

A Phase II Study of Celecoxib in Combination with Paclitaxel, Carboplatin, and Radiotherapy for Patients with Inoperable Stage IIIA/B Non – Small Cell Lung Cancer

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Abstract Purpose: Cyclooxygenase (COX)-2 up-regulation plays an important role in the pathogenesis of lung cancer. Selective COX-2 inhibitors have promoted chemosensitivity and radiosensitivity of tumor cells in preclinical trials.

Experimental Design: In a single-institution phase II study, we sought to determine the effectiveness of concurrent chemoradiation given with celecoxib and examined biomarkers to predict response to COX-2 inhibition.

Results: Seventeen patients with stage IIIA or IIIB non – small cell lung cancer (NSCLC) were enrolled in the study. All received 400 mg celecoxib twice daily continuously while on trial in addition to concurrent chemoradiation therapy with paclitaxel and carboplatin. Celecoxib was continued until disease progression. The overall objective response rate was 42.9%, and the median overall survival time was 203 days. In contrast to nonresponders, those patients with complete and partial responses had a significant decrease in the level of urinary 11 α -hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGE-M), the major metabolite of prostaglandin E₂, after 1 week of celecoxib administration. Patients with very high levels of PGE-M before initiation of therapy also responded poorly to therapy. Serum vascular endothelial growth factor levels did not predict response or survival.

Conclusion: The trial was terminated because it did not meet the predetermined goal of 80% overall response rate. In unselected patients, the addition of celecoxib to concurrent chemoradiotherapy with inoperable stage IIIA/B NSCLC does not improve survival. Urinary PGE-M is a promising biomarker for predicting response to COX-2 inhibition in NSCLC.

Lung cancer is the most prevalent cancer in the United States and the leading cause of cancer-related mortality, killing more patients than the next three deadliest malignancies (colorectal, breast, and prostate) combined (1). Approximately one third of non – small cell lung cancer (NSCLC) cases will present with locally advanced disease with mediastinum involvement either by metastatic lymph nodes or by primary (2). Because these patients are not good candidates for surgical resection, definitive treatment combining platinum-based chemotherapy and radiotherapy is considered the standard of care (3).

Cyclooxygenase (COX) is a key enzyme in arachidonic acid conversion to prostaglandins and other eicosanoids. There are

two isoforms of COX known as COX-1 and COX-2. Unlike constitutively expressed COX-1, COX-2 is inducible and up-regulated by a variety of factors, which include cytokines, growth factors, and tumor promoters (4). COX-2 is overexpressed by various human cancers, including lung. Furthermore, a growing body of evidence suggests that up-regulation of COX-2 and its product, prostaglandin E₂ (PGE₂), is important in the growth of lung cancer (5, 6). COX-2 and its derived prostaglandins play a role in stimulating angiogenesis and apoptosis inhibition and in suppressing the immune response (7). COX-2 overexpression may also enhance the metastatic potential of tumors (5). Therefore, the possibility of using COX inhibitors as part of oncologic therapy has gained interest. Research was also encouraged by large epidemiologic studies showing that nonsteroidal anti-inflammatory drugs can reduce the incidence of colorectal, breast, prostate, and lung cancers (8). This protective effect is presumed to be related to inhibition of COX activity by nonsteroidal anti-inflammatory drugs.

11 α -Hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGE-M) is the major urinary metabolite of PGE₂ (9). PGE₂ has not only been implicated in tumorigenesis, but evidence also suggests that prostaglandins are radioprotective and chemoprotective (7). We previously developed a novel and robust method of measuring urinary PGE-M using a relatively simple liquid chromatography/tandem mass spectrometry

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Translational Relevance

Despite recent improvements in chemotherapy and radiation therapy in lung cancer, novel treatment strategies are needed to further benefit patients. Cyclooxygenase (COX)-2 up-regulation plays an important role in lung cancer pathogenesis and, thus, represents an attractive therapeutic target. Preclinical findings have shown that COX-2 inhibition enhances tumor response to both radiation and chemotherapeutic agents. Based on these encouraging data, we conducted a phase II clinical trial of celecoxib in combination with paclitaxel, carboplatin, and radiotherapy for patients with inoperable stage IIIA/B non-small cell lung cancer (NSCLC). The trial was terminated because it did not meet the predetermined goal of 80% overall response rate. Interestingly, urinary 11 α -hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostanoic acid (PGE-M) was identified as a useful biomarker for predicting response to COX-2 inhibition in NSCLC. Therefore, this study suggests that future clinical trial design would benefit from patient selection based on their urinary PGE-M level.

(MS) assay. It is both a precise and accurate method of quantifying PGE₂ production in humans (10). We have had prior success at characterizing tumor-derived COX-2 activity before and after selective inhibition of COX-2 by measuring this excreted urinary prostaglandin metabolite (9, 11).

Measurement of vascular endothelial growth factor (VEGF) seems to also theoretically be a potentially useful marker of COX-2 activity, as COX-2 plays an important role in stimulating tumor angiogenesis (7, 12). VEGF is the most important of all growth factors involved in tumor angiogenesis, and levels of VEGF have been correlated with tumor response and survival in malignancies (13, 14). COX-2 inhibition has previously been shown to decrease the production of VEGF in tumors (7, 12). In addition, there is evidence that inhibition of neovascularization may be one of the primary mechanisms that COX-2 inhibitors may potentiate radiotherapy (15, 16).

Selective inhibition of COX-2 has been shown to impair the growth of tumors (17, 18), and preclinical studies showed that COX-2 inhibitors increased the chemosensitivity and radiosensitivity of tumor cells *in vitro* and *in vivo* (19, 20). Importantly, the enhanced cytotoxicity of COX-2 inhibitors on tumors expressing COX-2 when administered concurrently with chemotherapy and radiotherapy was not seen on normal tissues (15, 19, 21). It has therefore been suggested that selective COX-2 inhibitors have the potential to improve the therapeutic ratio of chemoradiotherapy (22–24).

We designed this phase II trial to determine whether celecoxib could improve the efficacy of concurrent chemoradiotherapy in locally advanced NSCLC and to assess the toxicity of this regimen (Fig. 1). In addition, we analyzed two biomarkers, PGE-M and VEGF, before and after COX-2 inhibition. We hypothesized that a decline in urinary PGE-M and serum VEGF levels might predict changes in tumor COX-2 activity after celecoxib administration, potentially enabling us to predict clinical response to COX-2 inhibition therapy.

