

# Meta-analysis of survival in patients with HNSCC discriminates risk depending on combined HPV and p16 status

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**Abstract** Data indicate a better prognosis for human papillomavirus (HPV)-associated head and neck squamous cell carcinoma (HNSCC). HPV and p16 detection are established markers for HPV-related HNSCC. Both are accepted as survival-independent predictors. Previous studies investigating the survival in HNSCC patients depending on HPV<sup>+/-</sup> and p16<sup>+/-</sup> status consistently found discordant results with p16<sup>-</sup>/HPV<sup>+</sup> and p16<sup>+</sup>/HPV<sup>-</sup>. However, no meta-analysis regarding the survival according to combined HPV/p16 status has been performed yet. The objective of this study was to discriminate the impact of combined HPV<sup>+/-</sup> and p16<sup>+/-</sup> status on survival. Data sources were identification and review of publications assessing survival of the distinct subgroups with both p16 and HPV investigated in HNSCC until February, 2015. A meta-analysis was performed to classify survival and clinical outcomes. 18 out of 397 articles (4424 patients) were eligible for the meta-analysis. The percent proportion of the subgroups was 25 % for HPV<sup>+</sup>/p16<sup>+</sup>, 61.2 % for HPV<sup>-</sup>/p16<sup>-</sup>, 7.1 % for HPV<sup>-</sup>/p16<sup>+</sup> and 6.8 % for HPV<sup>+</sup>/p16<sup>-</sup>. The meta-analysis showed a significantly improved 5-year overall survival (OS), 5-year disease-free survival and their corresponding hazard ratio for HPV<sup>+</sup>

p16<sup>+</sup> HNSCC in comparison to HPV<sup>-</sup>/p16<sup>-</sup>, HPV<sup>+</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup>. The 5-year OS of the HPV<sup>-</sup>/p16<sup>+</sup> subgroup was intermediate while HPV<sup>+</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>-</sup> HNSCC had the shortest survival. With current therapeutic strategies, survival of patients with HNSCC is better if associated with HPV<sup>+</sup>/p16<sup>+</sup> or HPV<sup>-</sup>/p16<sup>+</sup>. Clinical trials are needed to confirm the distinct survival pattern and to investigate possible differences in survival for HPV<sup>+</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup> HNSCC. To further differentiate p16<sup>+</sup> HNSCC, HPV testing may be advisable.

**Keywords** p16 · Human papillomavirus · Head and neck cancer · Survival · Meta-analysis

## Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide with 350,000 deaths per year [1]. Despite modern treatment strategies, local recurrence rates, metastases, and overall survival (OS) have not changed much in the last 30 years [21, 31, 51]. In recent years it has been accepted that HNSCC etiologically associated with excessive alcohol and tobacco consumption is characterized by specific p53 mutations. This has to be distinguished from HNSCC etiologically associated with persistent infection with the oncogenic high-risk human papillomaviruses (HR-HPV). These generally contain wild-type p53 [24] as well as an increased expression of p16, a marker for the oncogenic activity of HR-HPV [24, 33]. Proteins derived from the viral oncogenes HPV-E6 and HPV-E7 form complexes with tumor suppressor gene products leading to p53 degradation and pRb inactivation, respectively [70, 81]. This is followed by a suppression of apoptosis and initiates a transition to active cell cycling.

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The aforementioned oncogenes also prevent a physiological immune response to virus-infected tumor cells preventing their elimination [5, 22]. Overall, HR-HPV-associated HNSCC amount currently to 25 % and in oropharyngeal HNSCC (OSCC) up to 70 % [23, 44]. The incidence of HPV-associated HNSCC is expected to rise further [42]. Evidence-based clinical guidelines recommend HPV testing in HNSCC, especially for those arising in the oropharynx. HPV status may provide prognostic information and in future, it may also guide specific treatment decisions [17, 19]. However, a standardized procedure for HPV testing remains to be established [6, 9, 38]. The HPV protein E7 leads to pRb degradation which in turn overexpresses p16. Consequently, overexpressed p16 has been used as a surrogate marker for transcriptionally active HPV in OSCC [50, 79]. All HPV-specific tests, including p16-IHC, show a strong correlation with patient survival [e.g., presence of HPV-RNA by RT-PCR, HPV-RNA by in situ hybridization (ISH), and p16-IHC] [6, 50, 63, 67, 79]. And the expression of both markers correlates significantly [12]. So far, all major multivariate survival analyses have demonstrated that a positive p16-IHC status, independently, correlated with better survival [6, 50, 64, 67, 79]. Thus, HPV-DNA-positivity and p16 overexpression represent independent prognostic risk factors for improved survival [77].

Robinson et al. found by HPV-DNA testing in a study including 496 patients that only 5 % of cases were p16<sup>+</sup>/HPV<sup>-</sup> and that 8 % were p16<sup>-</sup>/HPV<sup>+</sup> [60]. Other, studies confirmed this finding by HPV E6/E7 mRNA testing and defined the range for p16<sup>+</sup> tumors to be HPV-RNA<sup>-</sup> between 1 and 7 % and of p16<sup>-</sup> tumors to be HPV-RNA<sup>+</sup> between 2 and 7 % [50, 63, 65]. At this time, it remains unclear whether these consistently made observations have an underlying and so far unknown biological cause or represent an artifact due to insufficient sensitivity and specificity of existing HPV- and p16-testing.

## Objectives

Despite the relatively low rate of discrepant cases (p16<sup>-</sup>/HPV<sup>+</sup> or p16<sup>+</sup>/HPV<sup>-</sup>), the aim of the present study was to perform a meta-analysis on the survival of these distinct groups in comparison to the p16<sup>+</sup>/HPV<sup>+</sup> and p16<sup>-</sup>/HPV<sup>-</sup> groups.

## Methods

### Literature search strategy and selection criteria

We searched for published literature evaluating the survival of subgroups according to the detection of both

HPV and p16 markers three databases: PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), EMBASE ([“http://www.embase.com/”](http://www.embase.com/)) and Wanfang (<http://www.wanfangdata.com.cn>) up to February 14th, 2015. The following search terms were used “HPV, p16, head neck”. Additionally, we checked references cited in original or review articles that were not retrieved from the database by the initial literature search. The manuscripts were screened and all studies on HNSCC patients investigating survival rates according to the tumors’ p16 and HPV status were included. Exclusion criteria were missing information on patient survival and investigation of only one marker (HPV or p16), primary cancer different from HNSCC (nasopharyngeal carcinoma, skin cancer, pre-cancer), cell culture or animal models, and reviews or case reports. In a more detailed second round of selection, we excluded studies with duplicate patient data from the same or similar population according to authors’ names and institution. In these instances, for further analysis the more recent study or the study with a larger patient number was selected. Additionally, we excluded studies with insufficient survival data for this meta-analysis. Finally, this meta-analysis includes studies with the following criteria: (1) in numbers the portion of the subgroups HPV<sup>+</sup>/p16<sup>+</sup> versus HPV<sup>-</sup>/p16<sup>-</sup> versus HPV<sup>+</sup>/p16<sup>-</sup> versus HPV<sup>-</sup>/p16<sup>+</sup> in HNSCC patients; (2) in numbers survival data of these subgroups [hazard ratio (HR); overall survival (OS); disease-free survival (DFS)] or Kaplan–Meier curves of the subgroups of OS or DFS.

