFS and disease control rate for those who had high COX-2 expression in their tumors. Our observations are similar to the findings of Edelman et al. Their trial did not show increased efficacy from adding celecoxib to second-line chemotherapy in unselected patients with NSCLC. However, subset analysis revealed improved survival in celecoxib-treated patients with high expression of intratumoral COX-2 compared with patients that received chemotherapy plus a lipoxygenase inhibitor (19, 20). Both studies used comparable thresholds for high COX-2 expression based on an index score that reflected both COX-2 frequency of staining and intensity.

Table 3. Summary of toxicity

	Grades 1-2	Grade 3	Grade 4
Diarrhea	17	0	1
Skin	21	0	0
Fatigue	8	1	0
Pulmonary	0	1	0
GIB	1	3	1

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Table 4. Summary of phase II trials of EGFR TKIs combined with COX-2 inhibitors

Investigator	Regimen	Patients	Complete response + partial response (%)	Stable disease (%)	PFS/TTP (mo)	Survival (mo)
O'Byrne (23)	Gefitinib-rofecoxib	42	3 (7%)	12 (29%)	1.8	4.8
Agarwala (22)	Erlotinib-celecoxib	31	5 (16%)	8 (25%)	2.8	7.2
Gadgeel (24)	Gefitinib-celecoxib	27	2 (7%)	6 (22%)	2.2	4.6
Fidler	Erlotinib-celecoxib	26	2 (8%)	8 (30%)	2.0	9.2

Intriguing subset analyses have also been reported for studies combining COX-2 inhibitors with chemotherapy. Csiki et al. found no indication of improved effectiveness with adding celecoxib to docetaxel for second line therapy in unselected NSCLC patients. However, they observed significantly longer survival in a subset of patients who experienced >72% decrease in excretion of the urinary metabolite of prostaglandin E receptor 2 (11α-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostan-1,20-dioic acid, PGE-M) during the first 5 to 10 days of treatment with celecoxib alone (26). Because PGE-M is a metabolite of intratumoral prostaglandin E receptor 2, the investigators suggested that this decrease of PGE-M reflected celecoxib's inhibition of intratumoral PGE-2 synthesis. Using the same dose of celecoxib, another group of investigators has reported data which supports this hypothesis. Altorki et al. compared cohorts of NSCLC patients treated with preoperative chemotherapy plus celecoxib to patients given chemotherapy alone. They found that intratumoral prostaglandin E receptor 2 levels were reduced after treatment with chemotherapy and celecoxib, but not after chemotherapy alone (28, 29).

Traditional nonsteroidal antiinflammatory drugs inhibit both COX-1 and COX-2 and are associated with gastrointestinal ulceration and perforation. This is thought to be due to inhibition of COX-1 and the resultant loss of gastro-protective prostaglandins. Selective COX-2 inhibitors have been shown to have fewer gastrointestinal side effects than traditional nonsteroidal antiinflammatory drugs (38, 39). In the study of Reckamp et al. on combined celecoxib and erlotinib therapy, only 1 of 22 patients had grade 3 GIB. This trial, though, excluded patients with both a history of GIB and patients with coagulation variables of >1.5 times normal (25). In our trial, four patients had grades 3 to 4 GIB. Three of the four had endoscopies and were found to have peptic ulcers. All patients that bled were either on anticoagulation or had a history of peptic ulcer disease, and two had international normalized ratio tests in the supratherapeutic range. In this trial, patients were monitored with weekly international normalized ratio testing if on warfarin therapy. Supratherapeutic international normalized ratio values could have resulted from decreased clearance of warfarin, as erlotinib *in vitro* studies showed metabolism by CYP3A4 and to a lesser extent by CYP1A2, enzymes within the P450 cytochrome isoenzyme family (Erlotinib package insert). Similarly, celecoxib is hepatically metabolized by the cytochrome P450 2C9 system, and serious bleeding events associated with increased prothrombin time have been reported in patients taking warfarin and celecoxib concurrently (Celecoxib package insert). Based on GIB toxicity, we closed this trial, although, in retrospect, it would have been appropriate to complete the trial, excluding patients with a history of peptic ulcer disease or those requiring anticoagulation.

In summary, our observations with erlotinib and celecoxib combined with the collective results from other phase II studies suggest that adding COX-2 inhibitors to either EGFR TKIs or to chemotherapy is unlikely to improve outcomes in unselected NSCLC patients. However, it seems reasonable to continue to study celecoxib combined with EGFR TKIs or chemotherapy in patient subsets defined by high intratumoral COX-2 expression or in patients who experience significant decrease of PGE-M with a short course of a COX-2 inhibitor. Our data and that of Edelman et al. suggest that using a threshold of COX-2 index around 4 may be useful for future randomized trials to predict which patients may have improved outcomes with COX-2 inhibitors. In our study, EGFR expression did not significantly correlate with primary or secondary end points and sample size was too small to comment on excluding patients with low EGFR expression for trials combining EGFR TKI and COX-2 inhibitors. Based on the high rate of GIB in our patients, these trials should exclude patients with a previous history of peptic ulcer or those who are anticoagulated.

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