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p16 expression in oropharyngeal cancer: its impact on staging and prognosis compared with the conventional clinical staging parameters

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Background: Currently, staging of head neck squamous cell carcinoma (HNSCC) is on the basis of primary tumor extension (cT), lymph node involvement (cN) and distant metastasis (cM). The aim of cancer staging was to improve diagnosis, prognosis and to compare outcome results. A new subgroup of oropharyngeal squamous cell carcinoma (OPSCC) induced by human papillomavirus (HPV) infection is reported to show an increasing incidence. These HPV-positive OPSCC show distinct molecular differences, specific p16 overexpression and a significantly better prognosis. Therefore, the aim of this study was to evaluate the prognostic influence of p16 expression in OPSCC and compare its relevance with the established prognostic markers cT and cN classification and the clinical stages I–IV.

Patients and methods: Immunohistochemistry for p16 was carried out on the basis of a tissue microarray including 102 OPSCC patients with corresponding retrospective clinicopathological and follow-up data.

Results: p16 is the strongest independent prognostic marker in OPSCC, surpassing the significance of cT and cN classification as well as the clinical stages I–IV. Prognosis of p16-positive OPSCC of an advanced stage reached or even exceeded prognosis of the next clinically smaller conventionally staged group of tumors.

Conclusion: p16 is the most relevant prognostic marker in OPSCC and should be considered for inclusion into the official staging system of HNSCC.

Key words: HPV, oropharyngeal carcinoma, p16, prognosis, staging

introduction

The aim of cancer classification is to better understand prognosis of cancer, to improve diagnosis and compare outcome results for a consecutive improvement of treatment recommendations of distinct cancers at a specific stage of disease. Current staging of head neck squamous cell carcinoma (HNSCC) is primarily based on clinical primary tumor extension (cT), lymph node involvement (cN) and distant metastasis (cM). For surgically treated tumors, two histopathological parameters tumor grading (G) and radicality of resection (R) are added. Risk factors for locoregional relapse such as vascular invasion (V), lymph node capsular spread and tumor-free margin size of the resected tumor were recently identified. Locoregional control is significantly improved when these HNSCC patients are treated with postoperative concurrent radiotherapy (RT) with platinum-based chemotherapy [1, 2]. Currently, no parameters representing information neither on tumor biology nor on tumor behavior under therapy are included nor in HNSCC staging nor for treatment modality choice. Small tumor stages I and II, including primary tumors <4 cm without locoregional lymph node metastases, are treated by single modalities, surgery or RT alone, whereas advanced tumor stages III and IV undergo a combined modality treatment with either radical surgery plus adjuvant RT or concomittant radiochemotherapy (RCT), combined with a platinum-based chemotherapy.

Cancer statistics report an increased incidence of oral and oropharyngeal squamous cell carcinoma (OPSCC) in the United States as well as in Europe [3–5]. This subgroup of OPSCC is characterized by human papillomavirus (HPV) 16 or 18 infections leading to distinct molecular characteristics and indicating a different pathway in carcinogenesis compared with HPV-negative HNSCC [6]. One particular molecular difference, among others, concerns p16 expression. Nuclear and cytoplasmic p16 overexpressions correlate precisely with HPV positivity and are indicated to be specific for HPV-positive OPSCC [7, 8]. Patients with OPSCC overexpressing HPV with or without p16 overexpression as well as those p16-positive OPSCC without HPV detection show a significantly improved

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prognosis when compared with patients with HPV- and p16-negative OPSCC [9], independent of the treatment modality chosen [6, 10–16].

In this study, we evaluate the prognostic influence of p16 expression in OPSCC and compare its relevance with the established prognostic markers cT and cN classification and the clinical stages I–IV. The importance of p16 status in OPSCC staging may be of decisive importance for staging as well as for treatment recommendations in the near future.

materials and methods

patient data and specimen characteristics

At the University Hospital in Basel, we constructed a tissue microarray (TMA) of previously untreated samples with complete medical history and follow-up data [17]. Hundred and two of the tumor specimens were located in the oropharynx. Small tumors with stages I and II were treated by single modality, either surgery or RT, advanced tumor stages III and IV with combined modalities, either surgery plus adjuvant RT or RCT.