### Data extraction

From all eligible publications, the relevant data were extracted by two of the authors (A.C. and A.E.A.) independently following to the inclusion criteria defined for this study. Discrepancies in findings were decided by a reanalysis of the study followed by a final decision by both authors. All relevant information from the manuscript, tables and figures of the incorporated studies, such as author information, date of publication, time frame of the study, country, tumor stage and localization, number of patients, study design, alcohol and tobacco consumption, number of HPV positive and negative patients, number of patients included in the subgroups HPV<sup>+</sup>/p16<sup>+</sup>, HPV<sup>-</sup>/p16<sup>-</sup>, HPV<sup>+</sup>/p16<sup>-</sup> or HPV<sup>-</sup>/p16<sup>+</sup>, HPV subtypes, HR-status, 5-year OS or DFS of the subgroups, p16 and HPV detection method were retrieved. In articles where the OS or DFS was displayed as Kaplan–Meier plot, the software GraphClick (Version 3.0.2, Arizona Software 2010, <http://www.arizona-software.ch/graphclick>) was used to compute the data.

## Statistical analysis

For statistical analysis, we used the relative risk (RR) to evaluate OS and DFS of all subgroups in correlation to the HPV<sup>+</sup>/p16<sup>+</sup> and HPV<sup>-</sup>/p16<sup>-</sup> groups. Summary RR estimates and 95 % confidence intervals (CI) were calculated using maximum-likelihood methods for linear mixed models. To assess study heterogeneity a Chi-squared-based *Q* test was used. A resulting *P* value above 0.05 indicated an absence of heterogeneity between the studies. In the meta-analysis, the *I*<sup>2</sup> index was used to search for any existing heterogeneity, which was estimated as percentage from 0 to 100 %. First, a fixed-effects model (Mantel–Haenszel method and Chi-squared test) was fitted to the data. If the heterogeneity was significant the random-effects model (DerSimonian–Liard method) was used. Depending on the extracted data of the individual publications, we studied the RR of the 5-year OS and DFS of all subgroups in correlation to the HPV<sup>+</sup>/p16<sup>+</sup>- and HPV<sup>-</sup>/p16<sup>-</sup> groups. The same analyses were performed if the HR was described in the studies. To compare all included studies a forest plot was used.

Analysis of publication bias was determined by using a funnel plot. All statistical analyses were performed using the computing environment R Version 3.1.0 (R Core Team [56]).

## Results

### Description of the included studies

A total of 18 out of 397 publications met the criteria for this analysis [11, 15, 25, 26, 29, 30, 34, 36, 43, 47, 48, 58, 62, 68, 69, 75, 80, 82] (Fig. 1; Table 1). The total number of patients included in the studies was 4424 (ranging from 34 to 841 patients per study). However, not for all of these patients the HPV/p16-status was complete, so finally the data of 2811 study patients (ranging from 34 to 723 patients per study) were analyzed in this meta-analysis. The main characteristics of the eligible studies are summarized in Tables 1, 2 and 3. In 14 articles patients with UICC tumor stage I–IV were investigated. In 8 studies OSCC and in 10 HNSCC was investigated (Table 1). HPV detection after preceding polymerase chain reaction (PCR) and in situ hybridization (ISH) without earlier PCR were used as HPV detection methods in 15 and 3 studies, respectively. Table 2 indicates the number of patients belonging to the different subgroups depending on the HPV and p16-status. The proportion of the subgroups was estimated from the 16 studies, which explicitly indicated the patient number [11, 15, 25, 26, 34, 36, 43, 47, 48, 58, 62, 68, 69, 75, 80, 82]. The subgroup of HPV<sup>+</sup>/p16<sup>+</sup> was 25 %, of

HPV<sup>-</sup>/p16<sup>-</sup> 61.2 %, of HPV<sup>-</sup>/p16<sup>+</sup> 7.1 % and of HPV<sup>+</sup>/p16<sup>-</sup> 6.8 %. Table 3 summarizes the studies investigating correlations between HPV status and clinicopathological characteristics like tumor size, lymph node involvement, localization in the OSCC, smoking, etc. 7 studies were performed in Europe, 7 studies in the United States of America (USA) and 4 in Asia.

### 5-year OS of the HPV/p16 subgroups

Thirteen studies determined 5-year OS of all distinct HPV/p16 subgroups. For the investigation of the 5-year OS, 12 studies (1961 patients) contained suitable data for performing a meta-analysis of HPV<sup>+</sup>/p16<sup>+</sup> and HPV<sup>-</sup>/p16<sup>-</sup> patients. The forest plot of this meta-analysis is shown in Fig. 2a and indicates that HPV<sup>+</sup>/p16<sup>+</sup> is associated with improved OS (fixed-effects model; RR of 2.45; 95 % CI 2.07–2.89; *P* = 0.8721).

