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Clinical Cancer Research

Tobacco Use in Human Papillomavirus–Positive Advanced Oropharynx Cancer Patients Related to Increased Risk of Distant Metastases and Tumor Recurrence

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Abstract

Purpose: The goal of this study was to examine the effect of tobacco use on disease recurrence (local/regional recurrence, distant metastasis, or second primary) among patients with human papillomavirus (HPV)–positive squamous cell carcinoma of the oropharynx (SCCOP) following a complete response to chemoradiation therapy.

Experimental Design: Between 1999 and 2007, 124 patients with advanced SCCOP (86% with stage IV) and adequate tumor tissue for HPV analysis who were enrolled in one of two consecutive University of Michigan treatment protocols were prospectively included in this study. Patients were categorized as never-, former, or current tobacco users. The primary end points were risk of disease recurrence and time to recurrence; secondary end points were disease-specific survival and overall survival.

Results: One hundred and two patients (82.3%) had HPV-positive tumors. Over two thirds (68%) of patients with HPV-positive tumors were tobacco users. Among HPV-positive patients, current tobacco users were at significantly higher risk of disease recurrence than never-tobacco users (hazard ratio, 5.2; confidence interval, 1.1-24.4; $P = 0.038$). Thirty-five percent of HPV-positive ever tobacco users recurred compared with only 6% of HPV-positive never users and 50% of HPV-negative patients. All HPV-negative patients were tobacco users and had significantly shorter times to recurrence ($P = 0.002$), and had reduced disease-specific survival ($P = 0.004$) and overall survival ($P < 0.001$) compared with HPV-positive patients. Compared with HPV-positive never-tobacco users, those with a tobacco history showed a trend for reduced disease-specific survival ($P = 0.064$) but not overall survival ($P = 0.221$).

Conclusions: Current tobacco users with advanced, HPV-positive SCCOP are at higher risk of disease recurrence compared with never-tobacco users. *Clin Cancer Res; 16(4); 1226–35. ©2010 AACR.*

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Head and neck squamous cell carcinoma is the eighth most common malignancy worldwide (1) and represents ~5% of new cancer diagnoses worldwide annually (2). Over the past three decades, there has been a steady increase in the incidence of tonsil and tongue squamous cell carcinomas (3, 4). Recent evidence has identified high-risk human papillomavirus (HPV), particularly HPV-16, as a causative agent for a subset of head and neck squamous cell carcinomas, accounting for over 50% of squamous cell carcinomas of the oropharynx (SCCOP) in the United States (5–9). HPV-positive SCCOP has a distinct risk factor profile (6) and oncogenic mechanism (10, 11), and likely portends a more favorable prognosis than HPV-negative SCCOP (5, 7, 12–17). Despite its effect on prognosis, tumor HPV status has not yet been used to alter therapeutic management. The most popular current treatment for advanced SCCOP, regardless of HPV status, involves concurrent chemoradiation

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Translational Relevance

This study shows that patients with advanced, HPV-positive squamous cell carcinoma of the oropharynx (SCCOP) who are current smokers are at higher risk of disease recurrence and tend to have worse disease-specific survival compared with never-smokers with human papillomavirus (HPV)–positive SCCOP. This supports the use of less aggressive treatment for never-smokers with HPV-positive cancer compared with their smoking counterparts. The development of clinical protocols to this effect is already under way at the University of Michigan based on the results of this study. This research is entirely original, applicable to clinical practice, and based on prior evidence that HPV-positive patients have better outcomes and are more likely to be nonsmokers than HPV-negative patients. We also test a well-supported hypothesis with reliable and robust statistics. We feel that this study will be of utmost interest to the readership of Clinical Cancer Research and trigger further research into the management of patients with SCCOP.

therapy (5, 18–22), which carries with it various morbidities (19, 20).

Conflicting data exist on the combined effect of HPV and tobacco use on prognosis. Some investigators have found that nonsmoking patients with HPV-positive tonsillar squamous cell carcinoma have a better disease-specific survival (DSS) rate than their smoking counterparts (13). Many studies, however, report no interaction between HPV and smoking on survival (6, 12, 23, 24). To the best of our knowledge, no studies have focused on tobacco and HPV in relation to tumor recurrence or DM.

The purpose of this investigation was to test the hypothesis that tobacco use among HPV-positive advanced SCCOP patients increases the risk of disease recurrence, including local/regional recurrence (LR), distant metastasis (DM), and second primary cancer (SP).

