# Elevated Levels of Urinary Prostaglandin E Metabolite Indicate a Poor Prognosis in Ever Smoker Head and Neck Squamous Cell Carcinoma Patients

Vikram D. Kekatpure,[1] Jay O. Boyle,[3] Xi Kathy Zhou,[2] Anna J. Duffield-Lillico,[3,4] Neil D. Gross,[6] Nancy Y. Lee,[5] Kotha Subbaramaiah,[1] Jason D. Morrow,[7] Ginger Milne,[7] Scott M. Lippman[8] and Andrew J. Dannenberg[1]

**Abstract** Cyclooxygenase (COX)-derived prostaglandin $E_2$ ($PGE_2$) plays a role in the development and progression of several tumor types including head and neck squamous cell carcinoma (HNSCC). Measurements of urinary PGE metabolite (PGE-M) can be used as an index of systemic $PGE_2$ production. In ever smokers, increased levels of urinary PGE-M reflect increased COX-2 activity. In this study, we determined whether baseline levels of urinary PGE-M were prognostic for ever smoker HNSCC patients. A retrospective chart review of ever smoker HNSCC patients treated with curative intent was done. Fifteen of 31 evaluable patients developed progressive disease (recurrence or a second primary tumor) after a median follow-up of 38 months. There were no statistically significant differences between patients with ($n$ = 15) or without disease progression ($n$ = 16) with regard to stage, site, treatment received, smoking status, and aspirin use during follow-up. Median urinary PGE-M levels were significantly higher in HNSCC patients with disease progression (21.7 ng/mg creatinine) compared with patients without (13.35 ng/mg creatinine; $P = 0.03$). Importantly, patients with high baseline levels of urinary PGE-M had a significantly greater risk of disease progression (hazard ratio, 4.76, 95% CI, 1.31-17.30; $P < 0.01$) and death (hazard ratio, 9.54; 95% CI, 1.17-77.7; $P = 0.01$) than patients with low baseline levels of urinary PGE-M. These differences were most evident among patients with early-stage disease. Taken together, our findings suggest that high baseline levels of urinary PGE-M indicate a poor prognosis in HNSCC patients. Possibly, HNSCC patients with high COX-2 activity manifested by elevated urinary PGE-M will benefit from treatment with a COX-2 inhibitor.

Cyclooxygenases (COX) catalyze the first step in the synthesis of prostaglandin $E_2$ ($PGE_2$) from arachidonic acid. There are two isoforms of COX, designated COX-1 and COX-2. COX-1 is constitutively expressed in most tissues and mediates various physiologic functions (1). In contrast, COX-2 is not detected in most normal tissues but is rapidly induced by a variety of mitogenic and inflammatory stimuli (2, 3) resulting in elevated levels of $PGE_2$ in neoplastic and inflamed tissues (4–7). Multiple lines of evidence suggest that COX-2 and $PGE_2$ play a significant role in carcinogenesis. Levels of COX-2 and $PGE_2$ are increased in a variety of malignancies including head and neck squamous cell carcinoma (HNSCC; refs. 3–6, 8). Tumor formation and growth are reduced in animals that are engineered to be COX-2 deficient (9–11). Treatment with selective inhibitors of COX-2, prototypic inhibitors of $PGE_2$ synthesis, or an anti-$PGE_2$ monoclonal antibody inhibited the growth of transplantable tumors including HNSCC (12–15). In humans, selective COX-2 inhibitors have proven chemopreventive efficacy in the management of colorectal polyps and may be beneficial in the treatment of non–small cell lung cancer (NSCLC; refs. 16–18).

Several mechanisms have been identified that can explain the link between COX-2, $PGE_2$, and malignancy. $PGE_2$ can stimulate cell proliferation, motility, and angiogenesis while inhibiting apoptosis and immune surveillance (3, 19–23). COX-2–derived $PGE_2$ may also promote metastasis by stimulating epithelial-mesenchymal transition and cell invasion (24,

**Authors' Affiliations:** Departments of [1]Medicine and [2]Public Health, Weill Cornell Medical College; Departments of [3]Surgery, [4]Epidemiology and Biostatistics, and [5]Radiation Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York; [6]Department of Otolaryngology-Head and Neck Surgery, Oregon Health and Science University, Portland, Oregon; [7]Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee; and [8]Department of Thoracic/Head and Neck Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas
Received 4/27/09; revised 9/4/09; accepted 9/6/09; published OnlineFirst 10/20/09.

**Grant support:** Flight Attendant Medical Research Institute; Memorial Sloan-Kettering Cancer Center Prevention Control and Population Research Program Pilot Project Award; American Society of Clinical Oncology Young Investigator Award; and NIH grants P01 CA77839, P01 CA106451, T32 CA09685, DK48831, GM15431, ES13125, and CTSC UL1-RR024996.

**Note:** J.D. Morrow is deceased. His passing is a great loss to all of us.

**Requests for reprints:** Andrew J. Dannenberg, Department of Medicine and Weill Cornell Cancer Center, 525 East 68th Street, Room F-206, New York, NY 10065. Phone: 212-746-4403; Fax: 212-746-4885; E-mail: ajdannen@med.cornell.edu.

©2009 American Association for Cancer Research.

doi:10.1158/1940-6207.CAPR-09-0093

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/2/11/957/1731621/957.pdf by guest on 17 July 2024

25). In HNSCC, high levels of intratumor COX-2 and $PGE_2$ have been associated with poor prognosis (26). Levels of COX-2 are also increased in the oral mucosa of seemingly healthy smokers (27).

Since it is rapidly catabolized in the lungs, $PGE_2$ in plasma does not accurately reflect endogenous production of PG (28). 15-Hydroxyprostaglandin dehydrogenase initiates the catabolism of $PGE_2$, leading to a stable end metabolite (PGE-M), or 11-α-hydroxy-9, 15-dioxo-2,3,4,5-tetranor-prostane-1, 20-dioic acid, which is excreted in the urine (29, 30). The value of urinary PGE-M as an index of systemic $PGE_2$ production has been shown in previous studies (31, 32). Urinary levels of PGE-M were found to be increased in non-small cell lung cancer (NSCLC) and colon cancer patients and in ever smokers without cancer or its history (33–35). HNSCC patients also were found to have a small, nearly significant increase in levels of urinary PGE-M (36). The suggestion that increased levels of urinary PGE-M reflect enhanced COX-2 activity has come from previous studies (33–35).

