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Review

Human Papillomavirus and Diseases of the Upper Airway: Head and Neck Cancer and Respiratory Papillomatosis

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ABSTRACT

Human papillomavirus (HPV) infection is causally associated with benign and malignant diseases of the upper airway, including respiratory papillomatosis and oropharyngeal cancer. Low-risk HPV types 6 and 11 are the predominant cause of papillomatosis, whereas only HPV16 definitively satisfies both molecular and epidemiological causal criteria as a carcinogenic or high-risk type in the upper airway. HPV16 E6/E7 mRNA expression and integration are observed predominantly among oropharyngeal cancers, and experimental models have shown E6/E7 expression to be necessary for the initiation and maintenance of the malignant phenotype of these cancers. From an epidemiological perspective, a strong and consistent association between markers of HPV16 exposure and oropharyngeal cancer has been demonstrated in numerous case-control studies. HPV-positive oropharyngeal cancers have also been shown to be distinct from HPV-negative head and neck squamous cell cancers with regard to risk-factor profiles, molecular genetic alterations, population-level incidence trends over time, and prognosis. Tumor HPV status (as determined by certain HPV16 *in situ* hybridization assays or certain p16 immunohistochemistry assays) is the strongest determinant of survival for patients with local-regionally advanced oropharyngeal cancer: patients with HPV-positive cancer have at least a 50% improvement in overall survival at 5 years, which is equivalent to an approximate 30% difference in absolute survival. Thus, HPV status determination is now part of the routine diagnostic evaluation for prognostication. Preliminary evidence indicates that a small proportion of head and neck cancers may be caused by additional HPV types (e.g., 18, 31, 33, 35) and that HPV-caused cancers may rarely arise from non-oropharyngeal sites (e.g., the oral cavity, nasopharynx, and larynx). Whether or not HPV vaccination has the potential to prevent oral HPV infections that lead to cancer or papillomatosis in the upper airway is currently unknown, as is the potential for secondary prevention with HPV detection.

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1. Causal criteria for an etiological association

The Cancer Etiology Branch of the United States National Cancer Institute has formulated criteria to guide establishment of a causal relationship between a carcinogen and the development of a specific cancer type [1]. These criteria are similar to those proposed by zur Hausen H [2] to establish involvement of an infectious agent, and include molecular-pathological, experimental and epidemiological evidence. For epidemiological evidence, the criteria originally established by AB Hill provide a useful framework for discussions of causal inference [3]. Thus, here we will review in sequence the relevant findings in the current literature regarding the causal association of Human papillomavirus (HPV) with head and neck squamous cell carcinoma (HNSCC).

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Table 1
Molecular criteria in support of a causal association.

Criterion	Feature	References
Molecular pathology	Regular presence in tumors	Kreimer AR *et al.* [6]
	Clonally related to tumors	Niedobitek G *et al.* [9] Gillison M *et al.* [10] Begum S *et al.* [11]
	Persistence in tumors	Yeudall WA *et al.* [150] Ferris RL *et al.* [12] Steenbergen RD *et al.* [13]
	Viral oncogene activity	Van Houten VM *et al.* [15] Jung A *et al.* [16] Hoffmann M *et al.* [17]
	Biological evidence on the basis of known viral oncogene functions	Gillison M *et al.* [10] Klussman JP *et al.* [24] Braakhuis BJ *et al.* [19] Westra W *et al.* [27] Hoffmann M *et al.* [17]
In vitro models	Capacity to transform respective target cells	Park NH *et al.* [35] Sexton CJ *et al.* [36] Chen RW *et al.* [38] Smeets SJ *et al.* [37] Lace MJ *et al.* [20]
	Dependence of transformed phenotype on functions exerted by viral oncogenes	Rampias T *et al.* [14]
Animal models	Capacity to induce respective tumors in transgenic mice	Strati K *et al.* [40] Strati and Lambert [151]

1.1. Causal criteria for an etiological association from a molecular perspective

As summarized in Table 1 and elaborated upon below, sufficient molecular and pathological evidence has now been collected to etiologically link infection by specific HPV types to a subset of HNSCCs, particularly oropharyngeal cancers (OPSCC) (see also reviews of Sudhoff HH *et al*., 2011 [4] and Chaturvedi A and Gillison M, 2010 [5]).

1.1.1. HPV detection, type distribution and clonality

As reviewed by Kreimer AR *et al.* [6], HPV DNA has been found by polymerase chain reaction (PCR) in HNSCC arising from various anatomic sites. Overall, HPV DNA prevalence was significantly higher in OPSCC (35%) than in oral cavity (23%) or laryngeal SCCs (24%). The prevalence of HPV DNA in OPSCC that arose from the tonsil has usually been higher than for other anatomic sites [7], as high as 90% in some series [8]. Moreover, HPV16 is the most dominant HPV type, accounting for 90% of HPV DNA-positive HNSCCs, whereas HPV18, 31, 33 and 35 constitute most of the remaining cases. Various studies, mainly involving HPV16, have shown that viral DNA is diffusely present in neoplastic cells throughout the tumor when detected by *in situ* hybridization (ISH), indicating clonality [9–11]. Demonstrated retention of viral DNA upon growth of tumor cells in culture, as shown for some oral cavity and oropharyngeal cancer cell lines [12–14], provides further evidence for viral clonality.

Detection of HPV DNA in HNSCC by PCR alone is, however, insufficient to prove causality. Expression of HPV E6/E7 region mRNA is far less common in HNSCC than viral DNA detection by PCR [15–17]. HPV E6/E7 oncogene expression is considered necessary for carcinogenesis, and therefore its absence points to absence of causality. Indeed, HPV DNA-positive tumors without E6/E7 mRNA are similar to HPV DNA-negative tumors in terms of p53 mutation status and number of chromosomal imbalances at 3p, 9p and 17p [15,18,19]. The majority of studies that have confirmed HPV E6/E7 expression in tumors have focused on HPV16.

In HNSCCs that can be attributed to HPV, the viral genome is often, but not exclusively, integrated into that of the host [20]. Moreover, viral physical status can be heterogeneous within one tumor, with parts harboring only episomal DNA and other parts only integrated viral DNA [21]. Therefore, similar to cervical cancer, viral integration cannot be considered a requirement for HPV-mediated HNSCC development.

1.1.2. Genetic alterations in HPV-positive vs. HPV-negative tumors

HPV E6/E7 mRNA expressing tumors have genetic alterations distinct from HPV E6/E7 mRNA-negative HNSCC that are indicative of viral oncoprotein function. Deregulated HPV E6/E7 activity in proliferating cells results in increased expression of $p16^{INK4A}$ triggered by the E7-mediated induction of the histone demethylase, KDM6B [22]. P16 expression is considered a marker for cervical pre(malignant) lesions harboring transforming HPV infections [23]. Indeed, HNSCCs displaying viral E6/E7 expression generally display diffuse $p16^{INK4A}$ immunostaining [17,24]. However, a subset of HPV DNA and mRNA-negative HNSCCs show diffuse $p16^{INK4A}$ staining, indicating expression is not specific for HPV activity [11,25]. The capacity of high-risk HPV E6 to inactivate p53 would, in theory, obviate the need for inactivating p53 mutations. Indeed, HPV-positive HNSCCs are less likely to contain inactivating p53 mutations than HPV-negative tumors [10,19,26,27].

Other molecular-genetic and chromosomal profiles differ between tumors with and without expression of HPV E6/E7 mRNA, particularly with regard to common regions of chromosomal loss previously identified in the molecular-progression of HNSCC, such as 9p, 3p and 17p [19,28]. Interestingly, HNSCCs with E6/E7 mRNA have chromosomal alterations in common with cervical carcinomas [29]. Moreover, HPV E6/E7 mRNA-positive HNSCCs are characterized by distinct microarray expression profiles [30–33], and exome sequencing studies have revealed that HPV-positive HNSCCs have fewer somatic mutations in coding regions than HPV-negative tumors [34].

1.1.3. Experimental evidence from in vitro and mouse models

Various studies have reported that oral and tonsillar epithelial cells can be immortalized by full-length HPV16 or its E6/E7 oncogenes [20,35–38]. Additionally, HPV16-containing oropharyngeal cancer cell lines are dependent upon E6/E7 expression for maintenance of the malignant phenotype: knockdown of E6/E7 expression by short hairpin RNA resulted in restoration of p53 and Rb pathways, leading to apoptosis [39]. These data indicate that HPV is involved in both the initiation and maintenance of the transformed phenotype in oral and oropharyngeal SCCs.

Finally, transgenic mouse models have revealed that HPV16 E6/E7 strongly increases susceptibility to oral and oropharyngeal carcinomas [40]. Although E7 was much more competent in inducing these tumors [40], a clear synergy between E6 and E7 in causing HNSCC was discovered [41].

1.1.4. Summary of molecular and experimental evidence

Based upon the data summarized above and listed in Table 1, from a molecular perspective, it can be concluded that HPV16 is etiologically involved in a substantial proportion of OPSCC and possibly a small proportion of oral cavity SCCs. For other high-risk HPV types such as 18, 31, 33 and 35, data beyond DNA detection and E6/E7 expression are lacking, which may reflect their rather low prevalence in HNSCC. Should larger series of cancers be analyzed, convincing molecular evidence might be collected for additional types.

1.2. Epidemiological evidence for a causal role for HPV in head and neck cancers

A number of epidemiological studies have evaluated potential associations between different measures of HPV exposure and HNSCC risk. In this section we summarize the accumulated epidemiological evidence derived from case-control and cohort studies.

1.2.1. Measures of HPV exposure in epidemiological studies

Several exposure measurements have been used to evaluate associations between HPV infection and HNSCC in case-control studies, including sexual behavior measures, HPV serology (L1/L2 and E6/E7 antibodies) and HPV DNA detection in oral exfoliated cells.

Sexually-transmitted oral HPV infection is thought to be the cause of HPV-associated HNSCC. Thus, sexual behavior measures should serve as a surrogate for oral HPV infection. Sexual behavior measures have some limitations such as information bias (recall bias and misreporting of sensitive information). Additionally, transmission may occur by means that are infrequently measured or difficult to measure, such as open-mouth kissing [42] or by hand to mouth auto-inoculation. HPV antibodies in serum are another surrogate of HPV exposure, and are produced against the viral capsid antigen L1 and the E6/E7 viral oncoproteins. Detection of HPV L1 antibodies in serum is a surrogate for lifetime-cumulative exposure to an HPV infection. However, only ~60–70% of individuals seroconvert after natural infection [43], and antibody levels are not always stable over time. Moreover, seropositivity is not specific to an anatomic site of infection or to a diagnosis of an HPV-caused cancer. In contrast, detection of antibodies against E6/E7 oncoproteins is highly specific to an HPV-caused cancer (although not of a particular organ site), as these proteins are overexpressed in the tumor. E6/E7 antibodies exhibit higher concordance than L1 antibodies with detection of HPV DNA in tumor tissues [44]. However, as with L1 antibodies, E6/E7 assay sensitivity is low. For HPV DNA detection in oral exfoliated cells, the oral sampling method used should be comparable in cases of HNSCC and their corresponding controls to ensure equivalent exposure measures. Studies have shown variable results regarding correlation (low-high) among HPV DNA detection in exfoliated cells and tumor tissue [7,44].