Median follow-up time was 48.5 months and overall 5-year survival rate (5-YSR) was 38% [95% confidence interval (CI) 28% to 48%]. Censored observations included patients who were alive at last possible follow-up. Of 102 patients evaluated, 68 patients (66.7%) died of disease and 34 (33.3%) were considered censored. Clinicopathological data are summarized in Table 1.

Table 1. Clinicopathological parameters in 102 oropharyngeal squamous cell carcinomas

	p16, *n* (%)		*P* value
	Positive	Negative	
Gender			
Female	12 (29)	11	0.183
Male	29 (71)	50	
Age, median (range) (years)	60.4 (39–78)	58 (36–91)	0.232
Tumor localization			
Palatine tonsil	19 (46)	28 (46)	0.32
Soft palate	3 (7)	11 (18)	
Base of the tongue	16 (40)	16 (26)	
Back wall	3 (7)	6 (10)	
cT classification			
cT1	9 (22)	10 (16)	0.189
cT2	22 (54)	24 (39)	
cT3	6 (14)	12 (20)	
cT4	4 (10)	15 (25)	
cN classification			
cN0	11 (26)	15 (25)	0.398
cN1	8 (20)	13 (21)	
cN2	22 (54)	29 (48)	
cN3	0 (0)	4 (6)	
c Stage			
Stage I	3 (7)	5 (8)	0.99
Stage II	6 (15)	8 (13)	
Stage III	8 (20)	11 (18)	
Stage IV	24 (56)	37 (61)	
Tumor grading			
Grade 1	1 (2)	4 (6)	0.646
Grade 2	23 (56)	37 (61)	
Grade 3	17 (42)	20 (33)	

immunohistochemical p16 analysis

For the immunohistochemical p16 staining and scoring, we followed the previously described procedure [13]. Failure of analysis (~7% of all cases, 8 from 110 samples) was related to TMA technology, including a fraction of missing samples (empty spot) and those spots containing no or only a few tumor cells.

statistical analysis methods

The association of p16 expression (negative <5% and positive ≥5% immunoreactivity in tumor cells) with clinicopathological features was carried out by the chi-square test, Fisher's exact test and Student's *t*-test, where appropriate. Univariate tumor-specific survival and recurrence-free survival were evaluated by the Kaplan–Meier method and log-rank test. The appropriate number of variables to be included into multivariable survival analysis using multiple Cox regression models was on the basis of the number of patient deaths. In order to prevent overfitting, we considered one variable per 10 patient deaths. Therefore, the prognostic impact of p16 expression in stage IV patients alone ($n = 44$ deaths) and stages III and IV patients ($n = 59$ deaths) was considered along with cT, cN and tumor grading. The hazard ratio (HR) and 95% CIs were obtained in order to determine the effect of each variable on outcome with HR < 1.0, indicating a negative effect on outcome with p16 positivity. The assumption of proportional hazards was first verified by analyzing the correlation of Schoenfeld residuals and the ranks of individual failure times. *P* values < 0.05 were considered statistically significant. All analyses were carried out using SAS (Version 9; Cary, NC).

results

patients

Distribution of the most relevant clinicopathological parameters between the p16-positive and -negative OPSCC is shown in Table 1. No significant differences were found; the proportion of larger primary tumors grew in favor of the p16-negative OPSCC.