Materials and Methods

Patient selection. To be eligible for this single-institution phase II study, patients had to have histologically or cytologically documented stage IIIA/IIIB NSCLC, measurable disease, an Eastern Cooperative Oncology Group performance status 0 or 1, no previous treatment with chemotherapy, lung radiation therapy, or totally resected lung tumor. Measurable disease was defined as any mass reproducibly measurable in two perpendicular diameters by chest X-ray, magnetic resonance imaging, or computed tomography (CT). All measurable lesions were measured in centimeters and had to be at least 1 cm in CT scan. Patients with measurable disease were evaluated using National Cancer Institute's Response Evaluation Criteria in Solid Tumors criteria (25) for measurable lesions. Unidimensional- and bidimensional-based criteria were used as baseline in this study to reduce the interobserver variability of measurements and the misclassification of some patients (26). Pleural effusions were not considered as measurable; therefore, patients with malignant pleural effusion were excluded from the study. In addition, initial laboratory values of granulocyte count $>2,000/\mu\text{L}$, platelet count $>100,000/\mu\text{L}$, hemoglobin ≥ 8 mg/dL, blood urea nitrogen $\leq 1.5\times$ upper limits of normal, calculated creatinine clearance >30 mL/min, FEV1 ≥ 800 mL, and bilirubin $<2.5\times$ upper limits of normal were required. No evidence of second cancer was allowed, except inactive nonmelanoma skin cancer or *in situ* cervical cancer. Previous nonlung malignancy was allowed only if the patient had no evidence of disease for >1 y. All patients were evaluated by radiation oncologist and were considered suitable for radiotherapy with boost volume of $<50\%$ of ipsilateral lung volume. The protocol was approved by the Institutional Review Board. All patients gave written informed consent before the study entry.

Treatment and evaluation. All patients received concurrent chemoradiotherapy with paclitaxel, carboplatin, radiation, and celecoxib. Involved nodes were all assessed by either CT or magnetic resonance imaging, and positive positron emission tomography and/or mediastinoscopy were required for documentation of N2/N3 involvement. The administration of celecoxib (400 mg, orally, twice daily) was started 5 d before initiation of concurrent chemotherapy and was continued without interruption until there was evidence of disease progression. The celecoxib dose was originally determined based on the highest Food and Drug Administration–approved dose of 800 mg/d. Several trials have reported the dose of 400 mg celecoxib twice daily in combination with chemotherapy as safe and effective in NSCLC treatment (27–30). A phase I study of thoracic radiotherapy for patients with unfavorable performance status inoperable/unresectable NSCLC also showed that celecoxib can be safely administered concurrently with radiotherapy when given up to 800 mg/d, without reaching a maximal tolerated dose (31).

For all patients, radiotherapy was delivered using a linear accelerator with effective photon energies of ≥ 6 MV. During simulation, each patient was positioned in an individualized immobilization device in the treatment position on a flat table. A volumetric treatment planning CT study was required to define gross tumor volume (GTV) and planning target volume (PTV). For this study, GTV was considered equal to the clinical target volume. The GTV, PTV, and normal organs, including both lungs and spinal cord, were delineated on all appropriate CT slices and displayed using beam's eye view. Volume and prescription dose definitions were in accordance with the 1993 ICRU Report 50 (International Commission on Radiation Units and Measurements, Bethesda, MD). GTV was defined by the physician as all known gross disease on the planning CT and clinical information and included the primary tumor and involved nodes and abnormally enlarged regional lymph nodes of >1.0 cm. PTV included GTV plus a margin of at least 20 mm (maximum, 25 mm) around the GTV to compensate for variability in treatment setup, breathing, or motion during treatment. Tumor and physiologic movements were assessed by fluoroscopy. The International Commission on Radiation Units and

Measurements reference point had to be located in the central part of PTV, typically at the intersection of the beam axis (isocenter). The PTV was treated with combination of three-dimensional conformal fields shaped to deliver the specified dose while restricting dose to normal tissues. Field arrangements were determined by three-dimensional planning to produce the optimal conformal plan in accordance with volume definitions. The 93% isodose curve was required to encompass the entire defined PTV. Dose inhomogeneity throughout target volumes was kept within 7%. No density correction was applied during treatment planning. The treatment plan selected and used for each patient was based on analysis of the volumetric dose, including dose-volume histogram analyses of PTV and critical normal structures. For each patient, mean lung dose and the percentage of lung receiving >20 Gy (V20) were determined. The lung volume was defined on CT scan as the volume of both lungs minus GTV. The spinal cord was excluded from the irradiated volume at 40 Gy. The initial radiation field received 1.8 Gy/d for 5 wk (total, 45 Gy). An additional radiation boost with 2 Gy/d to 18 Gy was delivered without a break during 2 wk. The total radiation dose received was 63 Gy. The boost target volume consisted of the primary tumor and primary involved nodes, as determined by imaging (including positron emission tomography scan) and mediastinoscopy, with at least 2.0-cm margins (maximum, 2.5 cm).

Chemotherapy consisting of paclitaxel (50 mg/m², weekly for 7 wk) and carboplatin (area under the curve, 2; weekly for 7 wk) was delivered concurrently with radiotherapy.

After completion of concurrent chemoradiotherapy, two additional cycles of paclitaxel (200 mg/m²) and carboplatin (area under the curve, 2) were given 3 wk apart. All patients were premedicated with dexamethasone, diphenhydramine, and ranitidine (or cimetidine) before each paclitaxel infusion. Patients were evaluated weekly while on treatment. Physical exam was done every week along with measuring vital signs. Complete blood count, platelet counts, blood urea nitrogen, and creatinine were measured weekly. During follow-up, patients were evaluated every 2 mo for 2 y, then every 6 mo for 3 to 5 y, and then yearly after 5 y. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0; ref. 32).

At the completion of chemoradiotherapy and on follow-up visits, the tumor response was measured using the National Cancer Institute's Response Evaluation Criteria in Solid Tumors criteria (25). Up to a maximum of 5 lesions per organ and 10 lesions in total were identified as target lesions and recorded and measured at the baseline. A sum of the longest diameter (SLD) for all target lesions was calculated and reported as the baseline SLD. Complete response (CR) was reported if all target lesions disappeared. Partial response (PR) was defined as at least a 30% decrease in the SLD from the baseline SLD. Progressive disease (PD) was defined as at least 20% increase in the SLD from the baseline SLD. Disease that showed neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD was recorded as stable disease (SD).

