

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

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### Abstract

Cyclooxygenase (COX)-derived prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) 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<sub>2</sub> 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<sub>2</sub> (PGE<sub>2</sub>) from arachidonic acid. There are two isoforms of COX, designated COX-1 and COX-2. COX-1 is constitutively expressed in most tissues and medi-

ates 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<sub>2</sub> in neoplastic and inflamed tissues (4–7). Multiple lines of evidence suggest that COX-2 and PGE<sub>2</sub> play a significant role in carcinogenesis. Levels of COX-2 and PGE<sub>2</sub> 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<sub>2</sub> synthesis, or an anti-PGE<sub>2</sub> 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<sub>2</sub>, and malignancy. PGE<sub>2</sub> can stimulate cell proliferation, motility, and angiogenesis while inhibiting apoptosis and immune surveillance (3, 19–23). COX-2-derived PGE<sub>2</sub> may also promote metastasis by stimulating epithelial-mesenchymal transition and cell invasion (24,

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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.

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

25). In HNSCC, high levels of intratumor COX-2 and PGE<sub>2</sub> 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<sub>2</sub> in plasma does not accurately reflect endogenous production of PG (28). 15-Hydroxyprostaglandin dehydrogenase initiates the catabolism of PGE<sub>2</sub>, leading to a stable end metabolite (PGE-M), or 11- $\alpha$ -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<sub>2</sub> 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  $-80^{\circ}\text{C}$ . 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 (<sup>2</sup>H<sub>6</sub>)PGE-M to quantify PGE<sub>2</sub> 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 (<sup>2</sup>H<sub>6</sub>)-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  $\rightarrow$  336 and *m/z* 391  $\rightarrow$  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  $\pm$  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

**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 $\pm$ SD	66.2 $\pm$ 8.9	64.2 $\pm$ 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 $\pm$ SD	30.2 $\pm$ 28.6	28.2 $\pm$ 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 pro-

gression 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

**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;  $P = 0.07$ ).

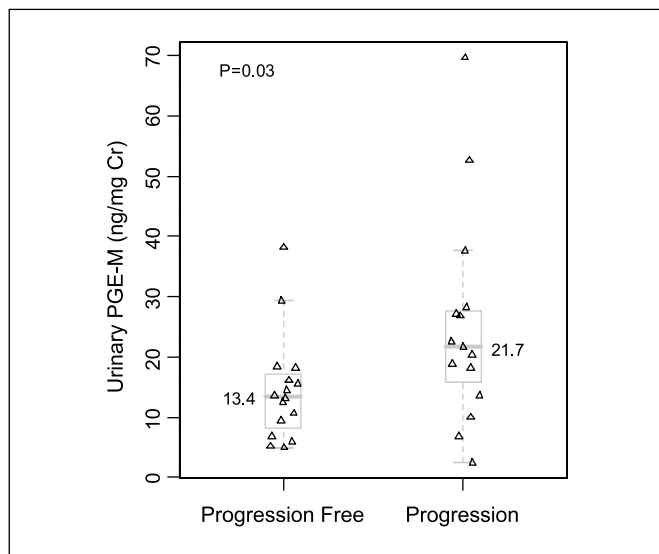
#### **Relationship between pretreatment urinary PGE-M and stage-specific progression-free and overall survival**

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/

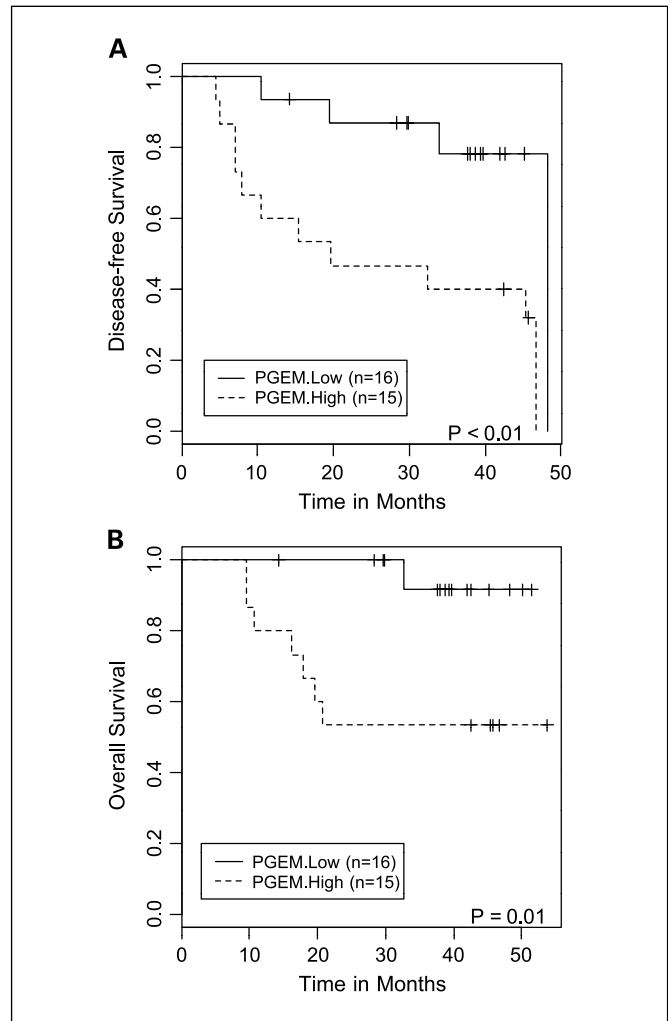
T2, node-negative, and tumor-node-metastasis stage I/II disease, high baseline urinary PGE-M was associated with increased risk of disease progression, and the association was significant or close to significance (*P* values were <0.01, 0.02 and 0.07, respectively; Fig. 3A, C, and E). Consistent with 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<sub>2</sub> 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