clinical staging parameters and overall survival

As the group of small OPSCC stages I and II included only 8 and 14 patients, respectively, these stages were analyzed together. Overall survival of the small stage tumors was significantly longer than that of the advanced stages ($P = 0.02$). 5-YSR for these was 63.3% (95% CI 39% to 80%) compared with 31% (95% CI 11% to 52%) for stage III, similar to stage IV [29.5% (95% CI 19% to 41%)] (Figure 1, panel A). The prognostic impact of the most relevant clinical prognostic parameter—cN classification, a significantly better overall survival for nodal-negative tumors with a 5-YSR of 56% (95% CI 35% to 72%) compared with 30% (95% CI 20% to 41%) for nodal-positive OPSCC ($P = 0.0097$) (data not shown). Among the advanced OPSCC, overall survival of stages III and IV depended highly on cT classification, tumors >4 cm had a significantly worse prognosis ($P = 0.001$) (Figure 1, panel C). No relevant influence was seen considering cN classification: as there were three OPSCC with cN0 classification, samples were grouped cN0 with cN1 versus cN2 with cN3 tumors. No differentiation in overall survival was achieved, 5-YSR was 27.9% and 30.7%, respectively ($P = 0.915$) (Figure 1, panel D).

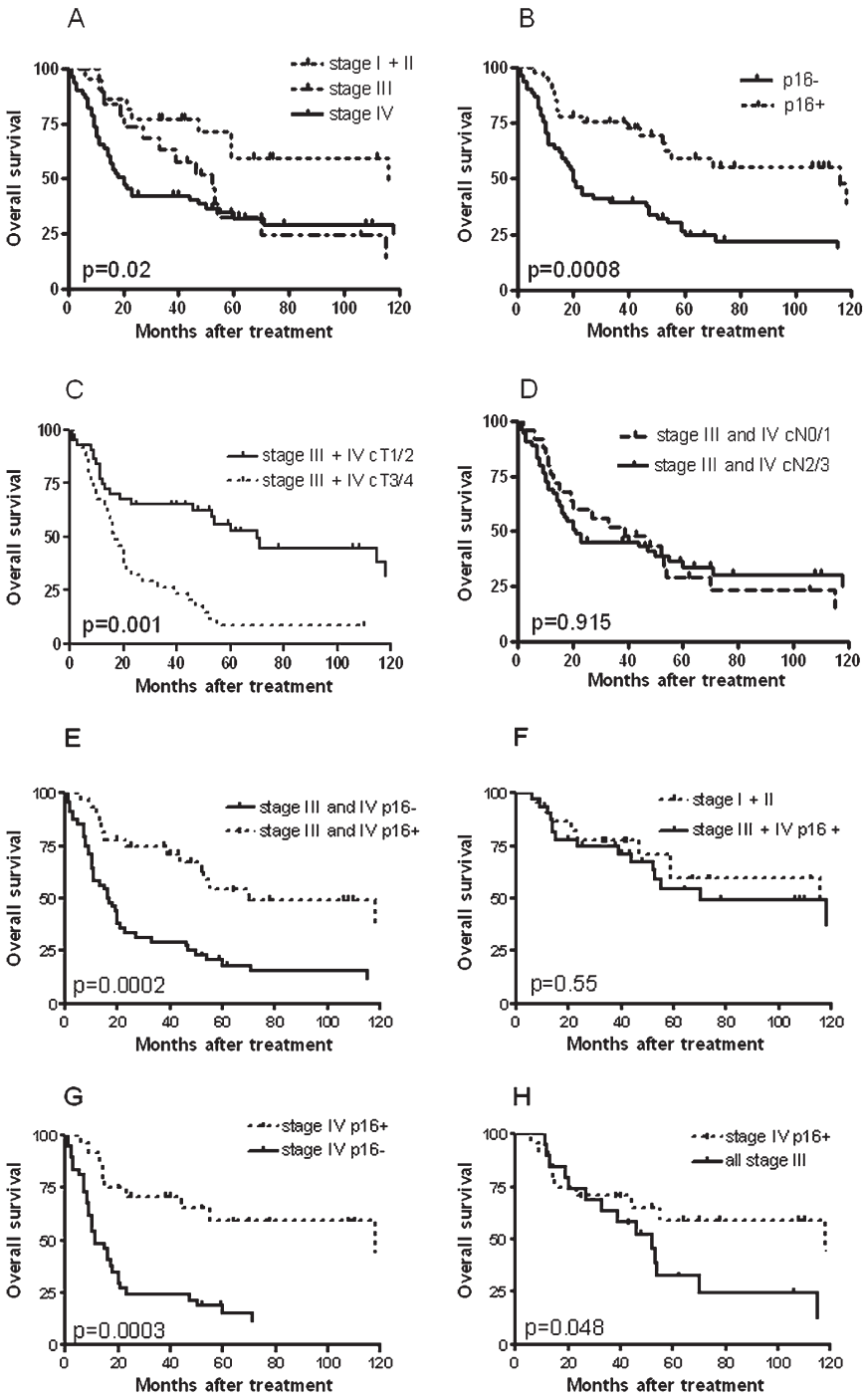

Figure 1. Kaplan–Meier survival curves of 102 oropharyngeal squamous cell carcinoma showing the overall survival differences between (A) the different clinical stages I + II, III and IV; (B) the p16-positive and p16-negative tumors, all stages included; (C) the primary tumor classifications cT1/2 and cT3/4 in the advanced stages III and IV; (D) the lymph node metastasis classifications cN0/1 and cN2/3 in the advanced stages III and IV; (E) the p16-positive and p16-negative advanced stages III and IV; (F) all the clinical stages I and II tumors and the p16-positive advanced stages III and IV; (G) the p16-positive and p16-negative advanced stage IV tumors; and (H) all the clinical stage III tumors and the p16-positive advanced tumors stage IV.

p16 expression and overall survival

Summarizing all four stages, p16-positive OPSCC had a better prognosis, 5-YSR 59.3% (95% CI 41% to 73%), than p16-negative tumors with 24.5% (95% CI 14% to 36%) (P = 0.0008) (Figure 1, panel B). This effect is seen even stronger in the advanced OPSCC stages III and IV: p16-positive tumors showed a 5-YSR of 54.1% (95% CI 34% to 71%) compared with 18% (95% CI 9% to 30%) for p16-negative OPSCC (P = 0.0002) (Figure 1, panel E). The p16-positive subgroup of the advanced stages III and IV showed the similar good overall survival as the small stages I and II with a 5-YSR of 59.4% (95% CI 34% to 78%) (P = 0.55) (Figure 1, panel F). Dividing the

