

Human Papillomavirus in HNSCC: Recognition of a Distinct Disease Type

Laura Vidal^a, Maura L. Gillison, MD, PhD^{b,*}

KEYWORDS

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The overall incidence of head and neck cancer has declined over the past two decades in the United States, likely because of a decline in smoking rates.¹ Concomitantly, however, an increase in the incidence of oropharyngeal cancer has been observed in the United States and some European countries, particularly among young men, nonsmokers, and nondrinkers.²⁻⁴ Human papillomavirus (HPV), a sexually transmitted infection, is now recognized as an etiologic factor for a subset of oral squamous cell carcinomas (OSCCs), in particular those that arise from the oropharynx.⁵⁻⁸ A recent analysis of data from the Surveillance, Epidemiology, and End Results registry demonstrated that the proportion of OSCCs arising from anatomic sites potentially related to HPV significantly increased from 1973 to 2004 in the United States, particularly among white men aged 40 to 59 years.⁹ Additionally, the age at diagnosis for HPV-related tumors declined during this time period. By contrast, the incidence for HPV-unrelated OSCCs sites decreased over time for both men and women over the age of 40. Although it has been estimated that 20% to 25% of head and neck cancers are attributable to HPV, the observed incidence trends may increase the HPV-attributable fraction over time. Mucosal HPVs are known to infect the upper aerodigestive tract.¹⁰ The oncogenic types (eg, HPV-16) are able to induce malignant transformation of an infected cell. Two viral oncoproteins, E6 and E7, promote tumor growth by inactivating the tumor suppressor pathways p53 and Rb.¹¹⁻¹⁴ As a consequence of the viral oncoproteins, HPV-positive tumors have a specific molecular and genetic signature distinct from that of HPV-negative tumors. In addition, recent data indicate that patients with HPV-positive OSCC seem to have a better prognosis and clinical outcome when compared with those with HPV-negative disease.^{5,15} All these

^a Department of Medical Oncology, Princess Margaret Hospital, Toronto, Ontario, Canada

^b Johns Hopkins Medical Institutions, 1650 Orleans Street, CRB-1 3M 54A, Baltimore, MD 21231, USA

* Corresponding author.

E-mail address: gillima@jhmi.edu (M.L. Gillison).

47 differential molecular, clinical, and pathologic characteristics indicate that HPV-posi-
 48 tive head and neck squamous cell carcinoma (HNSCC) is a distinctive disease entity.
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51 BIOLOGY OF HUMAN PAPILLOMAVIRUS

52 HPV belongs to the Papillomaviridae family, small DNA viruses that are widely distrib-
 53 uted, having been detected in species from mammals to birds.¹⁶ HPVs specifically in-
 54 fect humans. There are currently approximately 120 genotypes of HPVs based on the
 55 level of similarity among nucleotide sequences in specific genomic regions (ie, E6, E7,
 56 and L1).¹⁶ HPVs infect exclusively epithelial cells. Each HPV type is preferentially as-
 57 sociated with specific clinical lesions and has an anatomic site preference for either
 58 cutaneous or mucosal squamous epithelium.¹⁷ The cutaneous types are commonly
 59 found in the general population and cause common warts. HPV-5 and -8 are associ-
 60 ated with squamous cell carcinomas of the skin that arise in patients with epidermo-
 61 dysplasia verruciformis, a rare genodermatosis with a selective defect in cell-mediated
 62 immunity.^{18,19} The mucosotropic types are further classified into nononcogenic or
 63 low-risk types or as oncogenic or high-risk types, those HPV types with strong epide-
 64 miologic associations with cervical cancer²⁰ and the potential to induce malignant
 65 transformation in vitro.^{21,22} The most common low-risk types are HPV-6 and -11
 66 and are mainly responsible for benign lesions, such as anogenital warts or oral papil-
 67 lomas. High-risk types (eg, HPV-16, -18, -31, -33, and -45) are predominantly found in
 68 squamous cell carcinomas of the cervix,²³ other anogenital malignancies,²⁴⁻²⁶ and in
 69 a subset of head and neck cancers.⁵

