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ORIGINAL REPORT

Tobacco Smoking and Increased Risk of Death and Progression for Patients With p16-Positive and p16-Negative Oropharyngeal Cancer

Maura L. Gillison, Qiang Zhang, Richard Jordan, Weihong Xiao, William H. Westra, Andy Trotti, Sharon Spencer, Jonathan Harris, Christine H. Chung, and K. Kian Ang

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A B S T R A C T

Purpose

Tobacco smoking is associated with oropharynx cancer survival, but to what extent cancer progression or death increases with increasing tobacco exposure is unknown.

Patients and Methods

Patients with oropharynx cancer enrolled onto a phase III trial of radiotherapy from 1991 to 1997 (Radiation Therapy Oncology Group [RTOG] 9003) or of chemoradiotherapy from 2002 to 2005 (RTOG 0129) were evaluated for tumor human papillomavirus status by a surrogate, p16 immunohistochemistry, and for tobacco exposure by a standardized questionnaire. Associations between tobacco exposure and overall survival (OS) and progression-free survival (PFS) were estimated by Cox proportional hazards models.

Results

Prevalence of p16-positive cancer was 39.5% among patients in RTOG 9003 and 68.0% in RTOG 0129. Median pack-years of tobacco smoking were lower among p16-positive than p16-negative patients in both trials (RTOG 9003: 29 *v* 45.9 pack-years; $P = .02$; RTOG 0129: 10 *v* 40 pack-years; $P < .001$). After adjustment for p16 and other factors, risk of progression (PFS) or death (OS) increased by 1% per pack-year (for both, hazard ratio [HR], 1.01; 95% CI, 1.00 to 1.01; $P = .002$) or 2% per year of smoking (for both, HR, 1.02; 95% CI, 1.01 to 1.03; $P < .001$) in both trials. In RTOG 9003, risk of death doubled (HR, 2.19; 95% CI, 1.46 to 3.28) among those who smoked during radiotherapy after accounting for pack-years and other factors, and risk of second primary tumors increased by 1.5% per pack-year (HR, 1.015; 95% CI, 1.005 to 1.026).

Conclusion

Risk of oropharyngeal cancer progression and death increases directly as a function of tobacco exposure at diagnosis and during therapy and is independent of tumor p16 status and treatment.

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Maura L. Gillison and Weihong Xiao, The Ohio State University Comprehensive Cancer Center, Columbus, OH; Qiang Zhang and Jonathan Harris, Radiation Therapy Oncology Group Statistical Center, Philadelphia, PA; Richard Jordan, University of California at San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; William H. Westra and Christine H. Chung, Johns Hopkins Hospital, Baltimore, MD; Andy Trotti, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; Sharon Spencer, University of Alabama at Birmingham Medical Center, Birmingham, AL; and K. Kian Ang, University of Texas MD Anderson Cancer Center, Houston, TX.

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Corresponding author: Maura L. Gillison, MD, PhD, The Ohio State University Comprehensive Cancer Center, 420 West 12th Ave, Room 620, Columbus, OH 43210; e-mail: maura.gillison@osumc.edu.

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INTRODUCTION

Tobacco smoking remains the principal risk factor for head and neck squamous cell carcinoma (HNSCC) worldwide. In addition to its etiologic role, smoking status at diagnosis (never, former, current) is associated with treatment response,[1] risk of second primary cancers,[2-4] and survival.[5] Smoking during radiotherapy is also associated with treatment response and disease control,[6] albeit inconsistently.[7] However, the magnitude by which a patient's risk of cancer progression or death is affected by both cumulative measures of lifetime tobacco exposure at diagnosis and smoking during treatment is unknown.

Patients with oropharyngeal carcinoma (OPC) provide an opportunity to measure the impact of tobacco exposure before, during, or after treatment on outcomes, including progression-free survival (PFS), overall survival (OS), and risk of second primary tumors. Although tobacco smoking does not appear to be a strong cofactor for development of the human papillomavirus (HPV) –positive subset of OPC,[8,9] there is increasing evidence that it may nevertheless alter the behavior and treatment response of this cancer. In preliminary studies, patients who were HPV-positive and were smokers at diagnosis were reported to have worse survival than those who were not,[10,11] possibly because of an increased risk for both local recurrence and distant metastases.[12]