advanced stages in stages III and IV tumors, patients with p16-positive stage IV tumors survive 5 years in 59.2% (95% CI 36% to 77%) compared with 18.9% (95% CI 8% to 33%) for p16-negative stage IV OPSCC ($P = 0.0003$) (Figure 1, panel G). Patients with p16-positive stage IV OPSCC survived significantly longer than those conventionally staged III OPSCC, 5-YSR 32.5% (95% CI 13% to 55%) ($P = 0.048$) (Figure 1, panel H).

The impact of the clinical parameters and p16 expression in the advanced stages III and IV was analyzed and compared in a multivariable analysis (Table 2), demonstrating the strongest prognostic effect of p16, which is independent of the conventional staging parameters cT and cN classification as well as tumor grade.

discussion

The first classification of cancer stage was developed from 1943 to 1952 by Professor Pierre Denoix at the Institute Gustave Roussy in France, later establishing the Union Internationale Contre le Cancer (UICC). Since the fusion of the UICC and the American Joint Committee for Cancer in 1987, official and regularly updated staging guidelines for most malignant tumors are published. Standardized cancer staging and classification should lead to a more precise and comprehensive recording of the neoplastic disease as a reliable basis for comparison of treatment outcome of a distinct tumor with a defined extension. Furthermore, these data enable an improvement toward a more individualized patient-centered treatment.

We analyzed the impact of p16 overexpression in OPSCC. p16, a cell cycle checkpoint regulator functions as a tumor suppressor and is located on chromosome 9p21. It is overexpressed in HPV-positive OPSCC due to the degradation of pRb by the viral oncoprotein E7 [18, 19], as pRb normally functions as a negative regulator of p16 expression. Nuclear and cytoplasmic p16 overexpressions correlate precisely to HPV positivity in OPSCC and are indicated to be specific for these carcinomas. As p16 overexpression is very rarely seen in HPV-negative HNSCC, it is considered a surrogate marker for HPV positivity in OPSCC [7, 8]. The relevance of the positive prognostic effect of p16 expression has also been reported in HPV-negative OPSCC compared with HPV- and p16-negative tumors [9]. A possible reason could be an induction of p16 expression by E7 at a very early stage of disease, when HPV cannot yet be detected.