70 The HPV genome is a small double-stranded circular DNA molecule of approxi-
 71 mately 8000 base pairs. The genetic organization of all papillomaviruses is very simi-
 72 lar.²⁷ The coding information for HPV exists on one strand, and is divided into three
 73 major regions. The early region (E1-8) consists of genes responsible for transcription,
 74 plasmid replication, and transformation. The late region codes for the major (L1) and
 75 minor (L2) capsid proteins. The long-control region contains the regulatory elements
 76 for transcription and replication (Fig. 1). The E1 and E2 proteins are required for rep-
 77 lication and maintenance of the viral genome.²⁸ Although cooperation of E1 and E2 is
 78 required to initiate viral replication, E2 in addition plays an important role as a transcrip-
 79 tional repressor of both E6 and E7 oncogene expression.²⁹ E4 protein is encoded in
 80 the early region but expressed late in infection. The role of the E4 gene is still not
 81 known, but it is thought to promote the productive phase of the papilloma virus life cy-
 82 cle.^{30,31} E5 activates specific growth factor receptors^{32,33} and seems to be involved in
 83 the vegetative phase of the viral cycle.³⁴ E6 and E7 encode the papillomavirus major
 84 oncoproteins. The E6 protein of the high-risk papillomaviruses induces the degrada-
 85 tion of p53, the gatekeeper of the cell cycle, by ubiquitin-mediated process.³⁵ In ad-
 86 dition, E6 has been shown to activate telomerase in infected cells, prolonging the
 87 lifespan of the epithelial cells and enabling the production of viral progeny.³⁶ The E7

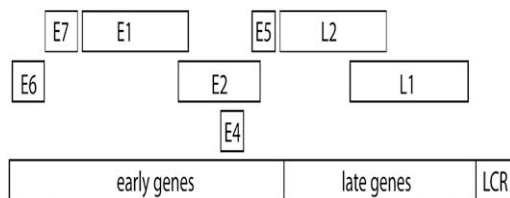


Fig. 1. Linear representation of the HPV genome.

[Q1]

98 protein binds the retinoblastoma tumor suppressor (pRB)¹⁴ and other proteins, such
99 as histone deacetylases, which control the activity of the essential transcription factor
100 E2F.³⁷ E7 liberates active E2F from an inactive pRB-E2F complex by targeting pRB,
101 allowing E2F to drive the cell into S phase and proliferation. E7 has also been
102 demonstrated to disrupt normal cell cycle control through blockade of specific cy-
103 clin-dependent kinases inhibitors.^{38,39} The late genes L1 and L2 encode viral capsid
104 proteins used in the construction of new viruses. The L1 protein alone, or L1 coex-
105 pressed with L2, is able to self-assemble into virus-like particles that are morpholog-
106 ically and immunologically similar to native virions, but lack potentially oncogenic
107 DNA.^{40,41} Virus-like particles are the basis of prophylactic HPV vaccines designed
108 to elicit virus-neutralizing antibodies to protect against initial HPV infection. L1 capsid
109 proteins are firstly synthesized in the cytoplasm before being transported to the nu-
110 cleus, to package viral chromatin. L2, the minor capsid component, seems to bind
111 specific sites of DNA replication in the nucleus and recruit L1 for new viral particles
112 to be assembled.^{42,43}

