UICC
global cancer control

IJC
International Journal of Cancer

Human papillomavirus and survival in patients with base of tongue cancer

Per Attner[1], Juan Du[2], Anders Näsman[2], Lalle Hammarstedt[1], Torbjörn Ramqvist[2], Johan Lindholm[2], Linda Marklund[1], Tina Dalianis[2] and Eva Munck-Wikland[1]

[1] Department of Oto-Rhino-Laryngology, Head and Neck Surgery, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[2] Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden

The incidence of base of tongue cancer is increasing in Sweden and the proportion of human papillomavirus (HPV) positive cancer has increased in Stockholm, Sweden. Between 2006 and 2007, 84% of base of tongue cancer cases in Stockholm were HPV-positive. The objective of this study was to assess the impact of HPV status on prognosis for base of tongue cancer patients. One-hundred and nine patients were diagnosed with base of tongue cancer between 1998 and 2007 in Stockholm County and 95 paraffin-embedded diagnostic tumor biopsies were obtained and tested for HPV by PCR. Eighty-seven patients had available biopsies, were treated with intention to cure and could be included in the survival analysis. Age, sex, TNM-stage, stage, treatment and survival were recorded from patient charts. Kaplan–Meier curves were used to present survival data. In multivariable analyses, a Cox proportional hazards model was used to adjust for covariates. In total 68 (78%) tumor biopsies from the 87 included patients were HPV DNA positive. Kaplan–Meier estimates showed that the overall survival for patients with HPV-positive cancer was significantly better ($p = 0.0004$), (log-rank test) than that of patients with HPV-negative cancer. Patients with HPV-positive tumors also had significantly better disease-free survival ($p = 0.0008$), (log-rank test) than those with HPV-negative tumors. These results further strengthen the option to consider HPV-status when planning prospective studies on treatment for base of tongue cancer.

Approximately 1,200 new cases of squamous cell carcinoma (SCC) in the head and neck region are diagnosed each year in Sweden. Tobacco use and alcohol consumption are well-known risk-factors for SCC, but infection with high-risk human papillomavirus (HPV) has in recent years become etiologically linked to oropharyngeal cancer[1–8] and is now recognized as a risk factor by the International Agency for Research on Cancer (IARC).

Base of tongue SCC is the second most common oropharyngeal cancer in Sweden and the incidence is increasing here as well as in other countries.[9,10] Symptoms and clinical findings may be insidious, and the majority of patients present with an advanced stage disease at diagnosis.[11] Treatment may include radiotherapy, brachytherapy, chemotherapy and surgery. All treatment modalities carry side-effects and sequelae such as problems with swallowing, aspiration and speech. Treatment today is based on tumor stage although the aggressiveness and response to treatment of the tumor varies greatly. Identification of predictive markers allowing a more individualized treatment would thus be of utmost clinical importance for patients with this cancer disease.

Studies on survival for patients with head and neck cancer are not always stratified for different sites and rarely evaluated for subsites[12,13] despite differences in clinical behavior. Furthermore, when base of tongue cancer has been investigated specifically, the studied groups were small and/or nonconsecutive.[14]

HPV has been shown to be a favorable prognostic factor for patients with oropharyngeal cancer in general[12,13,15] and more specifically for patients with tonsillar cancer.[16–18] Tonsils and base of tongue are both part of the Waldeyer ring and resemble each other histopatologically, but some studies show that prognosis and survival is worse for patients with base of tongue cancer than for patients with tonsillar cancer.[19–21] The reason for this difference is yet unknown.

The aim of this study was to examine if HPV implies better survival specifically for patients with base of tongue cancer.

Key words: HPV, survival, base of tongue cancer
Grant sponsors: The Swedish Cancer Foundation, The Stockholm Cancer Society, The Swedish Research Council, The Laryngeal Foundation, Henning and Ida Perssons Foundation, The Stockholm City Council, Karolinska Institutet, The ACTA Otolaryngologica Foundation
DOI: 10.1002/ijc.25625
History: Received 27 May 2010; Accepted 3 Aug 2010; Online 19 Aug 2010
Correspondence to: Per Attner, Department of Oto- Rhino-Laryngology, Head and Neck Surgery, Karolinska University Hospital, 171 76 Stockholm, Sweden, Tel.: +46-8-51771539, E-mail: per.attner@karolinska.se

Material and Methods

Patient data

The study comprises 87 of the 109 patients diagnosed 1998–2007 with base of tongue squamous cell carcinoma in the

County of Stockholm, Sweden. Inclusion criteria were that pretreatment biopsies could be obtained (95 patients) and that the patients were treated with intention to cure. The diagnosis was defined according to International Classification of Diseases (ICD) system 10, code C01.9. Presence of HPV in the biopsies was tested as described earlier.[9] The study was conducted according to ethical permissions 2005/431-31/4 and 2005/1330-32 from the Ethical Committee at Karolinska Institutet, Stockholm, Sweden.