Serum celecoxib measurement. Concentration of serum celecoxib was assessed after 5-d administration of single-agent celecoxib before concurrent chemoradiation therapy. Plasma or serum samples were analyzed for celecoxib by doing a protein precipitation step using an equal sample volume of cold methanol and separating the supernatant from the denatured proteins. Celecoxib in the supernatant was then extracted using a 5× volume of hexane/ethyl acetate (50:50, v/v) and evaporating the organic extraction solvent under a stream of nitrogen gas. The residue was reconstituted with methanol and 10 mmol/L ammonium acetate (pH 8.5) equal to the volume of sample extracted. The extracted samples were analyzed by liquid chromatography/MS/MS using electrospray negative ionization in multiple reaction monitoring mode, using a set of celecoxib reference standard to positively identify and quantify celecoxib in the samples. The lower limit of quantification was 1 ng/mL.

Urinary PGE-M assay. Urinary PGE-M levels were assessed within 4 wk before the study entry and after 5-d administration of single-agent celecoxib before concurrent chemoradiation therapy. PGE₂ production

in vivo was quantified by measuring urinary PGE-M by MS using stable isotope dilution methods with chemically synthesized [²H₆]PGE-M as an internal standard (10, 33). Briefly, the procedure is as follows: endogenous urinary PGE-M was converted to an unlabeled O-methoxyloxime derivative and extracted (34). The internal standard was prepared by converting the chemically synthesized PGE-M to an [²H₆]O-methoxyloxime derivative. During MS, precursor ions of the unlabeled (*m/z* 385) and ²H₆-labeled (*m/z* 391) O-methoxyloxime PGE-M were subjected to collision-induced dissociation, producing ion *m/z* 336 representing endogenous PGE-M and ion *m/z* 339 representing the deuterated internal standard. Levels of endogenous PGE-M in samples were calculated from the ratio of the mass chromatogram peak areas of the *m/z* 336 and *m/z* 339 ions.

Serum VEGF measurement. Serum VEGF levels were measured before and immediately after the first 4 d of celecoxib therapy using the Quantikine kit developed by R&D Systems. In summary, blood was obtained in a serum separator tube and allowed to clot for 30 min before centrifugation for 10 min at ~1,000 × *g*. The serum was removed and stored at -80°C. The reagents and standards were prepared after directions of the manufacturer.

Statistical analysis. A two-stage phase II design was used to ensure that the expected number of patients exposed to the therapy was minimized (35). We sought to show that the objective response rate of this combination therapy for stage III NSCLC was at least 80%. The study was designed so that if the combination therapy with celecoxib was inactive (<60% objective response rate), then it would be terminated early. Initially, 11 patients would be enrolled, of whom 8 would have to show objective response to initiate the second stage of the study to accrue 43 patients. If at least 31 of 43 showed a response, the study would be continued as a phase III trial. This design provided 80% statistical power to detect a difference of 20% with a type I error rate of <5%.

Analysis of study results focused on determining the overall response rate (both CR and PR considered as response) and was calculated with an exact two-sided 95% confidence interval. The urinary PGE-M level decline rate ($\frac{\text{post}-\text{pre}}{\text{pre}}$ %) was also compared between CR/PR and SD/PD patients. The Fisher's exact test was used to detect the association between objective response (CR/PR versus SD/PD) and PGE-M decline (dichotomized by decline rate of <10% and prelevel of <30 ng/mg creatinine). The cutoff point of 10% change from the baseline measurement was predetermined before analysis (prospectively set). The Wilcoxon rank sum test was used to determine whether there was an association between PGE-M levels and grade 3 or 4 pulmonary or esophageal toxicity. Overall survival was calculated for each patient from the date of diagnosis until the date of death or (for surviving patients) the date of last follow-up. Survival estimates were based on the Kaplan-Meier method. In addition, survival analysis was carried out for PGE-M decline with the exact permutation test conditioning on follow-up (36). All tests of significance were based on two-sided probabilities at *P* < 0.05. Statistical analyses were done using R (version 2.4.1) and Statistical Analysis System software (version 9.1).

Results

Patient characteristics and disposition. Between March 2001 and February 2004, 17 patients were enrolled, including 4 patients with stage IIIA and 13 patients with stage IIIB NSCLC. Patient demographics are summarized in Table 1. The median age of patients was 58 years.

The most common reason for patient study withdrawal was PD (7, 41%). Six patients (35%) were withdrawn due to excessive complication or toxicity, including pneumonia/respiratory failure/acute respiratory distress syndrome (four) and esophagitis (two). Three (18%) patients were not assessable for response: two died on treatment without PD,

Table 1. Characteristics of patients

Characteristic	No.
Sex	
Male	13
Female	4
Age (y)	
Median	58
Range	50-73
Race	
Caucasian	16
African-American	1
ECOG performance status	
0	5
1	11
Stage	
IIIA	4
IIIB	13
Histology	
SCC	7
ADC	3
LC	1
NOS	6

Abbreviations: ECOG, Eastern Cooperative Oncology Group; SCC, squamous cell carcinoma; ADC, adenocarcinoma; LC, large cell carcinoma; NOS, NSCLC not otherwise specified.

whereas one patient withdrew on the second day of study after experiencing nausea and bone pain after administration of celecoxib, before initiation of concurrent chemoradiation. Therefore, a total of 14 patients were assessable for response.

Serum celecoxib levels. Steady-state serum concentration of celecoxib was assessed after 5-day administration of single-agent celecoxib and before concurrent chemoradiotherapy to assess both absorption from gastrointestinal tract and compliance. Celecoxib inhibits recombinant COX-2 with an IC_{50} of 0.4 $\mu\text{mol/L}$ (21). Each patient had detectable levels of celecoxib in their serum. The levels ranged from 0.52 to 5.48 $\mu\text{mol/L}$, with a mean of 3.30 $\mu\text{mol/L}$ among all patients. No correlation was seen between plasma celecoxib concentration and PGE-2 levels as determined by urinary PGE-M (data not shown).