113 The papillomavirus life cycle is closely tied to the epithelial differentiation program.
114 Papillomaviruses replicate exclusively in keratinocytes. Keratinocyte stem cells are the
115 initial target of papillomavirus infections.⁴⁴ Microtraumas (small wounds) in the skin or
116 mucosal surface allow the virus to access the basal layer of the epithelium. No single
117 receptor for HPV entry has been definitively identified to date. Some data support
118 a role for $\alpha 6$ integrin, which is expressed primarily during wound healing, as a candi-
119 date receptor.⁴⁵ Additionally, a ubiquitous polysaccharide expressed on the cell sur-
120 face, glycosaminoglycan heparan sulfate, may play a role in the initial attachment
121 required for HPV infection.⁴⁶ HPV uses the host cell DNA machinery to perpetuate
122 the production of viral progeny. Following infection, HPVs establish their DNA genome
123 within the host cell nucleus, expressing early HPV proteins. In this phase, the HPV ge-
124 nome is maintained at a low copy number and provides a reservoir of viral DNA for fur-
125 ther cell divisions. As basal cells and viral DNA divide, some daughter cells may persist
126 in the basal layers, whereas other daughter cells move toward the upper layers of the
127 epithelium and begin to differentiate. It is during this differentiation process (vegetative
128 phase) that the viral genome replicates to a higher copy number and the capsid pro-
129 teins are expressed and that virions are assembled and eventually shed.⁴⁴

131 HUMAN PAPILLOMAVIRUS INFECTION AND MALIGNANT TRANSFORMATION

132 Mucosal HPVs are commonly transmitted by close contact, in particular sexual con-
133 tact, although other routes of infection have been documented.^{47–49} Most individuals
134 (80%–85%) acquire an HPV infection at some stage in their lives, generally in child-
135 hood for cutaneous types (hand and plantar warts) and in early adulthood for mucosal
136 types by sexual transmission. Like many other viral infections in healthy individuals,
137 most (around 80%) HPV infections clear spontaneously.⁵⁰ progression to malignancy
138 is relatively rare.⁵¹ In a large follow-up study of approximately 600 women, it was ob-
139 served that among HPV infections that persisted at least 12 months, the risk of cervical
140 invasive neoplasia diagnosed by 30 months was 21% and increased to 53% for
141 women younger than 30 years old.⁵²

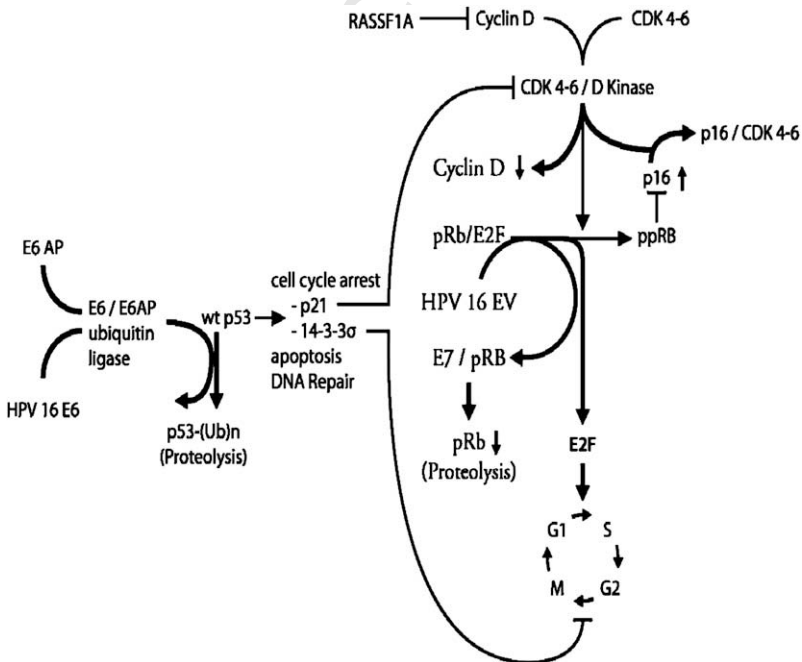
142 Cervical cancer serves as a model for HPV-mediated pathogenesis. In cervical can-
143 cers, the HPV viral genome frequently integrates into the host-cell genome, preferen-
144 tially, although not exclusively, at common fragile sites.⁵³ Viral integration may disrupt
145 the E2 coding region, resulting in the loss of E2-mediated transcriptional control and
146 dysregulated expression of the E6 and E7 oncoproteins. As for cervical cancer, the
147 HPV genome may be episomal, integrated, or both in oropharyngeal carcinomas.^{54,55}

149 Although some of HNSCC, primarily tonsillar carcinomas,⁵⁵ do not contain integrated
 150 HPV DNA, expression of the viral oncogenes can still be detected, indicating viral
 151 integration is not a necessary step for carcinogenesis.