All patients' charts were analyzed and age, sex, TNM-stage, stage, treatment and survival were recorded. HPV-status was defined as positive or negative. Treatment was categorized as radiotherapy, including both external and interstitial (brachytherapy) radiation sources, with conventional and hyperfractionated doses up to 68 Gray, or chemoradiotherapy, where patients in addition to above radiotherapy also were administered two cycles of induction chemotherapy; Fluorouracil (5FU) 1000 mg/m^2 and Cisplatin 100 mg/m^2 as intravenous infusion prior to radiotherapy.

Treatment response was evaluated by clinical examination and/or by histopathological evaluation of biopsies taken under general anesthesia after completed treatment. Patients were evaluated for disease progression every three months the first 3 years, then every six months for a total of 5 years after treatment. All patients have been evaluated for a minimum of 2 years and the follow-up period for the patients ranged from 3 to 122 months. For the survival analysis, age was categorized in two groups, either under or over the age of 62 (mean age for the whole group). Patients categorized in Stage 1 or 2 at time of diagnosis were pooled into one group and compared to patients categorized in Stage 3 and 4.

Detection of HPV DNA

Detection of HPV DNA was performed by PCR using the general primer pairs GP5+/6+ and CPI/IIG. Presence of HPV-16 and HPV-33 DNA was tested in all samples by type-specific primers and of other HPV types by sequencing. To minimize over-diagnosis of HPV positivity, we also assayed for E6 and E7 RNA in 20 samples and could verify that HPV really was active in the vast majority of these samples.

These methods and the results are described in more detail in our previous study.[9]

Statistical analyses

Overall survival was defined as the time from the date of diagnosis to the date of death or to the date of censorship (the last day of follow-up). Disease-free survival was defined as the time from the date of diagnosis to the date of the last known occasion that the patient was disease-free or the date of disease recurrence (local, regional or distant recurrence). Death without documented recurrence was censored at the date of death.

Kaplan–Meier curves were used to present survival data for patients with HPV-positive and HPV-negative tumors and the log-rank test was used in univariate analysis. In multivariable analyses, a Cox proportional hazards model was used to adjust for covariates. The proportional hazards assumption was evaluated with Schoenfeld residuals. A p value of ≤ 0.05 was considered statistically significant. Two-sided p-values were reported. An independent, two-sided t-test was performed to compare the mean age between patients with HPV-positive and HPV-negative tumors. All analyses were performed in a statistical computer program (Stata).

The associations of HPV status with TNM status, stage or histopathological differentiation were calculated using the Freeman-Halton extension of Fisher's exact test (two-tailed) utilizing VassarStats web site for statistical calculations (http://faculty.vassar.edu/lowry/VassarStats.html).

Results

In our previous study, 95 pretreatment biopsies (65 male and 30 female patients) were obtained from the 109 patients diagnosed 1998–2007 with base of tongue SCC in the Stockholm area.[9] The clinical data and TNM stage for the patients with the missing 14 biopsies was random (data not shown). Of the 95 patients where tumor biopsies were available, 87 were treated with curative intent and hence included in the survival analysis in the present study. Of the 87 tumor biopsies from the included patients, 68 were previously identified[9] as HPV DNA positive (78%); 58 were identified as HPV-16 (85%), 7 as HPV-33 (10%), 2 as HPV-35 (3%) and 1 as HPV-58 (2%). None of the samples were positive for more than one HPV type.

The characteristics of the patients and their tumors are listed in Table 1. Age at the time of diagnosis ranged from 41 to 85 years. No significant differences regarding mean age, sex or tumor differentiation were observed between HPV-positive and HPV-negative groups. Patients with HPV-positive tumors were higher staged ($p < 0.01$) and showed more advanced N-classification ($p < 0.02$), than patients with HPV-negative tumors.