Toxicity. The main side effects of radiotherapy with carboplatin, paclitaxel, and celecoxib treatment were pulmonary and gastrointestinal (Table 2). Grade ≥ 3 radiation pneumonitis/

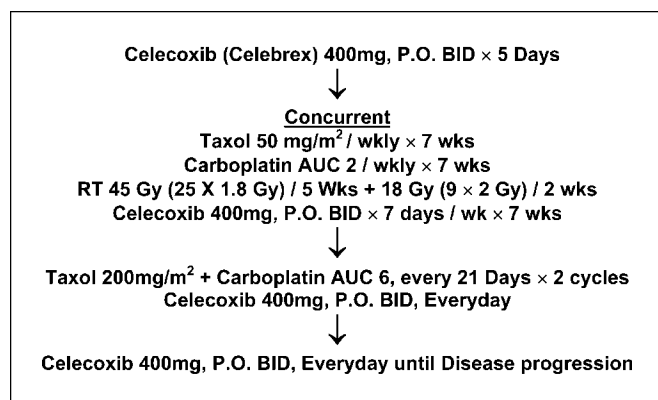


Fig. 1. Phase II treatment schema of celecoxib in combination with paclitaxel, carboplatin, and radiation.

pulmonary infiltrate (National Cancer Institute Common Toxicity Criteria version 2) was observed in one (6%) patient. In addition, two (12%) patients presented with concurrent pulmonary infiltrates and infection, whereas two (12%) patients presented essentially with severe pneumonia/sepsis and developed respiratory failure. Two occurrences of dysphagia/esophagitis/odynophagia (12%), two occurrences of grade 3 nausea, and two occurrences of grade 3 vomiting were observed. These all occurred in the same two patients but were reported as distinct events. Data on grade 3 and 4 toxicity are presented in Table 2.

As noted above, two patients died on treatment without PD. One was hospitalized with pneumonia and died of respiratory failure and hemodynamic compromise 3 months after initiation of therapy, whereas the other succumbed to a pulmonary embolism during hospitalization for percutaneous endoscopic gastrostomy tube placement at day 11 of the trial.

To determine whether there was any association between initial PGE-M level or PGE-M drop rate after 5 days of celecoxib with toxicity, we dichotomized patients into two groups: those with grade 3/4 pulmonary or esophageal toxicity and those without. Using the two-sample Wilcoxon rank sum test, we did not detect a significant association between PGE-M prelevel or decline and toxicity ($P = 0.852$ and 0.662 , respectively; data not shown).

Response and survival. Fourteen of 17 patients were assessable for response to treatment. There was one CR and five PRs, resulting in an overall objective response rate of 42.9% (95% confidence interval, 17.7-71.1%). Five patients had SD, and three had PD. The median overall survival time was 203 days, and the 1-year overall survival rate was at least 32.1%. The median progression-free survival time was 138 days.

Table 2. Acute high-grade (≥ 3) toxicity

Complications	Patient no.
Hematologic	
Neutropenia/granulocytes	3
Leukocytes	2
Platelets	1
Gastrointestinal	
Anorexia	1
Constipation	1
Dysphagia/esophagitis/odynophagia	2
Nausea	2
Vomiting	2
Pulmonary	
Pneumonia	2
Pneumonitis/pulmonary infiltrates	1
Both	2
Infection/febrile neutropenia	
With ANC < 1.0	2
Infection without neutropenia	1
Metabolic	
Hyperglycemia	4
Hypokalemia	1
Hyponatremia	1
Other	
Chest pain	1
Radiation dermatitis	2
Weight loss	2
Fatigue	1

Abbreviation: ANC, absolute neutrophil count.

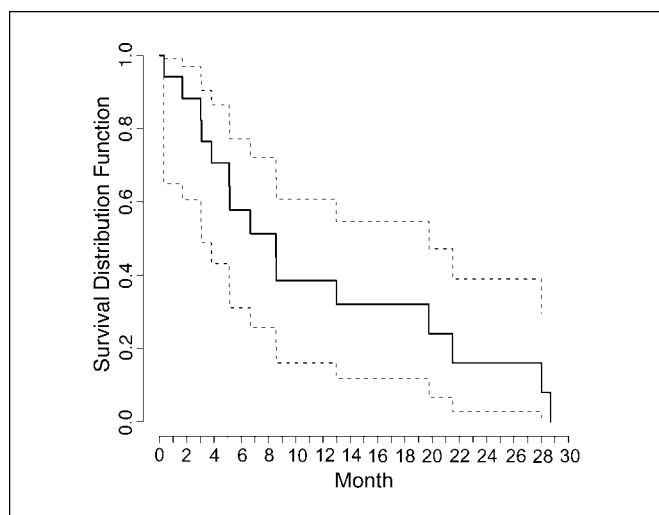


Fig. 2. Solid line, Kaplan-Meier survival curve for overall survival; dashed lines, confidence intervals.

Kaplan-Meier estimates of overall survival are shown in Fig. 2, and progression-free survival is shown in Fig. 3.

Effect of celecoxib on serum angiogenic factors. COX-2-derived eicosanoids stimulate angiogenesis by increasing VEGF expression, and several *in vitro* and *in vivo* studies have shown associations between COX-2 expression and levels of VEGF production in human cancers (7, 37). Based on these studies, we analyzed serum levels of VEGF in patients immediately before and after the first 5 days of celecoxib therapy to see if we might be able to predict response to COX-2 inhibition in combination with chemoradiotherapy. We expected to see a decrease in VEGF, particularly in those patients that responded to therapy. However, in contrast to urinary PGE-M, we observed no significant decrease in the average serum VEGF levels after celecoxib therapy. In addition, there was no correlation in VEGF levels with response or survival (data not shown).

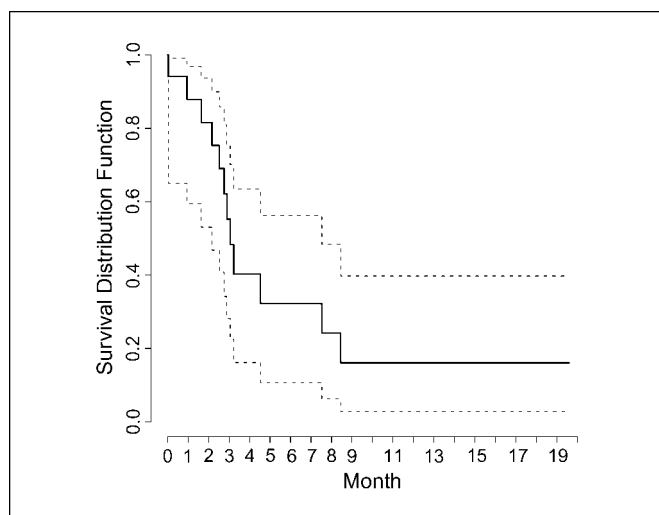


Fig. 3. Solid line, Kaplan-Meier survival curve for progression-free survival. Time on study was used as progression-free survival time. Dashed lines, confidence intervals.