152 The mechanism of viral-induced cell growth is well established and analogous to
 153 other tumor viruses that deregulate cell growth.⁵⁶ Both E6 and E7 oncoproteins inter-
 154 fere with well-established tumor suppressor pathways, such as p53 and Rb, among
 155 others,^{57,58} leading to a disturbance of cell cycle control and a deficiency in DNA repair
 156 (Fig. 2).⁵⁹ E7 also disrupts centrosome duplication resulting in genomic instability and
 157 aneuploidy, one of the hallmarks of a cancer cell.⁶⁰

158 On the basis of epidemiologic and molecular evidence, in 1995 the International
 159 Agency for Research on Cancer recognized that the high-risk HPV types 16 and 18
 160 were carcinogenic in humans.⁶¹ The low-risk HPV E6 proteins do not seem to affect
 161 p53 levels,⁶² whereas the low-risk HPV E7 proteins bind to the pRB proteins with
 162 much lower efficiency and are unable to induce genomic instability.⁶³

163 Establishing the link between HPV and a subset of HNSCC was initially difficult be-
 164 cause of the heterogeneity of HNSCC, and the fact that only a fraction of cases are
 165 HPV-associated. Syrjänen and colleagues⁶⁴ was the first to observe that some
 166 OSCC have morphologic and immunohistochemical features indicative of HPV infec-
 167 [Q7] tion. Since the first report of HPV DNA detection in HNSCC,⁶⁵ high-risk HPV (predom-
 168 inantly types 16, 31, and 33) have been repeatedly detected in a variable proportion of
 169 HNSCC and the viral genome has been specifically found localized to the tumor cells
 170 and transcriptionally active.^{66,67} The transforming potential of HPVs in the upper
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182 Fig. 2. HPV-mediated oncogenesis through inactivation of p53 and pRb by HPV oncoproteins
 183 E6/7. (From Gillison M. Human papillomavirus-associated head and neck cancer is a distinct
 184 epidemiologic, clinical, and molecular entity. *Semin Oncol* 2004;31:744-54; with permission.)
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airway has been supported by in vitro studies demonstrating HPV immortalization of oral keratinocytes.^{68–71} Additionally, E6 and E7 have been identified in vivo in a transgenic mouse model for HPV-associated HNSCC as the major transforming oncogenes,⁷² as has been previously described for cervical cancer.⁷³

Although expression of high-risk oncogenes can result in immortalization of an infected cell, their expression alone is not sufficient for transformation of human keratinocytes in culture.⁷⁴ For cervical cancers, other cofactors, such as smoking^{75,76} or long-term use of oral contraceptives, have been shown to modify the probability of HPV infection persistence and progression to cancer.⁷⁷ Immunosuppression is also associated with persistence of HPV infection at cervical and anal sites. Studies derived from immunocompromised patients suggest that a defective immune response may contribute to the progression of HPV-associated tumors.^{78–80} Contrary to other viruses, HPV do not infect and replicate in antigen-presenting cells located in the epithelium, nor cause cell lysis, so there is no opportunity for antigen-presenting cells to present virion-derived antigens to the immune system. Furthermore, there is no blood-borne phase of infection, so the immune system outside the epithelium has little opportunity to detect the virus.⁸¹ Although seroconversion occurs in 50% to 70%⁵⁰ of patients with HPV infection, the development of antibodies occurs late in infection, often months after the initial infection.⁸¹

HPV-induced cancers seem to retain viral oncogene expression for years or decades,⁸² so strategies to target gene expression to prevent further tumor growth could be used even at later stages of disease. More importantly, because HPV-induced cancers seem to appear years after the initial infection,^{8,83,84} it provides the excellent, but at this time theoretic, opportunity to implement adequate screening strategies to diagnose cancers at an earlier stage.