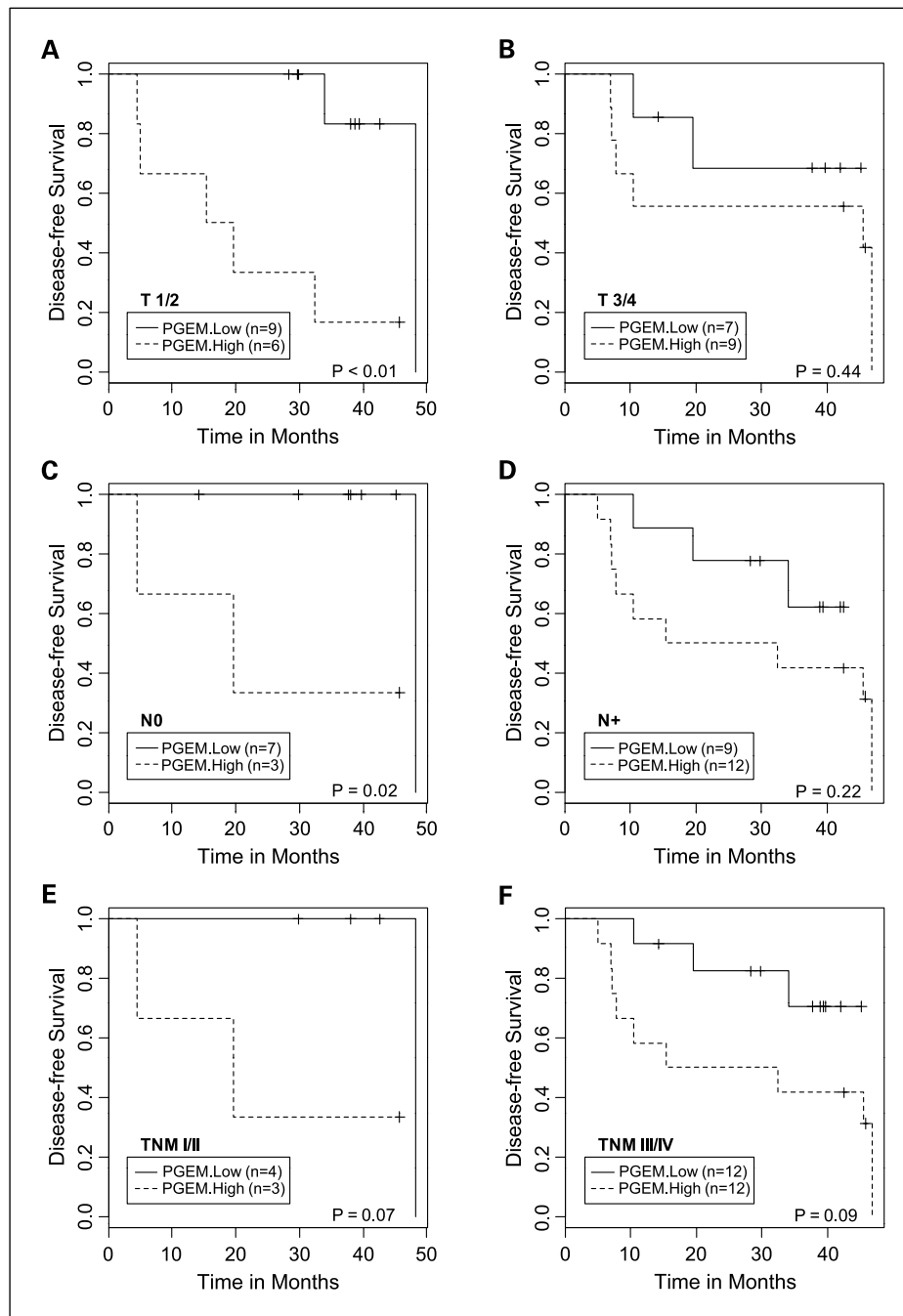
**Fig. 1.** Significantly higher baseline urinary PGE-M levels were observed for patients developing disease progression (median, 21.7; range, 2.4-69.7) compared with those without progression (median, 13.4; range, 4.9-38.2; *P* = 0.03 Wilcoxon rank-sum test). Distribution of urinary PGE-M levels in 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.