Effect of celecoxib on urinary levels of PGE-M. PGE-M is the major urinary metabolite of PGE₂, one of the synthesized products of arachidonic acid generated by COX activity. We have previously reported normal levels of PGE-M in healthy men (10.4 ± 1.5 ng/mg creatinine) and women (6.0 ± 0.7 ng/mg creatinine; ref. 10). We and others have shown COX-2 up-regulation in NSCLC, among many other tumors, and we have reported that levels of PGE-M were markedly increased in patients with unresectable disease (10). Here, we found that in those patients with CR or PR, the average pretreatment level of PGE-M was 15.2 ng/mg creatinine. Those who experienced PD or SD had higher average precelecoxib levels of 50.8 ng/mg creatinine (Fig. 4). Patients with CR or PR tended to have robust decreases in PGE-M levels after 5 days of celecoxib therapy (Table 3). The decrease in PGE-M after celecoxib was statistically significant ($P = 0.020$, paired *t* test; Fig. 5). Furthermore, we prospectively dichotomized the patients into a “bad group” (precelecoxib level of PGE-M of ≥30 ng/mg creatinine and a rate of decline of PGE-M of ≤10%) as well as a “good group” (precelecoxib level of PGE-M of <30 ng/mg and a rate of decline of PGE-M of >10%). Using Fisher’s exact test, we detected a robustly significant association between objective response (CR/PR versus SD/PD) and the initial PGE-M levels and rates of decline outlined above ($P = 0.005$). Exact test results did not reach statistically significant levels for overall and progression-free survival and local failure ($P = 0.114, 0.296, \text{ and } 0.1026$, respectively).

Discussion

Despite much effort on the part of physicians and scientists, treatment for lung cancer remains largely unsatisfactory, demanding novel agents with radiation-enhancing properties and novel biomarkers to predict response to therapy. This phase II clinical study examined the efficacy and toxicity of selective COX-2 inhibition with celecoxib in combination with chemoradiotherapy for patients with inoperable stage III lung cancer. Indeed, several *in vitro* and *in vivo* studies had shown

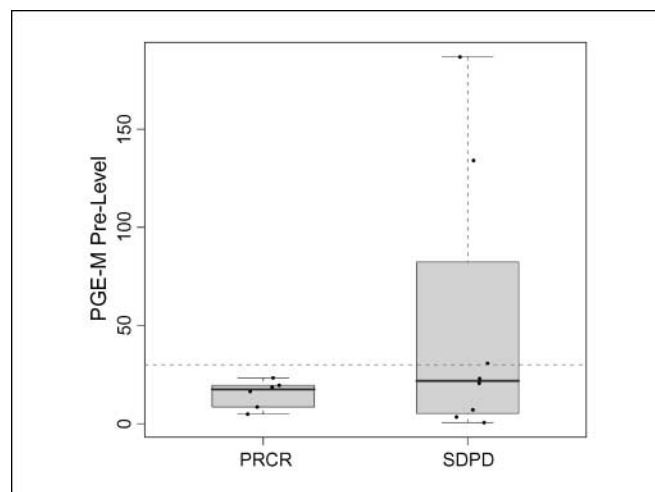


Fig. 4. PGE-M levels before celecoxib administration. A cutoff level of 30 ng/mg creatinine was used to dichotomize PGE-M groups for the Fisher’s exact test. Patients are grouped in the figure according to response (CR/PR versus and SD/PD).

Table 3. Urinary PGE-M levels (ng/mg creatinine) before (Pre) and after (Post) 7 d of celecoxib administration, as well as the rate of decrease in PGE-M (post-pre/pre)

	Pre	Post	Drop rate (post-pre/pre)
PR + CR			
1	16.41	10.08	-0.386
2	4.86	4.03	-0.171
3	23.31	4.74	-0.797
4	18.54	9.36	-0.495
5	8.49	4.15	-0.511
6	19.53	7.51	-0.515
SD + PD			
1	0.50	3.11	5.220
2	20.62	19.19	-0.069
3	134.08	74.74	-0.442
4	3.39	7.48	1.206
5	186.86	3.70	-0.980
6	30.65	8.79	-0.713
7	22.92	18.25	-0.203
8	7.03	20.04	1.851

NOTE: Patients are grouped according to response: PR/CR and SD/PD.

the ability of COX-2 inhibition to enhance tumor response to radiation and chemotherapeutic agents (7). Furthermore, an enhanced response was shown when celecoxib was combined with these agents preoperatively in early-stage NSCLC (30).

Notwithstanding such promising preclinical and clinical data, in this study of unselected patients, we did not observe an improved response rate or survival with concurrent chemoradiation therapy and celecoxib. Six patients had objective responses to therapy, and the median overall survival was 203 days (mean, 323 days). These values were below those previously reported with radiotherapy combined with carboplatin/paclitaxel in the absence of celecoxib for stage III NSCLC treatment, and our study was stopped early because it did not meet the predetermined goal of 80% overall response rate (38–40).

Importantly, however, we showed that an accurate and relatively simple urinary assay developed at our institution for PGE-M predicted response to COX-2 inhibition with celecoxib in combination with chemoradiotherapy. Pretreatment PGE-M levels were elevated in a majority (75%) of patients that entered the study relative to reported controls of healthy men and women, a finding we had previously observed in lung cancer patients (10, 11, 41). Consistent with our previous observations, we found that celecoxib significantly decreased PGE-M production in patients with NSCLC, suggesting that COX-2 upregulation is responsible for much of the excess PGE₂ production in this population (10, 11, 42–44). It was noteworthy that patients with the best response were not those with higher levels of COX-2 activity and PGE₂ as assessed by urinary PGE-M. In fact, those patients who did not respond to therapy had on average greater than thrice the measured PGE-M immediately before study entry as those that responded. This finding is consistent with studies showing COX-2 overexpression to be associated with more aggressive disease, diminished control after therapy, and worse prognosis (7, 42–44). In addition to initial PGE-M levels, measured decrease in PGE-M

after just 7 days of celecoxib was predictive of clinical outcome. Indeed, there was a significant drop in PGE-M after celecoxib in those patients with CR or PR ($P = 0.020$).