HUMAN PAPILLOMAVIRUS IN HEAD AND NECK CANCER

Prevalence of Human Papillomavirus in Head and Neck Cancers

Since the first description of a potential link between HPV infection and head and neck cancer,⁶⁴ several studies have strongly supported an etiologic role for HPV in cancers arising from specific mucosal sites within the head and neck.^{5,8,85} Detection of HPV genomic DNA has been found in approximately 25% of all HNSCC using sensitive polymerase chain reaction–based methods.⁸⁶ The association is strongest for oropharynx cancers, with detection rates of 50% or more,^{7,66,87,88} possibly because of a facilitated viral access to basal mucosal cells in the tonsillar crypts and an apparent predilection of this anatomic site to transformation by HPVs, analogous to the cervical transformation zone. In a meta-analysis, within the oropharyngeal subsite, the association between HPV-16 and cancer was strongest for tonsil (odds ratio, 15.1; 95% confidence interval [CI], 6.8–33.7). When the meta-analysis included data for the oropharynx as a whole, the odds ratio was 4.3 (95% CI, 2.1–8.9) compared with the oral cavity (odds ratio, 2; 95% CI, 1.2–3.4) and larynx (odds ratio, 2; 95% CI, 1.0–4.2).⁸⁹

Prevalence rates of HPV in OSCC seem to vary within studies, however, depending on ethnic and geographic location,^{85,86,90,91} tumor subsite,^{85,87,91,92} and HPV detection methods.^{86,92–99} Polymerase chain reaction has been widely used to identify and type the HPV DNA genome because of high sensitivity.^{84,87,88,93,100} Polymerase chain reaction assay has been found to be subject to contamination, however, which can yield false-positives results, so careful sample acquisition and processing are required.¹⁰¹ Recent studies^{8,15,102} have shown that in situ hybridization, using a signal amplification system,¹⁰³ is very sensitive, relatively inexpensive, and permits visualization of single copies of HPV-16 in an infected cell. Moreover, it can be successfully

used in clinical samples, including tumor cells from fine-needle aspirated neck masses, which makes this approach easily applicable to clinical practice.¹⁰⁴ Among all detected types, high-risk HPV-16 is the most common type identified in all head and neck cancers^{5,84,105–108} and accounts for 90% to 95% of HPV-related oropharyngeal cancer.⁶⁸ A number of other high-risk types (18, 31, 33, and 35) account for the remaining 5% to 10% of HPV-positive HNSCC.

Despite the prevalence of HPV observed in a large number of epidemiology studies, to establish a pathogenic role for the HPV in HNSCC it is not sufficient to find HPV-DNA in cancer specimens, but identification of markers of viral oncogene expression within tumor cell nuclei is also required. For HPV-positive HNSCC there are some studies that have demonstrated the presence of viral characteristics that are considered major determinants of HPV-associated carcinogenesis, such as viral oncoprotein expression;^{109–111} integration into cell genome;^{5,55} and high (greater than or equal to one copy per cell) viral load.^{66,112} In addition, the specific presence of antibodies to HPV-16 E6 and E7 in sera of HPV HNSCC patients has provided evidence for expression of these viral oncoproteins.¹¹³ Several case-control studies have been able to correlate the presence of HPV-DNA in specimens of oropharyngeal cancers with high prevalence of antibodies for oncoproteins E6, E7, or both.^{114,115} Furthermore, case-control studies have reported strong associations between HPV seropositivity and OSCC.^{8,85,116,117} In particular, the prospective study by Monk and colleagues⁸⁴ demonstrated a 14-fold increased risk for subsequent development of oropharyngeal cancer among HPV-16 seropositive individuals.