When assessing differences in associations between HPV exposure measures and HNSCC, it is also important to take into consideration the influence of other study features. These include the study design and population, the distribution of the anatomic subsite of the cancer cases (i.e., oral cavity, pharynx, and larynx), the characteristics of the assays used to detect HPV antibodies or HPV DNA, as well as the type of adjustments used to control for potential confounding factors (e.g., tobacco and alcohol consumption habits).

1.2.2. HNSCC risk associated with sexual behavior indicators

A summary of the evidence for an association between sexual behavior and HNSCC from 14 case-control studies is shown in Table 2. At least one measure of sexual behavior was found to be associated with HNSCC in eight of these studies. Four of these reported statistically significant associations with overall HNSCC without specifying the anatomic subsite of the cancers, whereas three found associations with oropharyngeal cancer [45,46] and one with oral cavity cancer [47]. In contrast, sexual behaviors were not associated with oral cavity cancer in two studies [7,46] nor with laryngeal cancer in the only study that specifically examined this association [46]. Sexual behaviors most consistently associated with HNSCC risk include increasing lifetime number of genital and oral sexual partners. Factors less frequently associated with HNSCC risk have included younger age at first intercourse, history of genital warts or sexually transmitted infection, lack or rare use of condoms, same-gender sexual contacts and oral-anal sex.

The largest epidemiological study reported to date compared sexual behavior measures among 5642 cases of HNSCC to 6069 control subjects [46]. This pooled analysis of four population-based and four hospital-based, case-control studies was conducted by the International Head and Neck Cancer Epidemiology (INHANCE) consortium, with participants from Argentina, Australia, Brazil, Canada, Cuba, India, Italy, Spain, Poland, Puerto Rico, Russia and the USA. In this study, a history of six or more lifetime sexual partners (odds ratio [OR] 1.3, 95% confidence interval [CI]: 1.0–1.5) or four or more lifetime oral sex partners (OR 2.3, 95% CI: 1.4–3.6) were associated with oropharyngeal cancer. Four or more lifetime oral sex partners (OR 3.4, 95% CI: 1.3–8.5), and ever having had oral sex (OR 1.6, 95% CI 1.1–2.3) and earlier age at sexual debut (OR 2.4, 95% CI: 1.4–5.1) among men were associated with tonsillar cancer. Ever having oral sex among women (OR 4.3, 95% CI: 1.1–17.6), having two sexual partners in comparison with only one (OR 2.0, 95% CI: 1.2–3.5) and a history of same-gender sexual contact among men (OR 8.9, 95% CI: 2.1–36.8) were associated with base of tongue cancer. It is important to note that no associations were found with cancers of the oral cavity or larynx. The authors concluded that sexual behaviors were associated with cancer risk at the head and neck cancer subsites most strongly associated with HPV E6/E7 mRNA expression as noted above, namely oropharyngeal cancer (OPC) (e.g., tonsil and base of the tongue cancers).

1.2.3. HNSCC risk associated with HPV16 type-specific L1 serology

Results from 16 epidemiological studies that assessed associations between seroreactivity to HPV L1 and HNSCC are shown in Table 3. Thirteen of these studies reported at least one positive association between HPV16 L1 seropositivity and HNSCC overall or for one or more anatomic subsites. Among studies reporting statistically significant associations, the odds of HNSCC observed in association with seropositivity varied from 1.7 to 7.5 for HNSCC overall, from 1.5 to 2.8 for oral cavity cancer, and from 2.3 to 182.3 for oropharyngeal cancer. Out of the four studies specifically assessing the association with laryngeal cancer, only two reported a statistically significant OR (of 2.4 and 2.7) [48,49].

Two large, multicenter, case-control studies have come to contradictory conclusions with regard to associations between HPV16 L1 serology and HNSCC. In the first, a total of 1537 oral cavity and oropharynx cancer cases and 1568 controls were enrolled in Italy, Spain, Northern Ireland, Poland, India, Cuba, Canada, Australia, and Sudan from 1996 through 1999 [7]. Seropositivity to HPV16 L1 was associated with oropharyngeal (OR 3.5, 95% CI: 2.1–5.9) and oral cavity cancer (OR 1.5, 95% CI: 1.1–2.1). In contrast, another large hospital-based case-control study that enrolled 2042 oral cavity, oropharynx or hypopharynx/larynx cases and 3080 controls between 1998 and 2003 from Latin America (Argentina, Brazil, and Cuba) and Central Europe (Russia, Slovakia/Czech Republic, Romania, and Poland) found no statistically significant associations between HPV16 L1 serology and HNSCC, either overall, by region, or by anatomic subsite, including the larynx [50]. HPV DNA was detected in only 0.6% of 507 tumors available for analysis in this study, as compared to 6.6% of 852 cases in the prior study, likely explaining the contradictory conclusions.

A single nested case-control study has provided significant evidence that HPV exposure precedes the development of HNSCC [48], and therefore must be emphasized. In this cohort study conducted in Nordic countries, serum samples were prospectively collected from 292 persons who subsequently developed HNSCC and from 1568 age- and gender-matched controls [48]. After adjustment for serum cotinine levels (a strong surrogate for recent use of tobacco products), HPV16-seropositive individuals had a two-fold increase in risk for HNSCC overall (OR 2.1, 95% CI: 1.4–3.2), 14-fold for

Table 2
Case-control studies on head and neck cancer – Measurement of exposure: Sexual behavior indicators.

Author, year	Study location	Design	Cases/controls (n)	Associations with all HNSCCs	Associations with oral cavity cancer	Associations with oropharyngeal cancer	Associations with laryngeal cancer	Adjustment factors
Maden C *et al.* 1992 [152] [a]	Washington State (USA)	Population-based case-control	131/136	Association in males with 30 or more partners; and protective effect of the ones ever having had oral sex	-	-	-	Age, tobacco, alcohol
Schwartz SM *et al.* 1998 [51] [a]	Washington State (USA)	Population-based case-control	Males:154/294 Females: 112/171	Association in males, with decreasing age at first intercourse, increasing number of partners and prior diagnosis of GW	-	-	-	Age, tobacco, alcohol, race, income, education
Talamini R *et al.*, 2000 [153] [a,b]	Friuli-Venezia Giulia (North-East Italy)	Hospital-based case-control	132/148	Increased risk with mouth candidiasis	-	-	-	Age, gender, fruit/vegetable intake, tobacco, alcohol
Fernandez-Garrote LF *et al.*, 2001 [154] [a,b]	Habana (Cuba)	Hospital-based case-control	200/200	No associations	-	-	-	Age, gender, residence area, education, tobacco, alcohol
Rajkumar T *et al.* 2003 [47] [a,b]	Bangalore, Madras, Trivandrum (India)	Hospital-based case-control	591/582	-	Association in males, with oral sex practices	-	-	Age, gender, residence area, education, tobacco, alcohol
Herrero R *et al.*, 2003 [7] [a]	Italy, Spain, Northern Ireland, Poland, India, Cuba, Canada, Australia, and Sudan (Multicenter)	Hospital-based case-control	1670/1732	-	No associations	No associations	-	Age, gender, country, tobacco, paan chewing, alcohol
Lissowska J *et al.*, 2003 [155] [a,b]	Warsaw (Poland)	Hospital-based case-control	122/124	No associations	-	-	-	-
Smith EM *et al.* 2004 [57] [c]	Iowa State (USA)	Hospital-based case-control	201/333	No associations	-	-	-	Age, tobacco, alcohol, HPV in exfoliated cells
Smith EM *et al.* 2006 [52] [c]	Iowa State (USA)	Hospital-based case-control	204/326	No associations	-	-	-	Age, tobacco, alcohol
D'Souza G *et al.*, 2007 [45] [c]	Baltimore (USA)	Hospital-based case-control	100/200	-	-	Association with lifetime vaginal and oral partners, casual-sex partner, never or rarely use of condoms	-	Age, gender, tobacco, alcohol, dentition status and family history of head and neck cancers

Table 2 (*Continued*)

Author, year	Study location	Design	Cases/controls (n)	Associations with all HNSCCs	Associations with oral cavity cancer	Associations with oropharyngeal cancer	Associations with laryngeal cancer	Adjustment factors
Gillison M *et al.* 2008 [44] [c]	Baltimore (USA)	Hospital-based case-control	HNSCCs: 148[d]/296 OPC: 92[e]/184	No associations	-	Association ≥11 vaginal partners; ≥6 oral sex partners; never or rarely use of condom in vaginal sex; never or rarely use of barrier en oral sex; past history of STDs	-	Age, sex, race, tobacco, alcohol, marijuana use, oral hygiene
Pintos J *et al.* 2008 [53] [a,b]	Montreal (Canada)	Hospital-based case-control	72/129	No associations	-	-	-	-
Tachezy R *et al.* 2009 [54] [c]	Prague (Czech Republic)	Hospital-based case-control	86/111	Association with oral-anal sex	-	-	-	Age, tobacco, alcohol, HPV in exfoliated cells
Heck JE *et al.* 2010 [46] [a,c]	Multicenter study IARC INHANCE	Population and hospital- based case-control	5642/6069	-	No associations	OPC: Association with lifetime vaginal and oral partners TO: Association with lifetime oral partners, ever having oral sex-males, younger age at first intercourse-males Base of the TG: Association with ever having oral sex-women, same sex contact-males	No associations	Age, gender, race, education, tobacco, alcohol

[a] Structured in-person interview.
[b] From IARC study (Herrero R *et al*, 2003 [7]).
[c] Self-administered questionnaire.
[d] Cases: Selection of HPV16 negative (78% non-oropharyngeal cancers).
[e] Cases: Selection of HPV16 positive (90% oropharyngeal cancers).
GW: Genital Warts; HNSCC: Head and neck cancers; OPC: Oropharynx; STDs: Sexual Transmitted Diseases; TG: Tongue; TO: Tonsil; Bold face figures indicate statistical significance.

Table 3
Case-control studies on head and neck cancer – Measurement of exposure: HPV type-specific L1 serology.