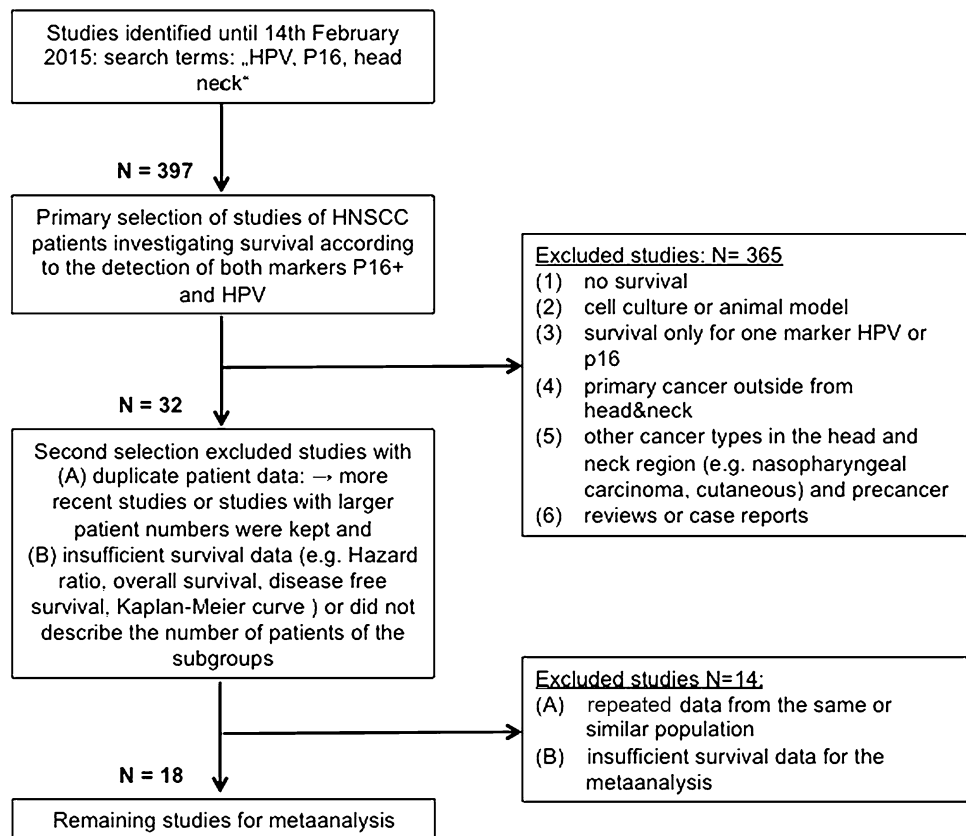
Eight studies (511 patients) included data for the 5-year OS of HPV<sup>+</sup>/p16<sup>+</sup> and HPV<sup>-</sup>/p16<sup>+</sup> patients and 6 studies (373 patients) of HPV<sup>+</sup>/p16<sup>+</sup> and HPV<sup>+</sup>/p16<sup>-</sup> patients. The forest plot of these meta-analyses is shown in Fig. 2b, c and indicates that HPV<sup>+</sup>/p16<sup>+</sup> has an improved OS compared with both the HPV<sup>-</sup>/p16<sup>+</sup> subgroup (fixed-effects model; RR of 1.87; 95 % CI 1.40–2.51; *P* = 0.6343) and the HPV<sup>+</sup>/p16<sup>-</sup> subgroup (fixed-effects model; RR of 2.53; 95 % CI 1.96–3.26; *P* = 0.075).

Eight studies (1465 patients) included data for the 5-year OS of HPV<sup>-</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup> patients and 4 studies (421 patients) of HPV<sup>-</sup>/p16<sup>-</sup> and HPV<sup>+</sup>/p16<sup>-</sup> patients. The forest plot of the meta-analysis of HPV<sup>-</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup> is shown in Fig. 3a and indicates that the OS of HPV<sup>-</sup>/p16<sup>-</sup> does not significantly differ from the OS in the HPV<sup>+</sup>/p16<sup>-</sup> subgroup (fixed-effects model; RR of 0.97; 95 % CI 0.81–1.16; *P* = 0.8222). However, the OS in the HPV<sup>-</sup>/p16<sup>-</sup> subgroup was significantly lower compared to the HPV<sup>-</sup>/p16<sup>+</sup> (fixed-effects model; RR of 0.82; 95 % CI 0.67–0.99; *P* = 0.5043; Fig. 3b).

Furthermore, 4 studies (149 patients) included data of HPV<sup>+</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup> patients for 5-year OS (Fig. 3c). The subgroup of HPV<sup>+</sup>/p16<sup>-</sup> had a reduced OS compared with the subgroup of HPV<sup>-</sup>/p16<sup>+</sup> (fixed-effects model; RR of 0.56, 95 % CI 0.42–0.73, *P* = 0.0809).

### HR of the OS of the HPV/p16 subgroups

The HR for the OS could be determined from data included in 10 studies. Four studies used HPV<sup>+</sup>/p16<sup>+</sup> and 6 used HPV<sup>-</sup>/p16<sup>-</sup> as a reference marker. The results of the individual meta-analyses are summarized in Table 4. The HRs for the OS of the HPV<sup>+</sup>/p16<sup>+</sup> subgroup were significantly increased compared to the HPV<sup>-</sup>/p16<sup>-</sup> subgroup regardless whether HPV<sup>+</sup>/p16<sup>+</sup> or HPV<sup>-</sup>/p16<sup>-</sup> was used

**Fig. 1** Literature search strategy and selection of articles**Table 1** Main characteristics of the eligible studies

References	Time	Total	Men/women	UICC stage	Localization	Mean age (years)
1 Wittekindt et al. [80]	–	34	25/9	I–IV	OSCC	61
2 Smith et al. [68]	1994–2004	301	188/113	I–IV	All	
3 Kong et al. [34]	–	99	69/13	II–IV	All	
4 Weinberger et al. [75]	1980–1999 Yale, 2004–2007 Georgia	140	106/34	I–IV	All	60.3
5 Heath et al. [25]	2004–2007	83	48/35	I–IV	All	64
6 Park et al. [48]	2002–2007	93	80/13	I–IV	OSCC	62.1
7 Holzinger et al. [29]	1990–2008	199	146/50	I–IV	OSCC	57
8 Liang et al. [36]	1999–2003	844		I–IV	All	
9 Park et al. [47]	2000–2008	142	73/6	III–IV	OSCC	54
10 Evans et al. [15]	2001–2006	147	104/34	I–IV	All	58.1
11 Melkane et al. [43]	2007–2009	133	94/39	I–IV	OSCC	59
12 Stephen et al. [69]	1986–2003	80	66/14	I–IV	All	
13 Rietbergen et al. [58]	2000–2006	841		I–IV	OSCC	
14 Huang et al. [30]	1999–2009	66		I–IV	OSCC	59
15 Salazar et al. [62]	–	158	110/43	I–IV	OSCC <sup>a</sup>	
16 Xu et al. [82]	2004–2013	278	229/27	I–IV	All	
17 Heiduschka et al. [26]	2002–2013	103	46/17	II–IV	OSCC	60.5
18 Chung et al. [11]	–	683		III–IV	All non-OSCC	58

<sup>a</sup> The original study included all HNSCC regions, but for this study only the data derived from OSCC were used