Survival analysis

The mean follow-up time for the study group was 50.4 months and the median follow-up time was 50 months (range 3–122 months). There were 18 deaths among patients with HPV-positive cancer and 13 deaths among patients with HPV-negative cancer. Four patients with HPV-positive cancer and two patients with HPV-negative cancer died free of disease.

Based on Kaplan–Meier estimates, the overall survival for patients with HPV-positive cancer was significantly better ($p = 0.0004$), (log-rank test) compared to patients with HPV-negative cancer (Fig. 1). Patients with HPV-positive tumors also had a significantly better disease-free survival ($p = 0.0008$), (log-rank test) than patients with HPV-negative cancer (Fig. 2).

Table 1. Characteristics of patients and tumor samples between 1998 and 2007

Patient and tumor characteristics[1]	All patients (n = 87)		Patients with HPV-positive[2] tumors (n = 68)		Patients with HPV-negative tumors (n = 19)		p-value[3]
	N	%	*n*	%	*n*	%	
Sex							
male	59	68	46	68	13	68	ns
female	28	32	22	32	6	32	
TNM classification[4]							
T1	25	29	19	28	6	32	ns
T2	24	28	22	32	2	11	
T3	10	11	5	7	5	26	
T4	28	32	22	32	6	32	
N0	26	30	15	22	11	58	<0.02
N1	13	15	11	16	2	11	
N2a-c	44	50	39	57	5	26	
N3	4	5	3	4	1	5	
M0	85	98	66	97	19	100	ns
M1	2	2	2	3	0	0	
Stage							
I	5	6	1	1	4	21	<0.01
II	8	9	6	9	2	11	
III	15	17	10	15	5	26	
IV	59	68	51	75	8	42	
Histopathology[5]							
Well	5	6	4	6	1	5	ns
Medium	25	29	17	25	8	43	
Poor	54	62	45	66	9	47	
Not assessed	3	3	2	3	1	5	
Age							
Mean	62		62		61		ns
Median	62		62		61		ns
Treatment							
Radiotherapy	53	61	37	54	16	84	<0.05
Chemoradiotherapy	34	39	31	46	3	16	

ns = not significant.
[1]At time of diagnosis. [2]Presence of HPV DNA by PCR. [3]p-values obtained comparing HPV+ and HPV- groups. [4]TNM classification and stage of cancer according to International Union Against Cancer, UICC, 2002. [5]Tumors graded according to a three-tier grading scheme.

The estimated 1- and 2-year overall survival rates were 90% (95% CI = 80–95%) and 84% (95% CI = 73–91%), respectively, among patients with HPV positive tumors, compared to 74% (95% CI = 48–88%) and 58% (95% CI = 33–76%), respectively, among patients with HPV negative tumors.

For disease-free survival the estimated 1- and 2-year survival rates were 92% (95% CI = 83–97%) and 86% (95% CI = 76–93%), respectively, among patients with HPV positive tumors, compared to 75% (95% CI = 49–88%) and 60% (95% CI = 35–77%), respectively, among patients with HPV negative tumors.

To evaluate factors potentially associated with overall and disease-free survival, univariate analyses were performed and HPV-status, age, sex, tumor stage and treatment were analyzed individually. HPV-status was associated with better both overall and disease-free survival. Chemoradiotherapy treatment was associated with favorable overall survival (Table 2).

To assess the independent prognostic value of these variables for both overall and disease free survival, we performed a multivariable analysis using the Cox proportional hazards model. The results are shown in Table 2. HPV-status was

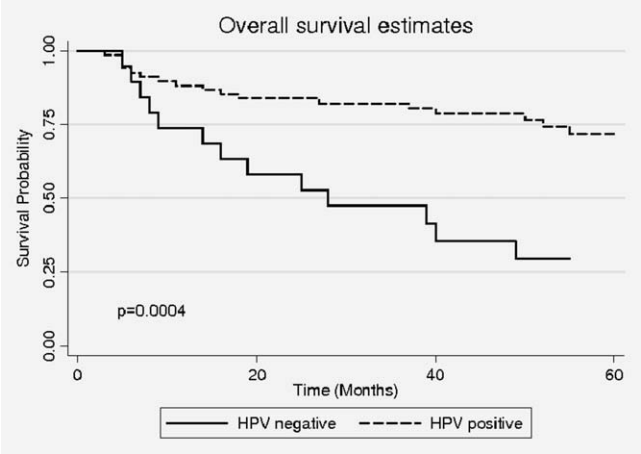

Figure 1. Overall survival illustrated with Kaplan–Meier curve stratified by HPV status.