Author, year	Study location	Design	Cases/controls (n)	All HNSCCs OR (95% CI)	Oral cavity cancer OR (95% CI)	Oropharyngeal cancer OR (95% CI)	Laryngeal cancer OR (95% CI)	Adjustment factors
Dillner J *et al.* 1995 [156]	Finland	Nested case-control in a serum cohort study	165/327	-	L+TG+Salivary: 0.6 (0.2-2.1) Other oral: 0.4 (0.0-7.1)	-	0.2 (0.0-2.0)	Tobacco
Schwartz SM *et al.* 1998 [51]	Washington State (USA)	Population-based case-control	259/446	**2.3 (1.6-3.3)**	FM: 1.1 (0.5-2.5) **TG: 2.4 (1.5-3.8)**	**TO: 3.9 (2.0-7.8)**	-	Age, gender, tobacco, alcohol, race, income, education
Mork J *et al.* 2001 [48] [a]	Norway, Finland and Sweden	Nested case-control in a serum cohort study	292/1568	**HPV16: 2.2 (1.4-3.4)** HPV18: 1.0 (0.6-1.8) HPV33: 0.8 (0.5-1.3) HPV73: 0.6 (0.4-1.2)	L: 0.5 (0.1-2.1) **TG: 2.8 (1.2-6.6)** NOS: 3.6 (0.5-26.3)	**OPC: 14.4 (3.6-58.1)** **TO: 10.2 (2.4-42.9)** **Base TG: 20.7 (2.7-160.1)**	**2.4 (1.0-5.6)**	Age, gender, tobacco cotinine levels in serum, time storage
Herrero R *et al.* 2003 [7]	Italy, Spain, Northern Ireland, Poland, India, Cuba, Canada, Australia, and Sudan (Multicenter)	Hospital-based case-control	1537/1527	-	**1.5 (1.1-2.1)**	**3.5 (2.1-5.9)**	-	Age, gender, country, tobacco, paan chewing, alcohol
Dahlstrom KR *et al.* 2003 [157]	Houston, Texas (USA)	Hospital-based case-control	120/120	**6.7 (3.0-14.9)**	-	**59.5 (5.7-620.2)**	-	Age, gender, tobacco, cotinine, alcohol
Van Doornum GJ *et al.* 2003 [158]	Amsterdam (The Netherlands)	Nested case-control in a serum cohort study	48/100	-	-	**2.3 (1.0-4.9)**	-	-
Smith EM *et al.* 2006 [52] [b]	Iowa State (USA)	Hospital-based case-control	204/326	**HPV16: 1.7 (1.1-2.5)** HPV18: 1.2 (0.7-2.0) HPV31: 1.2 (0.8-1.9) HPV33: 1.1 (0.7-1.8)	HPV16: 1.2 (0.7-2.0) HPV18: 1.2 (0.7-2.1) HPV31: 1.2 (0.8-2.0) HPV33: 0.8 (0.5-1.4)	**HPV16: 3.5 (1.9-6.5)** HPV18: 1.5 (0.7-3.3) HPV31: 1.4 (0.7-2.7) **HPV33: 2.2 (1.1-4.2)**	-	Age, tobacco, alcohol, sex and lifetime sex partners
D'Souza G *et al.* 2007 [45]	Baltimore (USA)	Hospital-based case-control	100/200	-	-	**32.2 (14.6-71.3)**	-	Age, gender, tobacco, alcohol, dentition status and family history of head and neck cancers
Applebaum K *et al.* 2007 [49] [c]	Boston (USA)	Population-based case-control	485/549	**4.5 (3.1-6.5)**	**HPV16: 1.7 (1.0-2.8)** HPV18: 0.7 (0.3-1.4) HPV6: 1.4 (0.9-2.1) HPV11: 1.0 (0.5-2.0)	**HPV16: 10.0 (6.6-15.3)** HPV18: 1.3 (0.7-2.3) **HPV6: 1.6 (1.0-2.5)** HPV11: 0.9 (0.5-1.9)	**HPV16: 2.7 (1.5-5.1)** HPV18: 0.8 (0.3-2.0) HPV6: 1.1 (0.6-2.0) HPV11: 0.7 (0.2–2.0)	Age, gender, race, tobacco, alcohol, education (for HPV18/6/11 also adjusted by HPV16)

Table 3 (*Continued*)

Author, year	Study location	Design	Cases/controls (n)	All HNSCCs OR (95% CI)	Oral cavity cancer OR (95% CI)	Oropharyngeal cancer OR (95% CI)	Laryngeal cancer OR (95% CI)	Adjustment factors
Sitas F *et al.* 2007 [159]	Johannesburg (South Africa)	Hospital-based case-control	102/2055	Medium titers (0.45-0.77): 1.1 (0.7-1.9) High titers (>0.77): 1.5 (0.9-2.5)	-	-	-	Age, gender, race, tobacco, alcohol, education, bithplace and current residence
Gillison M *et al.* 2008 [44] [d]	Baltimore (USA)	Hospital-based case-control	HNSCCs: 148[e]/296 OPC: 92[f]/184	HPV16: 0.9 (0.4-2.2) HPV18: 1.5 (0.7-3.1) HPV31: 1.0 (0.5-2.2) **HPV35: 3.1 (1.5-6.3)**	-	**HPV16: 18.3 (6.8-49.0)** HPV18: 2.0 (0.9-4.4) **HPV31: 2.8 (1.4-5.8)** **HPV35: 3.8 (1.8-7.9)**	-	Age, gender, race, tobacco, alcohol, marijuana use, oral hygiene
Pintos J *et al.* 2008 [53] [g]	Montreal (Canada)	Hospital-based case-control	72/128	**HPV16: 7.5 (2.1-27.2)** HPV18: 2.3 (0.4-13.4) HPV31: 2.2 (0.8–6.3)	HPV16: 3.9 (0.9-17.5) Other types: no association	**HPV16: TO and base TG: 182.3 (7.0-4753.0)** Other types: no association	-	Age, gender, shooling, religion, language, tobacco, alcohol
Ji X *et al.* 2008 [64]	Houston, Texas (USA)	Hospital-based case-control	188/342	-	-	**5.7 (3.7-8.7)**	-	Age, gender, tobacco, alcohol
Chen X *et al.* 2008 [160]	Houston, Texas (USA)	Hospital-based case-control	326/349	**3.4 (2.3-5.1)**	-	-	-	Age, gender, tobacco, alcohol
Tachezy R *et al.* 2009 [54] [h]	Prague (Czech Republic)	Hospital-based case-control	86/104	**HPV16: 6.2 (2.9-13.4)** **HR: 3.8 (1.9-7.4)**	HPV16: 5.5 (0.6-52.6) HR: 1.8 (0.2-14.0)	**HPV16: 6.3 (2.9-13.9)** **HR: 3.3 (1.6-6.7)**	-	Age, tobacco, alcohol
Ribeiro K *et al.* 2011 [50] [i]	Latin American countries and Central Europe (Multicenter)	Hospital-based case-control	2042/3080	HPV16: 1.0 (0.8-1.3) **HPV6: 1.3 (1.1-1.5)** Other types: no association	HPV16: 0.9 (0.7-1.3) **HPV6: 1.4 (1.2-1.8)** Other types: no association	HPV16: 1.1 (0.7-1.7) Other types: no association	HPV16: 1.2 (0.9-1.6) **HPV6: 1.2 (1.0-1.5)** Other types: no association	Age, gender, tobacco, alcohol, country

[a] HPV 16,18,33 L1/L2 antibodies and HPV 73 L1 antibodies.
[b] HPV 16,18,31,33 L1 antibodies.
[c] HPV 6,11,18 L1 antibodies from an extended analysis, Furniss CS *et al.* [55].
[d] HPV 16,18,31,35 L1 antibodies.
[e] Cases: Selection of HPV16 negative (78% non-oropharyngeal cancers).
[f] Cases: Selection of HPV16 positive (90% oropharyngeal cancers).
[g] HPV 16,18,31 L1/L2 antibodies.
[h] HPV 16,18,31,33,6,11 L1/L2 antibodies.
[i] HPV 16,6,11,1,4,8,38,49,77 L1 antibodies.

FM: Floor of the mouth; HNSCC: Head and neck cancers; L: Lips; NOS: Not otherwise specified; OPC: Oropharynx; OR: Odds ratio; TG: Tongue; TO: Tonsils; 95% CI: 95% Confidence interval.
Bold face figures indicate statistical significance. Note that if HPV type is not specified, results relate to HPV16 type.

oropharyngeal cancer (OR 14.4, 95% CI: 3.6–58.1), three-fold for tongue cancer (OR 2.8, 95% CI: 1.2–6.6) and two-fold for laryngeal cancer (OR 2.1, 95% CI: 1.0–5.6). The increased HNSCC risk was observed up to 15 years prior to cancer diagnosis, indicating that HPV exposure preceded cancer development by many years. No associations were observed between seroreactivity to HPV18, 33 and 73 and HNSCC in this study.

Among the studies presenting ORs for HPV16 L1 serology by cancer subsite, all but one [50] reported much stronger associations for oropharyngeal cancer than those for oral and laryngeal cancers [7,48,49,51–54]. These results are consistent with the much greater prevalence of HPV DNA and HPV E6/E7 expression in oropharyngeal cancers compared to other HNSCC sites. Antibodies against other HPV types have been evaluated in seven of the selected studies with different results. Statistically significant associations were found between HPV31 and HPV33 with OPC [44,52]; HPV35 with HNSCC and OPC [44]; and HPV6 with HNSCC and different sites [50,55]. Associations with HPV6 may be explained by co-infection or residual confounding by exposure to other HPV types.

1.2.4. HNSCC risk associated with HPV16 E6/E7 serology

Data from five studies that have assessed the association between HPV16 E6/E7 serology and HNSCC risk are summarized in Table 4. All five studies found statistically significant associations for HNSCC overall or for one or more anatomic subsites. In the study by Herrero R *et al.*, seropositivity to both HPV16 E6 and E7 was associated with a 67-fold (OR 67.1, 95% CI: 12.9–348.2) increase in odds for oropharyngeal cancer and a non-significant four-fold increase in oral cavity cancer (OR 4.3, 95% CI: 0.80–23.2) [7]. In the study by Ribeiro K *et al.* [50], joint E6/E7 seropositivity was associated with HNSCC (OR 19, 95% CI: 5.24–69.0). By anatomic subsite, significant associations were found for oropharynx, larynx, and hypopharynx cancers, but not for cancer of the oral cavity. In all five studies, associations were stronger for oropharyngeal cancer than for the other cancer sites, as has been observed for HPV16 L1 seropositivity. Regarding other HPV types, Ribeiro K *et al.* also analyzed E6/E7 from HPVs 6,11,18,31,33,35,45,52,58 and, after exclusion of subjects that were seropositive for the homologous HPV16 proteins, the only remaining significant association was found for antibodies to HPV52 E6 and oropharyngeal cancer (OR 9.2, 95% CI: 1.9–44.8).