**Table 2** Number of patients according to the subgroups depending on the detection of HPV and p16

References	Patients extracted for analysis	Number of HPV <sup>+</sup> /p16 <sup>+</sup> (%)	Number of HPV <sup>-</sup> /p16 <sup>-</sup> (%)	Number of HPV <sup>-</sup> /p16 <sup>+</sup> (%)	Number of HPV <sup>+</sup> /p16 <sup>-</sup> (%)
1	Wittekindt et al. [80]	34	16 (47.1)	16 (47.1)	0 (0)
2	Smith et al. [68]	301	62 (20.6)	175 (58.1)	43 (14.3)
3	Kong et al. [34]	82	30 (36.6)	36 (43.9)	10 (12.2)
4	Weinberger et al. [75]	102	25 (24.5)	44 (43.1)	0 (0)
5	Heath et al. [25]	60	18 (30.0)	34 (56.7)	8 (13.3)
6	Park et al. [48]	93	46 (49.5)	40 (43.0)	0 (0)
7	Holzinger et al. [29]	178	42 (23.6)	85 (47.8) <sup>a</sup>	50 (28.1)
8	Liang et al. [36]	121	31 (25.6)	43 (35.5)	7 (5.8)
9	Park et al. [47]	79	50 (63.3)	12 (15.2)	13 (16.5)
10	Evans et al. [15]	138	69 (50.0)	59 (42.8)	4 (2.9)
11	Melkane et al. [43]	126	61 (48.4)	40 (31.7)	4 (3.2)
12	Stephen et al. [69]	80	12 (15.0)	31 (38.8)	9 (11.3)
13	Rietbergen et al. [58]	723	152 (21.0)	545 (75.4)	26 (3.6)
14	Huang et al. [30]	66	9 (13.6)	43 (65.2)	14 (21.2) <sup>b</sup>
15	Salazar et al. [62]	36	15 (41.7)	17 (47.2)	3 (8.3)
16	Xu et al. [82]	256	9 (3.5)	239 (93.4)	8 (3.1)
17	Heiduschka et al. [26]	63	25 (39.7)	25 (39.7)	13 (20.6)
18	Chung et al. [11]	273	20 (7.3)	213 (78.0)	33 (12.1)

<sup>a</sup> Number of patients representing subgroups of HPV<sup>-</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup>

<sup>b</sup> Number of patients representing subgroups of HPV<sup>-</sup>/p16<sup>+</sup> and HPV<sup>+</sup>/p16<sup>-</sup>

as reference value. The further meta-analyses included only 2–3 studies. Thus they are more limited in their value for general conclusions. The HRs for the OS of the HPV<sup>+</sup>/p16<sup>+</sup> subgroup was significantly increased compared to the HPV<sup>-</sup>/p16<sup>+</sup> subgroup; however, it did not significantly differ from the HPV<sup>+</sup>/p16<sup>-</sup> subgroup. The HRs for the OS of the HPV<sup>-</sup>/p16<sup>-</sup> subgroup did not significantly differ from the HPV<sup>-</sup>/p16<sup>+</sup> and HPV<sup>+</sup>/p16<sup>-</sup> subgroup.

### 5-year DFS of the distinct HPV/p16 subgroups

5-year DFS was investigated in 6 studies. The results of the individual meta-analyses of the different subgroups in relation to the individual HPV and p16-status are summarized in Table 5. Six studies, including 1430 patients, showed suitable data to perform a meta-analysis on the 5-year DFS of HPV<sup>+</sup>/p16<sup>+</sup> and HPV<sup>-</sup>/p16<sup>-</sup> patients. The HPV<sup>+</sup>/p16<sup>+</sup> subgroup was associated with a significantly improved DFS (fixed-effects model; RR 2.06; 95 % CI 1.73–2.44;  $P = 0.1116$ ). The meta-analyses included 3–4 studies and are summarized in Table 5. However, the studies did not have sufficient data to perform a meta-analysis of the subgroups HPV<sup>+</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup>. The HPV<sup>+</sup>/p16<sup>+</sup> subgroup was associated with a significantly improved DFS compared to both the HPV<sup>-</sup>/p16<sup>+</sup> DFS (fixed-effects model; RR 1.4; 95 % CI 1.02–1.92;  $P = 0.2697$ ) and HPV<sup>+</sup>/p16<sup>-</sup> subgroup DFS (random-

effects model; RR 2.52; 95 % CI 1.11–5.74;  $P < 0.001$ ). The HPV<sup>-</sup>/p16<sup>-</sup> subgroup was associated with an improved DFS compared to the HPV<sup>+</sup>/p16<sup>-</sup> DFS (random-effects model; RR 1.69; 95 % CI 1.06–2.69;  $P = 0.0034$ ).

The HR for the DFS was investigated in 6 studies. The HPV<sup>+</sup>/p16<sup>+</sup> subgroup was associated with a significantly improved DFS (random-effects model; RR 2.63; 95 % CI 2.60–2.67;  $P < 0.001$ ).

### Sensitivity analysis

We performed a sensitivity analysis in order to test for a possible bias resulting from the low numbers of available eligible publications. Therefore, we divided the meta-analysis of the 5-year OS of the HPV<sup>+</sup>/p16<sup>+</sup> and HPV<sup>-</sup>/p16<sup>-</sup> subgroups according to the continent where the study had been performed. These sub-meta-analyses from the US, Europe and Asia showed comparable results to the whole meta-analyses with the complete data set of all international studies (Fig. 2a). Due to non-significant heterogeneity ( $P > 0.05$ ), the fixed-effects model was used in all sub-meta-analyses (data not shown), indicating that our results were statistically robust. The RR and CI were essentially not altered compared with the whole meta-analyses.

Finally, to test for a possible bias resulting from differences of the HPV detection methods used in the



**Table 3** Selected characteristics of the 18 studies

Study design	
Case–control	12/18 (66.7 %)
Cohort	6/18 (33.3 %)
Continent	
USA	7/18 (38.8 %)
Europe	7/18 (38.8 %)
Asia	4/18 (22.2 %)
HPV detection method	
PCR-based	15/18 (83.3 %)
ISH (without PCR)	3/18 (16.7 %)
Correlation with HPV <sup>+</sup> HNSCC	
Tumor size ( <i>T</i> )	4/7 (57.1 %)
Cervical lymph node metastases ( <i>N</i> )	5/7 (71.4 %)
Advanced UICC tumor stage	5/9 (55.6 %)
OSCC localization	5/5 (100 %)
Low grading of cancer	4/6 (66.7 %)
Non-drinking	3/9 (33.3 %)
Non-smoking	6/11 (54.5 %)
p16+	10/11 (90.9 %)
Younger age	6/10 (60 %)
Gender	1/8 (12.5 %)

*UICC* Union for International Cancer Control, *OSCC* oropharyngeal squamous cell carcinoma, *SCC* squamous cell carcinoma

included studies, a sensitivity analysis was performed. We divided the meta-analysis of the 5-year OS of the HPV<sup>+</sup>/p16<sup>+</sup> and HPV<sup>-</sup>/p16<sup>-</sup> subgroups according to the HPV detection methods into two groups: one group using PCR and one group using ISH without PCR. These two meta-analyses showed comparable results to the meta-analyses where all data were included (Fig. 2a). As before due to non-significant heterogeneity ( $P > 0.05$ ), the fixed-effects model was used (data not shown), indicating that our results were statistically robust. The RR and CI were essentially not altered compared with the meta-analyses of all data.

### Publication bias

The shape of the funnel plots did not reveal obvious evidence of asymmetry.