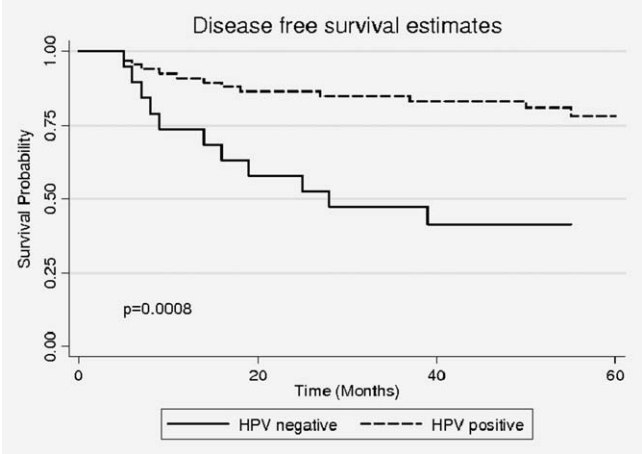

Figure 2. Disease-free survival illustrated with Kaplan–Meier curve stratified by HPV status.

Table 2. Univariate and multivariable models for overall and disease-free survival

	Univariate		Multivariable	
Characteristic	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
Overall survival				
Age (>62 *vs.* <62 years)	1.99 (0.94–4.24)	0.073	1.83 (0.81–4.13)	0.15
Sex (male *vs.* female)	1.03 (0.48–2.18)	0.94	0.92 (0.42–2.05)	0.85
Stage (1+2 *vs.* 3)	0.33 (0.10–1.12)	0.075	0.51 (0.14–1.92)	0.32
Stage (1+2 *vs.* 4)	0.46 (0.19–1.09)	0.078	0.88 (0.31–2.45)	0.80
HPV status (pos *vs.* neg)	0.30 (0.14–0.61)	0.001	0.31 (0.14–0.70)	0.005
Treatment (RT *vs.* CRT)	0.37 (0.15–0.89)	0.027	0.59 (0.22–1.59)	0.30
Disease free survival				
Age (>62 *vs.* <62 years)	1.71 (0.76–3.88)	0.20	1.68 (0.68–4.13)	0.26
Sex (male *vs.* female)	0.87 (0.39–1.97)	0.74	0.81 (0.34–1.95)	0.65
Stage (1+2 *vs.* 3)	0.47 (0.13–1.75)	0.26	0.66 (0.16–2.76)	0.57
Stage (1+2 *vs.* 4)	0.54 (0.20–1.47)	0.23	1.00 (0.30–3.31)	1.0
HPV status (pos *vs.* neg)	0.28 (0.13–0.62)	0.002	0.27 (0.11–0.66)	0.004
Treatment (RT *vs.* CRT)	0.47 (0.19–1.19)	0.11	0.77 (0.27–2.16)	0.62

HR, hazard ratio; CI, confidence interval; HPV, human papilloma virus; RT, radiotherapy; CRT, chemoradiotherapy.

found to be an independent and significant positive prognostic factor both for overall survival ($p = 0.005$) and for disease-free survival ($p = 0.004$). Other variables, including treatment, did not affect prognosis significantly.

Discussion

In this study, including 87 patients with base of tongue cancer, after adjustment for age, sex, tumor stage and treatment, we found that patients with HPV-positive base of tongue cancer had a significantly better overall and disease-free survival compared to those with HPV-negative tumors. This, despite the fact that patients with HPV-positive tumors were higher staged than those with HPV-negative ones.

To our knowledge, this is the first, consecutive study on base of tongue cancer alone and our results are in accordance with what others have shown for oropharyngeal cancer in general.[12]

In our material we found that significantly more patients with HPV-positive tumors received chemoradiotherapy compared to those with HPV-negative tumors. However, when compensating for this difference in the statistical models, it did not seem to influence outcome. The reason why more patients with HPV-positive tumors received chemoradiotherapy could be that this treatment was introduced to all patients with base of tongue cancer from 2005, and since then the vast majority of patients with base of tongue cancer have had HPV-positive tumors.

One limitation of our study is that the sample size is relatively small. However, the material is consecutive over a 10-year period, the disease is not so common and this is, to our knowledge the largest study performed so far exclusively on patients with base of tongue cancer.

Another limitation is that our study is retrospective which limits the number of variables that could be included in the statistical analyses and there is always a risk of residual confounding. For example, data on patient performance status, smoking habits and co-morbidity were not available.