1.2.5. HNSCC risk associated with HPV DNA in oral exfoliated cells

Data derived from the 11 case-control studies that have assessed associations between HPV DNA detection in exfoliated oral cells and HNSCC risk are shown in Table 5. The most frequent method used for collection of exfoliated cells from the oral cavity and pharynx was the use of saline oral rinse with or without cytology brush or toothbrush. One study used, in addition, cotton-tipped swabs to sample the tumors and the tonsillar fossa [56]. As shown in the table, nine of the 11 studies reported associations between HPV DNA detection in exfoliated cells and HNSCC. Associations were consistently stronger with cancer of the oropharynx than with cancer of the oral cavity and other anatomic sites. None of the studies assessed associations with laryngeal cancer, as this cancer site cannot be sampled with non-invasive methods. Although the largest study exploring oral HPV DNA observed no association with oral cavity or oropharyngeal cancer, methods used in this study demonstrated poor correlation between HPV detection in exfoliated cells and detection in biopsy specimens [7]. Three out of four studies reporting ORs stratified by low- versus high-risk HPV types found associations only with high-risk HPV types [53,56,57]. Thus, there is a strong and consistent association between oral infection by high-risk HPV types and oropharyngeal cancer.

While these studies clearly demonstrate a strong and consistent association between HPV and oropharyngeal cancer, for oral cavity carcinomas the association remains somewhat controversial because of the lack of consistency and absence of compelling molecular data as described above. Syrjanen S *et al.* [58] recently reported a meta-analysis of the association between oral HPV infection and oral cavity squamous cell carcinoma and oral potentially malignant disorders. Thirty-nine case-control studies out of 1,121 manuscripts met quality criteria for inclusion in the analysis, and yet none of these were deemed at low-potential for bias based upon study design. A pooled analysis of 1885 cases and 2248 controls estimated any oral HPV infection (OR 3.98, 95% CI: 2.62–6.02) or HPV16 infection (OR 3.86, 95% CI: 2.16–6.87) to confer a four-fold increase in odds of oral cavity cancer. A similar four-fold increase (OR 3.87, 95% CI: 2.87–5.21) in odds of oral potentially malignant lesions was observed, which on subgroup analysis was significant for all four lesion types (i.e., leukoplakia, erythroplakia, oral lichen planus and oral proliferative leukoplakia). Stratification by sample collection method revealed significant associations among studies with biopsy samples from cases and controls, but not with oral exfoliative cytology samples or in studies in which the sampling method used for cases and controls differed. The study authors acknowledge that misclassification of OSCC as oral cavity cancers could not be excluded and that a causal association between HPV and the premalignant lesions could not be assumed from the data, as abnormal oral epithelium or its treatment (e.g., steroids for oral lichen planus) might increase susceptibility to HPV infection.

1.2.6. Cofactors and interactions with other head and neck cancer risk factors

HNSCC has other well-established risk factors, in addition to HPV infection. Tobacco and alcohol use have independent as well as synergistic effects on risk, and multiple studies support associations with dietary factors, oral hygiene, and common genetic variation [59–61]. Whether these factors also increase the risk of HPV-positive HNSCC by acting as co-factors to HPV infection, as well as whether the effects of HPV infection are modified by these non-viral factors, or vice versa, has been addressed by means of two strategies (Table 6). First, formal assessments of effect modification have been performed in the context of case-control studies in which exposure to HPV has been measured using serologic assays or oral HPV DNA detection (e.g., using exfoliated cells from a rinse or a brush). In these analyses, the observed magnitude of joint effects was gauged against the predicted joint effects under a particular model (additive or multiplicative). In the second strategy, cases were stratified by the presence or absence of HPV DNA in tumors or by HPV serology and then associations with non-viral factors were evaluated separately in the two groups in comparison to controls. While these stratified analyses provide the opportunity to directly quantify a risk factor's role as a co-factor, these analyses do not formally test for the presence of effect modification/interaction. Alternatively, in the absence of control groups, the prevalence of non-viral characteristics is compared between patients with and without HPV. These case-case analyses provide the opportunity to identify non-viral co-factors that also qualify as effect modifiers.

1.2.6.1. Tobacco and alcohol. The literature is inconsistent with regard to whether tobacco and/or alcohol use are associated with increased risk of HPV-positive OPSCC (i.e., co-factors) and whether tobacco/alcohol can act synergistically with HPV infection in increasing OPSCC risk (i.e., effect modification). Of the case-control studies listed in Tables 3 and 4, and 5, seven studies have addressed the question of tobacco/alcohol as co-factors and effect modifiers of the HPV-OPSCC association using the strategies noted above (Table 6). Pertaining to co-factors, four studies [7,50,62,63] reported positive associations of tobacco use, alcohol use, or use

Table 4
Case-control studies on head and neck cancer – Measurement of exposure: HPV16 E6/E7 serology.

Author, year	Study location	Design	Cases/controls (n)	All HNSCCs OR (95% CI)	Oral cavity cancer OR (95% CI)	Oropharyngeal cancer OR (95% CI)	Laryngeal cancer OR (95% CI)	Adjustment factors
Herrero R *et al.* 2003 [7] [a]	Italy, Spain, Northern Ireland, Poland, India, Cuba, Canada, Australia, and Sudan (Multicenter)	Hospital-based case-control	1652/1607	-	**2.9 (1.7-4.8)**	**9.2 (4.8-17.7)**	-	Age, gender, country, tobacco, paan chewing, alcohol
Smith EM *et al.* 2006 [52] [a]	Iowa State (USA)	Hospital-based case-control	204/326	**28.8 (9.9-84.0)**	**4.9 (1.4-17.2)**	**231.0 (62.0-859.0)**	-	Age, tobacco, alcohol, sex and lifetime sex partners
D'Souza G *et al.* 2007 [45] [a]	Baltimore (USA)	Hospital-based case-control	100/200	-	-	**58.4 (24.2-138.3)**	-	Age, gender, tobacco, alcohol, dentition status and family history of head and neck cancers
Tachezy R *et al.* 2009 [54] [a]	Prague (Czech Republic)	Hospital-based case-control	86/104	**14.4 (5.2-39.7)**	0.9 (0.03-24.9)	**16.3 (5.9-45.0)**	-	Age, tobacco, alcohol
Ribeiro K *et al.* 2011 [50] [b]	Latin American countries and Central Europe (Multicenter)	Hospital-based case-control	2110/3235	**19.0 (5.2-69.0)**	1.8 (0.1-37.1)	**179.0 (35.8-899.0)**	**14.9 (3.0-76.1)**	Age, gender, tobacco, alcohol, country

[a] Antibodies: OR positive for either or both compared to negative category.
[b] Antibodies: OR positive for both; Also analyzed E6/E7 from other HPV types: 6,11,18,31,33,35,45,52,58.
HNSCC: Head and neck cancers; OR: Odds ratio; 95% CI: 95% Confidence interval.
Bold face figures indicate statistical significance.

Table 5
Case-control studies on head and neck cancer – Measurement of exposure: HPV DNA in oral exfoliated cells.

Author, year	Study location	Design	Cases/controls (n)	Sampling method	HPV PCR-genotyping	HPV types	All HNSCCs OR (95% CI)	Oral cavity cancer OR (95% CI)	Oropharyngeal cancer OR (95% CI)	Adjustment factors
Maden C *et al.* 1992 [152]	Washington State (USA)	Population-based case-control	118/112	Toothbrush (oral cavity)	PCR E6/E7 for 6/16	6/16	**HPV6: 2.9 (1.1-7.3)** HPV16: 6.2 (0.7-52.2)	-	-	Age, tobacco, alcohol, lifetime sexual partners
Schwartz SM *et al.* 1998 [51]	Washington State (USA)	Population-based case-control	237/435	Toothbrush (oral cavity) Tap water oral rinse	MY09/MY11 (L1) and E6 for 6/11/16/18 [Hybridization-Southern blot]	16/18/31/ 33/35/6/11	Any: 0.9 (0.5-1.6) HPV6/11: 0.5 (0.2-1.4) HR: 1.3 (0.6-2.9)	-	-	Age, gender, tobacco, alcohol, race/income/education
Smith EM *et al.* 1998 [161]	Iowa State (USA)	Hospital-based case-control	93/205	Saline oral rinse	MY09/MY11 (L1) [Sequencing]	-	**Any: 3.7 (1.5-9.3)**	-	-	Age, gender, tobacco, alcohol
Herrero R *et al.* 2003 [7]	Italy, Spain, Northern Ireland, Poland, India, Cuba, Canada, Australia, and Sudan (Multicenter)	Hospital-based case-control	601/613	Toothbrush (oral cavity) Saline oral rinse	GP5+/6+ (L1) [EIA-Southern blot]	16/18/31/33/35 /39/45/51/52/ 56/58/59/66/ 68/6/11/40/42/ 43/44	-	0.6 (0.3-1.1)	1.0 (0.4-2.5)	Age, gender, country, tobacco, paan chewing, alcohol
Smith EM *et al.* 2004 [57]	Iowa State (USA)	Hospital-based case-control	201/333	Saline oral rinse	MY09/MY11 and GP5+ [Sequencing]	-	**Any: 1.8 (1.1-2.7)** LR: 0.8 (0.4-1.7) **HR: 2.6 (1.5-4.2)**	-	**HR: 3.6 (1.8-7.1)**	Age, tobacco, alcohol
Hansson BG *et al.* 2005 [56]	Southern region of Sweden	Population-based case-control	131/320	Tumour and tonsillar fossa using cotton-tipped swabs Saline oral rinse	MY09/MY11 and GP5+/6+[Sequencing]	-	LR: 1.4 (0.5-4.3) **HR: 63.0 (14.0-280.0)**	TG: LR: 2.4 (0.5-11.0) **HR: 24 (3.2-180.0)** FM: LR: 3.3 (0.7-17.0) **HR: 51 (3.2-810.0)** OC NOS: **HR: 22 (2.8-170.0)**	LR: 0.8 (0.1-6.9) **HR: 230.0 (44.0-1200.0)**	Age, gender, tobacco, alcohol

Table 5 (*Continued*)