Materials and Methods

Study population. One hundred and twenty-four patients treated at the University of Michigan between 1999 and 2007 with stage III or IV SCCOP were prospectively included in this study. All patients were consented for and participated in the University of Michigan Head and Neck Specialized Programs of Research Excellence program, had a pretreatment biopsy with adequate tumor tissue for DNA extraction and HPV assessment, and were treated according to one of two consecutive prospective clinical trial protocols (described as "cohorts" throughout the article). Forty-one patients were treated on a phase II trial [University of Michigan Cancer Center (UMCC)-9921] with induction chemotherapy (cisplatin and 5-fluorouracil) followed by concomitant cisplatin and full course radiation [70 Gray (Gy)], or surgical resection and full course radiation for nonresponders to induction chemotherapy as previously reported (5); 83 were treated with concomitant carboplatin, docetaxel, and intensity-modulated radiation therapy (70 Gy) on a more recent UMCC-0221 trial. A few patients who did not participate in the UMCC-0221 trial but received identical treatment were classified as UMCC-0221–like patients, and therefore, UMCC-0221 and UMCC-0221–like were considered one cohort.

Clinical and pathologic characteristics. Clinical and pathologic characteristics of interest included gender, age, primary tumor site, tumor-node-metastasis staging as defined by the American Joint Committee on Cancer, treatment cohort, and patient status at last follow-up. Primary tumor site was determined by direct laryngoscopy and biopsy was done at the University of Michigan. Charts were reviewed for disease recurrence, including LR, DM, or SP tumors. A LR was defined as a positive biopsy in the area of the primary tumor (local) or the cervical lymphatic region after a complete response to treatment as determined by a negative posttreatment biopsy and/or scan. A DM was identified through biopsy or positron emission tomography scan and verified as such by the University of Michigan Multidisciplinary Tumor Board. A tumor was considered a related SP if it was squamous cell carcinoma, occurred over 2 y after the patient had been disease free from the original SCCOP, and was at least 3 cm distant from the original site.

Tobacco history. History of tobacco use was determined through two methods: chart review and self-reporting at the time of study enrollment. Patients were categorized according to their use of cigarettes, cigars, pipes, or chewing tobacco as current (including those who quit <1 y before diagnosis), former (those who quit ≥1 y before diagnosis), or never-tobacco users. Never-tobacco users were those who had never used chewing tobacco, cigars, or pipes in their lifetime and those who smoked less than the equivalent of one pack per day per year—one pack-year—in their lifetime. Former tobacco users were then separated into an early-cessation group (those who quit ≥20 y before diagnosis) and a late-cessation group (those who quit <20 y before diagnosis). To evaluate the cigarette dose-effect, a subgroup analysis was conducted on cigarette users.

HPV detection. Using DNA extracted from tumor within the pretreatment biopsy, HPV analysis was accomplished with a sensitive, specific, quantitative method developed in Dr. David Kurnit's laboratory at the University of Michigan as previously described (5, 25) or by SensiGen LLC of Ann Arbor, MI (now owned by Sequenom), using Dr. Kurnit's licensed technology. In brief, HPV analysis involved real-time competitive PCR and matrix-assisted laser desorption/ionization-time of flight mass spectroscopy separation of products on a matrix-loaded silicon chip array. This method uses primers unique to the HPV-E6 region of each of 13 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) as well as HPV-6 and HPV-11. HPV type positivity was scored as positive by the presence of a discrete mass

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Table 1. Clinical/pathologic characteristics (A) and disease recurrence by tobacco status (B) among all patients with SCCOP

A. Patient demographics, tumor pathology, tobacco use, and disease recurrence for oropharyngeal cancer patients

Demographics	Total	HPV+ (*n* = 102)	HPV– (*n* = 22)
		Number (percent)	
Gender			
Female	21	13 (12.8)	8 (36.4)
Male	103	89 (87.3)	14 (63.6)
Age	57.2	56.5	60.3
Race or Ethnicity			
White, non-Hispanic	116	98 (96)	18 (81.8)
Black, non-Hispanic	5	2 (2.0)	3 (13.6)
Unknown	3	2 (2.0)	1 (4.6)
Primary tumor site			
Base of tongue	62	51 (50)	11 (50)
Tonsil	57	49 (48)	8 (36.4)
Oropharynx Unspecified	5	2 (2.0)	3 (13.6)
Tumor stage			
III	17	13 (12.7)	4 (18.2)
IVA	92	76 (74.5)	16 (72.7)
IVB	15	13 (12.8)	2 (9.1)
Primary tumor (T class)			
1	13	13 (12.8)	0 (0)
2	38	35 (34.3)	3 (13.6)
3	35	27 (26.5)	8 (36.4)
4	38	27 (26.5)	11 (50)
Lymph nodes (N class)			
0	15	11 (10.8)	4 (18.2)
1	16	11 (10.8)	5 (22.7)
2a	17	13 (12.7)	4 (18.2)
2b	37	32 (31.4)	5 (22.7)
2c	24	22 (21.6)	2 (9.1)
3	15	13 (12.7)	2 (9.1)
Tobacco use			
Never	33	33 (32.4)	0 (0)
Former early-cessation	23	20 (19.6)	3 (13.6)
Former late-cessation	29	26 (25.5)	3 (13.6)
Current	39	23 (22.6)	16 (72.7)
Disease progression events			
LR	10	6 (5.9)	4 (18.2)
DM	17	12 (11.8)	5 (22.7)
SP	6	5 (4.9)	1 (4.6)