In this study, we determined whether baseline levels of urinary PGE-M were a prognostic factor for ever smoker HNSCC patients. Importantly, patients with high baseline levels of urinary PGE-M had a significantly greater risk of cancer progression and death than did patients with low baseline levels of urinary PGE-M. We speculate that HNSCC patients with elevated levels of urinary PGE-M may benefit from treatment with a COX-2 inhibitor as an adjunct to curative primary therapy and/or as adjuvant therapy to prevent second primary or recurrent cancer.

## Materials and Methods

### Study population

The HNSCC patients included in the current study have been described previously (36). Briefly, patients with histologically confirmed HNSCC (newly diagnosed or recurrent) were enrolled. Patients with any surgery, chemotherapy (including corticosteroids), hormonal and/or radiation therapy within 6 wk of enrollment, known unrelated malignancy, chronic inflammatory disease, renal disease or active infectious process, and patients on nonsteroidal anti-inflammatory drugs within 1 wk of enrollment to study were excluded. Participant exposure to known HNSCC risk factors, including tobacco and alcohol, were documented. Former smokers were defined as subjects who quit at least 12 mo before presentation. Daily 81-mg aspirin use, defined as routine intake including within 48 h of urine collection, was documented. Information regarding site and stage of disease was then extracted from the medical record. All tumors were staged according to the American Joint Committee on Cancer staging system. When available, pathologic staging was preferred over clinical staging. Previous cancer history and any applied therapeutic interventions were identified and recorded as applicable. Before initiation of cancer treatment, single void urine specimens were collected from each participant, aliquoted into 2-mL cryovials and stored at −80°C. An informed consent was obtained from each participant. The Institutional Review Board of Memorial Sloan-Kettering Cancer Center approved this study.

Forty of the 58 HNSCC patients in the original study were ever smokers, and 18 were never smokers (36). In this study, the 18 never smokers were excluded because the etiology and prognosis of HNSCC arising in never smokers are very different from those of smoking-related HNSCC, and it was not possible to meaningfully analyze such a small sample size (only 3 of the 18 progressed) in this study (37, 38). A retrospective chart review of 40 ever smokers was done by a head and neck surgeon who independently confirmed the status of each case. Patients who received treatment with curative intent, had documentation of disease-free status by clinical or radiological examination following completion of treatment, and had a minimum of 1 y follow-up after completion of treatment were included in the analysis, for a total of 31 patients. Fifteen of the 31 patients developed progressive disease (14 clinically classified recurrences, 1 second primary tumor); the remaining 16 patients were disease free. Data concerning smoking status during treatment, smoking status during follow-up, aspirin or nonsteroidal anti-inflammatory drug use, local failure, regional failure, distal failure, and death due to disease were obtained from the medical records. Details of surgery, radiotherapy, and chemoradiation were also obtained.

### Urinary PGE-M

Analyses of urine specimens were contemporaneous and blinded. As described previously, we measured urinary PGE-M via mass spectrometry using stable isotope dilution methodology with chemically synthesized ($^2H_6$)PGE-M to quantify $PGE_2$ production (36). We converted endogenous urinary PGE-M to an unlabeled *O*-methyloxime derivative and then extracted it. Mass spectrometry was done using a Thermo Scientific Quantum Ultra instrument fitted with an electrospray source and was operated in the negative ion mode using multiple reaction monitoring. The transition of the precursor ions for endogenous (*m/z* 385) and ($^2H_6$)-labeled (*m/z* 391) *O*-methyloxime PGE-M were collisionally activated at 21eV, and product ions *m/z* 336 and *m/z* 339 were monitored. We then calculated the specimen levels of endogenous PGE-M from the ratio of the mass chromatogram peak areas of the *m/z* 385 → 336 and *m/z* 391 → 339 transitions. The urinary creatinine concentration was used to normalize the results.

### Statistical analysis

The primary objective of this study was to evaluate the prognostic role of urinary PGE-M. Outcomes of interest include disease-free and overall survival. Variables describing patient demographics, smoking, aspirin use, disease, and treatment characteristics are summarized for cases with and without progression separately, in terms of mean ± SD and median (range) for continuous variables and count (proportion) for categorical variables. Differences in the means between the two groups were compared using a parametric *t* test, with log transformation of the data applied when the distribution of data deviated from the normality assumption. Differences in the medians and proportions between the two groups were compared using the nonparametric Wilcoxon rank-sum test and Fisher's exact test, respectively. Time-to-event data for subjects with high (above median) and low (below median) PGE-M levels are summarized using Kaplan-Meier curves. Two-year disease-free survival probability and 3-y overall survival probability and their respective 95% confidence intervals (CI) were determined. Log-rank test was used to examine the association between each of the independent variables and a time-to-event outcome univariately. The Cox proportional hazard model was used for multivariable analysis of the association between urinary PGE-M and the outcome adjusting for other covariates, which were identified using a significance level of 0.20 based on results from the univariate analyses. The hazard ratios (HR) with 95% CI and *P* values are reported. All tests were two sided, and *P* values of $<0.05$ were considered statistically significant.

## Results

### Patient and tumor characteristics

The characteristics of the 31 HNSCC patients are shown in Table 1. Patients were grouped as those with ($n = 15$) and without ($n = 16$) disease progression (recurrence or a second primary tumor). Patients who developed local, regional, or distant disease following treatment with curative intent were

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/2/11/957/1731621/957.pdf by guest on 17 July 2024

**Table 1.** Patient characteristics

| Variable | HNSCC cases with progression ($n$ = 15) | HNSCC cases without progression ($n$ = 16) | $P$ |
|---|---|---|---|
| Age, y | | | |
| Median (range) | 66.6 (53.9-81.0) | 64.7 (45.1-79.1) | 0.64 (Wilcoxon) |
| Mean ± SD | 66.2 ± 8.9 | 64.2 ± 10.9 | 0.57 ($t$ test) |
| Gender, $n$ (%) | | | 0.70 (Fisher's exact test) |
| Male | 11 (73.3) | 10 (62.5) | |
| Female | 4 (26.7) | 6 (37.5) | |
| Tobacco use at time of sample collection, $n$ (%) | | | 0.70 |
| Current | 4 (26.7) | 6 (37.5) | |
| Former | 11 (73.3) | 10 (62.5) | |
| Pack year exposure* | | | |
| Median (range) | 28.1 (2-100.5) | 25.0 (2.5-80.0) | 0.88 |
| Mean ± SD | 30.2 ± 28.6 | 28.2 ± 22.7 | 0.84 |
| Tobacco use during follow-up, $n$ (%) | | | 0.23 |
| Yes | 2 (13.3) | 0 (0) | |
| No | 13 (86.7) | 16 (100) | |
| Aspirin use before urine collection, $n$ (%) | | | 0.69 |
| Yes | 4 (26.7) | 3 (18.8) | |
| No | 11 (73.3) | 13 (81.2) | |
| Aspirin use during follow-up, $n$ (%) | | | 1.00 |
| Yes | 2 (13.3) | 2 (12.5) | |
| No | 13 (86.7) | 14 (87.5) | |