Author, year	Study location	Design	Cases/controls (n)	Sampling method	HPV PCR-genotyping	HPV types	All HNSCCs OR (95% CI)	Oral cavity cancer OR (95% CI)	Oropharyngeal cancer OR (95% CI)	Adjustment factors
D'Souza G *et al.* 2007 [45]	Baltimore (USA)	Hospital-based case-control	100/200	Cytology brush (posterior oropharyngeal wall) Saline oral rinse	PGMY09/11 Types-Linear probe array	-	-	-	**Any: 12.3 (5.4-26.4) HPV16: 14.6 (6.3-36.6)**	Age, gender, tobacco, alcohol, dentition status and family history of head and neck cancers
Gillison M *et al.* 2008 [44]	Baltimore (USA)	Hospital-based case-control	HNSCCs: 148[a]/296 OPC: 92[b]/184	Saline oral rinse	HPV16 Real-time PCR (E6)	16	1.1 (0.2-4.8)	-	**HPV16: 53.0 (8.5-333.0)**	Age, gender, race, tobacco, alcohol, marijuana use, oral hygiene
Pintos J *et al.* 2008 [53]	Montreal (Canada)	Hospital-based case-control	72/129	Toothbrush (oral cavity) Saline oral rinse	PGMY09/11 (L1) [Line blot]	16/18/31/33/35/39/45/51/52/56/58/59/68/73/82/6/11/26/40/42/53/54/55/66/83/84	Any: 3.1 (0.9-10.9) LR: 0.3 (0.0-4.4) **HR: 4.8 (1.2-19.4)**	Any: 1.3 (0.3-6.3) LR: 0.3 (0.0-5.5) HR: 2.1 (0.4-13.0)	TO and Base TG: **Any: 18.4 (2.2-154.5) HR: 19.3 (2.3-159.5)**	Age, gender, schooling, religion, language, tobacco, alcohol
Anaya-Saavedra G *et al.* 2008 [162]	Mexico city (Mexico)	Hospital-based case-control	62/248	Cytology brush from lesion and from same mucosal site in controls	MY09/11 and GP5+/GP6+[Sequencing]	-	-	**HR: 5.8 (2.4-13.8)**	-	Age, gender, familial history of cancer, tobacco, alcohol
Tachezy R *et al.* 2009 [54]	Prague (Czech Republic)	Hospital-based case-control	86/104	Saline oral rinse	MY09/11 and GP5+/GP6+[Sequencing]	-	**HR: 44.3 (13.2-148.7)**	HR: 0.9 (0.04-24.9)	**HR: 41.9 (12.6-139.0)**	Age, tobacco, alcohol

[a] Cases: Selection of HPV16 negative (78% non-oropharyngeal cancers).

[b] Cases: Selection of HPV16 positive (90% oropharyngeal cancers).

Any: Positive for any HPV type; FM: Floor of the mouth; HNSCC: Head and neck cancers; HR: High-risk HPV types; LR: Low-risk HPV types; NOS: Not otherwise specified; OR: Odds ratio; TG: Tongue; TO: Tonsils; 95% CI: 95% Confidence interval.

Bold face figures indicate statistical significance.

Table 6
Cofactors and interactions with other head and neck cancer risk factors.

Risk factor, Study	HPV exposure	Co-factor	Effect modification/interaction	Comments
Tobacco/alcohol				
Schwartz SM *et al.*1998 [51]	L1 serology	Yes	Yes [super-additive interaction]	Joint effects of HPV and tobacco/alcohol use higher than predicted.
Herrero R *et al.* 2003 [7]	L1 serology	Yes	No [on multiplicative scale]	Joint effects of HPV and tobacco use similar to predicted.
Smith EM *et al.* 2004 [57]	Oral HPV DNA	Yes	Yes [positive multiplicative interaction]	Joint effects of HPV and alcohol use higher than predicted.
Smith E *et al.* 2010 [63]	L1 serology	Yes	Yes [positive multiplicative interaction]	
Ribeiro KB *et al.* 2011 [50]	E6/E7 serology	Yes	Yes [negative multiplicative interaction]	Joint effects of HPV and tobacco use lower than predicted.
D'Souza G *et al.* 2007 [45]	Oral HPV DNA	No	No [on additive scale]	Joint effects of HPV and tobacco/alcohol use similar to predicted.
	L1 serology	No	No [on additive scale]	
Applebaum K *et al.* 2007 [49]	L1 serology	No	Yes [negative multiplicative interaction]	Joint effects of HPV and tobacco use lower than predicted.
Ji X *et al.* 2008 [64]	L1 serology	No	Yes [negative multiplicative interaction]	Joint effects of HPV and tobacco use lower than predicted.
Diet				
Meyer MS *et al.* 2008 [65]	L1 serology	Yes	Yes [positive multiplicative interaction]	Joint effects of HPV and higher fruit/vegetable consumption higher than predicted.
Arthur AE *et al.* 2011 [66]	HPV DNA in tissues	Yes	NR	Case-only design. Higher vitamin A, vitamin E, iron, ß-carotene, and folate associated with increased risk of HPV-positive HNSCC.
Host genetics				
Ji X *et al.* 2008 [64]	L1 serology	Yes	Yes [positive multiplicative interaction]	Joint effects of HPV and *p53* codon 72 polymorphism higher than predicted.
Chen X *et al.* 2010 [68]	L1 serology	Yes	Yes [positive multiplicative interaction]	Joint effects of HPV and MDM2 variants higher than predicted.
Yu H *et al.* 2012 [69]	L1 serology	Yes	Yes [positive multiplicative interaction]	Joint effects of HPV and MDM4 variants higher than predicted.
Marijuana use				
Gillison M *et al.* 2008 [44]	Oral HPV DNA	Yes	NR	Stratified case-control design.
Liang C *et al.* 2009 [72]	L1 serology	No	No [on multiplicative scale]	Marijuana use not associated with risk of HPV-positive HNSCC.
Oral hygiene				
Tezal M *et al.* 2009 [73]	HPV DNA in tissues	Yes	NR	Case-only design. Poor oral hygiene associated with risk of HPV-positive HNSCC.

NR: Not reported.

of both tobacco and alcohol with risk of HPV-positive OPSCC compared to controls. In contrast, three studies [44,49,64] reported the lack of association of tobacco/alcohol use with risk of HPV-positive OPSCC. Of note, no study to date has reported significant negative associations for tobacco/alcohol.

Pertaining to effect modification between tobacco/alcohol use and HPV infection, two studies reported significant positive interactions [51,63] (joint effects higher than predicted), two studies reported the lack of statistical interactions [7,45] (joint effects similar to predicted), and three studies reported significant negative interactions [49,50,64] (joint effects lower than predicted).

The differences across studies in HPV exposure measures, definitions of tobacco/alcohol use, and HNSCC sites included in the analyses likely contribute to the differences in results. Given these inconsistent associations in the literature, it is currently unclear whether tobacco/alcohol can act as co-factors and/or effect modifiers for risk of HPV-positive OPSCC. Studies reporting no effect of tobacco/alcohol and/or negative interactions for HPV-positive OPSCCs point to the possibility that HPV and tobacco/alcohol cause distinct diseases. These discrepancies in the literature notwithstanding, it can be concluded that HPV infection causes OPSCCs among both users and non-users of tobacco/alcohol [45].

1.2.6.2. Diet and nutrition. Most studies of HNSCC have observed lower cancer risk among individuals who report consuming larger amounts of fruits and vegetable [59,60]. A study of 270 cases and 493 controls that collected self-reported dietary data found that estimated intake of fruits and vitamin C modified the association between HPV16 seropositivity and OSCC [65]. There was a statistically significant inverse association between fruit and vitamin C consumption and risk among HPV16-seronegative subjects versus a positive association among HPV16-seropositive subjects. These results contrast with a case-only study that found patients with HPV DNA-positive HNSCC to have higher estimated intake of vitamin E, vitamin A, iron, and folate [66]. Thus, further study of diet-HPV interactions is warranted.

1.2.6.3. Genetic variation. Studies of polymorphisms in candidate host genes relevant to the mechanisms through which HPV contributes to HNSCC risk have focused primarily on the p53 pathway. A p53 sequence polymorphism encoding an arginine rather than a proline at position 72 is purportedly more susceptible to HPV-mediated degradation [67]. A case-case study found that patients with HPV16 or 18 DNA-positive tumors had a slight excess of Arg/Arg genotype (34% vs. 25%) compared to patients with HPV

DNA-negative OSCC. Like HPV16 E6, MDM2 degrades p53, and a case-control study observed a strong positive interaction between alleles of two promoter region single-nucleotide polymorphisms (SNPs) in this gene, HPV16 seropositivity, and oropharyngeal risk [68]. No evidence of interaction was found for oral cavity cancers. Similar results were observed for SNPs in MDM4, which suppresses p53 function without degrading the protein [69]. Thus, data are accumulating in support of a possible link between polymorphisms in the p53 pathway and susceptibility to HPV-associated HNSCC.

1.2.6.4. Other Characteristics. The possibility that marijuana use contributes to HNSCC risk was first raised over 25 years ago [70], and this drug's immunomodulatory as well DNA-damaging effects would be predicted to contribute to the development of HNSCC that are due to HPV infection and tobacco use, respectively. Although a pooled analysis of ~4,000 cases and ~5,000 controls from five studies failed to demonstrate an association between a history of marijuana use and HNSCC risk, including among never users of tobacco, no data were available on HPV [71]. Two case-control studies in which HPV was assessed have reported conflicting results. In a hospital-based study, the risk of HPV16 DNA-positive ($n = 92$), but not HPV16 DNA-negative ($n = 148$), HNSCC was increased among both current and past marijuana users, and increased with increasing intensity and duration of marijuana use [44]. Although formal analyses for statistical interaction with HPV were not conducted in this study, a case-case comparison of HPV-positive vs. HPV-negative HNSCCs did not reveal significant differences in marijuana use. In contrast, a community-based, case-control study found that the risk of HNSCC was reduced in marijuana smokers and did not vary according to HPV16 seropositivity status [72].

Poor dental hygiene, or associated dental conditions such as periodontitis that involve prolonged tissue damage and inflammatory response, have been hypothesized to contribute to HNSCC risk [61,73]. A case-case study of base of tongue cancers found that those containing HPV16 DNA ($n = 21$) were more likely to have periodontitis (86%) than those lacking HPV16 DNA ($n = 8$, 22%) ($p = 0.002$), and have greater alveolar bone loss [73].

In summary, larger comprehensive studies are needed to further investigate whether traditional non-viral HNSCC risk factors act as co-factors or effect modifiers on risk of HPV-positive OPSCC.