B. Patients with recurrent disease (LR, DM, and related SP) by HPV status and tobacco history (never, former, or current)

	HPV+ (*n* = 102)	HPV– (*n* = 22)
Never	2/33 (6.1%)	0/0
LR	0	0
DM	1	0
SP	1	0
Former	9/46 (19.6%)	3/6 (50%)
LR	3	1
DM	5	1
SP	1	1

(*Continued on the following page*)

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Table 1. Clinical/pathologic characteristics (A) and disease recurrence by tobacco status (B) among all patients with SCCOP (Cont'd)

B. Patients with recurrent disease (LR, DM, and related SP) by HPV status and tobacco history (never, former, or current)

	HPV+ (*n* = 102)	HPV– (*n* = 22)
Current	8/23 (34.8%)*	8/16 (50%)
LR	3	4
DM	6	4
SP	2	2
Total	19/102 (18.6%)	11/22 (50%)

NOTE: Table 1A only includes second primaries related to original SCCOP (this excludes a prostate cancer, B-cell lymphoma, and lung adenocarcinoma; see Table 2B).

Abbreviations: SCCOP, squamous cell carcinoma of the oropharynx, *n*, number of patients.

*Three HPV-positive current tobacco users suffered two events each (LR/DM; LR/DM; LR/SP), thus 11 events occurred in eight patients.

spectroscopy peak for the high-risk HPV type–specific E6 amplicon corres-ponding to the internal HPV type–specific competitor (see refs. 5, 26).

Immunohistochemistry. As previously described (26), tissue microarray slides were deparaffinized, rehydrated, and peroxidase quenched (DAKO Cytomation). For p16 staining, antigen retrieval using citrate buffer was used. Slides were blocked with horse serum, washed, incubated with primary antibody [16P04; Lab-Vision (UMCC-9921), or the CINtec p16ink4a antibody (MTM Laboratories, Inc. (UMCC-0221)], washed, and probed with avidin/biotin peroxidase (ABC kit; Vector Laboratories). Proportion and intensity of staining were scored by a pathologist (K.G.C., who was blinded to the clinical outcome) using a scale of 1 to 4 (1, < 5%; 2, 5-20%; 3, 21-50%; and 4, 51-100% tumor staining). Intensity scored as 1 indicates no staining, 2 indicates low intensity, 3 indicates moderate intensity, and 4 indicates high intensity. Scores for multiple cores from each patient were averaged. A retest confirmed that both antibodies give the same result on the same tumors.

Study end points. The primary end point was time to recurrence. Secondary end points were DSS and overall survival (OS). DSS was measured from the date of study enrollment to the date of death caused by an LR, DM, or SP. OS was measured from the date of study enrollment to the date of death from any cause.

Statistical analysis. The analysis assessed the tobacco effect among HPV-positive patients on time to recurrence, DSS, and OS. Patients with persistent disease following treatment were excluded from the analysis of time to recurrence but included in the analysis of DSS and OS. The Kaplan-Meier method was used to depict the survival estimates between categories of a single discrete variable, whereas the Cox proportional hazard models were used to assess single-variable and multivariable effects while adjusting for the cohort effect.

Patients from the two treatment cohorts were analyzed together. However, each reported statistical analysis accounted for the cohort effect. To investigate the association between discrete variables, such as gender and T class, Cochran-Mantel-Haenszel statistics were used. The association between HPV and continuous variables, such as age, was examined by logistic regression.

To analyze the prognostic effects of T class and tobacco status on outcome, three models were constructed as follows: (*a*) a model with T class and cohort effect, (*b*) a model with tobacco status and cohort effect, and (*c*) a model with both T class and tobacco status in addition to cohort effect. Models 1 and 3, as well as 2 and 3, were used to assess one variable effect beyond the effects of the other variable while controlling for cohort effect. Likelihood ratio statistics were used to compare the models. Including HPV-negative subjects, a similar analysis was used to assess the effect of tobacco beyond HPV status and T class. All statistical analyses were done using SAS v9.1. A two-tailed *P* value of 0.05 or less was considered to be statistically significant.