*Excludes one pipe smoker in both groups.

grouped as having progressive disease. The median ages at initial presentation for patients with and without tumor progression were 66.6 and 64.7 years, respectively ($P$ = 0.64). A greater proportion of patients were male and former smokers. However, there were no statistically significant differences in gender and smoking status between the two groups. Smoking status during treatment and follow-up and long-term aspirin or nonsteroidal anti-inflammatory drug use during follow-up was recorded. Two patients (13.3%) in the progression group continued smoking through last follow-up. Four patients (26.7%) in the progression group and three patients (18.8%) whose tumors failed to progress used 81 mg of aspirin before urine collection; two patients in each group used aspirin during follow-up. The differences between the two groups were not statistically significant.

Medical records were reviewed to obtain information regarding the known risk factors of progression in HNSCC. Summary statistics of variables that can affect the disease outcome, including stage, site, size of the tumor, and the treatment received, are listed in the Table 2. Most patients with newly diagnosed disease in both groups were in advanced stages, with stage III and IV accounting for 66.7% and 56.3% in the progression and progression-free groups, respectively. Seven patients with recurrent disease who were offered treatment with curative intent were included in the analysis. Three of these patients eventually developed progressive disease and four patients remained free of disease. The difference between the two groups was not statistically significant. Median tumor size was 2.9 cm with a range of 0 to 4.5 cm for the progression group and 1.7 cm with a range of 0 to 6.0 cm in the group that failed to progress. The difference between the two groups was not significant statistically. Tumor sites were well matched in the two groups; however, a higher percentage of cases of laryngeal cancer were found in the group that progressed versus remained progression free. All patients received current standard of care curative intent treatment, which include surgery alone, radiation alone, chemoradiation, or a combination of these three modalities. The difference in the type of treatment received between the two groups was not statistically significant.

### Pretreatment urinary PGE-M levels

Baseline levels of urinary PGE-M were compared in the two groups (progression versus progression free) of HNSCC patients. A significantly higher median baseline urinary PGE-M level was found in HNSCC patients who developed progressive disease compared with HNSCC patients whose disease did not progress (Table 2; Fig. 1). Notably, there was considerable variability in levels of urinary PGE-M within each of the two groups of patients. Several individuals with low baseline urinary PGE-M levels developed progressive disease. Two patients whose disease did not recur had high urinary PGE-M levels.

### Relationship between pretreatment urinary PGE-M and progression-free and overall survival

***Univariable analysis.*** The log-rank test was used to examine the association between urinary PGE-M and disease-free

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/2/11/957/1731621/957.pdf by guest on 17 July 2024

**Table 2.** Tumor characteristics

| Variable | HNSCC cases with progression ($n$ = 15) | HNSCC cases without progression ($n$ = 16) | $P$ |
|---|---|---|---|
| Stage, $n$ (%) | | | 1.00 |
| I/II | 2 (13.3) | 3 (18.7) | |
| III/IV | 10 (66.7) | 9 (56.3) | |
| Recurrent | 3 (20.0) | 4 (25.0) | |
| Tumor size (cm) | | | |
| Median (range) | 2.9 (0-4.5) | 1.7 (0-6.0) | 0.12 |
| Mean ± SD | 2.4 ± 1.3 | 1.9 ± 1.5 | 0.25 |
| Tumor site, $n$ (%) | | | 0.69 |
| Oral cavity | 2 (13.4) | 4 (25.0) | |
| Oropharynx | 3 (20.0) | 5 (31.1) | |
| Hypopharynx | 1 (6.8) | 1 (6.3) | |
| Larynx | 8 (53.0) | 4 (25.0) | |
| Unknown | 1 (6.8) | 1 (6.3) | |
| Others | 0 (0) | 1 (6.3) | |
| Treatment, $n$ (%) | | | 0.55 |
| Surgery | 3 (20) | 7 (43.8) | |
| Radiation | 2 (13.3) | 2 (12.5) | |
| Chemoradiation | 7 (46.7) | 5 (31.2) | |
| Surgery+RT/CRT | 3 (20) | 2 (12.5) | |
| PGE-M | | | |
| Median (range) | 21.7 (2.4-69.7) | 13.4 (4.9-38.2) | 0.03 |
| Mean ± SD | 25.1 ± 17.4 | 14.5 ± 8.8 | 0.04 |

Abbreviations: RT, radiotherapy; CRT, chemoradiation therapy.

survival. The result suggests that higher urinary PGE-M levels were associated with significantly increased risk of progression (HR, 1.03; 95% CI, 1.00-1.07; $P$ = 0.04). Dichotomizing PGE-M at the median (16.1 ng/mg creatinine), patients with high urinary PGE-M (above median) had significantly increased risk of progression compared with patients with low urinary PGE-M (HR, 4.76; 95% CI, 1.31-17.30; $P$ < 0.01). The 2-year disease-free survival probability for patients with high urinary PGE-M was estimated to be 46.7% (95% CI, 27.2-80.2%) and 87.1% (95% CI, 71.8-100%) for those with low urinary PGE-M (Fig. 2A). Similarly, the log-rank test was used to examine the association between urinary PGE-M levels and overall patient survival. The result suggests that higher urinary PGE-M was significantly associated with increased risk of death (HR, 1.05; 95% CI, 1.01-1.09; $P$ = 0.01). Patients with high urinary PGE-M (above median) had significantly increased risk of death compared with patients with low urinary PGE-M (HR, 9.54; 95% CI, 1.17-77.7; $P$ = 0.01). Specifically, the 3-year survival probability for patients with high urinary PGE-M and low urinary PGE-M was estimated to be 53.3% (95% CI, 33.2-85.6%) and 91.7% (95% CI, 77.3-100%), respectively (Fig. 2B).