We also used PCR methodology to detect HPV DNA in the tumors, which has been suggested to be too sensitive. However, we have previously shown that the vast majority of our HPV-positive tumors also express E6/E7 mRNA.[9] Moreover, had the HPV-DNA PCR method been oversensitive (i.e. produce false positive cases) this would in turn dilute the difference in survival between patients with HPV-positive and HPV-negative tumors, and here we still show significant difference between the groups.

In recent years, we have shown a gradual increase in the relative frequency of HPV-positive tonsillar cancer[8,22] and base of tongue cancer.[9] The present study shows that the improved survival is independent of type of oncologic treatment, which is in line with previous studies regarding tonsillar and oropharyngeal cancer.[23–25] The reason for the better response to treatment for patients with HPV positive tumors is not known. Previous studies have found an inverse relationship between tumor HPV status and presence of p53 mutations in head and neck cancer.[26,27] The improved response to oncological treatment observed for patients with HPV-positive tumors could therefore be explained by the presence of an intact p53-mediated apoptotic response in HPV-positive tumors. Another possibility is immunological factors related to HPV infection.[28] It has furthermore been shown that the improved survival in oropharyngeal cancer is independent of oncological or surgical treatment.[29] Nonetheless, the reason for the improved survival among patients with HPV-positive tumors needs to be investigated further.

In conclusion, our data show a beneficial survival outcome for patients with HPV-positive base of tongue cancer. This finding needs to be confirmed in prospective studies stratified for HPV-status as well as base of tongue subsite. In the future, as suggested by others,[30] this could lead to the identification of a subgroup of patients who will be cured by less aggressive treatment with less sequelae without compromising survival.

References

1. Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P, Moller B, Pukkala E, Schiller JT, Youngman L, Lehtinen M, Dillner J. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2001;344:1125–31.
2. Gillison ML, Shah KV. Human papillomavirus-associated head and neck squamous cell carcinoma: mounting evidence for an etiologic role for human papillomavirus in a subset of head and neck cancers. *Curr Opin Oncol* 2001;13: 183–8.
3. Alani RM, Munger K. Human papillomaviruses and associated malignancies. *J Clin Oncol* 1998;16:330–7.
4. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, Westra WH, Gillison ML. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007;356:1944–56.
5. Klussmann JP, Weissenborn SJ, Wieland U, Dries V, Kolligs J, Jungehuelsing M, Eckel HE, Dienes HP, Pfister HJ, Fuchs PG. Prevalence, distribution, and viral load of human papillomavirus 16 DNA in tonsillar carcinomas. *Cancer* 2001;92: 2875–84.
6. Smith EM, Ritchie JM, Summersgill KF, Klussmann JP, Lee JH, Wang D, Haugen TH, Turek LP. Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. *Int J Cancer* 2004;108:766–72.
7. Snijders PJ, Cromme FV, van den Brule AJ, Schrijnemakers HF, Snow GB, Meijer CJ, Walboomers JM. Prevalence and expression of human papillomavirus in tonsillar carcinomas, indicating a possible viral etiology. *Int J Cancer* 1992;51: 845–50.
8. Hammarstedt L, Lindquist D, Dahlstrand H, Romanitan M, Dahlgren LO, Joneberg J, Creson N, Lindholm J, Ye W, Dalianis T, Munck-Wikland E. Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. *Int J Cancer* 2006;119:2620–3.
9. Attner P, Du J, Nasman A, Hammarstedt L, Ramqvist T, Lindholm J, Marklund L, Dalianis T, Munck-Wikland E. The role of human papillomavirus in the increased incidence of base of tongue cancer. *Int J Cancer* 2010;126:2879–84.
10. Shiboski CH, Schmidt BL, Jordan RC. Tongue and tonsil carcinoma: increasing trends in the U.S. population ages 20-44 years. *Cancer* 2005;103:1843–9.
11. Cancer incidence in Sweden 2007. Swedish Cancer Registry. The National Board of Health and Welfare, The National Board of Health and Welfare, Sweden, 2008.
12. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A, Gillison ML. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst* 2008;100:261–9.
13. Reimers N, Kasper HU, Weissenborn SJ, Stutzer H, Preuss SF, Hoffmann TK, Speel EJ, Dienes HP, Pfister HJ, Guntinas-Lichius O, Klussmann JP. Combined analysis of HPV-DNA, p16 and EGFR expression to predict prognosis in oropharyngeal cancer. *Int J Cancer* 2007;120:1731–8.
14. Dahlgren L, Dahlstrand HM, Lindquist D, Hogmo A, Bjornestal L, Lindholm J, Lundberg B, Dalianis T, Munck-Wikland E. Human papillomavirus is more common in base of tongue than in mobile tongue cancer and is a favorable prognostic factor in base of tongue cancer patients. *Int J Cancer* 2004;112:1015–9.
15. Weinberger PM, Yu Z, Haffty BG, Kowalski D, Harigopal M, Sasaki C, Rimm DL, Psyrri A. Prognostic significance of p16 protein levels in oropharyngeal squamous cell cancer. *Clin Cancer Res* 2004;10:5684–91.
16. Lindquist D, Romanitan M, Hammarstedt L, Nasman A, Dahlstrand H, Lindholm J, Onelöv L, Ramqvist T, Ye W, Munck-Wikland E, Dalianis T. Human papillomavirus is a favourable prognostic factor in tonsillar cancer ant its oncogenic role is supported by the expression of E6 and E7. *Mol Oncol* 2007;1: 350–5.
17. Gillison ML. Human papillomavirus-associated head and neck cancer is a distinct epidemiologic, clinical, and molecular entity. *Semin Oncol* 2004;31: 744–54.

18. Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, Viscidi R. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst* 2008;100: 407–20.
19. Pedruzzi PA, Kowalski LP, Nishimoto IN, Oliveira BV, Tironi F, Ramos GH. Analysis of prognostic factors in patients with oropharyngeal squamous cell carcinoma treated with radiotherapy alone or in combination with systemic chemotherapy. *Arch Otolaryngol Head Neck Surg* 2008; 134:1196–204.
20. Johansen LV, Grau C, Overgaard J. Squamous cell carcinoma of the oropharynx--an analysis of treatment results in 289 consecutive patients. *Acta Oncol* 2000;39:985–94.
21. Makitie AA, Pukkila M, Laranne J, Pulkkinen J, Vuola J, Back L, Koivunen P, Grenman R. Oropharyngeal carcinoma and its treatment in Finland between 1995-1999: a nationwide study. *Eur Arch Otorhinolaryngol* 2006;263: 139–43.
22. Nasman A, Attner P, Hammarstedt L, Du J, Eriksson M, Giraud G, Ahrlund-Richter S, Marklund L, Romanitan M, Lindquist D, Ramqvist T, Lindholm J, et al. Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? *Int J Cancer* 2009;125:362–6.
23. Lindel K, Beer KT, Laissue J, Greiner RH, Aebersold DM. Human papillomavirus positive squamous cell carcinoma of the oropharynx: a radiosensitive subgroup of head and neck carcinoma. *Cancer* 2001;92: 805–13.
24. Mellin Dahlstrand H, Lindquist D, Bjornestal L, Ohlsson A, Dalianis T, Munck-Wikland E, Elmberger G. P16(INK4a) correlates to human papillomavirus presence, response to radiotherapy and clinical outcome in tonsillar carcinoma. *Anticancer Res* 2005; 25:4375–83.
25. Licitra L, Perrone F, Bossi P, Suardi S, Mariani L, Artusi R, Oggionni M, Rossini C, Cantu G, Squadrelli M, Quattrone P, Locati LD, et al. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. *J Clin Oncol* 2006;24:5630–6.
26. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 2000;92: 709–20.
27. Dai M, Clifford GM, le Calvez F, Castellsague X, Snijders PJ, Pawlita M, Herrero R, Hainaut P, Franceschi S. Human papillomavirus type 16 and TP53 mutation in oral cancer: matched analysis of the IARC multicenter study. *Cancer Res* 2004;64:468–71.
28. Spanos WC, Nowicki P, Lee DW, Hoover A, Hostager B, Gupta A, Anderson ME, Lee JH. Immune response during therapy with cisplatin or radiation for human papillomavirus-related head and neck cancer. *Arch Otolaryngol Head Neck Surg* 2009;135:1137–46.
29. Ritchie JM, Smith EM, Summersgill KF, Hoffman HT, Wang D, Klussmann JP, Turek LP, Haugen TH. Human papillomavirus infection as a prognostic factor in carcinomas of the oral cavity and oropharynx. *Int J Cancer* 2003;104: 336–44.
30. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol* 2010;11:781–9.