Results

Study population. The 124 patients from both cohorts were similar in age, race, primary tumor site, clinical stage, tumor classification (T class), and nodal classification (N class). The UMCC-9921 trial was completed before the start of the UMCC-0221 trial and therefore had longer follow-up times. The median follow-up time was 76 months [95% confidence interval (95% CI), 71-85 months] and 36 months (95% CI, 31-39 months) for UMCC-9921 and UMCC-0221, respectively. The incidence of HPV-positive tumors was significantly higher ($P = 0.001$) in the more recent UMCC-0221 trial (90% HPV positive) compared with the UMCC-9921 trial (66% HPV positive). In the more recent UMCC-0221 trial, there was a higher proportion of never-tobacco users and former tobacco users in the early cessation group (those who quit ≥20 years before diagnosis) compared with the UMCC-9921 trial ($P = 0.03$). In addition, the proportion of women (12%) in the UMCC-0221 trial was lower than in the UMCC-9921 trial (27%; $P = 0.04$). The patients in UMCC-9921 who did not respond

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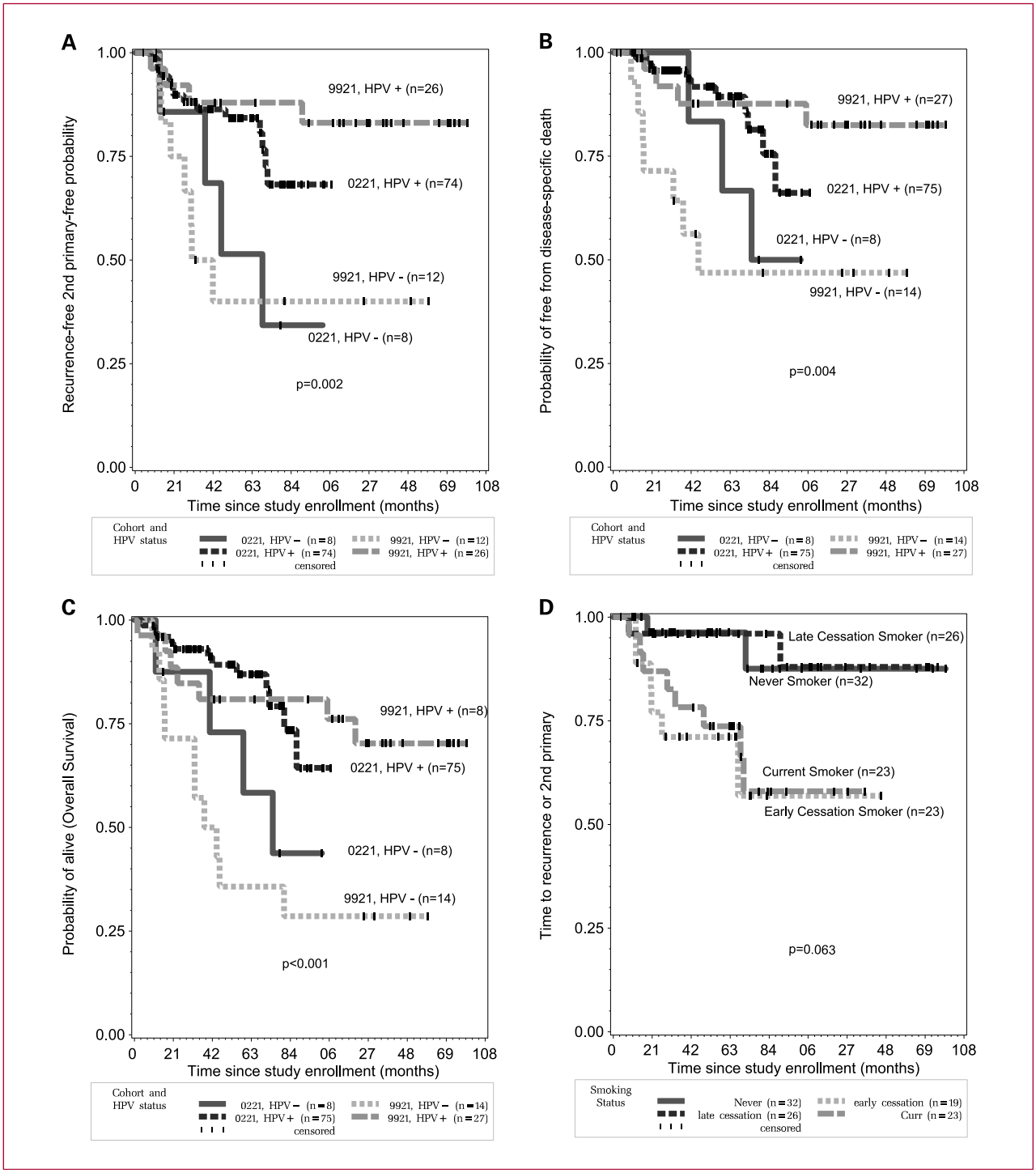

Fig. 1. A, time to recurrence by HPV status for each cohort; B, DSS by HPV status for each cohort; C, OS by HPV status for each cohort; D, time to recurrence by tobacco use among HPV-positive patients for each cohort.

to an initial cycle of neoadjuvant chemotherapy underwent definitive surgery with postoperative radiation, whereas all patients in UMCC-0221 completed definitive chemoradiation and were followed for recurrence and possible salvage surgery as necessary.