***Multivariable analysis.*** For disease-free survival, only tumor size and treatment showed significance at the level of 0.20. In multivariable analysis using the Cox proportional hazard model, elevated baseline urinary PGE-M appeared to be associated with increased risk of progression (HR, 1.03; 95% CI, 1.00-1.06; $P$ = 0.07) after adjusting for tumor size and treatment. Examining the association between disease-free survival and dichotomized PGE-M adjusting for these two variables suggested that patients with high PGE-M had increased risk of progression compared with patients with low PGE-M (HR, 3.93; 95% CI, 1.03-15.06; $P$ = 0.05). For overall survival, only tumor size showed association at significance level of 0.20. Multivariable analysis with the Cox proportional hazard model suggested that high urinary PGE-M remained significantly associated with increased risk of death (HR, 1.06; 95% CI, 1.02-1.12; $P$ = 0.007) after adjusting for tumor size. Examining the association between overall survival and dichotomized PGE-M adjusting for tumor size suggested that patients with high urinary PGE-M had increased risk of death compared with patients with low urinary PGE-M (HR, 7.49; 95% CI, 0.87-64.23; $P$ = 0.07).

### Relationship between pretreatment urinary PGE-M and stage-specific progression-free and overall survival

A prognostic biomarker, which indicates adverse prognosis for patients with early-stage disease, would be of significant clinical importance. It may help to stimulate appropriate therapies and screening for prevention and/or early detection of the recurrence. Therefore, we examined the association between pretreatment urinary PGE-M and stage-specific disease-free and overall survival. Because of the small sample size, the results of this analysis should be considered to be hypothesis generating. Elevated levels of urinary PGE-M were associated with increased risk of progression for patients with early-stage disease. For patients with primary T-stage T1/

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/2/11/957/1731621/957.pdf by guest on 17 July 2024

T2, node-negative, and tumor-node-metastasis stage I/II disease, high baseline urinary PGE-M was associated with increased risk of disease progression, and the association was significant or close to significance ($P$ values were <0.01, 0.02 and 0.07, respectively; Fig. 3A, C, and E). Consistent with increased risk for disease progression, patients with early-stage disease and high baseline urinary PGE-M had lower overall survival ($P$ values were <0.01, 0.03, and 0.07, respectively; Fig. 4A, C, and E). For patients with higher T-stage, nodal metastasis, and advanced stage III/IV disease, a trend toward higher risk of progression (Fig. 3B, D, and F) and poorer overall survival (Fig. 4B, D, and F) was suggested for those with higher baseline urinary PGE-M levels. Given the limited sample size of the study, this did not reach the level of statistical significance.

## Discussion

In the current study, high levels of urinary PGE-M marked a poor prognosis in HNSCC patients. This finding raises numerous issues. To begin with, the source of increased $PGE_2$ synthesis that resulted in high levels of urinary PGE-M in a subset of patients should be considered. One strong possibility is the tumor itself. Increased levels of urinary PGE-M have been found in both NSCLC and colorectal cancer patients (33, 34). We previously detected a nearly statistically significant increase ($P$ = 0.07) in levels of urinary PGE-M in HNSCC patients (36). Many of these HNSCC patients were smokers, an independent cause of elevated urinary PGE-M (35, 36). Our previous study was underpowered to detect a small increase in urinary PGE-M due to HNSCC. By contrast, increased levels of urinary PGE-M were readily detected in NSCLC patients, another smoking-related cancer. Taken together, this suggests that any increase in urinary PGE-M due to HNSCC would be smaller than the increase due to NSCLC. In fact, the mean levels of urinary PGE-M were 27.2 ng/mg Cr and 17.9 ng/mg Cr in NSCLC and HNSCC patients, respectively (36, 39). Hence, it seems likely that HNSCC contributes to increased urinary PGE-M levels in some patients. This is highly relevant because prior studies have shown that both elevated COX-2 and $PGE_2$ at the invasive front of the tumor predict for increased risk of lymph node metastases, local recurrence, and worse disease-free and overall survival in HNSCC patients (26, 40). In all likelihood, high levels of intratumor COX-2 cause elevated $PGE_2$ production in HNSCC patients, leading, in turn, to increased urinary PGE-M levels that mark a poor prognosis. Overexpression of microsomal prostaglandin E synthase-1, the enzyme that converts COX-derived $PGH_2$ to $PGE_2$, may

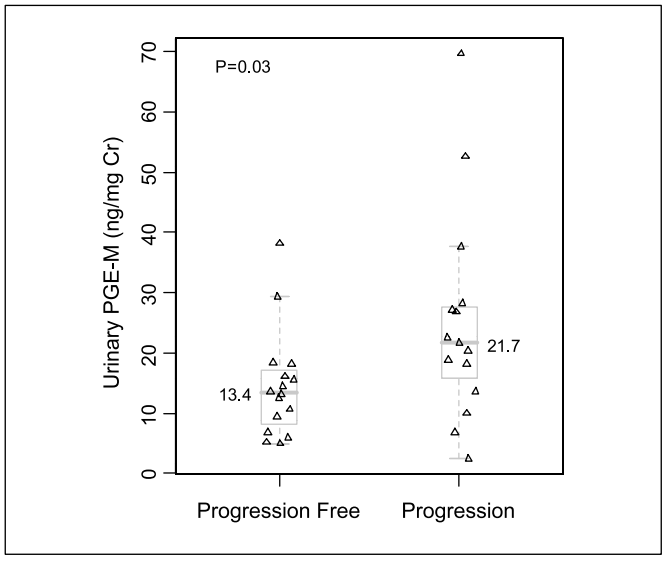


**Fig. 1.** Significantly higher baseline urinary PGE-M levels were observed for patients developing disease progression (median, 21.7; range, 2.4-69.7) compared with those without progression (median, 13.4; range, 4.9-38.2; $P$ = 0.03 Wilcoxon rank-sum test). Distribution of urinary PGE-M levels in patients with or without disease progression is illustrated with scatter diagram and the overlapping box plots. Levels of urinary PGE-M are expressed as ng/mg Cr.