Table 2. Prognostic effect of p16 expression after adjustment for effects of tumor grade, cT and cN classification in oropharyngeal squamous cell carcinoma

Feature	Stage IV		Stages III and IV	
	P value	HR (95% CI)	*P* value	HR (95% CI)
p16 positivity	0.006	0.34 (0.2–0.7)	0.002	0.39 (0.2–0.7)
Tumor grade	0.319	1.32 (0.7–2.3)	0.71	0.97 (0.8–1.2)
cT classification	0.073	1.39 (1.0–2.0)	0.005	1.49 (1.1–1.9)
cN classification	0.653	1.18 (0.6–2.4)	0.836	1.05 (0.7–1.6)

95% CI, 95% confidence interval; HR, hazard ratio.

Among the p16-positive and -negative OPSCC, no significant differences in the distribution of the clinical parameters was noticed, the p16-negative group including more advanced primary tumors, a result also presented by others [20]. Although some authors discuss HPV-induced OPSCC as carcinomas with more frequent lymph node metastases [21, 22], others could find no correlation to cN classification at all [11, 12], as it was also found in our study. Dividing OPSCC by clinical stages I–IV showed the expected prognostic division of the small stages I and II from the advanced stages III and IV. Similar to published results [21], a much more relevant division of OPSCC in terms of outcome differences was achieved not by clinical parameters but by grouping OPSCC in p16-positive and -negative tumors, the 5-YSR for the p16-positive OPSCC more than twice the rate of p16-negative OPSCC.

The advanced stages depend either on large primary tumors or on lymph node involvement. While primary tumor size had a highly significant impact on prognosis of stages III and IV OPSCC, cN classification did not. HPV- or p16-positive OPSCC is reported to show a more advanced cN classification lymphatic metastases at an earlier stage of the primary disease compared with p16-negative OPSCC [21, 22]. This led in our collective to the inclusion of more small primaries with advanced cN classifications in the stage IV group and more advanced primaries in the small stage III group, possible reasons for their similar long-term outcome (Figure 1A) and for the limited sample size of the cN0 group, which was too small to be analyzed separately and summarized with the cN1 tumors. Probably, a stronger differentiation could have been achieved if the cN0 tumors could be differentiated and compared separately with cN-positive tumors. Again, the influence of p16 expression in the advanced stages III and IV was the most relevant parameter and independent of the other clinical parameters in a multivariable analysis. Most interesting, 5-YSR of p16-positive advanced stage OPSCC was nearly as good as the survival rate of the clinically small stages I and II (54.1% versus 59.4%). We are aware of the fact that the advanced staged OPSCC were treated by a combined modality treatment, whereas small stages were treated either by surgery or RT alone. Therefore, the same examination was made among the advanced stages. The whole group was now treated by combined modalities, surgery plus adjuvant RT or RCT. p16-positive OPSCC clearly surpassed prognosis of the stage III tumors, the 5-YSR approaching the double survival rate of the stage III tumors (59.2% versus 32.6%). Again, the impact of p16 was shown to be the strongest prognostic marker in advanced OPSCC and independent of the clinical parameters in a multivariable analysis for stage IV tumors. A similar prognostic benefit of p16-expressing OPSCC was also reported by Weinberger et al. [21], who could show a significant prognostic effect not only on overall survival but also on locoregional tumor control and disease-free survival. Most recently, these data were confirmed in two large phase III trials: Gillison et al. [23] reported the prognostic advantage of HPV-positive OPSCC after RCT. Rischin et al. compared the prognostic effect of the four different combinations of HPV and p16 expression after RCT. They showed that the largest group of OPSCC with an

improved prognosis is identified by p16 positivity alone as the HPV-negative but p16-positive group of OPSCC had a better prognosis compared with the HPV- and p16-negative tumors [9].