HPV-positive versus HPV-negative patients. Controlling for the cohort effect, HPV-positive and HPV-negative patients were similar with respect to race, clinical stage, and N class. HPV-positive patients were significantly younger (P = 0.04), more likely to be male (P = 0.04), had less advanced T

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class ($P = 0.002$), and were more likely to be never-tobacco users ($P < 0.0001$; Table 1A). Compared with HPV-negative patients, those with HPV-positive tumors had significantly longer times to recurrence ($P = 0.002$; Fig. 1A) and more favorable DSS ($P = 0.004$; Fig. 1B) and OS ($P < 0.001$; Fig. 1C) among both UMCC-9921 and UMCC-0221 cohorts, regardless of tobacco history. HPV-negative patients were 3.5 times more likely ($P = 0.002$) to develop disease recurrence (hazard ratio, 3.5; 95% CI, 1.6-7.9) than HPV-positive patients. These findings are consistent with our previous reports (5, 26) and those of other groups (4, 12–16, 24, 27, 28).

HPV status and disease recurrence. Of the 124 patients, 102 (82%) had HPV-positive tumors (Table 1A). HPV-16 was detected in 95% of HPV-positive tumors, followed by HPV-35 in 3% and HPV-18 in 2% of tumors. Of the 102 HPV-positive patients, 33 (32.3%) were never-tobacco users and 69 (67.6%) had used tobacco in their lifetime. Thus, the majority of patients with HPV-positive tumors were tobacco users. In fact, 23 of 102 (22.6%) were current tobacco users and 46 of 102 (45%) were former users. The patient demographics, tumor pathology, tobacco status, and disease recurrence events (LR, DM, and histologically related SP) are depicted in Table 1A and B. Note that the study population consisted of patients with very advanced disease, with the majority, 107 of 124 (86.3%), having stage IV disease at diagnosis. Thirty patients (30 of 124; 24.2%) suffered disease recurrence events, including SP tumors of squamous cell histology (Table 2A). Of these 30 patients, 23 (76.7%) have died because of their SCCOP. Three other patients with histologically unrelated SP tumors were censored at the last follow-up for the recurrent disease analysis (Table 2B). Fourteen patients used cigars ($n = 7$), oral tobacco ($n = 4$), or pipes ($n = 3$) instead of or in addition to cigarettes. Three of the 14 (21.4%) suffered disease recurrence—1 LR and 2 DMs—and all 3 were cigar users.

HPV status, smoking, and disease recurrence. HPV-positive current tobacco users were over five times more likely to develop a recurrence compared with never users ($P = 0.038$; hazard ratio, 5.2; 95% CI, 1.1-24.4; Table 3). As depicted in Table 1B, the rate of disease recurrence among HPV-positive patients was lowest among never-tobacco users (6.1%), followed by former users (19.6%), and highest among current users (34.8%). Recurrence events occurred in 17 of 69 (24.6%) HPV-positive former ($n = 9$) or current ($n = 8$) tobacco users, a 3.7 times higher rate of recurrence compared with never-tobacco users. Three HPV-positive tobacco users suffered two distinct recurrence events each (LR/DM, LR/SP, and LR/DM; Table 2A, patients 14, 16, and 19). Among HPV-negative patients (all of whom were tobaccos users), 50% (11 of 22) had disease recurrence, among both former (3 of 6) and current (8 of 16) tobacco users.

Twenty-nine of 33 (87.9%) HPV-positive never-tobacco users are currently alive and disease free. Two HPV-positive never-tobacco users (Table 2A, patients 1 and 2) suffered disease recurrence; of these, one died and one is alive with disease. Patient no. 1 developed lung metastases and died 2 years following primary diagnosis; of note, he was a welder by occupation with a history of second-hand smoke exposure. Patient no. 2 developed a second primary squamous cell carcinoma of the gingiva and is alive with disease. Another two HPV-positive never-tobacco users died disease free from other causes: suicide and an idiopathic lung/kidney disorder. The fourth HPV-positive never-tobacco user who died had an undiagnosed dihydropyrimidine dehydrogenase deficiency and died 2 months after primary diagnosis from 5-fluorouracil toxicity; he was never disease free and therefore excluded from the time-to-disease recurrence analysis. One other HPV-positive, former tobacco user was never disease free and was excluded from the time-to-disease recurrence analysis.