In analyzing each patient's precelecoxib and postcelecoxib PGE-M levels closely, a clear pattern emerged between responders and nonresponders. Patients that did not respond to therapy had (a) very high initial PGE-M levels and/or (b) experienced only a small drop or an increase in PGE-M after 1 week of celecoxib. By dichotomizing patients into a bad group (precelecoxib level of PGE-M of ≥ 30 ng/mg creatinine and a rate of PGE-M decline of $\leq 10\%$) as well as a good group (precelecoxib level of PGE-M of < 30 ng/mg and PGE-M decline rate of $> 10\%$), we were able to detect a highly significant association with objective response (PR/CR) to therapy ($P = 0.005$).

Our study adds to the growing body of evidence suggesting the potential utility of PGE-M, an indirect biomarker of intratumoral COX-2 activity, in clinical oncology. We have previously shown that PGE-M may be useful for advanced colorectal cancer detection, and higher levels of PGE-M are strongly associated with risk of developing colorectal cancer in a prospective study (9). Finally, we have shown a significant survival benefit in patients with recurrent NSCLC treated with docetaxel and celecoxib who experienced the greatest decline in urinary PGE-M levels after celecoxib administration (11). The findings reported here are consistent with this prior study.

The predictive and prognostic value of the PGE-M assay was not paralleled in measuring levels of serum VEGF before and after celecoxib, likely because it is a less specific biomarker of COX-2 activity. Although COX-2 and PGE₂ stimulate angiogenesis, we did not see a significant decrease in VEGF levels after celecoxib administration. In addition, those patients who experienced a drop in serum VEGF did not have improved response rates or survival.

We are comfortable that patient compliance and gastrointestinal absorption of celecoxib here were adequate, specifically during the first week of therapy at which time serum and urinary biomarkers of COX-2 expression were assessed. All patients had levels greater than the IC₅₀ of celecoxib (45). A

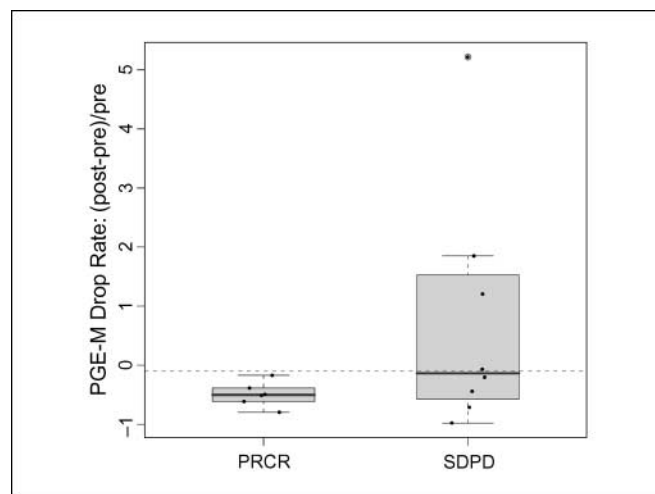


Fig. 5. PGE-M drop rate after administration of celecoxib (400 mg twice daily) for 7 d. A cutoff 10% drop rate level was used to dichotomize patients into groups for the Fisher's exact test. Patients are grouped in the figure according to response (CR/PR versus SD/PD).

prior study has shown that the dose of celecoxib used here (400 mg twice daily) is adequate to abrogate the increase in intratumoral PGE₂ seen after chemotherapy in NSCLC. This increase in PGE₂ after both radiotherapy and chemotherapy is believed to promote resistance to therapy (7, 30). We have also reported a significant decrease in intratumoral PGE₂ level in tumor biopsies of patients with NSCLC after celecoxib administration at this dose (11). In the present study, there was a clear association between PGE-M levels with response ($P = 0.005$) but not with survival ($P = 0.114$). Because radiographic response of the primary tumor is itself a poor surrogate for survival, PGE-M level is probably a biomarker for radiographic changes only and, therefore, not associated with survival. Therefore, we speculated that COX-2 inhibition may contribute to local control only by enhancing the targeted effects of radiotherapy and have less or no effect on subclinical systemic disease. However, our analyses showed that local failure was not found related to neither the dichotomous PGE-M level ($P = 0.1026$, Fisher's exact test) nor the PGE-M drop rate ($P = 0.5237$, Wilcoxon test).

Celecoxib has previously been shown to be safe in combination with paclitaxel in a phase II study and has been tested with other agents with similarly good safety results (46), including with radiation (31). Here, the most observed side effect was grade 3/4 pulmonary toxicity (which included pulmonary infections), affecting ~30% of patients. The patients ($n = 4$) with severe infectious pneumonia were included in this group because of the poor outcome they faced. The patients presenting high-grade pulmonary toxicity had a strong background of chronic obstructive pulmonary disease, lower lobe tumors, and a worse performance status and were thus more likely to develop pneumonitis. To determine if initial PGE-M level or PGE-M drop rate after 5 days of celecoxib therapy was associated with toxicity, we dichotomized patients into two groups: those with grade ≥ 3 pulmonary or esophageal toxicity and those without. We did not detect a significant association between PGE-M prelevel or decline and toxicity ($P = 0.852$ and 0.662 , respectively). In addition, we also analyzed several radiation factors, such as mean lung dose and V20, which may have contributed to the observed toxicities. There was no difference in mean lung dose (range, 7.7-20.4 Gy) in patients with grade ≥ 3 pulmonary toxicity compared with those without. In addition, patients with grade ≥ 3 pulmonary toxicity did not present an increased V20 compared with those without toxicity. Indeed, V20 was kept $<30\%$ for the majority of patients as required by our dosimetric guidelines and only one patient had a V20 of 40%. He did not develop any significant toxicity greater than grade 2. Although esophagus was not outlined at the time of the study, we retrospectively analyzed the length of esophagus included in the treatment field. Half of patients received 52 to 60 Gy on one third of esophagus, whereas the other half (including those two patients presenting high-grade toxicity) received 40 to 45 Gy on two thirds of

esophagus. Finally, toxicity was not associated with the dose prescribed for delivery to the tumor. Considering the small sample size, radiation factors analyzed, and clinical characteristics of those patients that experienced toxicity (chronic obstructive pulmonary disease, poor performance status, and lower lobe tumors), we cannot conclude that celecoxib or radiation delivery was associated with any unexpected toxicity. Nevertheless, it is prudent to consider our survival outcomes in the context of a study by Lilenbaum et al. (47). They compared docetaxel/irinotecan and gemcitabine/irinotecan with or without celecoxib in treatment of stage IIIB/IV NSCLC and observed a trend toward decreased survival in the celecoxib group that did not reach significance.