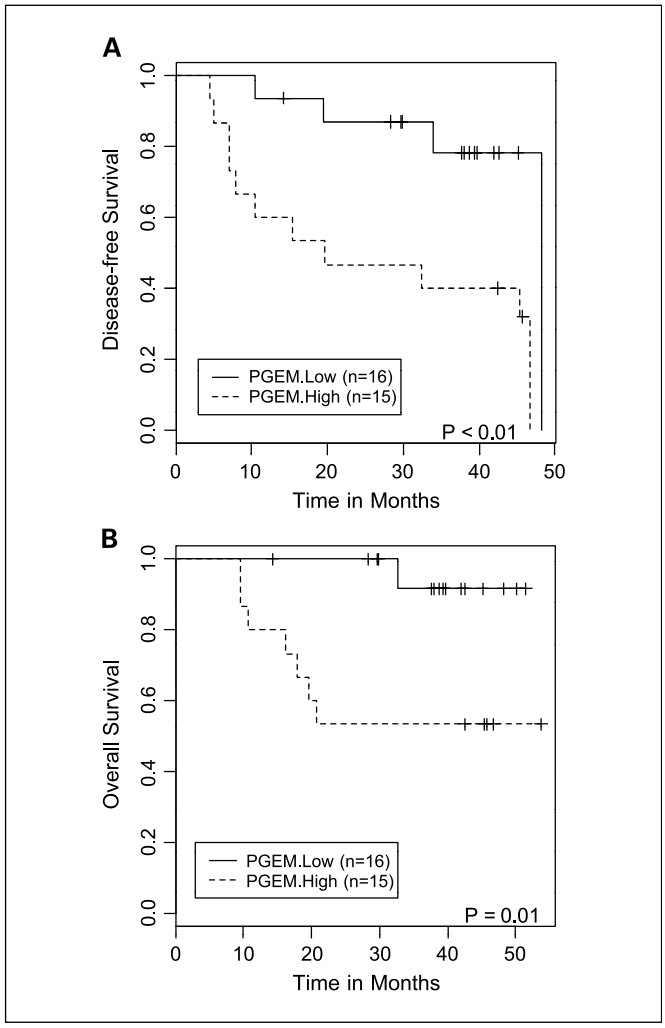


**Fig. 2.** Baseline urinary PGE-M levels predict disease-free and overall survival in patients with HNSCC. Kaplan Meier survival curves are illustrated. *A*, disease-free survival was significantly lower for patients with high baseline urinary PGE-M values ($P$ < 0.01). The 2-y disease-free survival probabilities were estimated to be 46.7% (95% CI, 27.2-80.2%) for patients with high urinary PGE-M, and 87.1% (95% CI, 71.8-100%) for those with low urinary PGE-M. *B*, overall survival was significantly lower for patients having high baseline urinary PGE-M values ($P$ = 0.01). The 3-y probability of survival for patients with high and low baseline urinary PGE-M values was estimated to be 53.3% (95% CI, 33.2-85.6%) and 91.7% (95% CI, 77.3-100%), respectively. The baseline urinary PGE-M values were dichotomized as high and low categories by using the median (16.1 ng/mg creatinine) as the cutoff. This definition of high and low PGE-M categories is used throughout this article.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/2/11/957/1731621/957.pdf by guest on 17 July 2024

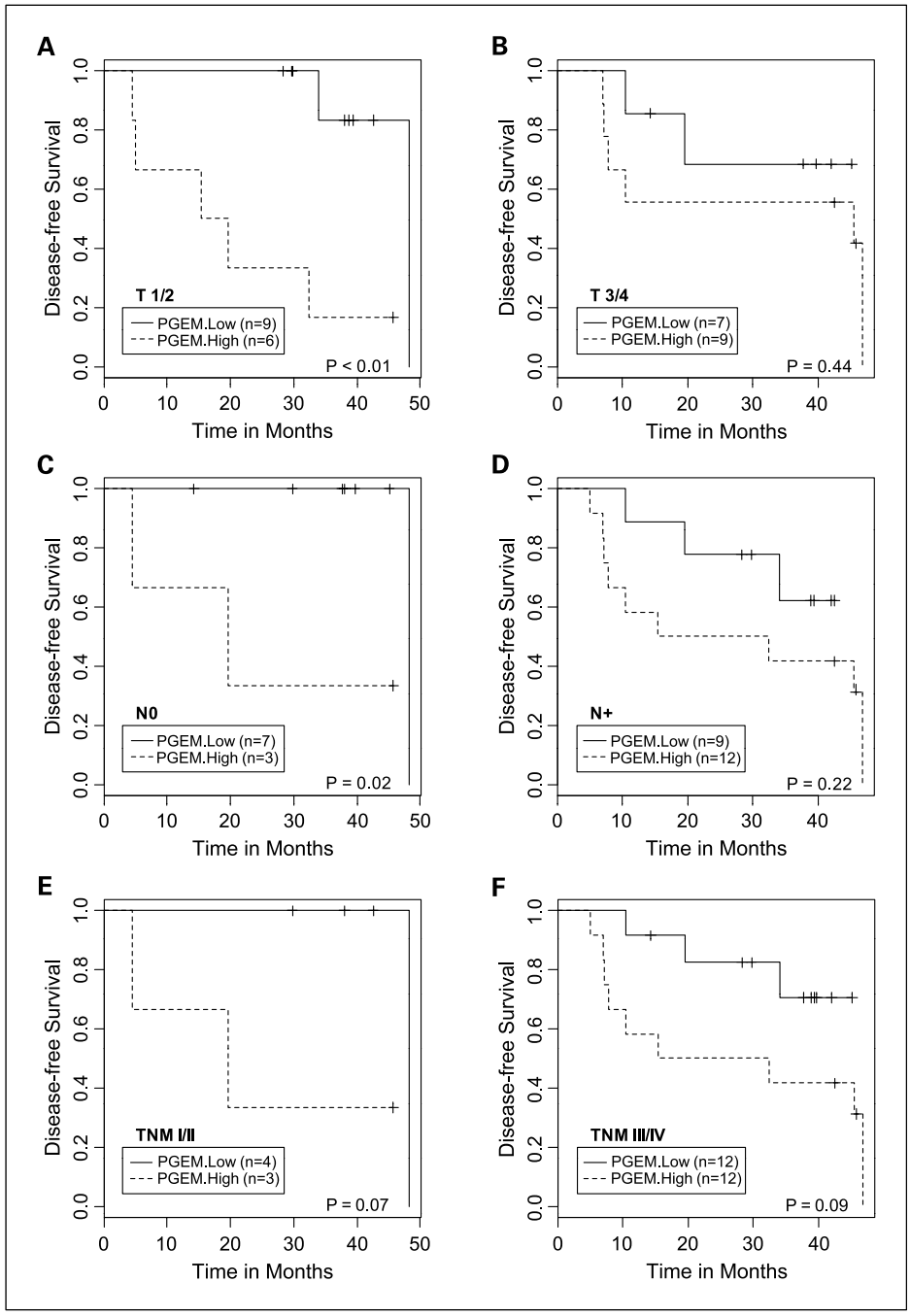


**Fig. 3.** Kaplan Meier curves illustrate a consistent pattern of lower disease-free survival probabilities for patients with high baseline urinary PGE-M levels compared with those with low baseline urinary PGE-M levels at the following disease stage categories: *A*, T1/T2 primary tumors; *B*, T3/T4 primary tumors; *C*, N0 neck; *D*, N+ neck; *E*, stage I/II; and *F*, stage III/IV.