### Discussion

Recent increases in incidence of HNSCC and survival of HNSCC patients, especially in countries with decreasing tobacco abuse, have been attributed to HPV-associated HNSCC. It is therefore of clinical relevance to increase current knowledge about (a) the incidence of HPV-

**Fig. 2** Meta-analysis for adjusted relative risk (RR) of the 5-year overall survival (OS) compared to the HPV<sup>+</sup>/p16<sup>+</sup> subgroup. Forest plot of RR among included studies for the 5-year OS of the HPV<sup>+</sup>/p16<sup>+</sup> subgroup compared to (a) HPV<sup>-</sup>/p16<sup>-</sup>, (b) HPV<sup>-</sup>/p16<sup>+</sup> and (c) HPV<sup>+</sup>/p16<sup>-</sup>. Combined RR was calculated by a random mode.

associated HNSCC and its surrogate markers; (b) the clinical course of the etiologically distinct HNSCC-types and (c) whether further subdivision of HNSCC according to their specific HPV/p16 status has any clinical relevance. The aim of such a subdivision would be to ultimately adapt therapy intensity to a specific HNSCC-subtype.

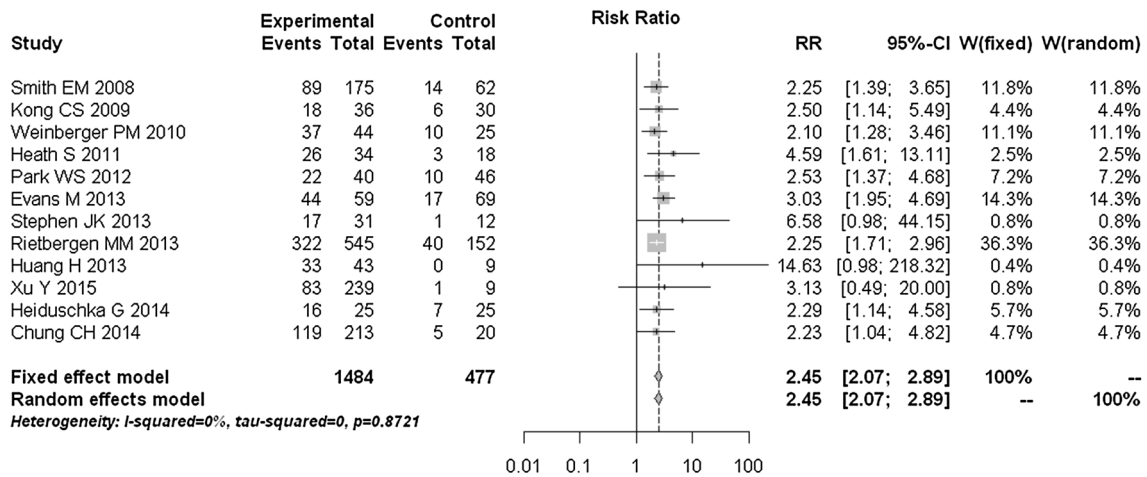
The expression of both markers p16-positive IHC and HPV-DNA positivity correlates significantly in HNSCC [12]. There is, however, a certain discrepancy rate of approximately 10 %. In these cases, HNSCC with the marker-combinations HPV<sup>+</sup>/p16<sup>-</sup> or HPV<sup>-</sup>/p16<sup>+</sup> are found [28, 38, 50, 63, 65]. In the present study, we therefore performed a meta-analysis in HNSCC to determine if the survival of these distinct subgroups is different from the more frequent HPV<sup>+</sup>/p16<sup>+</sup> and HPV<sup>-</sup>/p16<sup>-</sup> groups. To this date it is unclear if these discrepancies are real and have an as yet undefined biological explanation or if they are merely an artifact due to the limitations in detection specificity/sensitivity. To approach this question from an epidemiological perspective, we set out to investigate if there were differences in the clinical courses of the different patient groups.

A number of meta-analyses on HPV-associated HNSCC have been conducted so far, yet none has investigated the survival of patients by combining information about HPV status and p16 [12, 35, 54, 71]. Most of them showed the survival benefit of either HPV or p16 individually in two separate meta-analyses [55]. For this study, we searched for published studies evaluating the survival of subgroups according to the detection of both markers, i.e., HPV and p16. We did not subdivide the studies according to the percentage of p16 positive cells in order to increase the number of studies of the meta-analyses [37]. Ultimately, 18 studies out of 397 publications met the inclusion criteria, i.e., reporting the numbers of patients within the subgroups of HPV<sup>+</sup>/p16<sup>+</sup>, HPV<sup>-</sup>/p16<sup>-</sup>, HPV<sup>+</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup>, and their HR, OS or Kaplan–Meier curves of OS or DFS, respectively [11, 15, 25, 26, 29, 30, 34, 36, 43, 47, 48, 58, 62, 68, 69, 75, 80, 82] (Fig. 1). 14 studies investigated patients with UICC tumor stages I–IV. 8 studies investigated OSCC only and 10 investigated HNSCC. Despite the higher prevalence of HPV and p16 in OSCC [74], we included all HNSCC locations in this meta-analysis to increase the total number of patients and studies and to allow for a separate evaluation of the discrepant cases (HPV<sup>+</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup>). Therefore, the number of studies included in this analysis is smaller than in other

### 5-year OS

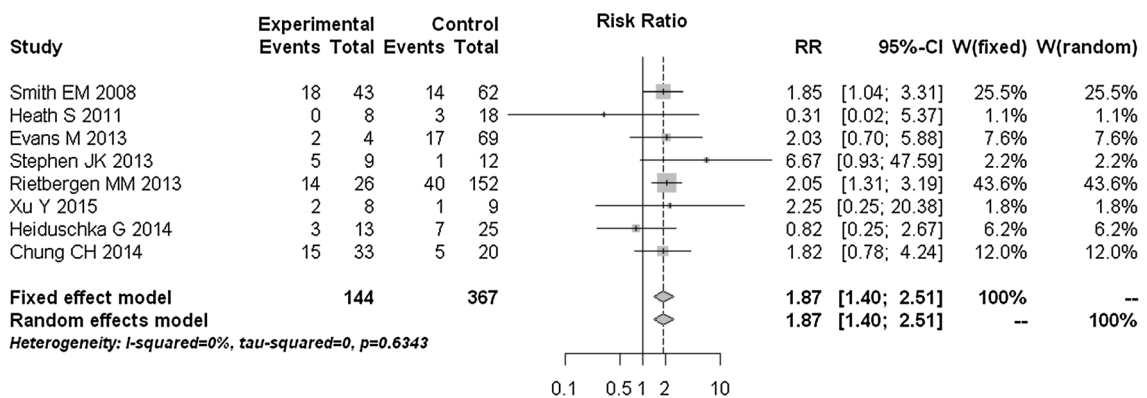
(A)

