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**Clinical and scientific impact of human papillomavirus on head and neck cancer**

Friedman JM *et al.* HPV transforms oropharyngeal squamous cell carcinoma

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**Abstract**

Head and neck cancer (HNC) arises from the skull base to the clavicles and is the fifth most common cancer in the world by incidence. Historically, in the developed world HNC was associated with tobacco use and alcohol consumption, and the combination of the two produced a synergistic increase in risk. However, beginning in 1983, investigators have found a significant and growing proportion of HNC patients with human papillomavirus-positive (HPV) tumors who neither drank nor used tobacco. Since that time, there has been increased interest in the molecular biology of HPV-positive HNC. Multiple studies now show that HPV has shifted the epidemiological landscape and prognosis of head and neck squamous cell carcinoma (HNSCC). These studies provide strong evidence for improved survival outcomes in patients with HPV-positive HNSCC compared to those with HPV-negative HNSCC. In many reports, HPV status is the strongest predictor of locoregional control (LRC), disease specific survival (DSS) and overall survival (OS). In response to these findings, there has been significant interest in the best management of HPV-positive disease. Discussions within major cooperative groups consider new trials designed to maintain the current strong survival outcomes while reducing the long-term treatment-related toxicities. This review will highlight the epidemiological, clinical and molecular discoveries surrounding HPV-related HNSCC over the recent decades and we conclude by suggesting how these findings may guide future treatment approaches.

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**Key words:**  Human papilloma virus; Head and neck cancer; Squamous cell carcinoma; Chemotherapy; Radiation; Molecular biology

**Core tip:** Head and neck squamous cell carcinoma (HNSCC) is the fifth most common cancer, and historically, in the developed world, was associated with tobacco and alcohol. However, beginning in 1983, investigators have found a growing proportion of HNSCC patients with human papillomavirus-positive (HPV) tumors who neither drank nor used tobacco. HPV has shifted the epidemiology and prognosis of HNSCC and HPV status is the strongest positive prognostic marker in patients with oropharyngeal SCC. This review will highlight the epidemiological, clinical and molecular discoveries surrounding HPV-related HNSCC over the recent decades and how these findings will guide future treatment approaches.

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**INTRODUCTION**

Head and neck cancer (HNC) describes a broad range of tumors that arise from the skull base to the clavicles. Worldwide, the incidence of HNC exceeds half a million annually, making it the fifth most common cancer in the world[[1](#_ENREF_1)]. In the year 2013, it is estimated that there will be 53640 new cases of HNC and 11520 deaths attributed to this disease in the United States alone[[2](#_ENREF_2)]. Anatomic sites of the head and neck include: the sinuses, orbits, nasopharynx, oropharynx, oral cavity, hypopharynx, and larynx. The primary risk factors for HNC include tobacco, alcohol, human papillomavirus (HPV) infection (for oropharyngeal cancer) and Epstein-Barr virus (EBV) infection (for nasopharyngeal carcinoma). The relative prevalence of these risk factors contributes to the various distributions of disease in different areas of the world.

Over the past 20 years there have been marked improvements in the diagnosis and treatment of HNC. Three-dimensional imaging including computed tomography (CT scans), positron emission tomography (PET), and magnetic resonance imaging (MRI) has allowed for better staging, target localization, and treatment planning. Advances in microsurgery and radiation therapy techniques have decreased treatment related morbidity and long term toxicities, while maintaining locoregional control (LRC)[[3](#_ENREF_3)].

Historically, in the developed world head and neck squamous cell carcinoma (HNSCC) was associated with tobacco use and alcohol consumption, and the combination of the two produced a synergistic increase in risk[[4](#_ENREF_4)]. However, a significant minority of patients (15%-20%) did not smoke or drink, indicating a different etiology existed. In 1983 investigators found immunohistochemical evidence of human papillomavirus (HPV) infection in tissue samples from 6 oral SCC[[5](#_ENREF_5)]. Since that time, there has been increased interest in the molecular biology of HPV-positive HNSCC. Multiple studies now show that HPV has clearly shifted the epidemiology and prognosis of head and neck squamous cell carcinoma (HNSCC). These studies provide strong evidence for improved survival outcomes in patients with HPV-positive HNSCC compared to those with HPV-negative HNSCC. In many reports, HPV status is the strongest predictor of locoregional control (LRC), disease specific survival (DSS) and overall survival (OS)[[6-8](#_ENREF_6)].