There are still many questions to be clarified as to the potential optimal use of COX-2 inhibition in NSCLC and other cancer therapy. When our trial began, selective COX-2 inhibitors were thought to be much better tolerated than nonselective nonsteroidal anti-inflammatory drugs because of lower rates of gastrointestinal toxicity. Despite the now known association of thrombotic events with their chronic use, it would be reasonable to assume the increased risk of myocardial infarction if COX-2 inhibitors were found to add greatly to tumor control (48). Our study and these prior findings underscore the importance of improving our understanding of tumor biology to individualize cancer therapies. Using urinary PGE-M levels to predict a subset of patients most likely to benefit from COX-2 inhibition may be one promising strategy of "triaging" patients toward therapies with the greatest potential of improving survival and quality of life (49). Furthermore, more research into the optimal biological dose of celecoxib in combination with chemoradiotherapy is also required. Reckamp et al. (50) recently reported that the optimal biological active dose of celecoxib in combination with erlotinib was 600 mg twice daily, suggesting that a higher dose than the one used in our study may be required for optimal response. It is unclear, however, whether this dose would be safely tolerated with chemoradiotherapy, again highlighting the need for further investigation.

In conclusion, the results of this study suggest that celecoxib at a dose of 400 mg twice daily has limited ability to induce objective responses and improve survival in randomly selected patients with NSCLC treated with carboplatin/paclitaxel and radiotherapy. Urinary PGE-M may be an effective biomarker at predicting and selecting patients that may respond to and benefit from COX-2 inhibition in combination with traditional therapies. We currently have clinical trials under way testing this novel strategy.

Disclosure of Potential Conflicts of Interest

D.P. Carbone, commercial research support, Genentech; consultant, Biondesix. H. Choy, commercial research grants, BMS, Eli-Lilly, GPC-Biotech; speaker's bureau, Eli-Lilly, Genentech; consultant, Eli-Lilly, Genentech, BMS, Pfizer, Inclone. The other authors disclosed no potential conflict of interests.

References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007;57:43-66.
2. Blackstock AW, Govindan R. Definitive chemoradiation for the treatment of locally advanced non small-cell lung cancer. *J Clin Oncol* 2007;25:4146-52.
3. Furuse K, Fukuoka M, Kawahara M, et al. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small-cell lung cancer. *J Clin Oncol* 1999;17:2692-9.
4. Williams CS, Mann M, DuBois RN. The role of