The overall effect of ever using tobacco exhibited a strong trend for increased risk of disease recurrence among HPV-positive patients but, after adjusting for cohort effect, did not reach statistical significance ($P = 0.063$; Fig. 1D). Similarly, the necessary adjustment for the cohort effect also influenced the significance level of tobacco use, which showed a strong trend for an adverse effect on DSS ($P = 0.064$), but not OS ($P = 0.221$), among HPV-positive tobacco users. Although HPV-positive current tobacco users had a 7.2 times greater risk of dying from their disease (DSS) than never-tobacco users, after adjusting for the cohort effect, this also fell slightly short of statistical significance ($P = 0.07$; Table 3).

Curiously, when HPV-positive former tobacco users were subdivided into an early-cessation group (those who quit ≥20 years before diagnosis; $n = 20$) and a late cessation group (those who quit <20 years before diagnosis; $n = 26$), the tobacco effect on time-to-disease recurrence was significant ($P = 0.043$) using the Cox model and adjusting for cohort effect. Among HPV-positive former tobacco users, early-cessation users were *five* times more likely to develop a recurrence than the never users ($P = 0.03$; hazard ratio, 5; 95% CI, 1.07-23.8). Conversely, former late-cessation users had nearly the same low risk of recurrence as never users ($P = 0.97$; hazard ratio, 1.05; 95% CI, 0.14-7.7).

HPV-positive status, T class, and pack-years. Among HPV-positive patients, more advanced T class was associated with current tobacco usage ($P = 0.02$). As a single variable, T class was a significant prognostic indicator for recurrence among HPV-positive subjects ($P = 0.01$; hazard ratio, 2; 95% CI, 1.2-3.5). Among both HPV-positive and HPV-negative patients, T class was significantly associated with a higher likelihood of recurrence ($P = 0.0004$). We explored the possible tobacco pack-year dose effect among combined former and current HPV-positive tobacco users on disease recurrence and did not find a dose-response relationship.

HPV status with p16 expression. Of the 124 patients tested for HPV, p16 staining data were available for 113. Of these, 11 of 113 (9.7%) had discrepancies between p16 expression and HPV status. Seven tumors were HPV positive/p16 negative and four were HPV negative/p16 positive. Of the HPV-positive/p16-negative cases, the majority (4 of 7) had low viral copy number. HPV-positive/p16-negative tumors are thought to contain transcriptionally inactive E7 (29), which our series suggests may be more common in tumors with low HPV copy number.

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Table 2. Clinical/pathologic characteristics of all patients with disease recurrence (A) and those with unrelated second primaries (B)

A. Clinical and pathologic characteristics of all patients who developed disease recurrence (LR, DM, and histologically related SP)

Patient no.	HPV status	Tobacco history	Pack-years (cigarette use only)	Primary tumor site	T class	N class	Disease recurrence event (LR, DM, or SP)	Site of disease recurrence event	Patient status at last follow-up (in reference to SCCOP)
1	Positive	Never*	0	Tonsil	4	2b	DM	Lung	Dead with disease
2	Positive	Never	0	Tonsil	3	3	SP	Mandibular gingiva	Alive with disease
3	Positive	Former cigarette; quit 37 y prior	10	BOT	4	3	DM	Lung, bone	Dead with disease
4	Positive	Former cigarette; quit 37 y prior	10	BOT	4	2b	LR	Suprasternal lymph node	No evidence of disease
5	Positive	Former cigarette; quit 35 y prior	6	Tonsil	4	3	DM	Lung	Dead with disease
6	Positive	Former cigarette; quit 28 y prior	12	BOT	2	2b	DM	Bone	Dead with disease
7	Positive	Former 1 cigar per day × 8 y; quit 25 y prior	0	Tonsil	1	3	LR	Level II lymph node	Dead with disease
8	Positive	Former cigarette; quit 21 y prior	120	Tonsil	2	1	LR	Tonsil, level III lymph node	No evidence of disease
9	Positive	Former cigarette; quit 20 y prior	12	Tonsil	2	2b	SP	Tongue	Alive with disease
10	Positive	Former cigarette; quit 4 y prior	100	BOT	4	2c	DM	Lung	Dead with disease
11	Positive	Former cigarette; quit 2 y prior	60	BOT	2	3	DM	Dermis, lung	Dead with disease
12	Positive	Current cigarette	35	BOT	4	0	DM	Liver	Dead with disease
13	Positive	Current cigarette	35	Tonsil	4	0	DM	Lung	Dead with disease
14	Positive	Current cigarette	30	BOT	4	2c	LR; DM	LR: retropharyngeal space DM: lung, bone	Dead with disease
15	Positive	Current cigarette	44	BOT	4	0	DM	Bone, dermis	Dead with disease
16	Positive	Current cigarette	35	Tonsil	3	0	LR; SP	LR: digastric lymph node SP: soft palate	Dead with disease
17	Positive	Current cigarette	45	BOT	4	2b	SP	Lung	No evidence of disease
18	Positive	Current 3 cigars per week × 15 y	0	BOT	4	2c	DM	Lung, bone	Dead with disease
19	Positive	Current 2-4 cigars per week × 20 y	0	Tonsil	4	2c	LR; DM	LR: tonsil DM: lung	Dead with disease
20	Negative	Former cigarette; quit 35 y prior	80	BOT	4	1	SP	Esophagus	No evidence of disease
21	Negative	Former cigarette; quit 25 y prior	50	Oropharynx†	3	2a	LR	Tongue	Dead with disease
22	Negative	Former cigarette; quit 20 y prior	40	BOT	4	1	DM	Lung	Dead with disease
23	Negative	Current cigarette	25	Tonsil	3	2a	DM	Lung, liver	Dead with disease