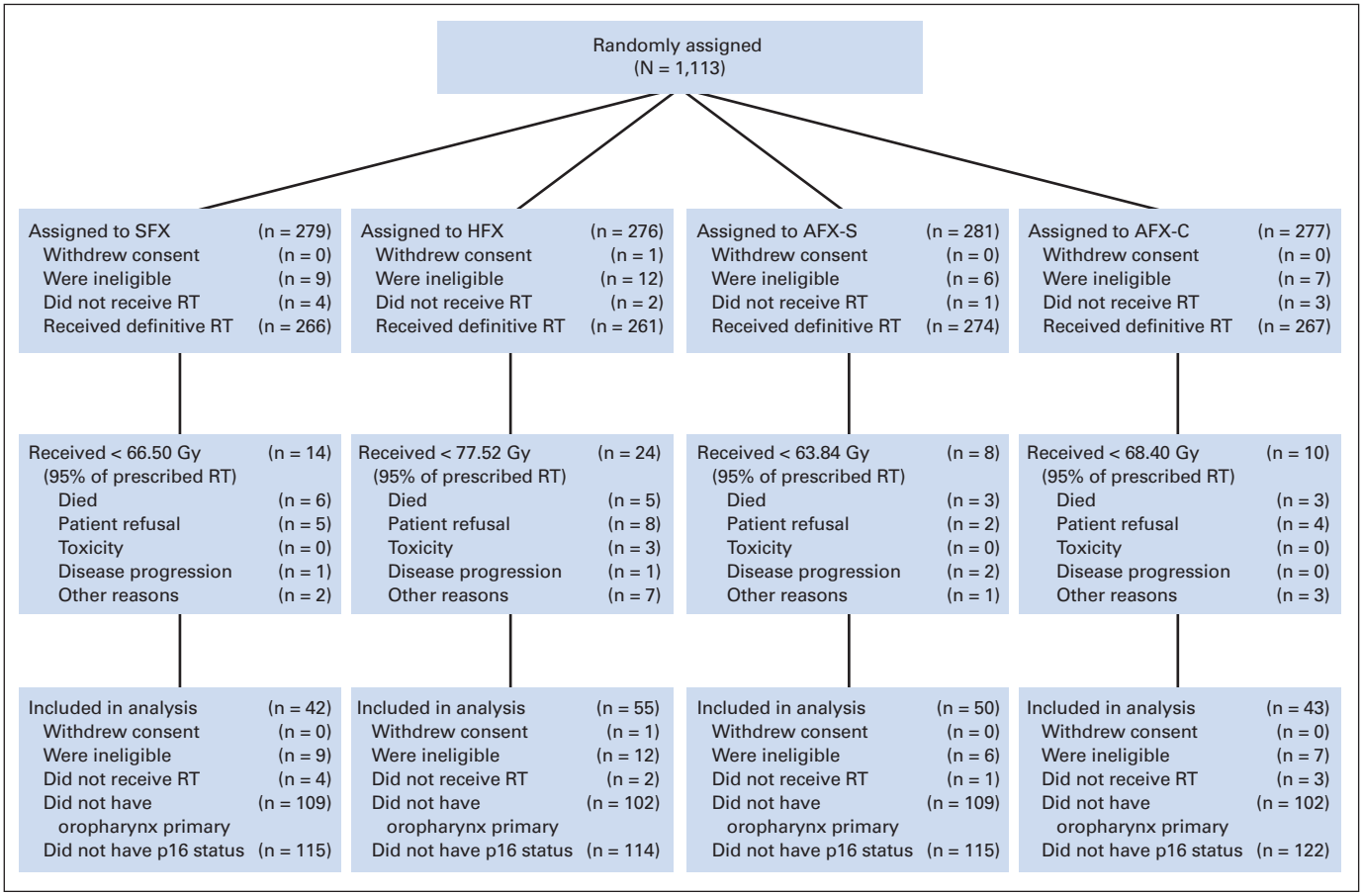

Fig 1. CONSORT diagram for Radiation Therapy Oncology Group (RTOG) 9003. AFX-C, accelerated fractionation with concomitant boost radiotherapy; AFX-S, accelerated fractionation with split radiotherapy; HFX, hyperfractionation radiotherapy; RT, radiotherapy; SFX, standard fractionation radiotherapy.

In a recent analysis,[13] we found that tumor HPV status and tobacco smoking (≤ 10 or > 10 pack-years) were the two strongest, independent determinants of PFS and OS for patients with OPC treated by chemoradiotherapy. In this study, we sought to further investigate the impact of quantitative measures of tobacco smoking on survival outcomes in two trials of the Radiation Therapy Oncology Group (RTOG): RTOG 9003 and RTOG 0129.

PATIENTS AND METHODS

Protocol

Patients eligible for RTOG 9003 (Fig 1) and RTOG 0129 (Fig 2) had untreated, pathologically confirmed, stage III to IV (M0)[14] squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, or larynx and were age ≥ 18 years. Patients with T1-2N1 or T1N2-3 were eligible for RTOG 9003 but were excluded from RTOG 0129. Karnofsky performance status was ≥ 60 for RTOG 9003 and more than 60 for RTOG 0129.[15]

In both trials, patients were stratified by tumor site, nodal stage, and performance status as previously reported.[13,16] Patients in RTOG 9003 were assigned to one of four radiotherapy regimens: standard fractionation, hyperfractionation, accelerated hyperfractionation with split, and accelerated fractionation with concomitant boost.[16] Patients in RTOG 0129 were assigned to cisplatin concurrent with either standard fractionation (cisplatin 100 mg/m^2 days 1, 22, and 43) or accelerated fractionation with concomitant boost (cisplatin 100 mg/m^2 on days 1 and 22).[13]

Lifetime cigarette exposure was prospectively determined at enrollment by use of a standardized questionnaire administered by clinical research staff. Surveys for both trials obtained data on ever use, current use, age at start, average number of cigarettes smoked per day, total years of smoking (RTOG 9003 only), or age stopped smoking (RTOG 0129 only). Data on current cigarette smoking (yes, no) were prospectively collected for patients in RTOG 9003 at the first follow-up visit after completion of radiotherapy.

To assess tumor status, physical examination and imaging studies (if indicated) were performed every 3 months for 18 to 24 months, every 4 to 6 months through year 3, every 6 months through year 5, and then annually. Both trials were approved by the institutional review boards of participating sites. Patients provided written informed consent.

Laboratory Studies

Formalin-fixed, paraffin-embedded tumor specimens were evaluated for tumor p16 expression, an established surrogate for tumor HPV status in OPC, by immunohistochemistry using a mouse monoclonal antibody (MTM Laboratories, Heidelberg, Germany) visualized with the Ventana XT autostainer using the one-view secondary detection kit (Ventana Medical Systems, Tucson, AZ).[17] p16 expression was scored as positive if strong and diffuse nuclear and cytoplasmic staining was present in $\geq 70\%$ of the tumor cells.[17]

Statistical Analysis

Our analysis was restricted to patients with OPC who had p16 determination because tumor p16 status is strongly associated with both smoking

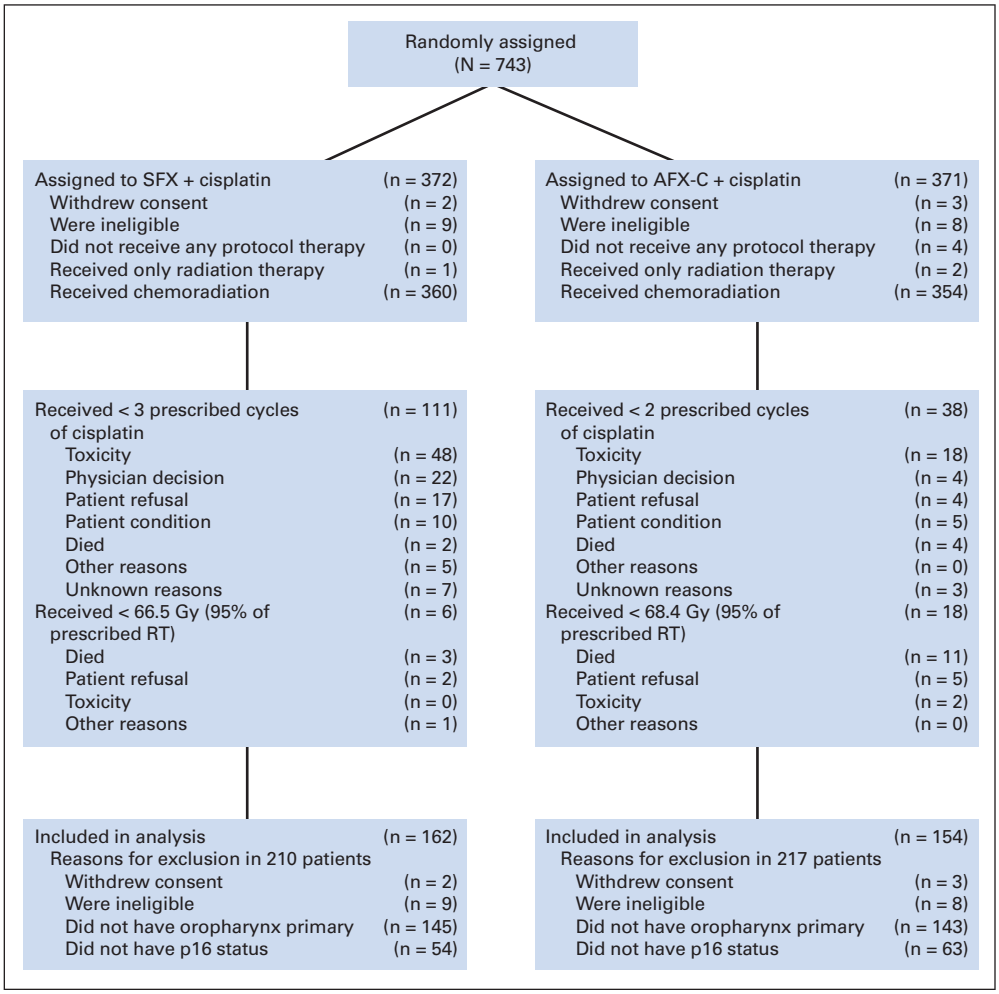

Fig 2. CONSORT diagram for Radiation Therapy Oncology Group (RTOG) 0129. AFX-C, accelerated fractionation with concomitant boost radiotherapy; RT, radiotherapy; SFX, standard fractionation radiotherapy.

and survival. This post hoc subset analysis was not part of the original study protocol.

Our primary outcome of interest was the effect of pack-years of tobacco exposure on OS, defined as time from random assignment to death due to any cause. PFS was defined as time from random assignment to death or first documented relapse, categorized as locoregional (primary site or regional nodes) failure (LRF) or distant metastases (DMs). Death from index cancer without documented site of recurrence was considered LRF. Second primary tumors (SPTs) were evaluated separately. PFS, LRF, and DM are reported here instead of protocol-specified secondary end points (eg, locoregional control) to be consistent with prior analyses of RTOG 0129.[13] Follow-up was calculated as days to the date of an event or last known date alive. OS and PFS rates were estimated by using the Kaplan-Meier method[18]and compared by log-rank test.[19] Karnofsky performance status was converted to Zubrod performance status to facilitate comparisons. The cumulative incidence method[20] and Gray's test[21] were used to estimate and compare rates of LRF, DM, and SPT. Cox proportional hazards models[22] were used to estimate hazard ratios (HRs); multivariable models were developed by minimizing Akaike information criteria[23] by using the method of Shtatland.[24] Cox regression was performed for patients with OPC with determined HPV status and smoking data. To investigate potential bias in estimates due to missing data, analyses were repeated for patients with OPC by using smoking values imputed with the Markov Chain Monte Carlo algorithm with a noninformative prior (SAS/STAT software, SAS Online Doc 9.2; SAS Institute, Cary, NC). Twenty data sets were created and the resulting analyses were combined per Rubin's formula.[25]

RESULTS

Patients were enrolled in RTOG 9003 from 1991 to 1997. Sixty percent (646 of 1,068) of the eligible patients had a diagnosis of OPC, and 29.4% (n = 190) of the 646 patients had tumor specimens available for p16 determination. No significant differences in baseline characteristics or outcomes were observed between patients with and without p16 determination (Appendix Table A1, online only). For RTOG 0129, 60% (433 of 721) of eligible patients enrolled from 2002 to 2005 had a diagnosis of OPC, and 73% (n = 316) of the 433 patients had p16 determination. The characteristics of the resulting study populations from RTOG 9003 and RTOG 0129 are listed in Table 1. Data on pack-years were missing for 15 and 56 patients for RTOG 9003 and 0129, respectively. Data on smoking during radiotherapy were missing for 15 patients for RTOG 9003.

In RTOG 9003, median age at start of smoking was 17 years (interquartile range [IQR], 15 to 20 years), and median cigarettes smoked per day was 20 cigarettes (IQR, 0 to 25 cigarettes). A median of 38 pack-years (IQR, 13 to 60 pack-years) and median 32 years of smoking (IQR, 21 to 44 years of smoking) were reported by patients with OPC.

Table 1. Baseline Patient and Tumor Characteristics for RTOG 9003 and RTOG 0129, Stratified by Tumor p16 Status

	RTOG 0129				RTOG 9003				
	p16 Positive (n = 215)		p16 Negative (n = 101)		p16 Positive (n = 75)		p16 Negative (n = 115)		
Characteristic	No.	%	No.	%	No.	%	No.	%	*P*[a]
Treatment assignment									.09[b]
SFX	114	53.0	48	47.5	14	18.7	28	24.3	
HFX	0	0.0	0	0.0	23	30.7	32	27.8	
AFX-S	0	0.0	0	0.0	26	34.7	24	20.9	
AFX-C	101	47.0	53	52.5	12	16.0	31	27.0	
Age, years									.48[c]
Median	53		57		57		59		
Range	31-78		37-82		40-82		40-84		
Q1-Q3	49-59		50-63		49-67		54-67		
Sex									.77[b]
Male	184	85.6	80	79.2	60	80.0	90	78.3	
Female	31	14.4	21	20.8	15	20.0	25	21.7	
Race									.08[d]
White	194	90.2	78	77.2	59	78.7	77	67.0	
Hispanic	0	0.0	0	0.0	6	8.0	5	4.3	
Black	14	6.5	21	20.8	8	10.7	31	27.0	
Asian	2	0.9	1	1.0	1	1.3	1	0.9	
Native American	3	1.4	1	1.0	1	1.3	0	0.0	
Other	0	0.0	0	0.0	0	0.0	1	0.9	
More than one race	1	0.5	0	0.0	0	0.0	0	0.0	
Unknown/prefers not to answer	1	0.5	0	0.0	0	0.0	0	0.0	
Zubrod performance status									.001[e]
0	145	67.4	59	58.4	55	73.3	57	49.6	
1	70	32.6	42	41.6	17	22.7	53	46.1	
2	0	0.0	0	0.0	3	4.0	5	4.3	
Anemia									< .001[b]
No	169	78.6	62	61.4	55	73.3	51	44.3	
Yes	46	21.4	39	38.6	20	26.7	64	55.7	
Primary site									.003[f]
Oropharynx, NOS	24	11.2	13	12.9	0	0.0	14	12.2	
Faucial arch	0	0.0	1	1.0	1	1.3	6	5.2	
Tonsillar fossa, tonsil	97	45.1	39	38.6	42	56.0	47	40.9	
Base of tongue	87	40.5	36	35.6	24	32.0	33	28.7	
Pharyngeal oropharynx	4	1.9	6	5.9	3	4.0	9	7.8	
Soft palate	3	1.4	6	5.9	5	6.7	6	5.2	
T stage									.003[g]
T1	0	0.0	0	0.0	9	12.0	2	1.7	
T2	73	34.0	22	21.8	17	22.7	21	18.3	
T3	87	40.5	38	37.6	36	48.0	55	47.8	
T4	55	25.6	41	40.6	13	17.3	37	32.2	
N stage									.77[g]
N0	15	7.0	8	7.9	12	16.0	19	16.5	
N1	27	12.6	19	18.8	14	18.7	28	24.3	
N2a	25	11.6	11	10.9	10	13.3	10	8.7	
N2b	80	37.2	26	25.7	16	21.3	23	20.0	
N2c	45	20.9	29	28.7	12	16.0	17	14.8	
N3	23	10.7	8	7.9	11	14.7	18	15.7	
AJCC stage									.82[h]
III	26	12.1	17	16.8	21	28.0	34	29.6	
IV	189	87.9	84	83.2	54	72.0	81	70.4	
Smoking history									< .001[i]
Never smoked	65	30.2	9	8.9	16	21.3	6	5.2	
Former smoker	115	53.5	44	43.6	31	41.3	28	24.3	
Current smoker	23	10.7	31	30.7	28	37.3	78	67.8	
Unknown	12	5.6	17	16.8	0	0.0	3	2.6	

(continued on following page)

Table 1. Baseline Patient and Tumor Characteristics for RTOG 9003 and RTOG 0129, Stratified by Tumor p16 Status (continued)

Characteristic	RTOG 0129 p16 Positive (n = 215) No.	%	RTOG 0129 p16 Negative (n = 101) No.	%	RTOG 9003 p16 Positive (n = 75) No.	%	RTOG 9003 p16 Negative (n = 115) No.	%	P[a]
Age started smoking, years	128	59.5	71	70.3	55	73.3	103	89.6	1.00[c]
Median	16.5		17		17		17		
Range	7-40		8-38		5-34		7-44		
Q1-Q3	15-19		15-20		15-20		14-20		
Cigarette use, years	189	87.9	77	76.2	72	96.0	106	92.2	.002[c]
Median	15		35		28		36		
Range	0-50		0-60		0-76		0-66		
Q1-Q3	0-30		24-40		4-37.5		26-47		
Cigarettes smoked per day	193	89.8	76	75.2	73	97.3	109	94.8	.06[c]
Median	12		20		20		20		
Range	0-76		0-60		0-80		0-60		
Q1-Q3	0-20		20-30		3-40		20-37		
Pack-years	187	87.0	73	72.3	72	96.0	103	89.6	.02[c]
Median	10		40		29		45.9		
Range	0-152		0-100		0-188		0-138		
Q1-Q3	0-33		21-54		1.125-56		23-60		

NOTE. For Radiation Therapy Oncology Group (RTOG) 9003, Karnofsky performance status was collected and converted to Zubrod performance status. For RTOG 0129, race and ethnicity were collected separately; 3.2% were Hispanic or Latino. Anemia is defined as hemoglobin ≤ 13.5 g/dL for men and ≤ 12.5 g/dL for women. A pack-year is defined as the equivalent of smoking one pack of cigarettes per day for 1 year. A former smoker is defined as someone who had not smoked for 12 months or more at enrollment.

Abbreviations: AFX-C, accelerated fractionation with concomitant boost radiotherapy [for RTOG 0129, includes concurrent cisplatin]; AFX-S, accelerated fractionation with split radiotherapy; AJCC, American Joint Committee on Cancer; HFX, hyperfractionation radiotherapy; NOS, not otherwise specified; Q1-Q3, quartile1 to quartile 3; SFX, standard fractionation radiotherapy [for RTOG 0129, includes concurrent cisplatin].

[a]Comparing p16-positive with p16-negative tumors in RTOG 9003.
[b]Pearson χ^2 test.
[c]Kolmogorov-Smirnov test.
[d]Pearson χ^2 test (white *v* nonwhite).
[e]Pearson χ^2 test (0 *v* 1-2).
[f]Pearson χ^2 test (tonsil and base of tongue *v* others).
[g]Kruskal-Wallis test.
[h]Pearson χ^2 test (II-III *v* IV).
[i]Pearson χ^2 test (never *v* former/current/unknown).

To examine the independent effect of smoking on outcomes for RTOG 9003, we must first account for the effect of an important confounder, p16 status, as previously reported for RTOG 0129.[13] p16 expression was found in 39.5% (95% CI, 32.5% to 46.4%) of patients with OPC. Characteristics of the p16-positive and p16-negative patients are listed in Table 1. p16-positive patients were more likely to be never smokers and had significantly lower cigarette smoking exposure, as measured by pack-years (median, 29 *v* 45.9 pack-years; $P = .02$) of smoking and cumulative years of smoking (median, 28 *v* 36 years of smoking; $P = .002$).

The median follow-up among surviving patients in RTOG 9003 was 9.3 years (range, 0.3 to 13.2 years) at the data cut point (August 11, 2005). The 5-year OS for patients with OPC in RTOG 9003 was 31.0% (95% CI, 24.3% to 37.7%; Fig 3A). There were 49 deaths among p16-positive and 104 deaths among p16-negative patients. In Kaplan-Meier analysis, patients with p16-positive tumors had better OS (Fig 3B) and PFS than patients with p16-negative tumors (log-rank test $P < .001$ for both). The 5-year OS rates were 49.0% (95% CI, 37.5% to 60.6%) and 19.6% (95% CI, 12.2% to 26.9%), and PFS rates were 43.6% (95% CI, 32.2% to 55.0%) and 19.0% (95% CI, 11.8% to 26.2%), respectively. LRF was lower for p16-positive patients (28.9% *v* 54.9% at 5 years; $P < .001$) but DMs (11.1% *v* 13.0% at 5 years; $P = .71$) and SPTs (13.8% *v* 11.4% at 5 years; $P = .40$) were not.

In the study population, 30 patients experienced SPTs, of which 14 were among p16-positive patients (n = 75) and 16 were among p16-negative patients (n = 115), respectively, at the data cut point. The only factor found to be significantly associated with risk of SPT was smoking exposure at diagnosis. The hazard of SPT increased 1.5% per pack-year (HR, 1.015; 95% CI, 1.005 to 1.026) or 1.5% per year of smoking (HR, 1.015; 95% CI, 0.994 to 1.037).

Smoking exposure at diagnosis was also strongly associated with OS in RTOG 9003. The hazard of death was more than two-fold higher among individuals with more than 10 versus ≤ 10 pack-years of tobacco smoking at diagnosis (HR, 2.10; 95% CI, 1.35 to 3.25; log-rank $P < .001$; Fig 3C). This was equivalent to an absolute difference in 5-year OS of 30% (95% CI, 6.9% to 53.1%) between the two smoking exposure groups.

Smoking exposures remained important predictors of survival, even after accounting for the strong effects of tumor p16 status and other important prognostic factors (Zubrod performance status, T stage, and N stage; Tables 2 and 3). When evaluated as a continuous variable, the hazard of death increased by approximately 1.0% per pack-year and by approximately 2% per year of smoking. The increased hazard of progression per pack-year and per year of smoking was quite similar to that for death (Table 3).

Smoking exposure was also an independent predictor of LRF in RTOG 9003. LRF was reported for 75 patients and was more common

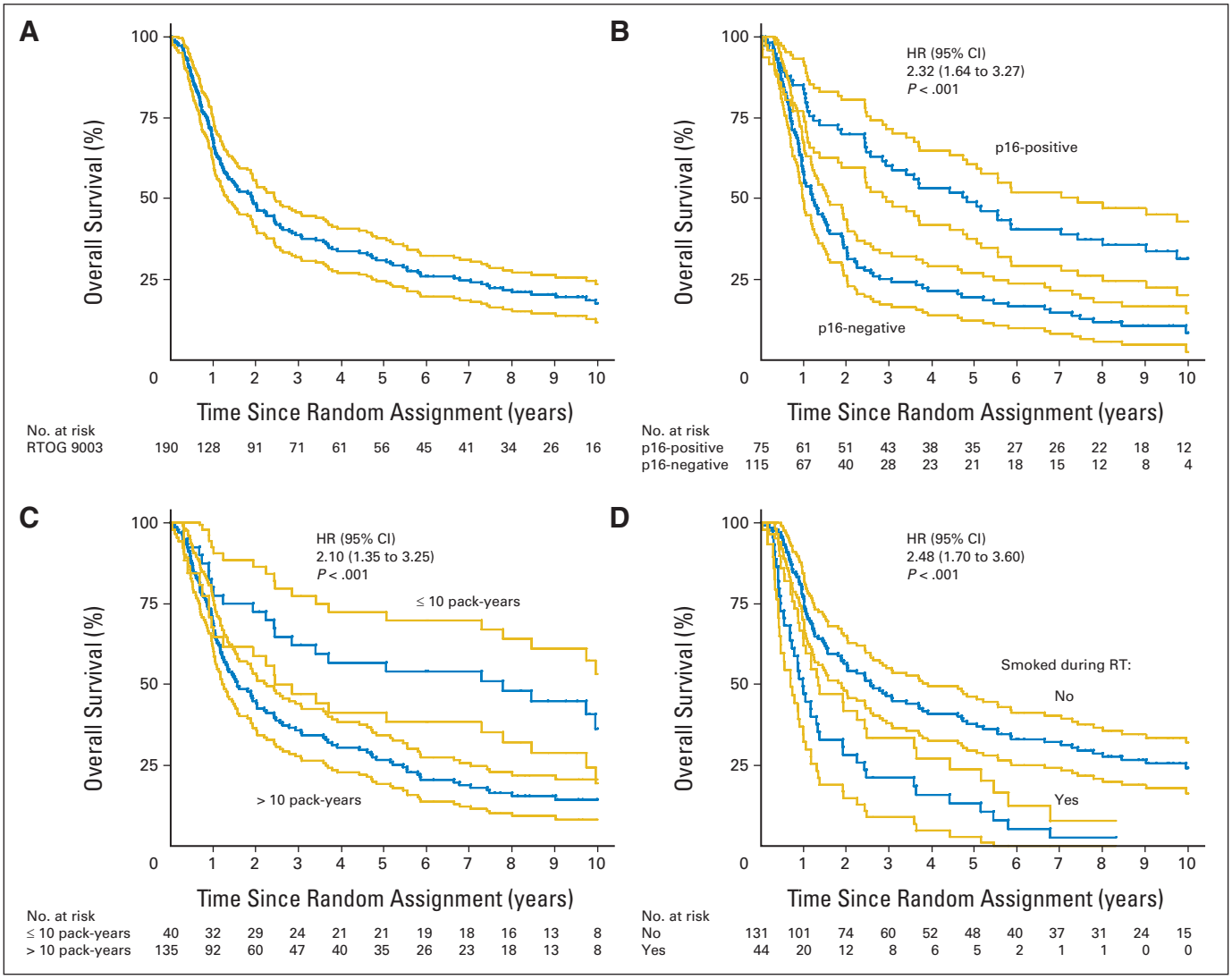

Fig 3. Survival outcomes for patients with oropharyngeal carcinoma (OPC) with known p16 status in Radiation Therapy Oncology Group (RTOG) 9003. Kaplan-Meier curves for overall survival (OS) for OPC with known p16 status enrolled in RTOG 9003 (A) overall, (B) stratified by p16 status, (C) smoking exposure, and (D) smoking during radiotherapy. (A) Median follow-up among surviving patients was 9.3 years (range, 0.3 to 13.2 years) and the 5-year OS was 31.0% (95% CI, 24.3% to 37.7%). (B) Patients with p16-positive OPC had significantly better OS when compared with patients with human papillomavirus –negative OPC (log-rank test $P < .001$). An absolute benefit in OS of 29.5% (95% CI, 15.8% to 43.2%) was observed at 5 years. (C) Patients with ≤ 10 pack-years had significantly better OS when compared with patients with more than 10 pack-years (log-rank test $P < .001$). An absolute benefit in OS of 30.0% (95% CI, 6.9% to 53.1%) was observed at 5 years. (D) OS stratified by smoking during radiotherapy. Patients who did not smoke during radiotherapy had significantly better OS compared with patients who did smoke during radiotherapy (HR, 2.48; 95% CI, 1.70 to 3.60; log-rank test $P < .001$). An absolute benefit in OS of 24.6% (95% CI, 5.9% to 43.3%) was observed at 5 years. Gold lines indicate 95% CIs for the survival estimates. HR, hazard ratio.

among individuals with more than 10 versus ≤ 10 pack-years of tobacco smoking at diagnosis (64 of 135 events *v* 11 of 40 events; 5-year LRF: 48.3% *v* 25.6%; Gray's $P = .01$). Even after adjustment for p16 status, performance status, and T stage, LRF was more common among individuals with more than 10 versus ≤ 10 pack-years of tobacco smoking (HR, 2.14; 95% CI, 1.09 to 4.18; $P = .03$). Age and treatment assignment were neither important predictors nor confounders in these multivariable analyses.

In RTOG 9003, current smoking status (yes, no) was available for the period of radiotherapy (accessed a median of 32 days [range, 0 to 105 days] after the end of radiotherapy). Smoking during radiotherapy significantly increased the hazard of death (Fig 3D), even after adjustment for pack-years and other factors (HR, 2.19; 95% CI, 1.46 to 3.28) or after adjustment for years of smoking and other factors (HR, 1.87; 95% CI, 1.23 to 2.84). Smoking during radiation similarly increased the hazard of progression (Table 3). No differences in rates of severe mucositis (grade ≥ 3) or radiotherapy treatment breaks (≥ 5 days) were observed in individuals who did or did not smoke during radiotherapy (data not shown).

To enhance our understanding of the effect of smoking on survival outcomes for patients with OPC, we examined the effect of several measures of tobacco exposure on survival outcomes in RTOG 0129. Medians of 20 pack-years (IQR, 0 to 40.5 pack-years) and 25 years of smoking (IQR, 0 to 35 years of smoking) were reported by patients with OPC who had p16 determination in that trial.

Table 2. Multivariable Cox Proportional Hazards Models for OS and PFS in RTOG 9003

Variable	Patients With p16 Status, Pack-Years, and Smoking Status During RT (n = 165)						Patients With P16 Status, With Imputations for Missing Pack-Years, and Smoking Status During RT (n = 190)		
	Model Without p16			Model With p16					
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
OS									
Zubrod PS (1-2 *v* 0)	2.18	1.51 to 3.14	< .001	1.90	1.30 to 2.79	< .001	2.03	1.43 to 2.88	< .001
T stage (T4 *v* T1-3)	2.01	1.35 to 3.00	< .001	1.78	1.18 to 2.68	.006	1.65	1.13 to 2.39	.009
N stage (N2-3 *v* N0-1)	1.55	1.07 to 2.24	.02	1.56	1.08 to 2.25	.02	1.39	1.00 to 1.94	.05
Pack-years (continuous)	1.01	1.00 to 1.01	.006	1.01	1.00 to 1.01	.009	1.01	1.00 to 1.01	.010
Smoked during RT (yes *v* no)	2.34	1.56 to 3.50	< .001	2.19	1.46 to 3.28	< .001	2.18	1.48 to 3.19	< .001
p16 status (positive *v* negative)	—		—	.62	.42 to .93	.02	.61	.42 to .89	.010
p16 status (negative *v* positive)	—		—	1.61	1.08 to 2.39	.02	1.63	1.12 to 2.36	.010
PFS									
Zubrod PS (1-2 *v* 0)	2.02	1.41 to 2.91	< .001	1.79	1.23 to 2.60	.002	1.93	1.37 to 2.73	< .001
T stage (T4 *v* T1-3)	1.96	1.33 to 2.88	< .001	1.76	1.19 to 2.61	.005	1.72	1.20 to 2.47	.003
N stage (N2-3 *v* N0-1)	1.47	1.02 to 2.10	.04	1.48	1.04 to 2.13	.03	1.34	.96 to 1.85	.08
Pack-years (continuous)	1.01	1.00 to 1.01	.003	1.01	1.00 to 1.01	.006	1.01	1.00 to 1.01	.008
Smoked during RT (yes *v* no)	2.12	1.42 to 3.16	< .001	2.02	1.36 to 3.01	< .001	2.04	1.39 to 2.97	< .001
p16 status (positive *v* negative)	—		—	.65	.44 to .95	.03	.65	.45 to .92	.02
p16 status (negative *v* positive)	—		—	1.54	1.05 to 2.27	.03	1.55	1.08 to 2.22	.02

NOTE. Estimates are adjusted for all other covariates listed for that endpoint. Missing pack-years was imputed for 15 patients. Missing smoking status during radiotherapy (RT) was imputed for 15 patients.

Abbreviations: HR, hazard ratio; OS, overall survival; PFS, progression-free survival; PS, performance status; RT, radiation therapy; RTOG, Radiation Therapy Oncology Group.

The proportion of patients with OPC in RTOG 0129 with p16-positive tumors was 68.0% (95% CI, 62.9% to 73.2%). p16-positive patients were more likely than p16-negative patients to be never smokers and had significantly lower cigarette smoking exposure, as measured by pack-years and cumulative years of smoking (Table 1).

The median follow-up among surviving patients in RTOG 0129 was 4.9 years (range, 1.6 to 6.4 years), and 5-year OS for patients with OPC was 66.8% (95% CI, 61.4% to 72.1%; Fig 4A). OS was significantly worse (HR, 2.81; 95% CI, 1.72 to 4.58; log-rank $P < .001$) for patients with OPC with more than 10 versus $\leq$ 10 pack-years of tobacco smoking (Fig 4B). This remained the case even after adjustment for other factors (Table 3).

The adjusted hazard of death or progression associated with several common measures of cumulative tobacco exposure for patients enrolled onto RTOG 0129 is provided in Table 3. As was observed for RTOG 9003, the hazard of death (OS) and progression (PFS) increased by approximately 1.0% per pack-year and by approximately 2% per year of smoking. Similarly, in RTOG 0129, LRF was more common among p16-negative than p16-positive patients (30 of 215 *v* 38 of 101 events; 5-year LRF, 38.6% *v* 14.3%; Gray's $P < .001$)

Table 3. Effect of Various Tobacco Exposure Measures on OS and PFS in RTOG 9003 and RTOG 0129

Variable	OS				PFS			
	RTOG 9003		RTOG 0129		RTOG 9003		RTOG 0129	
	HR*	95% CI	HR†	95% CI	HR*	95% CI	HR‡	95% CI
Smoking history (former/current *v* never)	2.419	1.288 to 4.543	1.969	1.048 to 3.703	2.258	1.231 to 4.141	2.549	1.425 to 4.559
Smoking history (former *v* never)	1.475	0.746 to 2.917	1.946	1.023 to 3.702	1.470	0.764 to 2.829	2.499	1.383 to 4.516
Smoking history (current *v* never)	3.875	2.009 to 7.474	2.048	0.975 to 4.302	3.398	1.804 to 6.401	2.733	1.371 to 5.447
Pack-years (> 5 *v* ≤ 5)	2.728	1.564 to 4.757	1.921	1.100 to 3.353	2.716	1.578 to 4.676	2.344	1.420 to 3.868
Pack-years (>10 *v* ≤ 10)	2.096	1.328 to 3.309	1.807	1.072 to 3.044	2.266	1.440 to 3.567	2.217	1.387 to 3.544
Pack-years (continuous)	1.007	1.002 to 1.012	1.008	1.000 to 1.017	1.007	1.003 to 1.012	1.008	1.001 to 1.015
Years smoked (continuous)	1.024	1.013 to 1.035	1.017	1.003 to 1.033	1.023	1.013 to 1.033	1.019	1.006 to 1.032
Cigarettes per day (continuous)	1.006	0.996 to 1.015	1.014	1.000 to 1.029	1.007	0.998 to 1.017	1.015	1.003 to 1.028
Smoked during RT (yes *v* no)	2.328	1.553 to 3.490			2.190	1.475 to 3.253		

Abbreviations: HR, hazard ratio [from Cox proportional hazards model]; OS, overall survival; PFS, progression-free survival; RT, radiation therapy; RTOG, Radiation Therapy Oncology Group.

*Adjusted for Zubrod performance status (PS), T stage, N stage, and p16 status.

†Adjusted for assigned treatment, age, race, T stage, N stage, and p16 status.

‡Adjusted for assigned treatment, age, race, Zubrod PS, T stage, N stage, and p16 status.

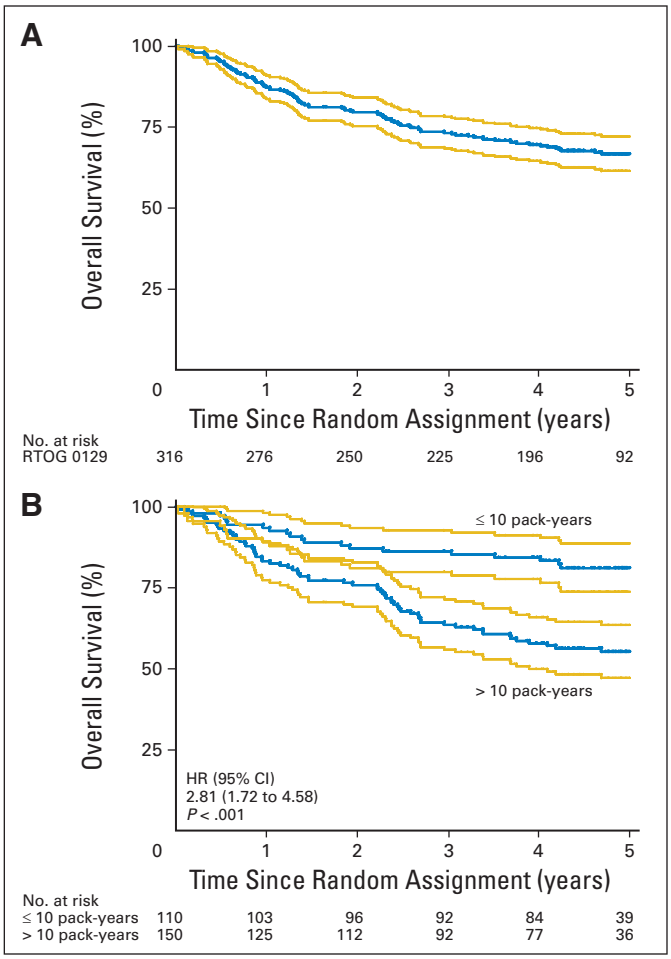

Fig 4. Survival outcomes for patients with oropharyngeal carcinoma with known p16 status in Radiation Therapy Oncology Group (RTOG) 0129. Kaplan-Meier curves for overall survival (OS) for oropharyngeal carcinoma with known p16 status enrolled onto RTOG 0129 (A) overall and (B) stratified by smoking exposure. (A) Median follow-up among surviving patients was 4.9 years (range, 1.6 to 6.4 years), and the 5-year OS was 66.8% (95% CI, 61.4% to 72.1%). (B) Patients with ≤ 10 pack-years had significantly better OS when compared with patients with more than 10 pack-years (log-rank test $P < .001$). An absolute benefit in OS of 25.9% (95% CI, 10.3% to 41.5%) was observed at 5 years. HR, hazard ratio.

and for those with more than 10 versus ≤ 10 pack-years of tobacco smoking at diagnosis (43 of 150 events *v* 13 of 110 events; 5-year LRF, 29.3% *v* 11.9%; Gray's $P = .001$). Even after adjustment for p16 status, performance status, and T stage, LRF was more common among individuals with more than 10 versus ≤ 10 pack-years of tobacco smoking (HR, 1.98; 95% CI, 1.03 to 3.80; $P = .04$).

DISCUSSION

In our prior analysis of RTOG 0129,[13] we demonstrated that tumor HPV status and tobacco exposure (≤ 10 or >10 pack-years) were the strongest determinants of survival for patients with OPC. Here we demonstrate that risk of cancer progression or death and SPTs increased as a direct function of quantitative measures of tobacco exposure at diagnosis and that the effect strength was independent of treatment by radiotherapy or chemoradiotherapy. Thus, significant changes in both the HPV-attributable proportion and tobacco exposure may, taken together, contribute to improvements in absolute survival over calendar time for patients with OPC. Furthermore, smoking during radiotherapy may further compromise treatment outcome.

The increased prevalence of p16-positive patients and the decline in tobacco exposure we observed when comparing the study population for RTOG 9003 with that for RTOG 0129 are consistent with increases in incidence for HPV-positive OPC[26,27] and declines in smoking prevalence at the population level in the United States.[28] Although the per pack-year increase in risk of progression or death was the same regardless of p16 status, declines in tobacco exposure were more marked for the p16-positive group, likely increasing their relative survival benefit over calendar time.

Smoking is known to increase all-cause[29] and cancer-specific mortality,[30] and therefore our findings are not unexpected. For patients with early stage HNSCC, risk of death has been associated with smoking status at diagnosis[2,4] and increased with increasing categories of exposure to tobacco as measured in pack-years or years of smoking.[31] Because tumor HPV status is strongly associated with both smoking status and survival, it is important to examine the effect of smoking on survival after accounting for the effect of tumor HPV status. Our data indicated that risk of cancer progression or death increased directly as a function of pack-years or total number of years of smoking, even after accounting for HPV status. Because deaths unrelated to cancer and from unknown cause are included as events in analyses of OS and PFS, competing causes of mortality reasonably expected to be more pronounced among heavy versus light or nonsmokers may account for associations between smoking and OS and PFS. However, the increased hazard of LRF we observed in association with smoking suggests a possible direct effect on treatment response and/or disease control. Interpreting our data from a molecular perspective, the probability that an OPC will acquire genetic hits imparting resistance to DNA-damage-induced cell death increases directly with smoking exposure. Further study is clearly warranted before incorporating measures of smoking exposure into treatment decision making.

Browman et al[6] originally reported that smoking during radiotherapy reduced response rates and 2-year survival for patients with head and neck cancer. However, in a subsequent report, smoking during radiotherapy was not an independent predictor of survival after accounting for prior tobacco use.[7] Chen et al[32] recently reported reduced 5-year rates of locoregional control, disease-free survival, and OS among patients with head and neck cancer who continued to smoke after diagnosis ("active smokers") who were matched to smokers who had quit. However, differences in baseline tobacco exposure could not be excluded as the explanation for these findings because median pack-years among the active smokers was twice that of the comparison group (40 *v* 20 pack-years). Although we accounted for prior exposure, we acknowledge that the excess mortality we observed in association with smoking during radiation therapy may not be independent of continued smoking beyond radiotherapy.[31]

There are several possible explanations for why smoking during radiotherapy might reduce effectiveness of therapy. Smoking during radiation therapy has been reported to increase the severity of mucositis, thus increasing the frequency of treatment breaks for smokers, which are known to decrease disease control (although not observed in this study).[32] Tissue hypoxia, commonly observed in head and neck

cancers, is known to be associated with reduced survival,[33] and hypoxic modification strategies have shown some benefit with regard to locoregional control.[34] Supporting evidence that smoking exacerbates tissue hypoxia includes smoking-induced tissue hypoxia in healthy human smokers[35] and reduced radiation control of cancers by carbon monoxide inhalation in animal models.[36] In addition, use of antioxidant vitamin supplementation during radiotherapy increased risk of disease recurrence only among those who smoked during radiotherapy[5] and not among those who smoked in the year prior to or subsequent to radiotherapy. Alternate biologic mechanisms in addition to hypoxia induction include nicotine interactions with both the mitogen-activated protein kinase and Akt pathways, which may inhibit apoptosis in response to therapy[37,38] and reduction by nicotine of the cytotoxic effects of cisplatin and radiation in head and neck cancer cell lines in vitro.[39]

Meta-analyses of randomized controlled trials for patients with locoregionally advanced HNSCC have estimated that cisplatin-based concurrent chemoradiotherapy confers an approximately 8% absolute improvement in 5-year survival when compared with radiotherapy alone.[40] When compared with HPV-negative patients, patients with HPV-positive tumors have increased response rates to cisplatin-based induction chemotherapy[41,42] and to radiotherapy. Whether p16-positive and p16-negative patients have a differential response to the addition of cisplatin to radiotherapy is unknown. Chaturvedi et al[27] recently reported that, from 1984 to 2004 in the United States, OS significantly increased for individuals with HPV-positive but not HPV-negative OPC. How the adoption of organ preservation chemoradiotherapy after 1999 may have contributed to this increase for the patient with OPC remains unknown.[43] Given the nonoverlapping time periods of enrollment and differences in eligibility criteria for RTOG 9003 and RTOG 0129, we are unable to inform this question. At this time, a randomized controlled trial for the HPV-positive patient comparing radiation versus concomitant cisplatin radiation appears unlikely to be performed.

Our data underscore the importance of measuring tobacco exposure in the context of clinical trials. Indeed, it has previously been recommended that all cooperative groups assess tobacco exposure via a standardized and centralized questionnaire.[44] In RTOG 1016, a phase III trial for HPV-positive patients with OPC that will compare accelerated radiotherapy in combination with either cisplatin or cetuximab, mandatory assessment of tobacco exposure will be performed by use of validated instruments.[45,46] Our data on smoking during radiotherapy also strongly support the implementation of smoking cessation programs and studies to evaluate the effect of smoking cessation on disease control.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Maura L. Gillison, Andy Trotti, Sharon Spencer, Christine H. Chung, K. Kian Ang
Provision of study materials or patients: Andy Trotti, Sharon Spencer, K. Kian Ang
Collection and assembly of data: Maura L. Gillison, Richard Jordan, Weihong Xiao, William H. Westra, Jonathan Harris, K. Kian Ang
Data analysis and interpretation: Maura L. Gillison, Qiang Zhang, Weihong Xiao, Jonathan Harris, K. Kian Ang
Manuscript writing: All authors
Final approval of manuscript: All authors

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