1.2.7. Summary of the epidemiological evidence of a causal association

The association of HPV16 with oropharynx cancers fulfills all of the criteria for causality as established by AB Hill (Table 7) [3]. All markers of HPV exposure (sexual behavior, serology, oral HPV infection) have been associated with HNSCC. Studies conducted in multiple populations have shown *a strong and consistent* association between HPV-exposed individuals and oropharyngeal cancer. Associations with oral cancer and other anatomic sites are neither strong nor consistent when compared to those for oropharyngeal cancer (*specificity*). At this time, it is unclear whether associations between HPV and laryngeal cancers (as well as very recent reports of HPV DNA in nasopharyngeal cancers) represent true primary cancers or are explained by anatomic continuity and misclassification (e.g., base of tongue with extension to the supraglottic larynx or palatine tonsil with extension to nasopharynx [74,75], respectively). Concerning HPV types, the majority of studies have reported significant associations with HPV16 serologic markers, and those assessing HPV DNA detection in exfoliated cells show associations with HPV16 alone or combined with other types classified as high-risk for cervical cancer. Only HPV16 exposure has been shown to precede the development of oropharyngeal cancer (*temporality*). Therefore, only HPV16 (*specificity*) satisfies epidemiological criteria (*strength and consistency, temporality*) as high-risk in the upper airway, with HPV types 18, 31, 33, 35, and 52 classified as potentially high-risk. Although increasing oropharyngeal cancer risk with increasing HPV antibody titers [76] suggests a dose-response effect (*biologic gradient* of increasing risk with increasing degree of exposure), there is little evidence that antibody titers to HPV antigens are indicative of increased exposure. Established epidemiological associations between HPV infection and cervical, anal, penile, vaginal, and vulvar carcinogenesis, as well as the molecular data noted above, lend support for *plausibility* and *analogy* [77,78]. Experimental evidence in the laboratory demonstrated HPV E6/E7 expression to be necessary for initiation and maintenance of the malignant phenotype. Although HPV vaccination has provided definitive *experimental* evidence in human subjects that prevention of anogenital HPV infection prevents anogenital dysplasias, this has not yet been demonstrated for oral HPV infections. Associations between sexual behavior and both oropharyngeal cancer and oral HPV infection are *coherent* with the sexual transmission framework of mucosal HPV infections.

2. The burden of HPV-positive head and neck cancers and oral HPV infection

2.1. Trends in incidence for head and neck cancer

Head and neck cancer incidence displays geographic and temporal heterogeneity, and has been historically correlated with patterns of tobacco use [79]. Regions of high head and neck cancer incidence include countries in Asia, where prevalence of chewing tobacco (and other products such as betel leaf/paan and areca nut) is high, as well as parts of central and Eastern Europe, and South America [79,80]. Despite overall declines in head and neck cancer incidence in most parts of the world, recent studies from several countries have shown that the incidence of oropharyngeal cancers, including cancers of the base of tongue, tonsil, and other parts of the oropharynx, has significantly increased over the past 20 years (Table 8).

Recent studies utilizing cancer registry data in Australia [81], Canada [82], Denmark [83], England [84], Finland [85], Japan [86], The Netherlands [87], Norway [88], Scotland [89], Sweden [90], and the United States (USA) [91] show rapid increases in oropharyngeal cancer incidence over the past 15–20 years (Table 8, annual percent changes ranging from 0.8–5.0% increase among men and 0.6–6.7% increase among women). In most (Australia, Canada, Norway, and USA), but not all countries (Denmark, England, Japan, and The Netherlands), incidence of non-oropharyngeal head and neck cancer sites significantly declined, consistent with recent declines in cigarette smoking. This rise in incidence of oropharyngeal cancers during an era of declining smoking has been attributed to HPV infection and characterized as a "virus-related epidemic [92]."

Notably, studies in Sweden [8], Australia [93], and USA [94] have complemented analyses of incidence trends with molecular testing of historical oropharyngeal cancer tumors for presence of HPV infection. These studies show that the prevalence of HPV DNA in tumors, as well as incidence of HPV-positive oropharyngeal cancers, has dramatically increased over time. For example, in Sweden, HPV prevalence in tonsil cancers increased from 23% during the 1970s to 68% during the early 2000s [90]. Likewise, in the USA, HPV prevalence in oropharyngeal tumors increased from 16% during the late 1980s to 72% during the early 2000s [94]. The results strongly support the hypothesis that HPV infection is the direct cause of rising oropharyngeal cancer incidence.

The rapid increases in oropharyngeal cancer incidence are believed to arise from changes in sexual behaviors, and thus increased oral HPV exposure, among recent birth cohorts. Indeed, in a majority of the studies noted in Table 8, rising oropharyngeal cancer incidence was predominantly observed among younger

Table 7
Epidemiological assessment of causality for HPV16 in oropharyngeal cancer.

Criterion	Evidence	References
Strength	Measures of HPV16 exposure (serologic or DNA-based) have been statistically associated in a range of 2.3–231 increased risk of oropharyngeal cancer in case-control studies.	Schwartz SM *et al.* [51]; Mork J *et al.* [48]; Herrero R *et al.* [7]; Dahlstrom KR *et al.* [157]; Van Doornum GJ *et al.* [158]; Smith *et al.* [52]; D'Souza G *et al.* [45]; Applebaum K *et al.* [49]; Gillison M *et al.* [44]; Pintos J *et al.* [53]; Ji X *et al.* [64]; Tachezy R *et al.* [54]; Ribeiro K *et al.* [50]
Consistency	HPV16 infection has been consistently associated with increased oropharyngeal cancer risk in studies conducted across different geographic locations/populations.	Schwartz SM *et al.* [51]; Mork *J et al.* [48]; Herrero R *et al.* 2003 [7]; Dahlstrom KR *et al.* [157]; Van Doornum GJ *et al.* [158]; Smith EM *et al.* [52]; D'Souza G *et al.* [45]; Applebaum K *et al.* [49]; Gillison M *et al.* [44]; Pintos J *et al.* [53]; Ji X *et al.* [64]; Tachezy R *et al.* [54]; Ribeiro K *et al.* [50]
Specificity	Across head and neck cancer anatomic subsites, the association of HPV seems specific for cancers arising in the oropharynx, including the base of tongue, lingual and palatine tonsil, and other parts of the oropharynx.	Schwartz SM *et al.* 1998 [51]; Mork J *et al.* 2001 [48]; Herrero R *et al.* 2003 [7]; Dahlstrom KR *et al.* [157]; Van Doornum GJ *et al.* [158]; Smith EM *et al.* [52]; D'Souza G *et al.* [45]; Applebaum K *et al.* 2007 [49]; Gillison M *et al.* 2008 [44]; Pintos J *et al.* 2008 [53]; Ji X *et al.* 2008 [64]; Tachezy R *et al.* 2009 [54]; Ribeiro K *et al.* 2011 [50]
Temporality	Only one nested case-control study generated within a serum cohort study has evaluated the association of HPV with prospective oropharyngeal cancer risk. HPV infection (measured by antibodies to HPV16 L1) precedes oropharyngeal cancer development by up to 15 years.	Mork J *et al.* 2001 [48]
Biologic gradient	Risk of oropharyngeal cancer increased significantly with increasing HPV16 L1 antibody titers indicating a dose-response effect.	Furniss CS *et al.* [76]
Plausibility	E6 and E7 proteins of HPV bind to and inactivate tumor suppressor proteins p53 and pRB, respectively, leading to malignant transformation of infected cells. Studies that evaluate HPV16 E6/E7 serology found stronger associations than other markers.	Herrero R *et al.* 2003 [7]; Smith EM *et al.* [52]; D'Souza G *et al.* [45]; Tachezy R *et al.* 2009 [54]; Ribeiro K *et al. 2011* [50]. *(For specific molecular evidence, see references from* Table 1*)*
Coherence	HPV-positive oropharyngeal cancers have evidence of integrated, high copy number HPV genomes in tumor cells as well as expression of E6 and E7 gene products. Consistent with HPVs being predominantly transmitted sexually, markers of sexual activity, including oral sex and number of lifetime oral sex partners have also been associated with increased oropharyngeal cancer risk in several studies.	D'Souza G *et al.* [45]; Gillison M *et al.* 2008 [44]; Heck JE *et al.* 2010 [46]. *(For specific molecular evidence, see references from* Table 1*)*
Experiment	Downregulation of E6 and E7 oncoproteins in HPV-positive cell lines resulted in increased apoptosis and reversal of malignant phenotype (as evidenced by increase in p53 and pRB levels).	*(For specific molecular evidence, see references from* Table 1*)*
Analogy	HPV-induced oropharyngeal carcinogenesis is analogous to HPV-induced cervical, anal, penile, vaginal, and vulvar carcinogenesis.	Bouvard V *et al.* [78]

individuals (recent birth cohorts). Consistent with this hypothesis, markers of high-risk sexual behavior—earlier age at sexual debut, practice of oral sex, premarital sex, and the average number of sex partners—have all increased among recent birth cohorts in countries that have experienced rising oropharyngeal cancer incidence, such as Australia, Sweden, and the USA [95]. Likewise, markers of HPV exposure, such as incidence of genital warts (in the USA and UK) and HPV seroprevalence (in Finland) have reportedly increased in recent years [96].

The increase in oropharyngeal cancer incidence appears to have largely occurred in developed countries (Europe, Australia, and North America, Table 8). Although data on incidence trends in developing countries are sparse, the incidence of oropharyngeal cancers has significantly declined in recent years in high incidence areas such as India, Thailand, and South American countries such as Colombia [80,97,98]. Differences in sexual behaviors, as well as in patterns of tobacco use, likely contribute to geographic differences in oropharyngeal cancer incidence trends. These factors could also contribute to differences in oropharyngeal cancer incidence trends by gender. For example, in the USA, whereas oropharyngeal cancer incidence increased among men, rates significantly declined among women [91], consistent with differences in overall as well as age-specific prevalence of oral HPV infection by gender in the US general population [99]. In contrast, in most other countries noted in Table 8, oropharyngeal cancer incidence increased among both men and women.

Oropharyngeal cancers could soon emerge as the dominant HPV-associated cancer in developed countries, where established screening programs have led to substantial declines in cervical cancer incidence. For example, in the USA, it is projected that the annual number of HPV-positive oropharynx cancers will surpass that of cervical cancers by the year 2020 [94].

2.2. Oral HPV infection

Given that high-risk HPV infection is a cause of oropharyngeal cancer based on a combination of molecular, epidemiological, and population trend data, the distribution of oral HPV infection in populations and risk factors for infection are of public health interest. To date, the literature has been quite limited relative to studies of cervical HPV infection and has comprised studies of small sample size with convenience samples, frequently conducted in high-risk populations, such as individuals attending a sexually transmitted disease clinic or human immunodeficiency virus (HIV)-infected individuals.