A thorough clinical staging evaluation, on which outcome comparisons and treatment recommendations are based, involves physical examination, endoscopy and radiological imaging. Physical examination and endoscopy depend primarily on the clinicians experience as does radiological imaging by computed tomography, magnetic resonance tomography or positron emission tomography depend on the radiologists interpretation [24]. Clinical and radiological evaluations bear limits and uncertainties. A corresponding example is the examination and interpretation of a possible bone invasion of the mandible by an advanced OPSCC, as bone invasion is a crucial factor for a cT4 classification of the primary tumor and a strong argument to treat this tumor by surgery instead of RT.

The lymph node status of HNSCC is prognostically even more important than the primary tumor size. Staging evaluation for the cN classification is not defined, clinical examination with ultrasound of the neck and radiological imaging are mandatory. But all three methods depend on the size and shape of the lymph nodes, sensitivity and specificity for a correct diagnosis of a lymph node metastasis in a cN0 neck are very low [25]. Despite a precise clinical neck staging, we miss in up to 30% of the clinically cN0 necks occult nodal disease, as reported by comparison with elective neck dissection or sentinel lymph node biopsy [26].

In conclusion, for conventional staging and pretreatment decision making, we still rely on results achieved by the 'best' staging methods/techniques available, in awareness of the bias included. p16 expression is a very sensitive and specific marker for HPV infection in HNSCC. p16 immunohistochemistry of HNSCC is carried out and evaluated in a very standardized way, it is cheap and easy to carry out also in smaller pathological centers. Interpretation of p16 expression furthermore is facilitated, as already 5% of p16 expressing tumor cells are defined as a positive result, indicating that this tumor is induced by HPV and has therefore a highly significantly better prognosis. Various publications reported this effect in tumors treated as well by surgery [12, 16] as by RT/RCT [9, 15, 23], it seems to be independent of the treatment modality chosen [13].

We are aware of the limitations of our study as the small sample size of evaluated OPSCC in a retrospective analysis. Despite these limitations, we present outcome data of a single multidisciplinary head and neck cancer institution, generated in a standardized way: clinical and radiological evaluation is coordinated and interpreted at a conjoint tumor board, treatment regimens chosen corresponding to published official guidelines. The study design was carried out according to the REMARK criteria [27] and biopsy specimens analyzed in a TMA, which allowed us a p16 staining under standardized circumstances and an evaluation of the immunohistochemistry by two independent observers.

Based on our and recently published results, we think that it could be time to reconsider staging of HNSCC and OPSCC especially, to combine clinical with molecular tumor-based information to improve validity and relevance of a modern accurate classification system, on which outcome comparison and treatment recommendations are built. In our view, future outcome comparisons of HNSCC and OPSCC, especially, should distinguish between p16-positive and -negative OPSCC. Although both tumors are squamous cell carcinomas, they present two different tumor entities of the same organ. As undifferentiated outcome results could lead to false prognostic assumptions for the conventional OPSCC, we recommend to introduce a third histopathological parameter 'p16 positive or negative' for all OPSCC. This will allow us in the future to improve treatment of these distinct HPV-positive OPSCCs, outside the main groups of nicotine- and alcohol-associated HNSCC, and spare these patients unnecessary treatment-related short- and long-term side-effects. We are aware, however, before implementation, our data need validation in large prognostic series.

disclosure

None of the authors declare conflicts of interest.