[Q8]

MOLECULAR CHARACTERISTICS

Although inactivation of p53 and pRb are both common findings in most HNSCC, their disruption occurs by different mechanisms in HPV-positive and -negative tumors. The molecular-genetic alterations found in HPV-positive tumors reflect the oncogenic function of E6 and E7. E6 inactivates p53 function,¹¹⁰ and several studies have found an inverse association between HPV-positive tumors and p53 mutations,^{110,118} whereas others have noted a reduced but persistent prevalence of p53 mutations in HPV-positive tumors. A recent study demonstrated the presence of p53 mutations in both HPV-16–positive and HPV-16–negative HNSCCs (25% versus 52%). Disruptive p53 mutations were only identified, however, in HPV-16–negative carcinomas. Specifically, none of the HPV-16–positive tonsillar cancers had functionally disruptive p53 mutations, in contrast to the 57% prevalence found in HPV-negative tonsillar cancers (0% versus 57%; $P = .008$).¹¹¹ Because of the ability of protein E7 to down-regulate pRb expression, HPV-positive HNSCC express decreased cyclin D and pRb.¹¹⁰ In addition, pRb negatively regulates the cyclin-dependent kinase inhibitor p16 (CDKN2A), which is often found up-regulated in HPV-positive HNSCC.^{119,120} Interestingly, p16 protein expression is lost in tobacco- and alcohol-associated HNSCC because of mutation, deletion, or gene methylation.¹²¹ Whereas HPV-positive HNSCC are associated with wild-type p53, down-regulation of cyclin D, pRb, and up-regulation of p16, HPV-negative HNSCC are characterized instead by p53 gene mutation, increased cyclin D, normal or increased levels of pRb, and decreased p16. These results support the existence of two distinct carcinogenetic pathways for the development of HNSCC: one driven by carcinogenic effects of tobacco, alcohol, or both and another by HPV-induced genomic instability.

Some studies have demonstrated an inverse correlation between HPV-positive tumors and epidermal growth factor receptor (EGFR) expression.^{122,123} Other markers for cell proliferation and apoptosis have been shown to be expressed differently in

HPV-positive and -negative HNSCC: PCNA, MIB-1, and surviving expression were found to be higher in HPV-negative tumors than in HPV-positive tumors.¹²⁴

RISK FACTORS

Emerging data indicate that risk factors for HPV-positive HNSCC are markedly different from those classically associated with HPV-negative HNSCC (eg, tobacco and alcohol). A recent case-control study showed that oral HPV infection was strongly associated with oropharyngeal cancers in cases with or without a history of significant exposure to tobacco and alcohol.⁸ Additionally, sexual behavior, a surrogate for exposure to HPV, was strongly associated with risk. For example, a high lifetime number of vaginal-sex partners was associated with oropharyngeal cancer (odds ratio, 3.1; 95% CI, 1.5–6.5), as was a high lifetime number of oral-sex partners (odds ratio, 3.4; 95% CI, 1.3–8.8). Another study by the same investigators demonstrated that sexual behavior and marijuana use were associated with HPV-16-positive HNSCC, whereas tobacco, alcohol, and poor oral hygiene were associated primarily with HPV-negative HNSCC.¹⁰² These results seem to contradict other studies, which showed an additive effect between oral HPV infection, tobacco or alcohol use, and oral cancer;^{115,117} larger studies are needed to evaluate possible interactions among these exposures.

In addition, defects in the immune system response seem to place subjects at higher risk of HPV-positive HNSCC and other HPV-associated malignancies.^{125,126} The recent observation that the use of marijuana, after adjusting for confounding factors, was strongly associated with HPV-16-positive OSCC, could be explained by an immunomodulatory effect of cannabinoids in HPV-mediated cancer.¹⁰²

CLINICOPATHOLOGIC CHARACTERISTICS

HPV-positive HNSCC patients tend to present with different clinical and histopathologic characteristics than patients with HPV-negative HNSCC (**Table 1**). Most of the studies have found that patients with HPV-positive oropharyngeal tumors tend to be younger (40–60 years) by approximately 5 years, on average, when compared with HPV-negative patients (≥ 60 years).^{6,8,15,117,127,128} Some studies support a predominance of HPV-positive HNSCC among men (3:1 ratio) as compared with women.^{8,15,102} In the univariate analysis in the study by Lowy and Gillison,¹²⁵ patients with HPV-positive tumors tended to be predominantly white and to have a higher education and economical status than those with HPV-negative HNSCC.