HPV+/p16<sup>+</sup> versus HPV-/p16<sup>-</sup>



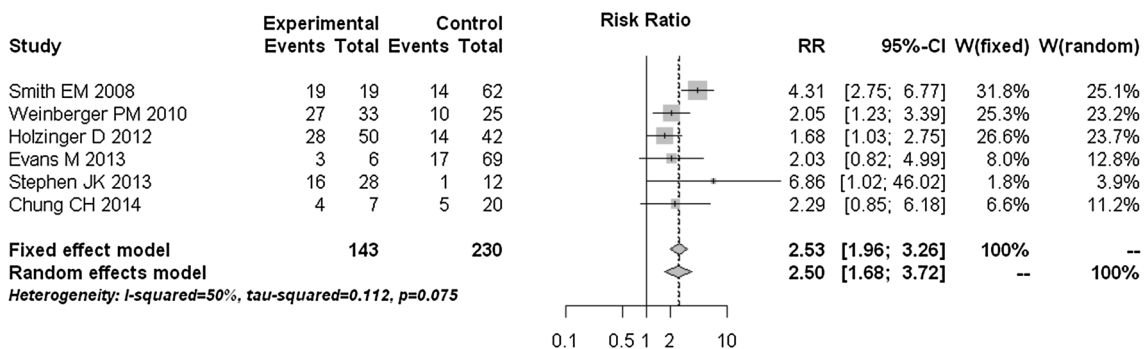
(B)

HPV+/p16<sup>+</sup> versus HPV-/p16<sup>+</sup>



(C)

HPV+/p16<sup>+</sup> versus HPV+/p16<sup>-</sup>



previously published meta-analyses with different inclusion criteria that investigated the markers independently [12, 35, 71].

Seven studies were performed in Europe, 7 in the US and 4 in Asia. Sensitivity analysis showed that the results of the 5-year OS were comparable in all continents. Therefore, a geographic differentiation of the study origin was not necessary. However, in future, as more data are available, such geographic distinctions may become relevant, since tobacco consumption and the incidence of HPV infection show differences between countries and continents [4, 10, 61]. Cofactors such as smoking behavior may impact the prevalence and presence of the two markers HPV and p16, but not the survival of the distinct subgroups [8, 72]. In 50 % of the studies the detection of HPV was associated with non-smoking. Smoking, however, has been shown to be an independent risk factor of reduced treatment efficiency and OS in HPV-related carcinomas [2, 20, 45]. Efforts to investigate prospectively relationships between tobacco consumption, HPV and p16 status and survival have been initiated at our institution [72]; however, data for the distinct subgroups are not yet available.

The method of HPV detection is critical for accurate assessment of the HPV status. HPV detection by PCR and by ISH only without PCR were used as HPV detection methods in 15 and 3 studies, respectively. Sensitivity analysis showed that the results of the 5-year OS were comparable using both HPV detection methods. In our meta-analysis, the subgroup categories of HPV<sup>+</sup>/p16<sup>+</sup> patients included 25 %, of HPV<sup>-</sup>/p16<sup>-</sup> 61.2 %, of HPV<sup>-</sup>/p16<sup>+</sup> 7.1 % and of HPV<sup>+</sup>/p16<sup>-</sup> 6.8 % patients, which is in line with earlier studies focussing on the number of discrepant cases without investigating the survival [3, 28, 38]. HPV testing is not yet standardized [6, 38]. Recently, Westra et al. [78] suggested one method for HPV detection. All HPV-specific tests including surrogate p16-IHC showed a strong and independent correlation with patient survival (e.g., HPV-RNA detection by RT-PCR, RNA detection by ISH, and p16-IHC) [6, 50, 63, 64, 67, 77, 79]. The actual prevalence of HPV in HPV-associated HNSCC depends on the sensitivity of the detection method. HPV testing by PCR has a slightly increased sensitivity over ISH [71]. The lack of data on the exact specificity of the PCR prevents a precise indication of the number of false-positive patients. HPV<sup>+</sup>/p16<sup>-</sup> cases are more similar to HPV<sup>-</sup>/p16<sup>-</sup> in terms of their genetic profiling, suggesting that HPV may be an innocent bystander in these samples and not directly involved in the process of the carcinogenesis [59, 76]. Expression of E6 and E7 mRNA is highly associated with p16 expression [27, 32, 33, 40, 53, 79]. However, the quality of results using mRNA detection is still controversial [14]. The number of studies investigating the survival of p16-positive HNSCC and E6/E7 oncoprotein

detection is not sufficient to perform a meta-analysis. Recently, E6/E7-specific antibodies have been developed that facilitate immunohistochemistry for detection of transforming infection. Those have not been used in conjunction with clinical studies. Also the measurement of activity of the viral oncoproteins has been used in experimental studies. Such methods, however, are not suitable for testing of clinical study materials yet. Therefore, the IHC for p16 may be a reasonable surrogate for detection of transcriptionally active HPV infection [67] if combined with HPV-DNA testing. In HPV<sup>-</sup> cases p53 mutations are common [7]. However, HPV-independent pathways of oncogenesis can also lead to an increased expression of p16. Moreover, p16 has been identified to have a distinct function in cellular transformation and is not just a surrogate marker. Knock out of p16 in HPV E7 expressing cells can lead to induction of apoptosis showing a physiological role in transformation despite its original role in the process of senescence [41, 49].

The specificity of the p16 IHC is only 79 % [3, 67]. Selecting for HPV16-DNA positive cases only, one study found a 93 % correlation between p16 expression and HPV status [66]. Some of the discordant results could be explained by the presence of HPV types other than type 16. HPV16 accounts for 78.6–100 % of HPV<sup>+</sup> OSCC cases [35]. However, there still remains a discordant group of p16-negative cases in the HPV16<sup>+</sup> compartment [3, 50, 63, 65].