In response to these findings, there has been significant interest in the best management of HPV-positive disease. Discussions within major cooperative groups including the Radiation Therapy Oncology Group (RTOG) and the Eastern Cooperative Oncology Group (ECOG) have considered new trial designs based on HPV-status including: induction chemotherapy with response adapted radiation; alternatives to concurrent cisplatin chemotherapy, de-escalation of radiation dose, and the integration of minimally invasive surgery into the treatment algorithm. The primary objective of these approaches is to maintain the current strong survival outcomes while reducing the long-term treatment toxicities. A detailed analysis and comparison of these techniques and results is beyond the scope of this review, but may be found elsewhere[[8](#_ENREF_8)]. There have been a number of reviews that have addressed HPV in HNSCC[[9](#_ENREF_9),[10](#_ENREF_10)]. Here we review the discoveries and observations (including clinical retrospective reviews, clinical prospective trials, and basic science research) that showed how HPV dramatically changed the epidemiologic, clinical, pathological and molecular landscape of head and neck cancer. In addition, we present hypotheses for the causes of these changes, and how they may guide future clinical trial design.

**EPIDEMIOLOGIC CHANGES**

Retrospective analyses have shown a clear change in the epidemiology of HNSCC in the United States over the last 4 decades. A review of 60 published studies using PCR-based methods to detect and genotype HNSCC biopsies demonstrate a higher prevalence of HPV-positivity in oropharyngeal SCC (OPSCC) *vs* oral or laryngeal SCCs (35.6% *vs* 23.5% *vs* 24.0%). HPV-16 was found in 86.7% of the HPV-positive OPSCCs, while the second most prevalent high risk type HPV-18 was found in only 2.8% of HPV-positive OPSCCs[[11](#_ENREF_11)]. A Surveillance, Epidemiology, and End Results (SEER) study of HNC cases between 1973-2003 showed a 1.85% annual reduction in smoking-related HNSCC, while HPV-related HNSCC had increased by 0.8% per year[[12](#_ENREF_12)]. HPV-related sites were defined as oropharyngeal, while smoking-related sites were defined as oral cavity, nasopharynx and larynx. The decrease in smoking-related HNSCC correlated with a decrease in smoking in the United States during that time[[13](#_ENREF_13)]. In addition, the age at diagnosis for HPV-related HNSCC decreased by 1.5 years while the age at diagnosis for HPV-unrelated HNSCC increased. Analysis of tissue from 271 cases in the SEER database between 1984 and 2004 revealed that HPV prevalence in OPSCC tumors increased from 16.3% during the 1980s to 72.7% during the 2000s[[14](#_ENREF_14)]. Table 1 shows the estimated number of HPV-positive and HPV-negative HNC cases in the United States in 2011 by site. Similar increases were shown in Swedish, Australian, and Canadian populations[[15-17](#_ENREF_15)].

Changes in sexual practices are potential causes for the observed increase in HPV-related HNSCC. HPV transmission occurs primary through direct sexual contact, most often during vaginal or anal intercourse, but may also occur during oral sex or other forms of mucosal contact As a general trend, Americans are reporting their first sexual encounter at a younger age, they are having more sexual partners, and are performing more oral sex[[18-20](#_ENREF_18)]. In particular, a French study showed the lifetime prevalence of oral sex increased from roughly 50% in 1970 to 90% in 2006[[19](#_ENREF_19)]. Two cross-sectional studies conducted in the United States confirmed that increased sexual promiscuity, such as lifetime number oral sex partners, was associated with an increased risk of oral HPV-16 infection[[21](#_ENREF_21),[22](#_ENREF_22)]. Additional epidemiologic studies have shown that exposure to HPV increases the risk of developing HNSCC, and HPV-16 seropositivity predates cancer development by 9 years[[23](#_ENREF_23),[24](#_ENREF_24)]. Finally, a case-control study by D’Souza *et al*[[25](#_ENREF_25)] revealed that the number of vaginal and oral sexual partners [odds ratios (or), 3.1 and 3.4] and HPV-16 seropositivity (OR, 32.2) correlated with an increased risk of developing OPSCC. Notably, alcohol and tobacco increased the risk of developing OPSCC primarily in those patients without HPV-16 exposure. Similar results have been substantiated in other studies of HNSCC[[26-28](#_ENREF_26)].