Table 1
Distinctive clinicopathologic characteristics for HPV-positive and HPV-negative HNSCC

	HPV-Positive	HPV-Negative
Anatomic site	Tonsil Base of tongue	All sites
Histology	Basaloid	Keratinized
Age	Younger	Older
Gender	3:1 men	3:1 men
Social economic status	High	Low
Risk factors	Sexual behavior	Alcohol and tobacco
Survival	Improved	Worse
Incidence	Increasing	Decreasing

Multiple studies have demonstrated that HPV-positive HNSCC tend to arise mostly from the lingual and palatine tonsils compared with other sites in the oropharynx,^{5,88,102,129,130} and frequently present with poorly differentiated nonkeratinizing, basaloid features^{5,15,109,131} compared with HPV-negative HNSCC, which presents with more differentiated and keratinized morphology.

[Q9] The presence of HPV has been found to correlate significantly with small tumor size and the presence of local metastases, and a more advanced AJCC TNM tumor stage (positive lymph nodes) at the time of diagnosis.^{88,132,133} In a study by Fakhry and colleagues, although nodal status and overall TNM stage did not differ by HPV status, HPV-positive tumors were more likely than HPV-negative tumors to have a tumor stage of T2 versus T3 to T4 ($P = .02$). In a study by Paz and colleagues, evaluating 167 patients with oropharyngeal cancer, only 4 of the 24 patients with HPV DNA in their tumors had negative lymph nodes (17%), whereas lymph nodes were negative in 73 of the 140 patients (56%) without HPV in their tumors. None of the HPV-positive HNSCC patients presented with early stage I disease, two presented with stage II, and the rest with stage III and IV (92%), whereas 50 (36%) of 141 of the HPV-negative patients were stage I or II. Other studies, however, have not found a correlation between nodal status, tumor stage, and HPV status.^{5,134} Recently, the presence of lymph node metastasis in neck dissection specimens was strongly associated with HPV-positive tonsillar cancers.¹³⁵

PROGNOSTIC AND TREATMENT IMPLICATIONS

A recent meta-analysis of papers reporting analysis of the impact of tumor HPV status on survival outcomes conducted by investigators at the University of Pittsburgh demonstrated that patients with HPV-positive HNSCC had a lower risk of dying (meta HR, 0.85; 95% CI, 0.7–1.0), and a lower risk of recurrence (meta HR, 0.62; 95% CI, 0.5–0.8) than HPV-negative HNSCC patients.¹³⁶

More recently, data from a new phase 2 prospective study conducted by the Eastern Cooperative Oncology Group (ECOG 2399) have confirmed that the presence of HPV infection actually heralds a better prognosis in patients with HPV-positive HNSCC. The ECOG 2399 trial assessed organ preservation, disease-free survival, and patterns of failure with taxane-based induction chemotherapy followed by taxane-based concurrent chemoradiation in 96 patients with resectable stage III and IV larynx and oropharyngeal cancer. HPV-positive tumors were detected in 40% of the patients by in situ hybridization and were oropharyngeal tumors. Tumor HPV status was statistically significantly associated with higher response rates after induction chemotherapy (82% versus 55%) and after chemoradiation treatment (84% versus 57%). Additionally, patients with HPV-positive tumors presented with improved 2-year overall survival (95% versus 62%) and a risk of disease progression that was 72% lower than that observed for the HPV-negative patients.¹⁵

A similar study by Worden and colleagues¹³⁷ that evaluated response to therapy and clinical outcomes in 66 patients with oropharyngeal cancer also found that the presence and titer of high-risk HPV-16 was associated with better response to chemotherapy ($P = .001$) and chemoradiotherapy ($P = .005$); better overall survival ($P = .007$); and better disease-specific survival ($P = .008$). The biologic reasons responsible for this survival difference remain unclear, but many hypotheses have been put forth and include the absence of field cancerization, immune surveillance to viral-specific tumor antigens, and an intact apoptotic response to radiation.^{6,138–140}