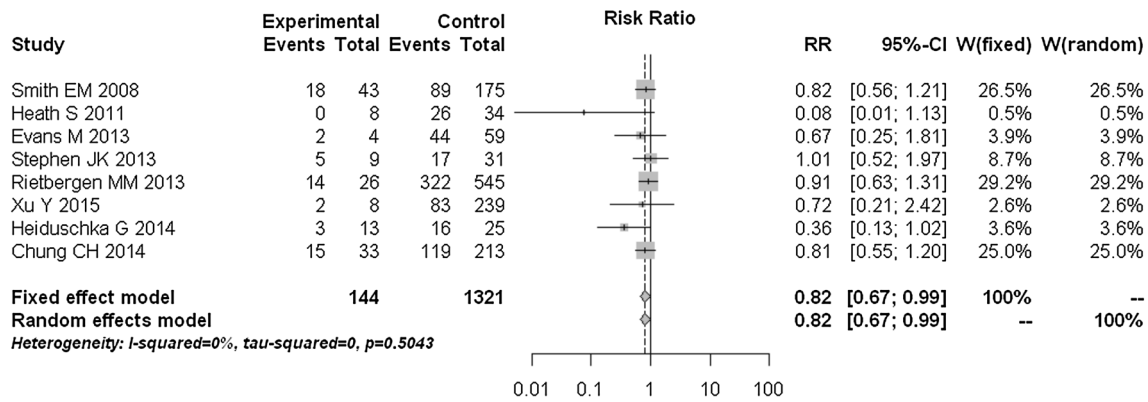
Patients with HNSCC that test HPV<sup>+</sup>/p16<sup>+</sup> have a significantly improved survival. This meta-analysis was able to demonstrate this for the 5-year OS, 5-year DFS and their corresponding HR. The same superiority in survival for HPV<sup>+</sup>/p16<sup>+</sup> was demonstrated in comparison with HPV<sup>+</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup> patients for the 5-year OS and DFS. The meta-analyses investigating OS of HPV<sup>-</sup>/p16<sup>-</sup> HNSCC compared to HPV<sup>+</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup> showed a significantly lower survival compared to the HPV<sup>-</sup>/p16<sup>+</sup> subgroup; however, the 5-year OS was comparable with the HPV<sup>+</sup>/p16<sup>-</sup> subgroup (Fig. 3). The meta-analysis comparing HPV<sup>-</sup>/p16<sup>+</sup> and HPV<sup>+</sup>/p16<sup>-</sup> showed that the 5-year OS of the subgroup of HPV<sup>-</sup>/p16<sup>+</sup> was increased compared with HPV<sup>+</sup>/p16<sup>-</sup> (Fig. 3c). The results confirm that the different HPV/p16 subgroups differ in their prognoses. The meta-analysis also proved that p16 can be used as a surrogate marker for HPV-related HNSCC. However, for a precise statement of the prognosis, each possible combination of p16/HPV has to be regarded as a separate group concerning prognosis. Thus, the detection of p16<sup>+</sup> HNSCC should be followed by a specific HPV detection method, especially if innovative therapy strategies are taken into account for cancer treatment [39]. Therefore, patient samples with detection of one or both markers for HPV or p16 should not be summarized into one single group [13, 29, 30]. The data from other groups and our own



### 5-year OS

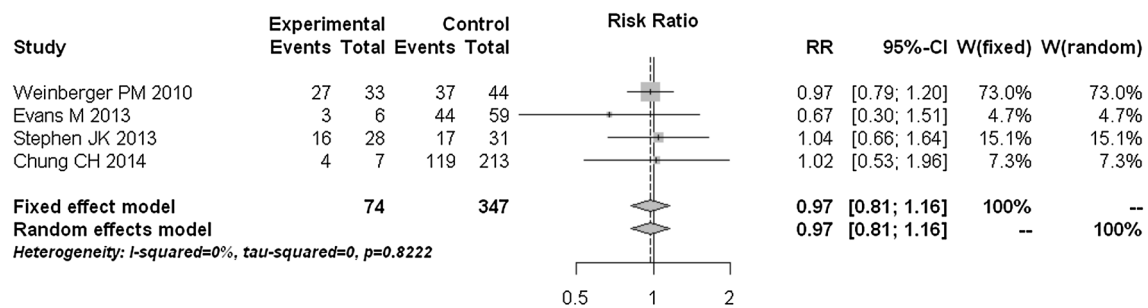
(A)

HPV-/p16<sup>-</sup> versus HPV-/p16<sup>+</sup>



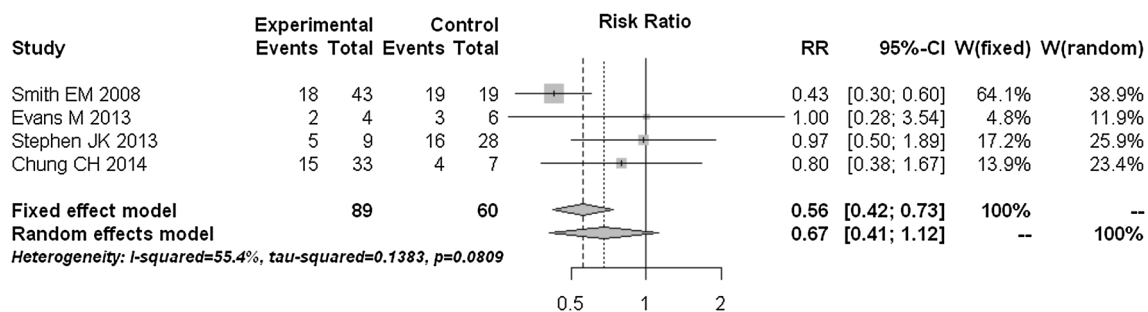
(B)

HPV-/p16<sup>-</sup> versus HPV<sup>+</sup>/p16<sup>-</sup>



(C)

HPV<sup>+</sup>/p16<sup>-</sup> versus HPV-/p16<sup>+</sup>



**Fig. 3** Meta-analysis for adjusted relative risk (RR) of the 5-year overall survival (OS) compared to the HPV-/p16<sup>-</sup> subgroup. Forest plot of RR among included studies for the 5-year OS of the HPV-/

p16<sup>-</sup> subgroup compared to (a) HPV-/p16<sup>+</sup> and (b) HPV<sup>+</sup>/p16<sup>-</sup>. (c) Forest plot of RR compares HPV-/p16<sup>+</sup> and HPV<sup>+</sup>/p16<sup>-</sup>. Combined RR was calculated by a random mode.

indicated that HPV-related HNSCC have a distinct biology [52], and are more responsive to treatment by radio- and chemotherapy [16, 17, 19].

The clinicopathological factors like tumor size, lymph node involvement, localization are comparable with those analyzed in other meta-analyses (Table 3) [12].

**Table 4** Meta-analyses on the hazard ratio of the overall survival of the subgroups of HPV<sup>+/-</sup> and p16<sup>+/-</sup>