also contribute to increased levels of $PGE_2$ in HNSCC (41). In addition to the tumor being a likely source of increased urinary PGE-M, smoking is an independent cause of increased COX-2 activity resulting in elevated urinary PGE-M (35). Although the location of the smoking-related increase in COX-2 activity has not been confirmed, the upper aerodigestive tract has been suggested to be a likely source (27, 35). Smoking-mediated increases in procarcinogenic $PGE_2$ levels might also have a negative impact on tumor progression in HNSCC patients. Additional studies will be required to determine the relative importance of intratumor $PGE_2$ versus smoking-related increases in mucosal $PGE_2$ as determinants of urinary PGE-M and disease progression. Perhaps the major overriding issue raised by our results is their implications for therapy and prevention in the setting of curatively treated HNSCC patients (discussed later).

As mentioned in the introduction, $PGE_2$ has numerous effects that can potentially explain the link between elevated urinary PGE-M levels and a poor prognosis. $PGE_2$ exerts its biological actions by binding to any one of four E-series

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/2/11/957/1731621/957.pdf by guest on 17 July 2024

of PG receptors in tumor or stromal cells resulting in increased cell proliferation, enhanced angiogenesis, reduced apoptosis, and inhibition of immune surveillance (3, 19–23). For example, stimulation of either $EP_2$ or $EP_4$ activates T-cell transcription factor/β-catenin–mediated transcription that leads, in turn, to enhanced expression of a variety of genes, e.g., *cyclin D1* and *c-myc*, which have been implicated in carcinogenesis (42). Cross-talk between E-series of PG receptors and the epidermal growth factor receptor may also contribute to increased cell growth (43). Recently, $PGE_2$ was found to activate Wnt signaling and thereby impact stem cell function, which could also be important for tumor progression (44). Additional known effects of $PGE_2$ that may contribute to tumor progression and metastasis include induction of vascular endothelial growth factor and matrix metalloproteinase-9, and stimulation of epithelial-mesenchymal transition (3, 24, 45). $PGE_2$ also exhibits potent immunosuppressive effects by modulating dendritic cell function and

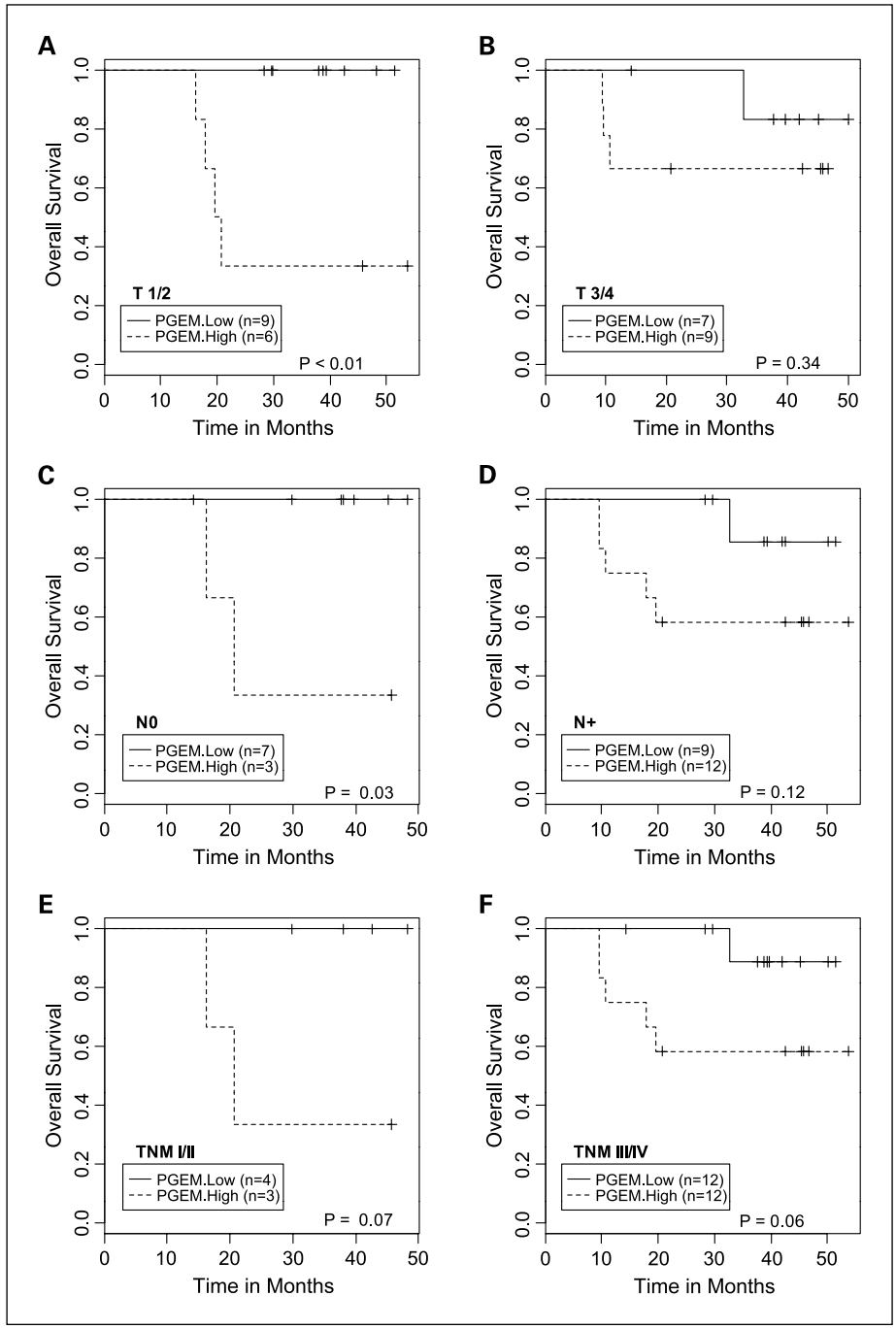


**Fig. 4.** Kaplan Meier curves illustrate a consistent pattern of lower overall survival probabilities for patients with high baseline urinary PGE-M levels compared with those with low baseline urinary PGE-M levels at the following disease stage categories: *A*, T1/T2 primary tumors; *B*, T3/T4 primary tumors; *C*, N0 neck; *D*, N+ neck; *E*, stage I/II; and *F*, stage III/IV.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/2/11/957/1731621/957.pdf by guest on 17 July 2024

causing an imbalance between type 1 and type 2 cytokines (46). In addition to explaining how increased levels of $PGE_2$ can contribute to disease progression, these effects of $PGE_2$ may also create resistance to chemotherapy and radiation therapy (47).