The results of these studies seem to confirm HPV status as a biomarker of prognosis in head and neck cancer. These findings will likely have important therapeutic

404 implications for treatment practices. Additionally, they may necessitate a reinterpretation of survival rates found in reported phase 3 trials. Are the improvements over time
405 a result of therapeutics or the result of tumor HPV status? Data from the Surveillance,
406 Epidemiology, and End Results registry demonstrated an absolute improvement of
407 23.1% during the period from 1973 to 2004 in 2-year overall survival for regional stage
408 HPV-related tumors treated with radiation compared with a 3.1% improvement for
409 HPV-unrelated OSCCs. Although these results could in part be attributable to better
410 use of radiation and concomitant chemotherapy,¹⁴¹ the significant improvement in
411 survival over time could also be explained in part by the increasing proportion of
412 HPV-positive tumors over time and their better response to radiation.^{138,142}

413
414 In a study of 100 patients with HPV-positive oropharyngeal tumors those patients
415 coexpressing both HPV and p16 had a significantly better overall survival when compared
416 with both tumors that were HPV-negative or HPV-positive but not expressing
417 p16.¹⁴³ A more recent study by Kumar and colleagues¹⁴⁴ examined the expression
418 of several biomarkers (eg, EGFR, p16, Bcl-xl, and p53) in the pretreatment biopsies
419 of 50 patients with locally advanced oropharyngeal cancer and their association
420 with high-risk HPV, response to induction chemotherapy, chemotherapy-radiation,
421 and survival. HPV DNA detection and p16 expression were strongly correlated.
422 When EGFR and p16 expression were combined, the clinical benefit was better for patients
423 with tumors expressing high p16 and low EGFR. Instead, high EGFR expression,
424 low HPV titer, and low p53/high Bcl-xL were associated with a significantly worse clinical
425 outcome. Interestingly, in patients with tumors with higher EGFR and p16 expression
426 (or HPV titers), the survival probability was improved when compared with those
427 without HPV DNA detection or p16 expression. Given that p16 and tumor HPV status
428 are highly correlated, it is not possible to evaluate the independent effects of either.

429 There is clearly the need to implement a reliable and easy diagnostic test to detect
430 HPV in paraffin-embedded tumor specimens of patients with HNSCC. In the absence
431 of direct measures of HPV, such as in situ hybridization, p16 expression is a reasonable
432 surrogate biomarker for HPV-associated oropharyngeal tumors.^{104,133,144,145} The expression
433 of p16 has shown to correlate strongly with in situ hybridization and HPV
434 gene expression and to distinguish polymerase chain reaction false-positives from
435 true-positives.¹⁴⁶

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SUMMARY

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440 The incidence of tonsillar cancer has increased in the United States, and recent data
441 suggest that this increase may be explained by HPV infection. In contrast to the
442 traditional profile of the elderly patient with head and neck cancer with a long history of
443 smoking and drinking, HPV infection is changing the demographic to include young,
444 nonsmoking, and nondrinking patients. Data indicate that patients with HPV-positive
445 HNSCC have a better prognosis and treatment responses to current standard of care
446 therapies compared with patients with HPV-negative HPV HNSCC. HPV-associated
447 disease may be having profound effects on past, current, and future clinical trial
448 results. Pretreatment screening for HPV disease should be implemented in future clinical
449 trial designs for HNSCC.

449 HPV status should now be considered a biomarker for prognosis in head and neck
450 cancer patients. Furthermore, the TNM classification may need to take into account
451 these biologic and molecular parameters to identify patients better who are more likely
452 to benefit from standard treatment; molecular targeted agents; and HPV-targeted
453 strategies, such as therapeutic vaccines currently undergoing clinical trials. Tailoring
454 individual treatment in tonsillar cancer may be of critical importance to increase

455 patient survival while at the same time attempting to reduce the long-term toxicities in
 456 a young patient population largely expected to survive their cancer.
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