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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> to PGE<sub>2</sub>, may

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**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.

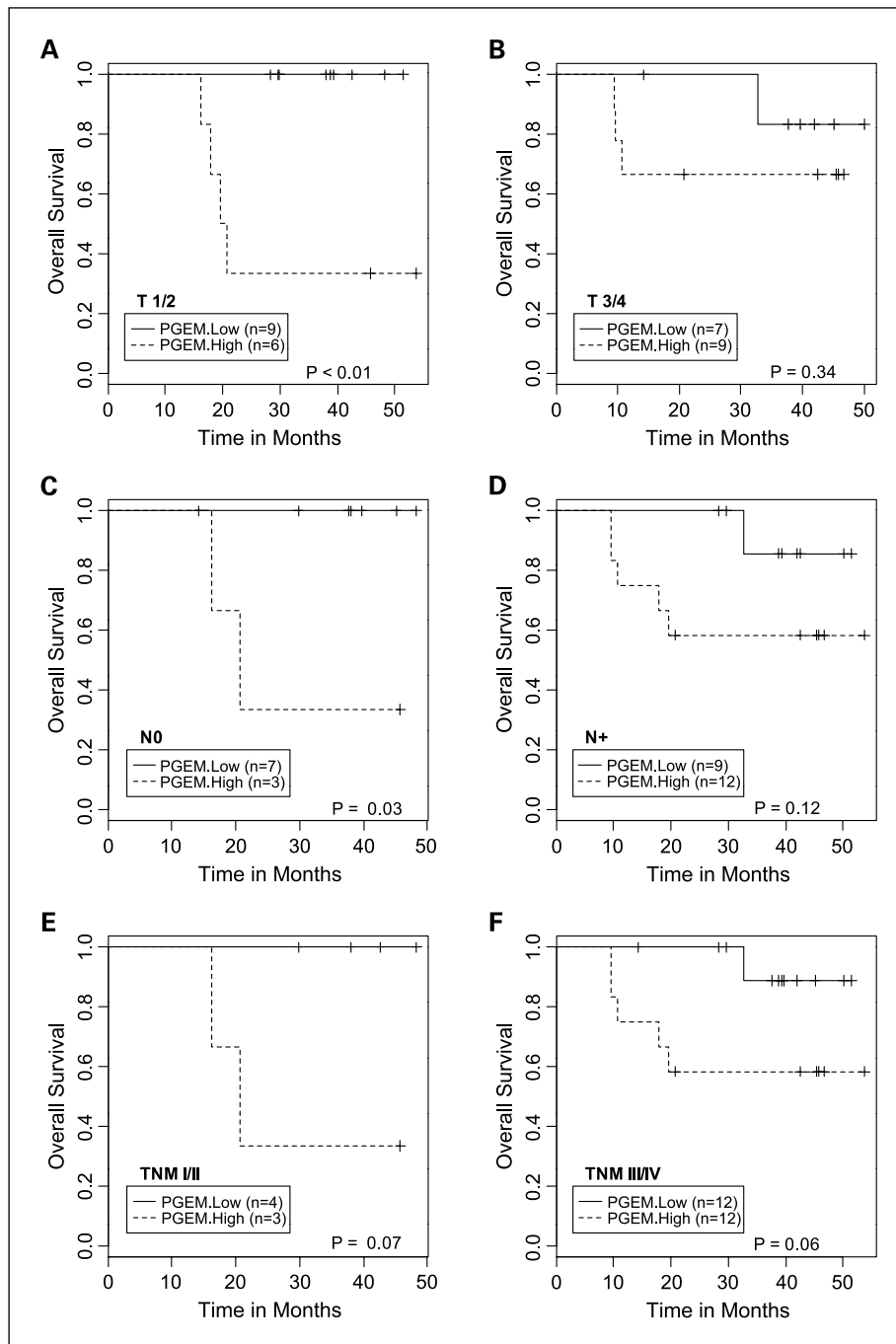
also contribute to increased levels of PGE<sub>2</sub> in HNSCC (41). In addition to the tumor being a likely source of increased urinary PGE-M, smoking is an independent cause of increased COX-2 activity resulting in elevated urinary PGE-M (35). Although the location of the smoking-related increase 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<sub>2</sub> 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<sub>2</sub> versus smoking-related increases in mucosal PGE<sub>2</sub> 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<sub>2</sub> has numerous effects that can potentially explain the link between elevated urinary PGE-M levels and a poor prognosis. PGE<sub>2</sub> exerts its biological actions by binding to any one of four E-series

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<sub>2</sub> or EP<sub>4</sub> activates T-cell transcription factor/ $\beta$ -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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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.

causing an imbalance between type 1 and type 2 cytokines (46). In addition to explaining how increased levels of PGE<sub>2</sub> can contribute to disease progression, these effects of PGE<sub>2</sub> 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<sub>2</sub> 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.

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