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Table 2. Clinical/pathologic characteristics of all patients with disease recurrence (A) and those with unrelated second primaries (B) (Cont'd)

A. Clinical and pathologic characteristics of all patients who developed disease recurrence (LR, DM, and histologically related SP)

Patient no.	HPV status	Tobacco history	Pack-years (cigarette use only)	Primary tumor site	T class	N class	Disease recurrence event (LR, DM, or SP)	Site of disease recurrence event	Patient status at last follow-up (in reference to SCCOP)
24	Negative	Current cigarette	30	BOT	4	3	DM	Bone, liver	Dead with disease
25	Negative	Current cigarette	50	BOT	4	0	DM	Bone	Dead with disease
26	Negative	Current cigarette	75	Tonsil	4	0	LR	Pretracheal tissue	Dead with disease
27	Negative	Current cigarette	35	Oropharynx†	4	2b	LR	Tonsil	No evidence of disease
28	Negative	Current cigarette	60	BOT	4	2c	LR	Tongue	Dead with disease
29	Negative	Current cigarette	40	BOT	4	2a	DM	Dermis, lung, abdomen, bone	Dead with disease
30	Negative	Current cigarette	59	BOT	2	2a	SP	Lung	Dead with disease

B. Clinical and pathologic characteristics of patients with histologically unrelated second primary tumors

31	Positive	Never	0	Oropharynx‡	2	3	SP	Prostate§	No evidence of disease
32	Positive	Former cigarette; quit 17 y prior	20	Tonsil	1	2b	SP	Lung (B-cell lymphoma)§	No evidence of disease
33	Negative	Former cigarette; quit 12 y prior	70	Oropharynx‡	3	1	SP	Lung (adenocarcinoma)§	Dead without disease

Abbreviation: BOT, base of tongue.

*This patient had daily exposure to second hand smoke and welding fumes.

†Tumor site within oropharynx unspecified.

‡Tumor site within oropharynx unspecified.

§SP tumor unrelated to original SCCOP based on location (patient 31) or tumor cell histology (patients 32 and 33). Patients 31 and 32 are alive with no evidence of SCCOP recurrence after 8 and 1.5 y of follow-up, respectively. Patient 33 died from the lung adenocarcinoma after 4 y without evidence of SCCOP recurrence. These three patients were censored at the last follow-up time for statistical analysis of time-to-disease recurrence.

Interestingly, more than one third of the discordant HPV/p16 results were HPV negative/p16 positive. Whether these represent tumors with high risk HPV types not represented in our panel or another mechanism, such as mutation or inactivation of the retinoblastoma protein, remains to be determined. Curiously, only 2 of 11 patients with discordant HPV/p16 died of their disease; both were in the UMCC 9921 cohort, developed DM, were current or former smokers, had less than one HPV copy/cell, and were p16 negative.

Discussion

It is now well established that HPV-positive SCCOP patients have a more favorable outcome and are more likely to be nonsmokers (4, 5, 9, 13, 23, 27, 28) than HPV-negative SCCOP patients. The patients in our study with HPV-positive tumors had a lower risk of disease recurrence and more favorable DSS and OS when compared with patients with HPV-negative tumors. However, never-tobacco users comprised only a minority of the HPV-positive patients. Over two thirds (68%) of our HPV-positive patients were former or current tobacco users. Strikingly, HPV-positive current tobacco users were five times more likely to develop disease recurrence, including LR, DM, or second primary tumor, compared with never-tobacco users. Former tobacco users were nearly three times more likely to have recurrence than never users in HPV-positive patients. Over the duration of this study, the recurrence rate was only 6% among HPV-positive never users, compared with 20% of former users and 35% of current users, all of which were lower than the recurrence rate of 50% among HPV-negative patients.