Numerous preclinical studies indicate that inhibiting COX-derived PG synthesis is useful for preventing or treating a variety of tumor types including HNSCC (9–15). Moreover, selective COX-2 inhibitors such as celecoxib have proven efficacy in the treatment of colorectal adenomas in humans (16, 17). The results of a small clinical trial suggested that indomethacin, a dual inhibitor of COX-1 and COX-2, reduced the growth of HNSCC (48). Prolonged use of selective COX-2 inhibitors led to a small increase in cardiovascular complications, which is a significant barrier for routine use in chemoprevention (49). Clearly, the risk versus benefit calculation is different in cancer patients. Nonetheless, it would be a major advance if a biomarker could be used to identify the subset of HNSCC patients who are most likely to benefit from treatment with a COX-2 inhibitor. In a phase II trial of celecoxib and docetaxel in NSCLC patients, patients who experienced the greatest proportional decline in urinary PGE-M following treatment with celecoxib were at significantly reduced risk of death relative to patients with no change or an increase in PGE-M levels (39). This result was also suggested in a second study of patients with unresectable NSCLC (50). Another recent trial involving NSCLC patients suggested that treatment with a selective COX-2 inhibitor was clinically beneficial in the subset of patients with the highest intratumor COX-2 levels (18). Encouraging responses were seen in a small trial of celecoxib plus gefitinib for the treatment of incurable HNSCC (51). If one can extrapolate from NSCLC to HNSCC, our results suggest that HNSCC patients with high baseline urinary PGE-M levels will be most likely to benefit from treatment with a COX-2 inhibitor. Given the findings in NSCLC, it will be worthwhile to determine whether the magnitude of decline in urinary PGE-M following treatment with a COX-2 inhibitor also predicts for clinical benefit in HNSCC patients.

It is important to acknowledge the potential limitations of our study. Although the important clinical, demographic, and tumor characteristics were well matched in patients with and without disease progression, our study had a small sample size. Moreover, determining whether levels of urinary PGE-M had prognostic significance was not a prespecified end point of the initial study, and our results are based on a retrospective chart review. A larger prospective study is warranted to validate and extend our findings. For example, a larger study is needed to determine the utility of urinary PGE-M as a prognostic biomarker for different tumor sites within the head and neck and stages of HNSCC. The current results suggest that urinary PGE-M may be a better prognostic biomarker for early- than late-stage disease. However, it is uncertain whether this will be true for all tumor sites within the head and neck. Due to the small size of the current study, the results should be viewed as hypothesis generating. If a larger study confirms that high levels of urinary PGE-M mark a poor prognosis in patients with early-stage disease, this biomarker could potentially be used to inform clinical decision making.

In conclusion, our finding that elevated levels of urinary PGE-M indicate a poor prognosis in HNSCC patients is consistent with previous data on the effects of $PGE_2$ on tumor progression and metastasis (52). Measurements of urinary PGE-M may provide insights that will enable the identification of subsets of patients who are most likely to benefit from primary or adjuvant/preventive treatment with a COX-2 inhibitor.

## Disclosure of Potential Conflicts of Interest

Andrew J. Dannenberg is a member of the Scientific Advisory Board of Tragara Pharmaceuticals Inc., a company that is developing a selective COX-2 inhibitor. The other authors revealed no potential conflicts of interest.

## References


1. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000;69:145–82.
2. DuBois RN, Awad J, Morrow J, Roberts LJ, Bishop PR. Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor-α and phorbol ester. J Clin Invest 1994;93:493–8.
3. Dannenberg AJ, Subbaramaiah K. Targeting cyclooxygenase-2 in human neoplasia: rationale and promise. Cancer Cell 2003;4:431–6.
4. Lupulescu A. Prostaglandins, their inhibitors and cancer. Prostaglandins Leukot Essent Fatty Acids 1996;54:83–94.
5. Jung TT, Berlinger NT, Juhn SK. Prostaglandins in squamous cell carcinoma of the head and neck: a preliminary study. Laryngoscope 1985;95:307–12.
6. Rigas B, Goldman IS, Levine L. Altered eicosanoid levels in human colon cancer. J Lab Clin Med 1993;122:518–23.
7. Sharon P, Ligumsky M, Rachmilewitz D, Zor U. Role of prostaglandins in ulcerative colitis. Enhanced production during active disease and inhibition by sulfasalazine. Gastroenterology 1978;75:638–40.
8. Chan G, Boyle JO, Yang EK, et al. Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. Cancer Res 1999;59:991–4.
9. Oshima M, Dinchuk JE, Kargman SL, et al. Suppression of intestinal polyposis in $Apc^{\Delta 716}$ knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 1996;87:803–9.
10. Tiano HF, Loftin CD, Akunda J, et al. Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis. Cancer Res 2002;62:3395–401.
11. Howe LR, Chang SH, Tolle KC, et al. HER2/neu-induced mammary tumorigenesis and angiogenesis are reduced in cyclooxygenase-2 knockout mice. Cancer Res 2005;65:10113–9.
12. Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res 1998;58:409–12.
13. 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.
14. Stolina M, Sharma S, Lin Y, et al. Specific inhibition of cyclooxygenase-2 restores antitumor reactivity by altering the balance of IL-10 and IL-12 synthesis. J Immunol 2000;164:361–70.
15. Zweifel BS, Davis TW, Ornberg RL, Masferrer JL. Direct evidence for a role of cyclooxygenase-2-derived prostaglandin $E_2$ in human head and neck xenograft tumors. Cancer Res 2002;62:6706–11.
16. Steinbach G, Lynch PM, Phillips RK, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000;342:1946–52.
17. Bertagnolli MM, Eagle CJ, Zauber AG, et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med 2006;355:873–84.
18. Edelman MJ, Watson D, Wang X, et al. Eicosanoid modulation in advanced lung cancer: cyclooxygenase-2 expression is a positive predictive factor for celecoxib + chemotherapy-Cancer and Leukemia Group B Trial 3023. J Clin Oncol 2008;26:848–55.
19. Sheng H, Shao J, Washington MK, DuBois RN. Prostaglandin $E_2$ increases growth and motility of colorectal carcinoma cells. J Biol Chem 2001;276:18075–81.
20. Buchanan FG, Wang D, Bargiacchi F, DuBois RN. Prostaglandin $E_2$ regulates cell migration via the intracellular activation of the epidermal growth factor receptor. J Biol Chem 2003;278:35451–7.
21. Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN. Modulation of apoptosis and Bcl-2 expression by prostaglandin $E_2$ in human colon cancer cells. Cancer Res 1998;58:362–6.


Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/2/11/957/1731621/957.pdf by guest on 17 July 2024

22. 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.
23. Huang M, Stolina M, Sharma S, et al. Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-regulation of interleukin 10 and down-regulation of interleukin 12 production. Cancer Res 1998;58:1208–16.
24. Dohadwala M, Yang SC, Luo J, et al. Cyclooxygenase-2-dependent regulation of E-cadherin: prostaglandin $E_2$ induces transcriptional repressors ZEB1 and Snail in non-small cell lung cancer. Cancer Res 2006;66:5338–45.
25. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci U S A 1997;94:3336–40.
26. Gallo O, Masini E, Bianchi B, Bruschini L, Paglierani M, Franchi A. Prognostic significance of cyclooxygenase-2 pathway and angiogenesis in head and neck squamous cell carcinoma. Hum Pathol 2002;33:708–14.
27. Moraitis D, Du B, De Lorenzo MS, et al. Levels of cyclooxygense-2 are increased in the oral mucosa of smokers: evidence for the role of epidermal growth factor and its ligands. Cancer Res 2005;65:664–70.
28. Piper PJ, Vane JR, Wyllie JH. Inactivation of prostaglandins by the lungs. Nature 1970;225:600–4.
29. Hamberg M, Samuelsson B. The structure of the major urinary metabolite of prostaglandin $E_2$ in man. J Am Chem Soc 1969;91:2177–8.
30. Hamberg M, Samuelsson B. On the metabolism of prostaglandins E1 and E2 in man. J Biol Chem 1971;246:6713–21.
31. Ferretti A, Flanagan VP, Roman JM. Quantitative analysis of 11 α-hydroxy-9,15-dioxo-2,3,4,5,20-pentanor-19-carboxyprostanoic acid, the major urinary metabolite of E prostaglandins in man. Anal Biochem 1983;128:351–8.
32. Seyberth HW, Sweetman BJ, Frolich JC, Oates JA. Quantifications of the major urinary metabolite of the E prostaglandins by mass spectrometry: evaluation of the method's application to clinical studies. Prostaglandins 1976;11:381–97.
33. Murphey LJ, Williams MK, Sanchez SC, et al. Quantification of the major urinary metabolite of $PGE_2$ by a liquid chromatographic/mass spectrometric assay: determination of cyclooxygenase-specific $PGE_2$ synthesis in healthy humans and those with lung cancer. Anal Biochem 2004;334:266–75.
34. Johnson JD, Schmidt CR, Shrubsole MJ, et al. Urine PGE-M: a metabolite of prostaglandin $E_2$ as a potential biomarker of advanced colorectal neoplasia. Clin Gastroenterol Hepatol 2006;4:1358–65.
35. Duffield-Lillico AJ, Boyle JO, Zhou K, et al. Levels of prostaglandin E metabolite and leukotriene E4 are increased in the urine of smokers. Evidence that celecoxib shunts arachidonic acid into the 5-lipoxygenase pathway. Cancer Prev Res 2009;2:322–9.
36. Gross ND, Boyle JO, Morrow JD, et al. Levels of prostaglandin E metabolite, the major urinary metabolite of prostaglandin $E_2$, are increased in smokers. Clin Cancer Res 2005;11:6087–93.
37. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007;356:1944–56.
38. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst 2008;100:261–9.
39. 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.
40. Gallo O, Franchi A, Magnelli L, et al. Cyclooxygenase-2 pathway correlates with VEGF expression in head and neck cancer. Implications for tumor angiogenesis and metastasis. Neoplasia 2001;3:53–61.
41. Cohen EG, Almahmeed T, Du B, et al. Microsomal prostaglandin E synthase-1 is overexpressed in head and neck squamous cell carcinoma. Clin Cancer Res 2003;9:3425–30.
42. Fujino H, West KA, Regan JW. Phosphorylation of glycogen synthase kinase-3 and stimulation of T-cell factor signaling following activation of $EP_2$ and $EP_4$ prostanoid receptors by prostaglandin $E_2$. J Biol Chem 2002;277:2614–19.
43. Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin $E_2$ transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Nat Med 2002;8:289–93.
44. Goessling W, North TE, Loewer S, et al. Genetic interaction of $PGE_2$ and Wnt signaling regulates developmental specification of stem cells and regeneration. Cell 2009;136:1136–47.
45. Pavlovic S, Du B, Sakamoto K, et al. Targeting prostaglandin $E_2$ receptors as an alternative strategy to block cyclooxygenase-2-dependent extracellular matrix-induced matrix metalloproteinase-9 expression by macrophages. J Biol Chem 2006;281:3321–8.
46. Sharma S, Stolina M, Yang SC, et al. Tumor cyclooxygenase-2-dependent suppression of dendritic cell function. Clin Cancer Res 2003;9:961–68.
47. Choy H, Milas L. Enhancing radiotherapy with cyclooxygenase-2 enzyme inhibitors: a rational advance? J Natl Cancer Inst 2003;95:1440–52.
48. Panje WR. Regression of head and neck carcinoma with a prostaglandin-synthesis inhibitor. Arch Otolaryngol 1981;107:658–63.
49. Solomon SC, Wittes J, Finn PV, et al. Cardiovascular risk of celecoxib in 6 randomized placebo-controlled trials. Circulation 2008;117:2104–13.
50. Mutter R, Lu B, Carbone DP, et al. 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. Clin Cancer Res 2009;15:2158–65.
51. Wirth LJ, Haddad RI, Lindeman NI, et al. Phase I study of gefitinib plus celecoxib in recurrent or metastatic squamous cell carcinoma of the head and neck. J Clin Oncol 2005;23:6976–81.
52. Klapan I, Katic V, Culo F, Cuk V. Prognostic significance of plasma prostaglandin E concentration in patients with head and neck cancer. J Cancer Res Clin Oncol 1992;118:308–13.


Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/2/11/957/1731621/957.pdf by guest on 17 July 2024