In a recent literature review, Kreimer AR and colleagues summarized results from 18 studies focused on "healthy populations" (e.g., subjects without cancer or immunosuppression), inclusive of a total of 4581 individuals [100]. Oral HPV infection prevalence was estimated to be 4.5% (95% CI: 3.9–5.1%) overall, for high-risk HPV types was 3.5% (95% CI: 3.0–4.1%), and for HPV16 was 1.3% (95% CI: 1.0–1.7%). Prevalence among men and women was similar. Subsequent to this analysis, two studies of relatively large sample size were published. In a study conducted in 1688 men aged 18 to 74 years from the USA, Mexico and Brazil, oral HPV prevalence was 4.0% (95% CI: 3.1–5.0%) [101]. Among 1000 men and women aged 18–30 attending a large, midwestern university in the USA, prevalence was 2.4% (95% CI: 0.8–2.0%) [102]. Low prevalence in these studies precluded analysis of factors independently associated with oral HPV infection. Preliminary associations

Table 8
Selected studies of incidence trends for head and neck cancers.

Country	Reference	Years	Incidence trends for oropharyngeal cancers		Incidence of oropharyngeal cancers by age	Incidence trends for other head and neck cancers	
			Men	*Women*		*Men*	*Women*
Australia	Hocking JS *et al.* [81]	1982-2005	Increase (APC = 1.4[a])	Increase (APC = 1.0[a])	Younger, 45–59 years	Decrease (APC = 1.7[a])	Stable
Canada	Auluck A *et al.* [82]	1980-2006	Increase (APC = 0.8[a])	Increase (APC = 0.6[a])	Younger, 55–64 years	Decrease (APC = 0.6[a])	Stable
Denmark	Blomberg M *et al.* [83]	1978-2007	Increase (APC = 4.4[a])	Increase (APC = 4.1[a])	Younger, <60 years	Increase (0.7[a])	Increase (0.9[a])
England	Reddy VM *et al.* [84]	1985-2006	Increase (APC = 2.5[a] to 6.7[a])	Increase (APC = 2.6[a] to 6.5[a])	Younger, 40–69 years	Increase (APC = 1.7[a])	Increase (APC = 2.8[a])
Japan	Ioka A *et al.* [86]	1965-1999	Increase (3.6-fold)	Increase (3-fold)	NR	Increase	Stable
The Netherlands	Braakhius BJ *et al.* [87]	1989-2006	Increase (APC = 2.6[a])	Increase (APC = 3.0[a])	Younger ages among men	Stable	Increase (APC = 2.0[a])
Norway	Mork J *et al.* [88]	1981-2005	Increase (APC = 5.0[a])	Increase (APC = 4.2[a])	Recent birth cohorts	Decrease	Stable/decrease
Sweden	Hammarstedt L *et al.* [90]	1970-2002	Increase (2.6- fold)	Increase (3.5-fold)	NR	NR	NR
USA	Chaturvedi AK *et al.* [91]	1973-2004	Increase (APC = 1.3[a])	Decrease	Younger, 40–59 years	Decrease	Decrease

NR: Not reported; APC: Annual percent change in incidence.
[a] Statistically significant at $p < 0.05$.

between current tobacco smoking and oral sexual behaviors (oral-genital contact and oral-oral contact) with prevalent oral HPV infection have been reported [42,100,102], but co-linearity of sexual behaviors and/or low prevalence limit study inferences. Oral HPV infection prevalence is higher in certain subgroups of the population. In particular, men and women with HIV-infection have oral HPV infection prevalence as high as 40% [103]. In these populations, oral HPV infection prevalence has been associated with severity of HIV-related immunosuppression.

The most comprehensive data on oral HPV infection prevalence were published by Gillison M *et al.*, who performed a population-based study of oral HPV infection among men and women aged 14–69 years in the USA [99]. Oral rinse samples were collected from 5579 individuals participating in the National Health and Nutrition Examination Survey 2009–2010, a statistically representative sample of the civilian US population. Oral HPV infection was detected in 6.9% (95% CI: 5.7–8.3%) and HPV16 in 1.0% (95% CI: 0.7–1.3%).

Factors found to be positively associated with oral HPV infection in both univariate and multivariable analysis included increasing age, male gender, an increasing number of sexual partners, and current tobacco smoking. Men had a three-fold higher prevalence of any HPV infection (prevalence ratio [PR] 2.80 [95% CI: 2.02–3.88]) and five-fold higher for HPV16 infection (PR 5.41, 95% CI: 2.12–13.83). Additionally, a bimodal pattern of infection with age was observed primarily among men with high-risk HPV infections. Infection was rare among sexually inexperienced individuals and significantly increased with number of sexual partners (p-trend < 0.001) and number of cigarettes smoked per day among current smokers (p-trend < 0.001). Although overall prevalence of oral HPV infection was relatively low, subgroups of the population, such as those with 20 or more lifetime sexual partners or current smokers of 20 or more cigarettes per day, had a prevalence of infection of 20%. These data therefore provide compelling evidence that oral HPV infection is predominantly sexually transmitted, and that the increased incidence of HPV-positive oropharyngeal cancer among men versus women in the USA [94] is explained in part by higher prevalence of infection among men. Natural history studies will be needed to demonstrate temporal associations between behaviors and infection, as well as to understand the effect of gender on the incidence and duration of oral HPV infection.

3. Clinical significance of HPV in head and neck cancers

The presence of HPV in HNSCC has recently become of clinical importance for reasons related to both diagnostics and prognostication.

3.1. HPV detection and diagnostics

From a histopathological perspective, HPV-positive HNSCC present most frequently as poorly differentiated, non-keratinizing (or basaloid) squamous cell carcinomas of the oropharynx [104], but may rarely present as lymphoepitheliomas [105] or small cell carcinomas [106]. HPV-positive cancers may present as cystic nodal metastases in the neck, and thus a cystic mass must be evaluated as a malignancy [107]. HPV detection in a cystic mass allows discrimination of malignant from benign lesions [108,109]. HPV presence in a cervical lymph node metastasis of an unknown primary is also strongly associated with a subclinical oropharyngeal cancer [110], and thus detection may guide radiation treatment portals to the oropharyngeal region. HPV detection in a metasynchronous solitary pulmonary nodule may also allow discrimination of metastases from second primary lung cancers [111].

3.2. HPV detection and prognostics

After the year 2000, numerous single institutional, retrospective case series reported an association between tumor HPV status and survival for patients with oropharyngeal cancer [10]. Despite substantial within- and between-study heterogeneity in patient characteristics and treatment, HPV presence in tumors was consistently associated with better survival. A meta-analysis of the literature estimated patients with HPV-positive OPSCC to have a 28% improvement in overall survival (hazard ratio [HR] 0.7, 95% CI: 0.5–1.0) and a 50% improvement in disease control (disease-free survival HR 0.5, 95% CI: 0.4–0.7) when compared to patients with HPV-negative OPC [112]. However, well established, favorable prognostic factors such as good performance status and absence of or minimal tobacco exposure were also consistently associated with HPV-positive OPC patients; therefore the possibility of residual confounding by these factors remained [113].

In the first prospective evaluation performed in the context of a clinical trial, Fakhry C *et al.* [114] observed patients with HPV-positive oropharyngeal cancer (positive by HPV16 ISH) to have a 64% (HR 0.36, 95% CI: 0.15–0.85) reduction in risk of death when compared to HPV-negative oropharyngeal and laryngeal cancer patients, after adjustment for age, tumor stage, and performance status. Subsequently, numerous retrospective analyses conducted within prospective, randomized controlled trials have consistently reported at least a 50% reduction in risk of death for the HPV-positive patient (Table 9) [115–119]. In the largest focused on oropharyngeal cancer, Ang K *et al.* [119] reported a 59% (HR 0.41, 95% CI: 0.29–0.57) reduction in risk of death after adjustment for age, race, performance status, tumor stage and nodal stage and treatment assignment. This was equivalent to a 25% difference in absolute survival between HPV16 ISH-positive and HPV16 ISH-negative patients at 3 years. In recursive partitioning analysis, tumor HPV status was the most influential prognostic factor. It is now generally accepted that tumor HPV status is the single greatest determinant of survival for patients with local-regionally advanced oropharyngeal cancer.

At this time, there appear to be multiple factors contributing to the marked difference in survival for the HPV-positive and negative patient populations. Ang K *et al.* estimated that the favorable prognostic factors associated with HPV-positive status (e.g., young age, good performance status) accounted for ~10% of the difference in survival between the two groups [119]. When compared to HPV-negative patients, patients with HPV-positive oropharynx tumors have increased response rates to platinum-based, induction chemotherapy [114,120]. Additionally, HPV status appears predictive of improved disease control within the radiation field (e.g., reduced local-regional failure rates), in studies utilizing either radiotherapy alone [115], chemoradiotherapy [116] or induction followed by chemoradiotherapy [117]. It remains unclear, however, whether the addition of chemotherapy provides benefit over radiotherapy alone. Some of the reduced risk of death among HPV-positive patients is also likely attributable to lower risk of distant metastases, although metastases among HPV-positive patients may occur at unusual anatomic sites (e.g., brain, muscle [121]). Second primary tumors associated with tobacco use are less common among HPV-positive patients, as tobacco exposure is less frequent in this group [119]. Additionally, comorbidities and competing risks for non-cancer associated deaths may be differential by tumor HPV status, particularly for smoking-associated illness. Indeed, quantitative measures of tobacco smoking (generally higher for the HPV-negative patient) have been associated with reduced overall and progression-free survival for patients with both HPV-positive and HPV-negative OPC [119].

Although HPV-positive and HPV-negative OPC are significantly different from one another with regard to frequency of several

Table 9
Selected studies investing association between tumor HPV status and overall survival in prospective clinical trials.