- cyclooxygenases in inflammation, cancer, and development. *Oncogene* 1999;18:7908–16.
5. Hida T, Yatabe Y, Achiwa H, et al. Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. *Cancer Res* 1998;58:3761–4.
 6. Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, Ristimäki A. Expression of cyclooxygenase-2 in human lung carcinoma. *Cancer Res* 1998;58:4997–5001.
 7. Choy H, Milas L. Enhancing radiotherapy with cyclooxygenase-2 enzyme inhibitors: a rational advance? *J Natl Cancer Inst* 2003;95:1440–52.
 8. Harris RE, Beebe-Donk J, Doss H, Burr Doss D, Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade (review). *Oncol Rep* 2005;13:559–83.
 9. Johnson JC, Schmidt CR, Shrubsole MJ, et al. Urine PGE-M: a metabolite of prostaglandin E2 as a potential biomarker of advanced colorectal neoplasia. *Clin Gastroenterol Hepatol* 2006;4:1358–65.
 10. Murphey LJ, Williams MK, Sanchez SC, et al. Quantification of the major urinary metabolite of PGE2 by a liquid chromatographic/mass spectrometric assay: determination of cyclooxygenase-specific PGE2 synthesis in healthy humans and those with lung cancer. *Anal Biochem* 2004;334:266–75.
 11. Csiki I, Morrow JD, Sandler A, et al. Targeting cyclooxygenase-2 in recurrent non-small cell lung cancer: a phase II trial of celecoxib and docetaxel. *Clin Cancer Res* 2005;11:6634–40.
 12. Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell* 1998;93:705–16.
 13. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;407:249–57.
 14. Folkman J. Angiogenesis: an organizing principle for drug discovery? *Nat Rev Drug Discov* 2007;6:273–86.
 15. Kishi K, Petersen S, Petersen C, et al. Preferential enhancement of tumor radioresponse by a cyclooxygenase-2 inhibitor. *Cancer Res* 2000;60:1326–31.
 16. Milas L, Kishi K, Hunter N, Mason K, Masferrer JL, Tofilon PJ. Enhancement of tumor response to γ -radiation by an inhibitor of cyclooxygenase-2 enzyme. *J Natl Cancer Inst* 1999;91:1501–4.
 17. Jacoby RF, Seibert K, Cole CE, Kelloff G, Lubet RA. The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. *Cancer Res* 2000;60:5040–4.
 18. Sheng H, Shao J, Kirkland SC, et al. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J Clin Invest* 1997;99:2254–9.
 19. Hida T, Kozaki K, Muramatsu H, et al. Cyclooxygenase-2 inhibitor induces apoptosis and enhances cytotoxicity of various anticancer agents in non-small cell lung cancer cell lines. *Clin Cancer Res* 2000;6:2006–11.
 20. Milas L. Cyclooxygenase-2 (COX-2) enzyme inhibitors and radiotherapy: preclinical basis. *Am J Clin Oncol* 2003;26:S66–9.
 21. Lipsky PE, Isakson PC. Outcome of specific COX-2 inhibition in rheumatoid arthritis. *J Rheumatol Suppl* 1997;49:9–14.
 22. Davis TW, Hunter N, Trifan OC, Milas L, Masferrer JL. COX-2 inhibitors as radiosensitizing agents for cancer therapy. *Am J Clin Oncol* 2003;26:S58–61.
 23. Saha D, Pyo H, Choy H. COX-2 inhibitor as a radiation enhancer: new strategies for the treatment of lung cancer. *Am J Clin Oncol* 2003;26:S70–4.
 24. Sweeney CJ. Why cyclooxygenase-2 inhibition plus chemotherapy? *Am J Clin Oncol* 2003;26:S122–5.
 25. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–16.
 26. Therasse P, Eisenhauer EA, Verweij J. RECIST revisited: a review of validation studies on tumour assessment. *Eur J Cancer* 2006;42:1031–9.
 27. Burton J, Badine E, El-Sayah D, et al. Update of a phase I/II trial of carboplatin/gemcitabine plus escalating doses of celecoxib for first-line treatment of stage IIIB/IV non-small cell lung cancer (NSCLC) [abstract]. *J Clin Oncol* 2004;22:7339.
 28. Gadgeel S, Thatai L, Kraut M, et al. Phase II study of celecoxib and docetaxel in non-small cell lung cancer (NSCLC) patients with progression after platinum-based therapy [abstract 2749]. *Proc Am Soc Clin Oncol* 2003;22:684.
 29. Stani S, Carillio G, Meo S, et al. Phase II study of celecoxib and weekly paclitaxel in the treatment of pretreated advanced non-small cell lung cancer (NSCLC) [abstract 7337]. *J Clin Oncol* 2004;22:145s.
 30. Altorki NK, Keresztes RS, Port JL, et al. Celecoxib, a selective cyclo-oxygenase-2 inhibitor, enhances the response to preoperative paclitaxel and carboplatin in early-stage non-small-cell lung cancer. *J Clin Oncol* 2003;21:2645–50.
 31. Liao Z, Komaki R, Milas L, et al. A phase I clinical trial of thoracic radiotherapy and concurrent celecoxib for patients with unfavorable performance status inoperable/unresectable non-small cell lung cancer. *Clin Cancer Res* 2005;11:3342–8.
 32. Trotti A, Byhardt R, Stetz J, et al. Common toxicity criteria: version 2.0, an improved reference for grading the acute effects of cancer treatment: impact on radiotherapy. *Int J Radiat Oncol Biol Phys* 2000;47:13–47.
 33. Taber DF, Teng D. Total synthesis of the ethyl ester of the major urinary metabolite of prostaglandin E(2). *J Org Chem* 2002;67:1607–12.
 34. Morrow JD, Prakash C, Duckworth TA, et al. A stable isotope dilution mass spectrometric assay for the major urinary metabolite of PGD2. *Adv Prostaglandin Thromboxane Leukot Res* 1991;21A:315–8.
 35. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989;10:1–10.
 36. Heinze G, Gnant M, Schemper M. Exact log-rank tests for unequal follow-up. *Biometrics* 2003;59:1151–7.
 37. Grosch S, Maier TJ, Schiffmann S, Geisslinger G. Cyclooxygenase-2 (COX-2)-independent anticarcinogenic effects of selective COX-2 inhibitors. *J Natl Cancer Inst* 2006;98:736–47.
 38. Choy H, Akerley W, Safran H, et al. Multiinstitutional phase II trial of paclitaxel, carboplatin, and concurrent radiation therapy for locally advanced non-small-cell lung cancer. *J Clin Oncol* 1998;16:3316–22.
 39. Choy H, Devore RF III, Hande KR, et al. A phase II study of paclitaxel, carboplatin, and hyperfractionated radiation therapy for locally advanced inoperable non-small-cell lung cancer (a Vanderbilt Cancer Center Affiliate Network Study). *Int J Radiat Oncol Biol Phys* 2000;47:931–7.
 40. Jeremic B, Milicic B, Acimovic L, Milisavljevic S. Concurrent hyperfractionated radiotherapy and low-dose daily carboplatin and paclitaxel in patients with stage III non-small-cell lung cancer: long-term results of a phase II study. *J Clin Oncol* 2005;23:1144–51.
 41. Seyberth HW, Segre GV, Morgan JL, Sweetman BJ, Potts JT, Jr., Oates JA. Prostaglandins as mediators of hypercalcemia associated with certain types of cancer. *N Engl J Med* 1975;293:1278–83.
 42. Gaffney DK, Haslam D, Tsodikov A, et al. Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) negatively affect overall survival in carcinoma of the cervix treated with radiotherapy. *Int J Radiat Oncol Biol Phys* 2003;56:922–8.
 43. Gaffney DK, Holden J, Davis M, Zempolich K, Murphy KJ, Dodson M. Elevated cyclooxygenase-2 expression correlates with diminished survival in carcinoma of the cervix treated with radiotherapy. *Int J Radiat Oncol Biol Phys* 2001;49:1213–7.
 44. Gaffney DK, Holden J, Zempolich K, Murphy KJ, Dicker AP, Dodson M. Elevated COX-2 expression in cervical carcinoma: reduced cause-specific survival and pelvic control. *Am J Clin Oncol* 2001;24:443–6.
 45. Hawkey CJ. COX-2 inhibitors. *Lancet* 1999;353:307–14.
 46. Gasparini G, Meo S, Comella G, et al. The combination of the selective cyclooxygenase-2 inhibitor celecoxib with weekly paclitaxel is a safe and active second-line therapy for non-small cell lung cancer: a phase II study with biological correlates. *Cancer J* 2005;11:209–16.
 47. Lilenbaum R, Socinski MA, Altorki NK, et al. Randomized phase II trial of docetaxel/irinotecan and gemcitabine/irinotecan with or without celecoxib in the second-line treatment of non-small-cell lung cancer. *J Clin Oncol* 2006;24:4825–32.
 48. Bresalier RS, Sandler RS, Quan H, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med* 2005;352:1092–102.
 49. Riesterer O, Milas L, Ang KK. Use of molecular biomarkers for predicting the response to radiotherapy with or without chemotherapy. *J Clin Oncol* 2007;25:4075–83.
 50. Reckamp KL, Krysan K, Morrow JD, et al. A phase I trial to determine the optimal biological dose of celecoxib when combined with erlotinib in advanced non-small cell lung cancer. *Clin Cancer Res* 2006;12:3381–8.