Tobacco use also exhibited a strong statistical trend for an adverse effect on DSS among HPV-positive patients. Consistent with our observations, Hafkamp et al. (13) found that nonsmoking HPV-positive tonsillar cancer patients had better DSS than their smoking counterparts. Although the sample size in that study was small (10 of 33 HPV-positive patients were never-smokers), and only

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Table 3. Risk of recurrence and DSS by tobacco use among HPV-positive patients

	Tobacco group	Hazard ratio	95% CI	*P*
Risk of Recurrence	Current vs never	5.2	(1.1-24.4)	0.038*
	Former vs never	2.9	(0.6-13.6)	0.18
	Current vs former	1.8	(0.7-4.8)	0.24
DSS	Current vs never	7.2	(0.88-58.4)	0.07
	Former vs never	3.6	(0.43-30.1)	0.24
	Current vs former	2	(0.66-6.03)	0.22

*Statistical significance.

3 of those 33 patients received some form of chemotherapy (most underwent surgery and/or radiation), nevertheless, similar effects of tobacco were observed. Moreover, Gillison et al. (30) reported at the American Society of Clinical Oncology this year (2009) that smoking >20 pack-years was associated with an increased hazard risk of death of 1.79 in HPV-positive oropharynx cancer patients treated on RTOG 0129 relative to HPV-positive oropharyngeal cancer with <20 pack-years. However, they did not assess risk of recurrence or DM as Worden et al. reported at the same meeting (31). Further studies investigating the prognostic effect of tobacco use in HPV-positive patients are warranted.

It is not surprising that current tobacco use increases the risk of recurrence among HPV-positive patients. However, the lack of a significant difference in risk of recurrence between current and former smokers in our two cohorts was puzzling. We noted a striking difference in risk of recurrence among the former early-cessation tobacco users and the former late-cessation users. This was unexpected and counterintuitive. Those who quit over 20 years before diagnosis had a much higher risk of recurrence than both the never-tobacco users and the late-cessation group. This suggests that early tobacco cessation in HPV-positive individuals does not lower the risk of recurrence to that of a never-tobacco user. Possibly, the mutagenic effects of tobacco exposure at the time of HPV infection, which likely occurs soon after becoming sexually active may create an environment more suitable for HPV DNA integration into the host genome as well as increase the risk of associated errors in the somatic DNA. Contrarily, these differences in recurrence rates among the early- and late-cessation groups may reflect other nontobacco-related biological factors or could be the result of a comparatively small sample size. It is also possible that because there were significantly more early-cessation users in the UMCC-0221 cohort, this treatment type may be less effective. Furthermore, the role of HPV integration in SCCOP remains unknown. Integration of HPV has been reported to occur in 40% to 50% of SCCOP (13); however, integration alone has not been linked to poorer outcome (13, 15).

There were several important differences in the two treatment cohorts that should be noted. For one, we observed a surprising increase in the proportion of HPV-positive oropharyngeal cancers among our two cohorts; from 66% HPV positive in the UMCC-9921 cohort to 90% in the more recent UMCC-0221 cohort. Similarly, the U.S. incidence of tonsil and tongue cancer has been increasing linearly at rates of nearly 4% and 2% per year, respectively, over the past 30 years (3). Our results indicate that HPV-related SCCOP may be increasing in incidence more rapidly in our patient population than has been reported in the literature. This is consistent with a recent report of changing incidence trends (32). Gender was also significantly different among the two cohorts, possibly reflecting changes in the epidemiology of SCCOP or changes in the patient population presenting at our institution. The UMCC-0221 trial had shorter follow-up times and slightly different treatments; therefore, it was important to take cohort effects into account during all statistical analyses.

In conclusion, a current tobacco history among HPV-positive SCCOP patients increases the risk of LR, DM, or SP tumors, compared with never-tobacco users. Our results also emphasize that the majority of HPV-positive SCCOP patients are current or former tobacco users and that an HPV-positive tumor does not necessarily confer as good a prognosis in smokers. Clinical trials are warranted to investigate whether targeting treatments with respect to tobacco history will improve survival and quality of life. Furthermore, it is yet to be determined whether reducing the intensity of treatment for some HPV-positive, nonsmoking, SCCOP patients will compromise prognosis.

Disclosure of Potential Conflicts of Interest

J. Stoerker: interest, Sequenom; T.E. Carey: medical advisory board member, honorarium and travel compensation, speaker, Sequenom. No additional potential conflicts of interest were disclosed.

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