	Number of studies	Total patient number	Number of patients of the control versus the experimental group	Fixed-effects model HR (95 % CI), <i>P</i> value	Random-effects model HR (95 % CI), <i>P</i> value	Quantifying heterogeneity	References
HPV <sup>-</sup> /p16 <sup>-</sup> versus HPV <sup>+</sup> /p16 <sup>+</sup>	5	401	195/206	0.1464 (0.1464–0.1465)	0.1815 (0.0431–0.7638)	$\tau^2 = 2.6876$ ; $I^2 = 100\%$ ; <0.0001	Kong et al. [34]; Liang et al. [36]; Evans et al. [15]; Melkane et al. [43]; Salazar et al. [62]
HPV <sup>+</sup> /p16 <sup>+</sup> versus HPV <sup>-</sup> /p16 <sup>-</sup>	4	435	145/290	2.4051 (2.2356–2.5875)	2.4051 (2.2356–2.5875)	$\tau^2 < 0.0001$ ; $I^2 = 0\%$ ; 0.6005	Smith et al. [68]; Weinberger et al. [75]; Park et al. [48]; Stephen et al. [69]
HPV <sup>+</sup> /p16 <sup>+</sup> versus HPV <sup>-</sup> /p16 <sup>+</sup>	2	126	74/52	3.3007 (1.4004; 7.7797)	3.3007 (1.4004–7.7797)	$\tau^2 < 0.0001$ ; $I^2 = 0\%$ ; 0.9764	Stephen et al. [69]; Smith et al. [68]
HPV <sup>-</sup> /p16 <sup>-</sup> versus HPV <sup>-</sup> /p16 <sup>+</sup>	2	75	55/20	1.2013 (0.725; 1.9903)	1.2013 (0.725–1.9903)	$\tau^2 < 0.0001$ ; $I^2 = 0\%$ ; 0.8863	Liang et al. [36]; Park et al. [47]
HPV <sup>+</sup> /p16 <sup>+</sup> versus HPV <sup>+</sup> /p16 <sup>-</sup>	3	179	99/80	2.5907 (0.3139; 21.3798)	2.5907 (0.3139–21.3798)	$\tau^2 < 0.0001$ ; $I^2 = 0\%$ ; 0.9653	Stephen et al. [69]; Smith et al. [68]; Weinberger et al. [75]
HPV <sup>-</sup> /p16 <sup>-</sup> versus HPV <sup>+</sup> /p16 <sup>-</sup>	3	160	95/65	0.6109 (0.5932; 0.6292)	0.7648 (0.3746–1.5616)	$\tau^2 = 0.2652$ ; $I^2 = 99.5\%$ ; <0.0001	Liang et al. [36]; Park et al. [47]; Melkane et al. [43]

HR hazard ratio

There are several limitations of our study: (a) the study is based on data extracted from the published literature and not from individual patient data. (b) Although a relatively high total number of patients were included in the meta-analysis, the number of the studies containing the data that were used to calculate specific associations was relatively small. A limitation that may only be overcome with access to primary data from all included publications and when further studies including more patients where HPV and p16 are determined simultaneously became available. (c) It is still necessary to determine the best method for tumor sampling and subsequent HPV and p16 detection. This will also help validating the role of p16-overexpression as surrogate marker for HPV-related HNSCC, as it is now in clinical practice of some centers. According to data from this study this practice has to be questioned. For now, HPV should be detected on a RNA or DNA level to further increase the sensitivity and to provide information about active transcription. (d) When selecting eligible studies for this analysis, an overlap of the same patient series included in multiple reports was observed in some instances. In these cases only the largest was included in our analysis.

In spite of the limitations inherent to this type of meta-analysis, our results are in line with published literature and our current understanding of the biology of HPV-related HNSCC. To advance current treatments, data from large randomized prospective clinical trials are needed to

determine the best treatment for patients based on the tumor's HPV- and p16-status. In our view, HNSCC with HPV<sup>+</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup>-status are of particular interest to clarify the prognosis and response to therapy and the necessity to determine the HPV status as discussed above in clinical routine. Also further confirmation of improved responses to current treatment modalities of patients with HPV<sup>+</sup>/p16<sup>+</sup> HNSCC may result in curative intent therapy regimen with a reduction in radiation- or chemotherapy dosage to reduce undesired side-effects [57]. Second, given the etiologic involvement of the HPV oncoproteins E6 and E7 during carcinogenesis of these tumors [73], it is also conceivable that intervention with drugs targeting E6 or E7 proteins or inhibiting virus-induced dysregulation (e.g., inactivation of p53, etc.) may enhance sensitivity of these tumors to cytotoxic drugs. HPV-16 is the most prevalent genotype in HPV-related HNSCC which may be prevented by a today's HPV-vaccination if applied in both sexes [18, 46].

## Conclusions

In conclusion, survival from HNSCC is improved in patients with HPV<sup>+</sup>/p16<sup>+</sup>-status, intermediate in patients of HPV<sup>-</sup>/p16<sup>+</sup> status and most limited in patients with the combinations of HPV<sup>-</sup>/p16<sup>-</sup> or HPV<sup>+</sup>/p16<sup>-</sup>. Larger trials

**Table 5** Meta-analyses of the 5-year disease-free survival of the subgroups of HPV<sup>+/-</sup> and p16<sup>+/-</sup>

	Number of studies	Total patient number	Number of patients of the control versus the experimental group	Fixed-effects model HR (95 % CI)	Random-effects model HR (95 % CI)	Quantifying heterogeneity	References
HPV <sup>+</sup> /p16 <sup>+</sup> versus HPV <sup>-</sup> /p16 <sup>-</sup>	6	1430	358/1072	2.06 (1.73–2.44)	2.13 (1.64–2.78)	$\tau^2 = 0.0437$ ; $I^2 = 44.1 \%$ ; $P = 0.1116$	Smith et al. [68]; Rietbergen et al. [58]; Weinberger et al. [75]; Kong et al. [34]; Evans et al. [15]; Chung et al. [11]
HPV <sup>+</sup> /p16 <sup>+</sup> versus HPV <sup>-</sup> /p16 <sup>+</sup>	3	336	234/102	1.40 (1.02–1.92)	1.37 (0.93–2.00)	$\tau^2 = 0.0282$ ; $I^2 = 23.7 \%$ ; $P = 0.2697$	Smith et al. [68]; Rietbergen et al. [58]; Chung et al. [11]
HPV <sup>+</sup> /p16 <sup>+</sup> versus HPV <sup>+</sup> /p16 <sup>-</sup>	4	258	149/109	2.04 (1.56–2.65)	2.52 (1.11–5.74)	$\tau^2 = 0.6096$ ; $I^2 = 87.8 \%$ ; $P < 0.001$	Smith et al. [68]; Weinberger et al. [75]; Holzinger et al. [29]; Chung et al. [11]
HPV <sup>-</sup> /p16 <sup>-</sup> versus HPV <sup>+</sup> /p16 <sup>-</sup>	3	491	432/59	2.10 (1.80–2.44)	1.69 (1.06–2.69)	$\tau^2 = 0.1365$ ; $I^2 = 82.4 \%$ ; $P = 0.0034$	Smith et al. [68]; Weinberger et al. [75]; Chung et al. [11]

DFS disease-free survival

are mandatory to investigate the survival of patients including those with HPV<sup>+</sup>/p16<sup>-</sup> and HPV<sup>-</sup>/p16<sup>+</sup> HNSCC more closely and should aim to adapt future therapeutic regimen according to the HPV/p16-status. The data indicate, that p16<sup>+</sup> HNSCC should be subdivided by HPV-typing to further identify HPV-associated HNSCC. Last, laboratory studies should aim to elucidate the underlying biological cause of the distinct HPV/p16 pattern.

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