references

1. Bernier J, Domenge C, Ozsahin M et al. Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med 2004; 350: 1945–1952.
2. Cooper JS, Pajak TF, Forastiere AA et al. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. N Engl J Med 2004; 350: 1937–1944.
3. Dahlstrand H, Nasman A, Romanitan M et al. Human papillomavirus accounts both for increased incidence and better prognosis in tonsillar cancer. Anticancer Res 2008; 28: 1133–1138.
4. Annertz K, Anderson H, Biorklund A et al. Incidence and survival of squamous cell carcinoma of the tongue in Scandinavia, with special reference to young adults. Int J Cancer 2002; 101: 95–99.
5. Shiboski CH, Schmidt BL, Jordan RC. Tongue and tonsil carcinoma: increasing trends in the U.S. population ages 20-44 years. Cancer 2005; 103: 1843–1849.
6. 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.
7. Hafkamp HC, Speel EJ, Haesevoets A et al. A subset of head and neck squamous cell carcinomas exhibits integration of HPV 16/18 DNA and overexpression of p16INK4A and p53 in the absence of mutations in p53 exons 5-8. Int J Cancer 2003; 107: 394–400.
8. 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.
9. Rischin D, Young R, Fisher R et al. Prognostic significance of HPV and p16 status in patients with oropharyngeal cancer treated on a large international phase III trial. J Clin Oncol 2009; 27: 6004.
10. Schwartz SR, Yueh B, McDougall JK et al. Human papillomavirus infection and survival in oral squamous cell cancer: a population-based study. Otolaryngol Head Neck Surg 2001; 125: 1–9.
11. Mellin H, Friesland S, Lewensohn R et al. Human papillomavirus (HPV) DNA in tonsillar cancer: clinical correlates, risk of relapse, and survival. Int J Cancer 2000; 89: 300–304.
12. Reimers N, Kasper HU, Weissenborn SJ et al. Combined analysis of HPV-DNA, p16 and EGFR expression to predict prognosis in oropharyngeal cancer. Int J Cancer 2007; 120: 1731–1738.
13. Fischer CA, Zlobec I, Green E et al. Is the improved prognosis of p16 positive oropharyngeal squamous cell carcinoma dependent of the treatment modality? Int J Cancer 2010; 126: 1256–1262.

14. Mellin DH, Lindquist D, Bjornestal L et al. P16(INK4a) correlates to human papillomavirus presence, response to radiotherapy and clinical outcome in tonsillar carcinoma. Anticancer Res 2005; 25: 4375–4383.
15. 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.
16. Licitra L, Perrone F, Bossi P et al. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. J Clin Oncol 2006; 24: 5630–5636.
17. Sauter G, Simon R, Hillan K. Tissue microarrays in drug discovery. Nat Rev Drug Discov 2003; 2: 962–972.
18. Wiest T, Schwarz E, Enders C et al. Involvement of intact HPV16 E6/E7 gene expression in head and neck cancers with unaltered p53 status and perturbed pRb cell cycle control. Oncogene 2002; 21: 1510–1517.
19. Li Y, Nichols MA, Shay JW, Xiong Y. Transcriptional repression of the D-type cyclin-dependent kinase inhibitor p16 by the retinoblastoma susceptibility gene product pRb. Cancer Res 1994; 54: 6078–6082.
20. Yuen PW, Man M, Lam KY, Kwong YL. Clinicopathological significance of p16 gene expression in the surgical treatment of head and neck squamous cell carcinomas. J Clin Pathol 2002; 55: 58–60.
21. 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.
22. Hafkamp HC, Manni JJ, Haesevoets A et al. Marked differences in survival rate between smokers and nonsmokers with HPV 16-associated tonsillar carcinomas. Int J Cancer 2008; 122: 2656–2664.
23. Gillison ML, Harris L, Westra W et al. Survival outcomes by tumor human papillomavirus (HPV) status in stage III-IV oropharyngeal cancer (OPC) in RTOG 0129. J Clin Oncol 2009; 27: 6003.
24. Ng SH, Yen TC, Liao CT et al. 18F-FDG PET and CT/MRI in oral cavity squamous cell carcinoma: a prospective study of 124 patients with histologic correlation. J Nucl Med 2005; 46: 1136–43.
25. de Bondt RB, Nelemans PJ, Hofman PA et al. Detection of lymph node metastases in head and neck cancer: a meta-analysis comparing US, USgFNAC, CT and MR imaging. Eur J Radiol 2007; 64: 266–272.
26. van den Brekel MW, van der Waal I, Meijer CJ et al. The incidence of micrometastases in neck dissection specimens obtained from elective neck dissections. Laryngoscope 1996; 106: 987–991.
27. McShane LM, Altman DG, Sauerbrei W et al. REporting recommendations for tumour MARKer prognostic studies (REMARK). Eur J Cancer 2005; 41: 1690–1696.