**CLINICAL CHANGES**

The changing epidemiology of HNSCC, particularly HPV-positive OPSCC, created a new patient profile in the clinic. Patients began presenting at a much younger age without a strong alcohol or tobacco history and with more advanced disease in the neck[[29](#_ENREF_29)]. The natural history of HPV-positive HNSCC began to unfold following several small retrospective studies reported in the late 1990s and early 2000s. In an analysis of 42 patients treated between 1975-1987, HPV-positive SCC of the tonsil showed improved survival compared to HPV-negative SCC[[30](#_ENREF_30)]. Furthermore, an incidental finding in a 1998 German study of 208 HNSCC samples (11 of 36 tonsil primaries tested positive for HPV DNA) revealed a better prognosis despite more adverse pathologic features for the HPV-positive samples[[31](#_ENREF_31)]. In a Swedish review of 60 patients treated with radiation with or without surgery between 1986-1996, HPV-positive OPSCC cases had better 5-year OS (53.5% *vs* 31.5%) and decreased risk of recurrence (OR, 4.1) regardless of age, stage or gender[[32](#_ENREF_32)]. These findings were supported by a Swiss study of 98 patients with OPSCC who received definitive radiation therapy from 1991-1997, which also showed that HPV-positive tumors (14% of the total) had better LRC and OS [risk ratios (RR), 0.33 and 0.35][[33](#_ENREF_33)]. A meta-analysis of 37 studies examining HPV and HNSCC from 2007 showed that HPV-positive OPSCC in particular had a 28% reduced risk of death [meta hazard ratio (HR) 0.72] and a 49% lower risk of disease-failure (HR, 0.51) than HPV-negative OPSCC. The prognostic benefits of HPV-positive tumors was not significant for other sites[[34](#_ENREF_34)].

These smaller studies led to larger retrospective analyses of prospective trials. The phase III TAX324 trial comparing two different induction chemotherapy regimens was retrospectively reviewed and included 111 OPSCC patients. HPV-positive patients had OS and locoregional failure (LRF) rates of 79% and 13% compared to 31% and 42% for HPV-negative patients[[35](#_ENREF_35)]. In the phase II ECOG 2399 trial involving induction chemotherapy followed by chemoradiation, HPV-positive patients (38 of 96) had a better response to induction chemotherapy (82% *vs* 55%) and chemoradiation treatment (84% *vs* 57%)[[8](#_ENREF_8)]. Moreover, the HPV-positive OPSCC patients had a 62% lower risk of progression (HR, 0.38) and a 61% lower risk of death (HR, 0.39) when compared to those with HPV-negative OPSCC[[6](#_ENREF_6)]. Around the time of ECOG 2399, the Danish DAHANCA 5 phase III clinical trial prospectively collected samples from 156 patients who underwent conventional radiation therapy for OPSCC or supraglottic SCC from 1986-1990. Of the 74 OPSCC samples, 24 were p16-positive (a surrogate marker for HPV-driven disease) and showed a LRC benefit (OR, 5.1) compared to those that were p16-negative[[36](#_ENREF_36)]. A phase II prospective trial of OPSCC from 2000-2002 using induction chemotherapy to stratify definitive treatment paths correlated HPV-16 to response and survival. Pre-treatment biopsies of 42 patients were analyzed and 67% were HPV-positive. HPV titer was associated with significantly improved response to induction chemotherapy (*P* = 0.001), improved response to chemoradiation therapy (*P* = 0.005), improved OS (*P* = 0.007), and improved DSS (*P* = 0.008)[[37](#_ENREF_37)]. When the Trans-Tasman Radiation Oncology Group (TROG) 20.02 trial comparing cisplatin-based chemoradiation with or without tirapazamine was retrospectively reviewed for HPV and p16 status, 57% of 185 patients with OPSCC were p16-positive. The p16-positive tumors showed better 2-year OS (91% *vs* 74%; HR, 0.36; *P* = 0.004) and failure-free survival (87% *vs* 72%; HR, 0.39; *P* = 0.003). Table 2 summarizes select studies that examined HPV status and prognosis for HNSCC.

It was evident that patients with HPV-positive HNSCC experienced improved OS compared to HPV-negative disease, but whether this was secondary to improved treatment sensitivity, locoregional control or distant metastasis was unclear. Princess Margaret Hospital reviewed the rates of distant metastases (DM) in HPV-positive OPSCC following radiation or chemoradiation therapy. They included 457 HPV-positive and 167 HPV-negative OPSCC cases, and while DM rates were similar at 3 years, 24 of 25 HPV-positive cases of DM occurred within 2 years while 7 of 54 HPV-negative cases were detected 3 years after treatment. The authors found that the post-DM survival rates were 11% *vs* 4% at 2-years in favor of the HPV-positive cases and interestingly, 5 of 6 HPV-positive patients with lung oligo-metastases had stable disease 2-years after salvage procedures, which included chemotherapy, resection, or radiation[[38](#_ENREF_38)]. Additional recent retrospective reviews, have shown similar unique patterns of distant metastatic spread including to bone, brain, and multiple organs, and increased post-DM OS for HPV-positive patients[[38-42](#_ENREF_38)]. The trend of better post-DM OS was not seen when analyzing data from the SPECTRUM trial of metastatic or recurrent HNSCC. Of 443 patients, 99 were p16-postive, and no significant difference was observed in OS for these patients when compared to p16-negative patients [[43](#_ENREF_43)]. Additionally, when Rischin *et al*[[44](#_ENREF_44)] analyzed the TROG 20.02 trial there was no difference in DM rates between HPV-positive and HPV-negative OPSCC patients.

As previously discussed, HPV-positive HNSCC generally presents in younger healthier patients with small primary tumors and advanced lymph node (LN) metastases. The analysis of the TAX324 trial confirmed the association with smaller primaries and improved performance status. The authors found that HPV-positive patients were more likely to have T1 or T2 tumors (49% *vs* 20%) and an ECOG performance status of 0 (77% *vs* 49%)[[35](#_ENREF_35)]. Similar results were found when the TROG 02.02 Phase III trial data was retrospectively reviewed; p16-positive tumors were more likely to have a lower T stage (*P* = 0.001), higher N stage (*P* = 0.001), and better performance status (*P* = 0.002)[[44](#_ENREF_44)]. The ECOG 2399 data showed that although nodal status and overall AJCC TNM stage did not differ by HPV status, HPV-positive tumors were more likely to have a tumor stage of T2 *vs* T3 or T4, and an ECOG performance status of 0 (66% *vs* 33%)[[6](#_ENREF_6)]. An analysis of cystic lymph node metastases (20 of 100) from neck dissections from 2002-2004 showed a strong association with HPV-positive SCC of the tonsil[[45](#_ENREF_45)]. Princess Margaret Hospital reviewed data from 493 N2-3 HNSCC patients between 2003-2009 and found that HPV-positive LNs (257) were larger (2.9 *vs* 2.5cm), were more likely to be cystic (38% *vs* 6%), regressed more often post treatment (36% *vs* 41% of initial size), and were more likely to resolve after 36 wk (90% *vs* 70%), when compared to LNs of HPV-negative patients[[46](#_ENREF_46)]. The etiology of these differences in clinical presentation is unknown, and do not seem to be prognostic when controlling for HPV status.

Microscopic pathologic differences have also been observed between HPV-driven HNSCC and tobacco and alcohol-related HNSCC. HPV-positive tumors are more likely than HPV-negative tumors to be poorly differentiated and to have basaloid features, though the relevance of this difference is still undetermined[[6](#_ENREF_6),[47](#_ENREF_47)]. The improved LRC observed in HPV-positive HNSCC may be due to a lack of field cancerization historically observed in smoking-related HNSCC causing a higher risk of both recurrence and development of a second primary tumor[[48-50](#_ENREF_48)]. A Germany study of 25 HPV-16 positive HNSCCs in 2001 showed that HPV-16 was more likely to localize to the tonsil and HPV DNA did not appear outside of the tumor, suggesting that HPV-positive patients may not have entire mucosal fields predisposed to tumor development[[51](#_ENREF_51)].

**MOLECULAR DIFFERENCES**

The molecular biology of high risk HPV-driven tumorigenesis has been thoroughly studied in cervical cancer. HPV is a small, nonenveloped double-stranded DNA virus that infects keratinocytes and promotes transformation by altering cell cycle control primarily through expression of the nuclear proteins E6 and E7. Normally, p53 levels increase in response to DNA damage and prevent entry into S phase at the G1 checkpoint. E6 prevents accumulation of p53 and p53-mediated cell cycle arrest by causing the ubiquitination and subsequent degradation of p53[[52-54](#_ENREF_52)]. E6 likely promotes immortalization through other mechanisms including increasing telomerase activity[[55](#_ENREF_55)].

E2F is a transcription factor necessary for DNA synthesis, and is inhibited by hypophosphorylated retinoblastoma protein (pRb). Mitogenic signals cause an increase in cyclin-dependent kinases (CDKs), which promote phosphorylation of pRb leading to the release of E2F allowing entry into S phase. E7 preferentially binds to hypophosphorylated pRb causing its degradation and uncoupling the G1 checkpoint from CDK control[[56-58](#_ENREF_56)]. The CDK inhibitor p16 is normally suppressed by pRb and E7-mediated pRb degradation causes p16 upregulation[[59](#_ENREF_59)]. However, E7 stimulates the S-phase cyclins E and A, which bypass the normal p16 cell cycle inhibitory effects[[60](#_ENREF_60)]. E7 also binds to another DNA damage checkpoint protein p21, a p53-induced CDK inhibitor, driving the cell cycle into S phase[[61](#_ENREF_61)].

Molecular investigations of HNSCC initially did not differentiate between HPV-positive and HPV-negative samples because they were conducted in the era when HPV-negative tumors were more prevalent. Studies showed that the *TP53* gene was mutated in 45% of HNSCC and *TP16* gene inactivation by either mutation, deletion, or promoter hypermethylation occurred in 80% of HNSCC[[62-65](#_ENREF_62)]. To determine the prognostic significance of *TP53* mutations, a large scale prospective study of 560 patients with HNSCC who were treated with primary surgery with curative intent was performed. Disruptive *TP53* mutations were found in 53% of patients and predicted for decreased OS (HR, 1.7)[[66](#_ENREF_66)]. The epidermal growth factor receptor (EGFR) pathway has been extensively studied in various cancers and several trials have shown clear clinical efficacy of a targeted EGFR blockade. In regards to HNSCC, EGFR is overexpressed in approximately 80% of cases, and EGFR overexpression and copy number both correlate with a poorer prognosis[[67-70](#_ENREF_67)].

A German study from 1998 examined the status of the retinoblastoma (Rb) pathway in 208 HNSCC that were treated surgically. The investigators found that 11% of the tumors had no or dramatically reduced levels of the pRb without genetic disruption of the *Rb1* gene, and these samples localized to the tonsil. They also overexpressed p16 and had wild type *p53*. The authors detected high risk HPV DNA in 11 of the 12 pRb-deficient tumors strongly suggesting that the E7 viral protein inactivated the Rb pathway. These tumors had poorer differentiation and they were all metastatic at the time of resection, yet they had better clinical outcomes post treatment compared to the rest of the cohort[[31](#_ENREF_31)]. Although this study had a very small number of HPV-positive patients, several additional studies examining HPV status and cell cycle regulators followed. A study in 2000 including 253 patients with HNSCC tested archived samples for HPV-status and *TP53* mutations. HPV was detected in 25% of the samples, and HPV-positive tumors were less likely to harbor *TP53* mutations (OR, 0.06)[[47](#_ENREF_47)]. A Dutch group investigating the relationship between HPV-status and *p53* mutational status examined 47 HNSCC in 2003 and found 10 were HPV-positive with 8 of those being OPSCC. All 10 HPV-positive cases overexpressed p16, while 8 of the 10 overexpressed p53, yet none harbored mutations in *p53*. They also noted an inverse relationship between smoking and HPV-positivity[[71](#_ENREF_71)]. The interest in EGFR prompted an examination of EGFR and p16 expression in 106 OPSCC patients, which showed a significantly higher 5-year disease-free survival (DFS) and OS for patients who overexpressed p16, but not EGFR when compared to those with tumors overexpressing EGFR, but not p16[[72](#_ENREF_72)]. Further analysis of a phase II trial for OPSCC showing HPV copy number correlated with prognosis, revealed that HPV copy number was associated with p16 expression (*P* < 0.0001), and p16 overexpressing tumors had a better response to therapy (*P* = 0.009) and OS (*P* = 0.001). In addition, EGFR expression correlated with worse OS (*P* = 0.001) and DSS (*P* = 0.002), and was inversely associated with HPV copy number and p16 expression[[73](#_ENREF_73)].

In 2005 investigators attempted to use gene expression analysis to accurately predict LN metastasis in oral and OPSCC. They included 45 tumors from patients who were N+ postoperatively or who subsequently developed LN metastasis and 37 tumors from individuals who were N0 postoperatively and remained metastasis-free. The 102 predictor genes outperformed the current clinical diagnosis when independently validated[[74](#_ENREF_74)]. Gene expression profiles for 8 HPV-positive and 28 HPV-negative HNSCC were generated and published in 2006. Statistical analysis based on HPV status identified 91 differentially expressed genes, which included HPV-related overexpression of *p16*, *p18*, and *CDC7*, and a significant proportion of the HPV-positive genes localized to 3q24[[75](#_ENREF_75)]. Recently, multi-institutional groups conducted whole-exome sequencing of 74 HNSCC tumor-normal pairs (14% HPV-positive) and of 32 HNSCC samples. The results identified previously known HNSCC genes (*TP53, CDKN2A, PTEN, PIK3CA*, and *HRAS*), and found mutations in genes that regulate squamous differentiation (*NOTCH1, IRF6*, and *TP63*). Compared with traditional tobacco-induced HNSCC, HPV-positive samples had one half the mutation rate (*P* = 0.004) and an inverse relationship with *TP53* mutations (*P* = 0.001)[[76](#_ENREF_76),[77](#_ENREF_77)]. These studies have not resulted in dramatic breakthroughs yet, but form an important foundation for elucidating critical pathways in HNSCC.

Molecular characterization and environmental history has allowed stratification of patients with OPSCC into risk categories. In 2006, the HPV and p16 status of 79 OPSCC patients with long term follow-up were determined leading to a three-class model based on p16 expression and the presence of HPV DNA. The class that was HPV-positive and overexpressed p16 had better OS (79% *vs* 20% and 18%; *P* = 0.0095), DFS (75% *vs* 15% and 13% (*P* = 0.0025), 5-year local recurrence (14% *vs* 45% and 74%; *P* = 0.03), and lower p53 and pRb expression (*P* = 0.017 and 0.001)[[78](#_ENREF_78)]. Ang *et al*[7] retrospectively reviewed 323 OPSCC patients from the phase III prospective trial RTOG 0129 comparing standard and accelerated fractionation for HPV status. They found that 63.8% of patients had HPV-positive tumors and these patients had better 3-year OS (82.4% *vs* 57.1%, *P* < 0.001). In addition, the risk of death significantly increased with each additional pack-year of tobacco smoking. Using recursive-partitioning analysis for HPV-status, pack-years of tobacco smoking (≤ 10 *vs* > 10), tumor stage (T2-T3 *vs* T4), and nodal stage (N0-N2a *vs* N2b-N3), patients were classified as having a low (3-year OS 93.0%), intermediate (3-year OS 70.8%), or high risk (3-year OS 46.2%) of death[[7](#_ENREF_7)].

One explanation for the improved LRC seen in HPV-driven HNSCC is an increased sensitivity to chemoradiation[[79](#_ENREF_79)]. E6-related degradation of p53 in HPV-positive cancers may be functionally inequivalent to HPV-negative *p53* mutations, and therefore, HPV-positive tumors may have an intact apoptotic response to radiation and chemotherapy. However, enforced expression of E6 and/or E7 in cell lines did not cause radiosensitization *in vitro*[[80](#_ENREF_80),[81](#_ENREF_81)]. Interestingly, p53 overexpression has been found in HPV-positive tumors, which indicates that another mechanism unrelated to E6 may be involved[[71](#_ENREF_71)]. Moreover, survival for patients with HPV-positive oropharyngeal cancers was improved relative to HPV-negative patients both with and without *p53* mutations in their tumors and in patients treated with and without radiation therapy[[82](#_ENREF_82)].

Pre-clinical studies show that successful chemoradiation depends on innate and adaptive antitumor immune responses, and the increased immunogenicity of HPV-infected tumor cells may contribute to their robust treatment response[[83](#_ENREF_83),[84](#_ENREF_84)]. As part of a prospective phase II trial for OPSCC, 47 patients had baseline immune cell counts in addition to assessment of EGFR and HPV status. The authors found that improved survival was associated with an elevated percentage of CD8 cells (*P* = 0.04), a low CD4:CD8 ratio (*P* = 0.01), low EGFR expression (*P* = 0.002), and HPV status (*P* = 0.02). The percentage of CD8 cells was significantly higher (*P* = 0.04) and the CD4:CD8 ratio was significantly lower (*P* = 0.02) in HPV-positive patients. A higher percentage of CD8 cells was associated with response to induction chemotherapy (*P* = 0.02) and complete tumor response after chemoradiotherapy (*P* = 0.045)[[85](#_ENREF_85)]. These associations were studied at a basic level in HPV-positive and HPV-negative OPSCC cell lines. *In vitro*, there was a decreased response to either cisplatin or radiation for the HPV-positive cell line. However, *in vivo*, there was an increased response to both cisplatin and radiation. The authors found this only to be true in immunocompetent mice and immune-deficient mice that had been injected with competent immune cells, suggesting more of an immunologic mechanism for HPV-driven disease response to therapy[[86](#_ENREF_86)].

**FUTURE DIRECTIONS**

There are clear clinical, epidemiologic and molecular differences between tobacco-driven tumorigenesis in the oropharynx and HPV-driven OPSCC. However, these complex interactions are inadequately described, and clinically, it appears that some OPSCCs are caused by a combination of both tobacco and HPV infection. While the relationship between HPV-induced oncogenesis, tumor sensitivity and improved clinical outcomes is still being investigated, we now know that approximately 50%-80% of OPSCC’s are associated with HPV-positivity. With these trends, it is estimated that HPV will eventually become the primary etiology for head and neck cancer in the United States.

Induction chemotherapy, alternative systemic regimens to cisplatin and other approaches have been investigated prior to the era of HPV-positive disease. The retrospective analyses of these trials, which we have discussed above, created the overwhelming body of evidence for the better prognosis of HPV-positive OPSCC. In response to the improved survival outcomes, several collaborative groups have considered dose reduction in patients with HPV-positive HNSCC. Patients receiving lower doses of radiation and/or chemotherapy should experience less acute and long-term toxicities. Because patients with HPV-positive HNC are typically younger and demonstrate excellent LRC and OS, the value of limiting long-term toxicities such as lymphedema, swallowing dysfunction, and xerostomia is particularly important. ECOG 1308, a phase II trial, recently reported excellent early outcomes in patients where radiation dose reduction was based on a complete response to chemotherapy[[87](#_ENREF_87)]. Several groups have initiated clinical trials investigating other forms of treatment de-intensification such as using less toxic radiosensitizers. For example, the RTOG 1016 phase III trial plans to compare concurrent cisplatin and radiotherapy *vs* concurrent cetuximab and radiotherapy for HPV-positive OPSCC. Three additional randomized trials investigating patients with HPV-positive disease are currently pending enrollment or underway in the United States[[88](#_ENREF_88)].

Molecular markers are being collected prospectively in these trials, and continued research will offer new insight into the oncogenic pathways that influence clinical outcomes. Many questions remain including: why do HPV-driven tumors present with smaller primary tumors and more advanced nodal involvement; why do HPV-driven OPSCCs respond better to locoregional chemoradiation and induction chemotherapy, but have similar distant metastasis rates; and would the molecular analysis of distant metastatic disease show similar differences in HPV- *vs* tobacco-driven OPSCC?

Perhaps HPV-driven tumors are more localized to the tonsillar crypts and the lack of mucosal field changes influences the size of the primary tumor. The lack of field cancerization may also contribute to the improved LRC, although it would not fully explain improvements seen in response to both induction and chemoradiation. Another possibility is that HPV-driven tumors are more immunogenic and are tempered by an active immune response. Perhaps the molecular characteristics of HPV-driven tumors predispose malignant cells to metastasize to LNs, and once HPV-driven disease enters the LNs it creates a more robust immune response. This response may be due to intracellular characteristics or cell surface markers on HPV-positive cells, such as E7.

Since metastasis rates are similar for HPV-positive and -negative tumors, it is possible that once an OPSCC cell becomes metastatic, it dedifferentiates to a particular molecular state regardless of HPV-status. However, recent reports point towards increased time to DM, and improved OS after DM for HPV-positive disease, which appears to contradict previous evidence of a comparable response to salvage/palliative chemotherapy. Both pre-clinical and correlative studies suggest that immunogenicity is an important component to HPV-driven disease response. Perhaps differences in clinical outcomes will be due to differential immune responses. HPV vaccines in adolescents promise to reduce the incidence in HPV-driven OPSCC in decades to come, but to date they have not shown efficacy as therapy for these tumors after they develop. We may discover clinical benefits using immunomodulators in HNSCC, just as we do with current trials in malignant melanoma and non-small cell lung cancer.

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**P-Reviewers:** Chen Z, Sharma DN, Viswanatha B **S-Editor:** Ji FF **L-Editor: E-Editor:**

**Table 1 Human papillomavirus-negetive vs human papillomavirus-positive tumors in 2011 based upon ACS estimates**

|  |  |  |  |
| --- | --- | --- | --- |
| **Head and neck cancers** | **Total** | **HPV-** | **HPV+** |
| Larynx | 12740 | 10192 | 2548 |
| OC/P: mouth | 11510 | 9208 | 2302 |
| OC/P: other | 2250 | 1800 | 450 |
| OC/P: tongue | 12060 | 4342 | 7718 |
| OC/P: pharynx | 13580 | 4889 | 8691 |
| **(Oropharyngeal Cancer)** | **(25640)** | **(9230)** | (16410) |
| **Total** | **52140** | **31456** | **20684** |

HPV-: Human papillomavirus-negetive; HPV+: Human papillomavirus-positive.

**Table 2 Selected references for association of tumor human papillomavirus status with prognosis**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Country** | **N** | **Site** | **Detection (PCR, p16, ISH)a** | **Prevalence of HPV-positive disease b** | **Follow-up timec (1median, 2mean, 3range)** | **Significantly improved prognosis for HPV-positive tumor status? (Yes/No)** | **Prognosis for HPV-positive**  ***vs* HPV-negative diseased** | **Factors adjusted for** |
| Andl *et al* [31] 1998 | Germany | 31 | tonsil | PCR, p16 | 48% | 28 mo2 | Yes | **OS:** improved (*P* = 0.0071) **DFS:** median 61.1 mo *vs* 25.8 mo (*P* = 0.028) | Overall stage |
| