Published in final edited form as:

Laryngoscope. 2010 August ; 120(8): 1531–1537. doi:10.1002/lary.20984.

HPV-Active Head and Neck Cancer and Ethnic Health Disparities

Paul M. Weinberger, MD, Mark A. Merkley, BS, Sunny S. Khichi, BS, Jeffrey L. Lee, MD, Amanda Psyrri, MD, Lana L. Jackson, MD, and William S. Dynan, PhD

Department of Otolaryngology, Medical College of Georgia (PWM, LLJ), Department of Pathology, Charlie Norwood Veterans Administration Medical Center (JL), Department of Medical Oncology, Yale University School of Medicine (AP), and Institute of Molecular Medicine and Genetics, Medical College of Georgia (MAM, SSK, WSD)

Abstract

OBJECTIVES/HYPOTHESIS—Mortality for black males with head and neck squamous cell carcinoma (HNSCC) is twice that of white males or females. Human papillomavirus (HPV)-active HNSCC, defined by the concurrent presence of high-risk type HPV DNA and host cell p16^{INK4a} expression, is associated with decreased mortality. We hypothesized that prevalence of this HPV-active disease class would be lower in black HNSCC patients compared to white patients.

STUDY DESIGN—Multi-institutional retrospective cohort analysis.

METHODS—Real-time PCR was used to evaluate for high-risk HPV DNA presence. Immunohistochemistry for p16^{INK4a} protein was used as a surrogate marker for HPV oncoprotein activity. Patients were classified as HPV-negative (HPV DNA-negative, p16^{INK4a} low), HPV-inactive (HPV DNA-positive, p16^{INK4a} low) and HPV-active (HPV DNA-positive, p16^{INK4a} high). Overall survival and recurrence rates were compared by Fisher's exact test and Kaplan-Meier analysis.

RESULTS—There were 140 patients with HNSCC who met inclusion criteria. Self-reported ethnicity was white (115), black (25), and other (0). Amplifiable DNA was recovered from 102/140 patients. The presence of HPV DNA and the level of p16^{INK4a} expression were determined and the results were used to classify these patients as HPV-negative (44), HPV-inactive (33) and HPV-active (25). Patients with HPV-active HNSCC had improved overall 5-year survival (59.7%) compared to HPV-negative and HPV-inactive patients (16.9%) ($P=0.003$). Black patients were less likely to have HPV-active disease (0%) compared to white patients (21%), $P=0.017$.

CONCLUSIONS—The favorable HPV-active disease class is less common in black than in white patients with HNSCC, which appears to partially explain observed ethnic health disparities.

Keywords

Head and neck squamous cell carcinoma; human papillomavirus; etiology; ethnic disparities

INTRODUCTION

Black males have a higher incidence of head and neck squamous cell carcinoma (HNSCC) than any other racial/gender group and suffer a mortality rate that is twice that observed in

Send correspondence to: William S. Dynan, Institute of Molecular Medicine and Genetics, Medical College of Georgia, Room CA 3002, 1120 15th Street, Augusta, GA, 30912. wdyan@mcg.edu.

Conflict of interest: none

white males or females^{1–5}. A disparity in access to care has been hypothesized as an explanation for the higher HNSCC mortality in black patients.^{3,4} Recently, however, Settle et al. published a landmark study linking ethnic health disparities in HNSCC to a positive test for human papillomavirus (HPV) DNA in tumor samples⁶. HPV infection is a favorable prognostic indicator for HNSCC^{7–10}, and differential overall survival for black patients in this study was driven by lower prevalence of HPV infection⁶.

We previously demonstrated that high levels of p16^{INK4a}, a cell cycle regulator linked to biological activity of HPV oncoproteins (reviewed in ¹¹), was a more robust prognostic indicator than the presence of HPV DNA alone⁸. The work led to the definition of three classes of tumors that are histopathologically similar but differ in molecular phenotype: (1) HPV-negative (DNA negative, p16^{INK4a} negative), (2) HPV-inactive (DNA positive, p16^{INK4a} negative), and (3) HPV-active (DNA positive, p16^{INK4a} positive)^{8,12}. Only HPV-active (and not HPV-inactive) HNSCC is associated with improved overall and disease-specific survival⁸. Here, we apply the three-class model to the investigation of ethnic health disparities for HNSCC. In agreement with Settle et al.⁶, we find that the prevalence of HPV DNA is lower in black HNSCC patients than in white, although the difference was not significant in our cohort ($P=0.12$). However, the difference between black and white patients was significant when p16^{INK4a} status was also considered ($P=0.01$). Correlation of molecular data with clinical outcomes indicates that HPV-active status, not the presence of HPV DNA *per se*, drove overall survival and recurrence rates in this group of HNSCC patients. This immunohistochemically-based p16^{INK4a} detection procedure is widely available and the results should thus be transposable to clinical practice in a variety of settings.

MATERIALS AND METHODS

Patients

Inclusion criteria at Institution 1 (Yale University) were presentation with primary or recurrent histologically confirmed squamous cell carcinoma of the oropharynx between 1980 and 1999 and treatment with primary external beam radiotherapy (RT) or gross total surgical resection and postoperative RT at Yale-New Haven Hospital, a 900+ bed academic medical center which serves as a tertiary referral center in the northeastern United States. Exclusion criteria included presentation with metastatic disease, failure to receive full course of radiation therapy and unavailability of archival tumor tissue. Inclusion criteria at Institution 2 (Medical College of Georgia (MCG)) were presentation with histologically confirmed HNSCC between 2004 and 2007 and enrollment in a voluntary tissue/tumor banking registry at MCG Hospital, a 600+ bed academic medical center that serves as a tertiary referral center in the southeastern United States. Biopsy specimens were obtained pre- chemotherapy and/or radiotherapy. The project was reviewed and approved by the Institutional Review Board at each institution.

HPV DNA Detection

For the Yale cohort, DNA was extracted from 0.6 mm tissue cores via a Chelex-100/Proteinase K protocol as previously described¹³. For the MCG cohort, frozen sections (5 μ m) were stained with hematoxylin and enriched for tumor cells by Laser Capture Microdissection (LCM) using an Arcturus PixCell Ile microscope (Molecular Devices, Sunnyvale CA). The polymer film with adherent tumor cells were placed into a microcentrifuge tube containing 20 μ g/ml Proteinase K (Gentra PureGene DNA Extraction Kit, Qiagen Corporation, Valencia CA) and incubated for 3 hours at 60 °C. All specimens were analyzed for amplifiable genomic DNA by SYBR Green qPCR using primers targeting a 161 nucleotide (nt) region of the β -globin gene (refer to Supporting Information

Supplementary Table I). For the Yale cohort, HPV detection was by SYBR Green qPCR using primers directed against a 109 bp region of the HPV16 E6 gene as described^{8,14} (Supplementary Table I). For the Georgia cohort, an HPV type-specific TaqMan-based qPCR assay was performed using primers directed against an 82 nt region of the HPV16 E7 gene and an 81 bp region of the HPV18 E7 gene (Supplementary Table I). Details are given in Supporting Information.

p16^{INK4a} Expression Status

For the Yale cohort, two-fold redundant tissue microarray (TMA) was constructed as previously described⁸ and included cores from HPV 18-positive HeLa and HPV 16-positive Caski cell lines fixed in formalin and embedded in paraffin as positive controls. For the MCG cohort, individual sections (4 μ m) were cut from paraffin blocks and mounted. Immunohistochemical detection of p16^{INK4a} expression using anti-p16^{INK4a} mouse monoclonal antibody was performed as described in Supporting Information. Slides were read independently by two investigators (PMW and MAM for the Georgia cohort and as previously described⁸ for the Yale cohort) and consensus scoring was used to classify samples as p16^{INK4a}-positive (strong, diffuse staining) or p16^{INK4a}-negative (weak or absent staining)¹⁵.

Tumor Classification and Statistical Analysis

Comparison of classification status with the clinical and demographic variables was made by contingency table analysis with Fisher's Exact test (ethnicity, gender, alcohol use, tobacco use) and Mantel-Haensel Chi Square (Tumor-Node-Metastasis (TNM) stage and histologic grade). Comparison of classification status with age was made by one-way analysis of variance (ANOVA). Disease-free survival, overall survival and local recurrence were assessed by Kaplan-Meier estimates of the survivor function with log-rank for determining statistical significance. Relative risk and prognostic independence was assessed by a multivariate Cox proportional hazards model with clinical and demographic variables, including HPV classification, TNM stage, histologic grade and ethnicity. Analysis was performed using a forward progression model with dummy variable encoding for all categorical variables. All statistical calculations were performed with SPSS 14.0 for Windows (SPSS Inc., Chicago IL) and where appropriate were two-tailed.

RESULTS

Patient demographics and HPV status

There were 140 patients with HNSCC who met inclusion criteria, drawn from two separate hospitals in geographically distinct regions of the United States. Table I indicates the demographic characteristics of the study cohort, including age, gender, ethnicity, TNM stage, grade, and primary versus recurrent tumor type. Institution 1 (Yale) had 116 patients included in this study, and Institution 2 (MCG) had 24 patients. There were no significant differences in demographic or clinical variables between institutions (data not shown).

The presence of high-risk type HPV 16 or HPV 18 DNA was determined by real-time polymerase chain reaction using primers directed against E6 and E7 genes as described in Materials and Methods. The presence of amplifiable DNA in the samples was determined based on the ability to detect the cellular β -globin gene. Amplifiable DNA was obtained from 106/140 (76%) of patients. Expression of p16^{INK4a} was determined for 102 of these 106 samples (96%). Based on these results, patients were classified using the 3-class system described previously (Table II)⁸. There were 44/102 (43.1%) HPV-negative (HPV16 DNA-negative, p16^{INK4a}-negative), 33/102 (32.4%) HPV-inactive (HPV16 DNA-positive,

p16^{INK4a}-negative) and 25/102 (24.5%) HPV-active (HPV16 DNA-positive, p16^{INK4a}-expressing) patients.

Several significant correlations were observed between HPV class and the demographic and clinical variables in Table I. Notably, white patients were significantly more like to have HPV-active disease than black patients (29% versus 0%, $P=0.017$). Increasing TNM stage was also associated with HPV-active classification ($P=0.008$), as was poorly differentiated grade ($P=0.014$).

We also observed a novel relationship between HPV class and primary versus recurrent tumor type. The majority of recurrent tumors were in the HPV-inactive class, where viral DNA was detected but p16^{INK4a} overexpression was not. There were 9/12 (75%) recurrent tumors assigned to this class, versus 24/88 (27%) primary tumors. The biological significance of this observation remains to be ascertained.

Survival and recurrence by ethnicity

We estimated overall survival and local recurrence using Kaplan-Meier estimates of the survival function with log-rank for determining statistical significance (Figure 1). The median survival for the entire cohort was 26.2 months (95% CI = 21.6–30.8 months), and the 5-year local recurrence rate was $49.3 \pm 11.7\%$. When data were stratified by ethnicity, median survival was found to be longer for black than for white patients (44.9 months versus 25.7 months) but the difference was not significant ($P=0.26$). The 5-year local recurrence rates for black versus white patients were very similar ($52.5 \pm 14.4\%$ versus $47.2 \pm 23.0\%$ respectively).

Survival and recurrence by HPV classification

We performed similar analyses of survival and local recurrence for the subset of 102 patients who could be classified by HPV status (Figure 2). Consistent with prior data from our group and others^{8,16,17}, HPV-active classification (HPV DNA and p16^{INK4a} overexpression) was associated with significantly greater median survival than the other two groups (median not reached at 60 months, versus 24.8 months for HPV-inactive and 16.8 months for HPV-negative, $P=0.009$). HPV-active classification was associated with a similar advantage in local recurrence rate ($21.2\% \pm 18.6\%$ for HPV-active, versus $73.2\% \pm 26.1\%$ for HPV-inactive and $45\% \pm 20.0\%$ for HPV-negative, $P=0.039$).

Relationship between ethnicity and HPV status

Figures 3A and 3B show the prevalence of HPV DNA in white and black patients. There was a trend for higher prevalence in white patients (60.2%) compared to black patients (38.8%), but the difference was not significant ($P=0.12$). When compared using the three-class model, however, the fraction of patients in the HPV-active class (concomitant presence of HPV16 DNA and p16^{INK4a}) differs markedly in the two ethnic groups. Whereas nearly half of the HPV-positive white patients fell into the HPV-active class (p16^{INK4a}-positive) (Figure 2C) none of the black patients were p16^{INK4a}-positive (Figure 2D), a significant difference ($P=0.017$).

Survival and recurrence by ethnicity

Kaplan-Meier analysis was performed to analyze survival and local recurrence rates stratified by a combination of ethnicity and HPV class (Figure 4). The HPV negative/inactive white patients (who were pooled for the analysis in Figure 4) had the shortest median survival of any group (19.2 months, 95% CI 12.9–25.6 months) and the highest recurrence rate ($64.8 \pm 23.8\%$). The HPV negative/inactive black patients actually fared somewhat better (median survival 44.9 months, 95% CI 4.9–85.0 months), although the

difference was not significant ($P = 0.11$). As indicated previously (Figure 2) the HPV-active white patients had the best outcomes (median survival not reached at 60 months, local recurrence rate $21.2\% \pm 18.6\%$). The difference between HPV-active and HPV-inactive/negative white patients was significant ($P=0.001$), although the difference with HPV-inactive/negative black patients was not, perhaps reflecting the small number of black patients in the cohort ($P= 0.23$).

Multivariate analysis of the relationship between outcomes, ethnicity and other clinical variables

We investigated potential confounding effects due to clinical parameters other than ethnicity on survival and recurrence by performing a Cox multivariate analysis. Clinical variables considered were ethnicity, TNM stage, histologic grade, primary versus recurrent tumor type, and HPV class. HPV-active class (compared to both HPV-negative, $P=0.002$, and HPV-inactive, $P=0.015$) and white ethnicity ($P=0.050$) were independently associated with improved survival. We further explored this relationship by testing interaction terms within the Cox model. The interaction term for ethnicity*HPV-active was significant in univariate analysis ($P=0.002$) but because of co-linearity with HPV-class it could not be included in the multivariate model. Similarly, for local recurrence recurrent tumor type ($P=-0.004$) and HPV-inactive versus HPV-active class ($P=0.034$) were independently associated with increased local recurrence but ethnicity was not ($P=0.71$). These data are summarized in Table III.

DISCUSSION

The present work demonstrates the striking absence of a favorable subset of HPV-associated HNSCC among black patients, concordant across two academic medical centers in different geographic regions of the United States. We used a three-class model based both on physical presence of HPV DNA and on biological activity of HPV oncoproteins, as evidenced by overexpression of p16^{INK4a}. This model distinguishes between HPV-active disease, HPV-negative disease, and a separate category of HPV-inactive disease, where viral DNA is present but the program of viral oncogenesis is not engaged or where there is a concomitant p16^{INK4a} deletion. HPV-active disease was significantly less prevalent in black patients than in white patients (0% versus 29%). By contrast, HPV-inactive disease was common in patients of both black and white ethnicity (31% and 38%, respectively). Significant interaction between HPV status, ethnicity, and clinical outcomes was evident only when HPV-active and HPV-inactive subsets were distinguished. Multivariate analysis shows that HPV-active status, and not ethnicity, is a principal contributor to differences in overall survival and recurrence rates among patient groups.

The mechanism underlying the different prevalence of HPV-active disease in different ethnic groups is not understood. However, occurrence of HPV-inactive disease in both the black and white groups indicates that the patients in both groups suffer exposure to, and initial infection with, HPV. The data suggest, therefore, that white patients may be more susceptible to progression from HPV infection to HPV-associated HNSCC, or alternatively that black patients may be protected. It does not appear that this differential susceptibility extends to cervical cancer, a disease that is universally HPV-associated. Black women in the United States have an increased prevalence of both high-risk HPV¹⁸ and cervical cancer¹⁹ compared to hispanic and white women.

It is likely that the immune response plays an important role in controlling susceptibility to progression of HPV-related disease (reviewed in ²⁰). In this context, it is notable that several studies have shown that white males and females are more likely to engage in oral sex compared to blacks^{21,22}. Conversely, black females are more likely to engage in early

intercourse compared to white and hispanic females²³. Whether early genital HPV infection may lend protective immunity against subsequent oropharyngeal HPV infection, or vice versa, remains to be investigated.

A better understanding of the interaction between HPV status, ethnicity, and clinical outcomes is important to the optimal design of prevention and therapeutic strategies. HPV-targeted therapeutic vaccines are currently undergoing clinical trials in head and neck cancer patients. Currently, these trials stratify patients based only on HPV DNA presence. The use of p16^{INK4a} immunohistochemistry to existing protocols for identification of HPV active disease would seem to be of vital importance to identify those patients who are most likely to benefit from HPV-targeted therapeutic strategies.

CONCLUSION

The prevalence of a more favorable, HPV-active disease subset is lower in black HNSCC patients than in white patients. This is the second report of ethnic differences in prevalence of HPV-associated HNSCC. Although the two studies differ in methodology and patient populations, they reach the same overall conclusion that a lower incidence of a more favorable HPV-associated disease subset contributes to the well-established ethnic disparity in HNSCC outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by a US Public Health Service Award, CA 95941. WSD is an Eminent Scholar of the Georgia Research Alliance.

BIBLIOGRAPHY

1. Al-Othman MO, Morris CG, Logan HL, Hinerman RW, Amdur RJ, Mendenhall WM. Impact of race on outcome after definitive radiotherapy for squamous cell carcinoma of the head and neck. *Cancer* 2003;98:2467–2472. [PubMed: 14635082]
2. Goodwin WJ, Thomas GR, Parker DF, et al. Unequal burden of head and neck cancer in the United States. *Head Neck*. 2007
3. Gourin CG, Podolsky RH. Racial disparities in patients with head and neck squamous cell carcinoma. *Laryngoscope* 2006;116:1093–1106. [PubMed: 16826042]
4. Yen KL, Horner MJ, Reed SG, et al. Head and neck cancer disparities in South Carolina: descriptive epidemiology, early detection, and special programs. *J S C Med Assoc* 2006;102:192–200. [PubMed: 17319230]
5. Molina MA, Cheung MC, Perez EA, et al. African American and poor patients have a dramatically worse prognosis for head and neck cancer: an examination of 20,915 patients. *Cancer* 2008;113:2797–2806. [PubMed: 18839393]
6. Settle K, Posner MR, Schumaker LM, et al. Racial Survival Disparity in Head and Neck Cancer Results from Low Prevalence of Human Papillomavirus Infection in Black Oropharyngeal Cancer Patients. *Cancer Prev Res (Phila Pa)*. 2009
7. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst* 2008;100:261–269. [PubMed: 18270337]
8. Weinberger PM, Yu Z, Haffty BG, et al. Molecular classification identifies a subset of human papillomavirus-associated oropharyngeal cancers with favorable prognosis. *J Clin Oncol* 2006;24:736–747. [PubMed: 16401683]

9. Ritchie JM, Smith EM, Summersgill KF, et al. Human papillomavirus infection as a prognostic factor in carcinomas of the oral cavity and oropharynx. *Int J Cancer* 2003;104:336–344. [PubMed: 12569557]
10. Li W, Thompson CH, O'Brien CJ, et al. Human papillomavirus positivity predicts favourable outcome for squamous carcinoma of the tonsil. *Int J Cancer* 2003;106:553–558. [PubMed: 12845651]
11. Human papillomaviruses. IARC Monogr Eval Carcinog Risks Hum 2007;90:1–636. [PubMed: 18354839]
12. Weinberger PM, Yu Z, Kountourakis P, et al. Defining molecular phenotypes of human papillomavirus-associated oropharyngeal squamous cell carcinoma: Validation of three-class hypothesis. *Otolaryngol Head Neck Surg* 2009;141:382–389. e381. [PubMed: 19716018]
13. Coombs NJ, Gough AC, Primrose JN. Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. *Nucleic Acids Res* 1999;27:e12. [PubMed: 10454649]
14. Koch WM, Lango M, Sewell D, Zahurak M, Sidransky D. Head and neck cancer in nonsmokers: a distinct clinical and molecular entity. *Laryngoscope* 1999;109:1544–1551. [PubMed: 10522920]
15. Weinberger PM, Yu Z, Haffty BG, et al. Prognostic significance of p16 protein levels in oropharyngeal squamous cell cancer. *Clin Cancer Res* 2004;10:5684–5691. [PubMed: 15355894]
16. Klussmann JP, Gultekin E, Weissenborn SJ, et al. Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. *Am J Pathol* 2003;162:747–753. [PubMed: 12598309]
17. Kong CS, Narasimhan B, Cao H, et al. The relationship between human papillomavirus status and other molecular prognostic markers in head and neck squamous cell carcinomas. *Int J Radiat Oncol Biol Phys* 2009;74:553–561. [PubMed: 19427557]
18. Kahn JA, Lan D, Kahn RS. Sociodemographic factors associated with high-risk human papillomavirus infection. *Obstet Gynecol* 2007;110:87–95. [PubMed: 17601901]
19. Walker B, Figs LW, Zahm SH. Differences in cancer incidence, mortality, and survival between African Americans and whites. *Environ Health Perspect* 1995;103 (Suppl 8):275–281. [PubMed: 8741798]
20. Frazer IH. Interaction of human papillomaviruses with the host immune system: a well evolved relationship. *Virology* 2009;384:410–414. [PubMed: 18986661]
21. Gates GJ, Sonenstein FL. Heterosexual genital sexual activity among adolescent males: 1988 and 1995. *Fam Plann Perspect* 2000;32:295–297. 304. [PubMed: 11138866]
22. National Survey of Family Growth [Centers for Disease Control and Prevention Web site]. [Accessed September 3, 2009]. Available at: <http://www.cdc.gov/nchs/NSFG.htm>
23. Hobfoll SE, Jackson AP, Lavin J, Britton PJ, Shepherd JB. Safer sex knowledge, behavior, and attitudes of inner-city women. *Health Psychol* 1993;12:481–488. [PubMed: 8293732]

Fig. 1. Overall survival and local recurrence rates

(A, B). Overall survival and local recurrence rates for the entire cohort (n=140) for a five year period post-diagnosis. (C, D) Overall survival and local recurrence rates stratified by patient ethnicity. Differences between ethnic groups were not significant ($P=0.26$, $P=0.96$ respectively).

Fig. 2. Survival and local recurrence rates stratified by HPV class

(A) Overall survival rates for HPV-active, HPV-inactive, and HPV-negative patients as indicated. (B) Local recurrence rates for patients in each class as indicated.

Fig. 3. Comparison of HPV prevalence and ethnicity

(A, B), Prevalence of HPV 16 DNA in patients stratified by ethnicity. A, white, B, black. Difference was not significant ($P=0.12$). (C, D), HPV status using 3-class system, stratified by ethnicity. C, white, D, black. Difference in the frequency of the HPV-active class between white and black patients was significant ($P=0.017$).

Fig. 4. Survival and local recurrence rates stratified by both ethnicity and HPV class
 (A) Overall survival rates for black and white patients, stratified by HPV-active versus HPV-negative/inactive as indicated. (B) Local recurrence rates for patients in each class as indicated.

Table 1

Patient demographics and comparison by HPV class.

	All patients	p16 ^{INK4a} /HPV available ^f	HPV-negative	HPV-inactive	HPV-active	P
Age, years	41 – 79 (mean 60.3)	102	41–79 (mean 60.4)	43–77 (mean 60.7)	41–71 (mean 58.0)	0.53
Gender						
Male	106	74	28	25	21	0.19
Female	34	28	16	8	4	
Ethnicity						
White	115	86	34	27	25	0.017 *
Black	25	16	10	6	0	
Other	0	0	0	0	0	
TNM stage						
Stage 1	2	1	0	1	0	0.008 **
Stage 2	18	12	9	2	1	
Stage 3	40	32	8	17	7	
Stage 4	80	57	27	13	17	
Grade ^g						
Well diff.	8	7	3	4	0	0.014 *
Moderately diff.	65	44	23	16	5	
Poorly diff.	50	40	14	10	16	
Tumor type ^h						
Primary	119	88	41	24	23	0.006 **
Recurrent	17	12	2	9	1	

* Significant at $P=0.05$ level,

** significant at $P=0.01$ level.

^f DNA extraction and HPV detection was successful in 106 patients, p16^{INK4a} expression was determined in 126 patients, and there were 102 patients with both HPV and p16^{INK4a} data available for classification by the 3-class system.

^g Excludes 17 patients (11 with p16^{INK4a}/HPV data available) where grade was not recorded.

^h Excludes 4 patients (2 with p16^{INK4a}/HPV data available) where tumor type was not recorded. Diff. = differentiated.

Table II

Classification system

	HPV DNA negative	HPV DNA-positive
p16^{INK4a} low	HPV-negative	HPV-inactive
p16^{INK4a} high	undefined (not observed)	HPV-active

Table III

Multivariate survival analysis (Cox regression)

Variable		Hazard Ratio	95% CI	P
<i>Overall Survival</i>				
HPV/p16 class	HPV active	(reference category)		
	HPV-inactive	4.2	1.3 – 13.4	0.015*
	HPV-negative	5.6	1.9 – 16.6	0.002**
TNM stage	stage 1	1.2	0.1 – 12.1	0.86
	stage 2	0.5	0.2 – 1.3	0.14
	stage 3	0.4	0.2 – 0.9	0.032*
	stage 4	(reference category)		
Histologic grade	poorly diff.	1.0	0.4 – 2.7	0.96
	moderately diff.	0.6	0.2 – 1.7	0.37
	well diff.	(reference category)		
Tumor type (recurrent vs primary)		1.1	0.4 – 3.2	0.89
Ethnicity (white vs black)		0.4	0.2 – 1.0	0.05*
<i>Local Recurrence</i>				
HPV/p16INK4a class	HPV active	(reference category)		
	HPV-inactive	4.2	1.0 – 18.4	0.056
	HPV-negative	4.4	1.1 – 17.3	0.034*
TNM stage	stage 1	0.0	0.0 – 0.0	0.98
	stage 2	0.6	0.2 – 2.3	0.45
	stage 3	0.9	0.4 – 2.2	0.80
	stage 4	(reference category)		
Grade	poorly diff.	1.2	0.4 – 4.0	0.74
	moderately diff.	0.4	0.1 – 1.5	0.18
	well diff.	(reference category)		
Tumor type (recurrent vs primary)		4.2	1.6 – 11.2	0.004**
Ethnicity (white vs black)		0.8	0.3 – 2.5	0.71

* Significant at $P=0.05$ level,** significant at $P=0.01$ level. Diff .= differentiated. For TNM staging dummy variable coding, TNM stage 4 was used as the reference category as this was the most common stage cancer.