Study	Design	Cancer Site	Sample Size	Treatment	HPV testing method	Hazard Ratio for OS: positive vs. negative		Follow-up time	Proportion surviving by HPV status	
						HR	95%CI		Positive	Negative
Fakhry C *et al.* 2008 [114]	Phase II	Oropharynx, Larynx	96	Induction and ChemoRT	HPV16 ISH	0.36	0.15–0.85[a]	2-year	95%	62%
Lassen P *et al.* 2009 [115]	Phase III control arm	Pharynx, Larynx	156	Radiotherapy	P16 IHC	0.44	0.28–0.68[b]	5-year	62%	26%
Ang K *et al.* 2010 [119]	Phase III	Oropharynx	323	ChemoRT	HPV16 ISH	0.42	0.27–0.66[c]	3-year	82%	57%
Rischin D *et al.* 2010 [116]	Phase III	Oropharynx	172	ChemoRT	P16 IHC	0.45	0.21–0.96[d]	2-year	91%	74%
Lassen P *et al.* 2011 [118]	Phase III	Larynx, Pharynx, Oral Cavity	794	Radiotherapy	P16 IHC	0.54	0.42–0.68[e]	5-year	62%	47%
Posner MR *et al.* 2011 [117]	Phase III	Oropharynx	111	Induction and ChemoRT	P16 IHC	0.20	0.10–0.38[f]	5-year	82%	35%

[a] Adjusted for ECOG performance status.
[b] Adjusted for T stage and N stage.
[c] Adjusted for age, race, T stage, N stage, tobacco exposure, treatment assignment.
[d] Adjusted for hemoglobin, T stage, N stage, ECOG performance status.
[e] Adjusted for T stage, N stage, performance status, radiation fractionation schedule.
[f] Unadjusted.
HR: Hazard ratio; 95%CI: 95% Confidence interval; ISH: *In situ* hybridization; IHC: Immunohistochemistry; RT: Radiotherapy.

molecular alterations (as noted above), specific molecular differences necessary for the improved survival for the HPV-positive patient remain undefined. Defining these changes could result in rationally-targeted therapy for both the HPV-positive patient (perhaps with reduced toxicity) and the HPV-negative patient (perhaps by altering pathways that confer chemotherapy and radiation responsiveness for the HPV-positive patient).

Because of its prognostic import, testing of tumor HPV status is now recommended as part of the routine histopathological evaluation of OPC [122]. Clinical diagnostic testing methods are currently highly variable across pathology laboratories due to the absence of validated commercial assays for this indication. Validation would include evaluation of assay performance in comparison to high-risk HPV E6/7 oncogene expression, inter-reader agreement on histopathological interpretation (e.g., for HPV ISH and p16 immunohistochemistry [IHC]) and prognostic discrimination in prospective clinical trials. Only certain HPV16 ISH and p16 IHC currently meet this standard.

Laboratory methods currently include HPV DNA detection in tumors, usually by PCR or ISH, or detection of p16 expression by IHC, a surrogate for HPV E7 oncoprotein function [123]. HPV DNA detection by PCR alone has poor specificity and poor prognostic discrimination due to false-positive tests in comparison to HPV E6/E7 message expression [16]. When compared to HPV16 E6/E7 mRNA expression in oropharynx cancers, commercially available ISH assays intended for cervical cancer diagnostics (e.g., INFORM® HPVIII Family 16 Probe, Ventana Medical Systems Inc, Tuscon AR) had moderate-to-poor sensitivity (38–67%) or poor-to-high specificity (30–100%) in one study [124] but moderate-to-high (88%) sensitivity and specificity in another study [125], both of which had small sample size. An HPV16 ISH assay with moderate-to-high agreement (86%) with HPV16 mRNA expression [126] has been used in US cooperative group trials [114,119] that established HPV as a strong prognostic factor, but is not commercially available for this indication, and its sensitivity is limited by its HPV type-specificity [127]. p16 IHC alone has demonstrated high sensitivity (≥90%) and moderate-to-high (>80%) specificity for HPV16 E6 mRNA expression, high inter-reader agreement on interpretation by pathologists [124,128] and high agreement in comparison to HPV ISH [108]. However, ∼5–10% of oropharynx cancers are p16-positive and HPV-negative, thus limiting specificity of p16 IHC testing [108], and the prognostic outcome of this subgroup is unclear. In experienced research laboratories, sequential testing for HPV DNA by consensus PCR followed by p16 IHC in formalin-fixed tissue has high sensitivity and specificity (100%) in comparison to HPV16 E6/E7 oncogene expression, but this algorithm has not been validated in an independent sample set [25]. Although such a consensus PCR assay on fresh tissue provides information about HPV types beyond HPV16, it may not be feasible in all clinical pathology laboratories. Sequential or combined testing with p16 IHC and HPV ISH is rapidly becoming a clinical standard because it allows for visualization of HPV DNA within tumor cell nuclei, together with biological evidence of oncoprotein function (manifest by p16 overexpression). However, consensus standards for specific laboratory methods and interpretation have yet to be established [108,129].

3.3. HPV detection and therapeutics

While tumor HPV status clearly has important diagnostic and prognostic implications, at this time it is not used for therapeutic decision-making outside of the context of clinical trials. Because of prognostic implications, randomized clinical trials must be stratified by tumor HPV status to ensure balance between treatment arms. Additionally, the marked difference in survival between patients with HPV-positive and HPV-negative tumors indicates that the risks and benefits of intensive multimodality therapy are different in the two populations. Clinical trials for the HPV-positive patient population, the majority of whom are expected to survive, are now designed to evaluate whether the intensity of treatment and its associated morbidity can be reduced without compromising survival. In contrast, survival for the HPV-negative patient remains quite poor. Thus, clinical trials for this group are investigating the addition of novel targeted therapies or the addition of multi-agent induction chemotherapy to the treatment platform of concomitant cisplatin and radiotherapy.

4. Respiratory papillomas

Recurrent respiratory papillomatosis (RRP) is caused primarily by HPVs 6 and 11, with a small fraction (less than 5%) caused by HPV16 or other types [130]. The disease is characterized by growth of multiple papillomas, usually arising from the larynx.

RRP can manifest in early childhood (juvenile onset) or in adulthood and is a rare disorder, with an estimated incidence of approximately 3 per 1 million person-years in children, and a prevalence of 3 to 7 per 100,000 for both pediatric and adult disease [131,132].

There is no clear difference in prevalence based on race or ethnicity. Juvenile-onset disease usually presents between the ages of 1 and 4 years, and is equally distributed between males and females. Adult-onset disease has a broad peak between ages 20 and 40 years, but can occur even later, and shows a 2:1 bias toward males [130].

The strongest risk factor for juvenile onset RRP is a maternal history of genital warts in pregnancy. Offspring of women with genital warts are estimated to have over 200 times the risk of women without genital warts [133]. A triad of first-born children delivered vaginally to teenage mothers has been linked with increased risk [134], as has prolonged labor. Cesarean delivery has been associated with reduced [135] risk in epidemiological studies, but whether or not it is protective against RRP among women with genital warts at delivery is controversial [136]. Adult-onset RRP has been associated with lifetime number of sexual partners and oral genital sex [137].

The majority of patients present with hoarseness or stridor due to growth of papillomas on the larynx. Young age at onset (<3 or 4 years) has consistently been associated with increased severity of disease, as measured by number of required surgical procedures, severity of hoarseness, or airway obstruction [138–140]. HPV type (6 or 11) is inconsistently associated with disease severity. The natural history of disease is highly variable and can either persist for the life of the patient or be interspersed with short or long periods of remission. Recurrence rates vary between patients, with the worst cases requiring surgery under general anesthesia as frequently as every 3 weeks to maintain an airway.

Respiratory papillomas are primarily located in the larynx, but approximately 17% of patients have tracheal disease and 5% have pulmonary involvement [139]. The papillomas are usually benign, but malignant conversion has been documented to occur rarely (approximately 2%) [141], but may be as high as 80% among those with extensive pulmonary involvement, with most of the carcinomas containing HPV11 [142]. Moreover, radiation therapy in particular is contraindicated, because it increased the risk of malignant conversion in the larynx to 15–20% of patients [143]. Therefore, the concept that HPV6/11 are “low-risk” viruses that do not usually cause malignancies is appropriate for the genital track and oropharynx, but perhaps not for the lower airway in rare circumstances.

Risk factors for developing RRP among children of women with genital warts are poorly understood. Patients have widespread latent HPV infection in clinically normal tissues of the larynx, trachea and bronchi [144]. In latency, the viral DNA is present but with essentially no expression of viral RNA and no clinical or histologic

evidence of disease. Recurrent papillomas are thought to be due to repeated activation of this latent infection, rather than re-infection or "seeding" of the virus during surgery, as was originally thought by many physicians. However, a significant fraction of the normal population carries latent HPV DNA in their airway but with no history of disease, suggesting a role for additional risk factors [145]. One such factor would be a failure of the immune system to respond to viral proteins expressed by the activated virus. In fact, such responses are inadequate and ineffective in patients with recurrent disease, and most impaired in patients with the most frequent recurrences [146]. Recent studies have shown that the enzyme cyclooxygenase-2 (COX-2) is constitutively expressed in the airway of patients with RRP [147], and the downstream product of COX-2, prostaglandin E_2, can alter local immune responses. Thus, constitutive expression of COX-2 might be a risk factor for the disease.

Many therapies have been tried for RRP, with limited success and often with severe side effects. These include various surgical approaches, topical and systemic treatment with immunomodulators, antivirals, and chemotherapeutic drugs. Unfortunately, with the exception of the two large interferon studies, most reports have been either case reports or small clinical series with no controls [148]. Large controlled trials of potential therapeutic agents are badly needed. It may be that the most effective long-term therapy will be one that stimulates an effective, persistent immune response in patients with RRP. Of interest, a drug that inhibits COX-2 appears to be a potential therapy for the disease [147], and a large multicenter, randomized trial of COX-2 inhibition is in progress.

5. Primary and secondary prevention of HPV-associated diseases of the upper airway

The potential for clinical intervention into the natural history of HPV-positive oropharyngeal cancer is unknown at this time. While HPV vaccines have been shown to prevent incident and persistent anogenital infection, as well as anogenital precancers associated with HPV16 and 18, vaccine efficacy in preventing oral HPV infections has not been investigated. Observed associations between sexual behavior and both oropharyngeal cancer and adult onset respiratory papillomatosis suggest that vaccination prior to onset of sexual behavior would be protective.

Although detection of oral HPV DNA is associated with oropharyngeal cancer, its utility as a mechanism for secondary cancer prevention through screening is unknown. Preliminary studies have observed no association between HPV16 DNA detection by PCR in brush cytology samples and cytopathology, arguing against the potential utility of a 'Pap-smear' equivalent [149].

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Disclosed potential conflicts of interest

MLG: Has had scientific collaborations and has received research funding from Merck. She has acted as a consultant for Merck.

MLG: Have consulted for GSK.

XC: Institutional support: HPV vaccine trials and epidemiological studies sponsored by GlaxoSmithKline, Merck and Sanofi Pasteur MSD. Screening and HPV testing trials partially supported by Qiagen. Personal support: Travel grants to scientific meetings and honorarium for consultancy are occasionally granted by either GlaxoSmithKline, Merck, Sanofi Pasteur MSD.

LA: Institutional support: HPV vaccine trials and epidemiological studies sponsored by GlaxoSmithKline, Merck and Sanofi Pasteur MSD. Screening and HPV testing trials partially supported by Qiagen. Personal support: Travel grants to conferences occasionally granted by Merck and Sanofi Pasteur MSD.

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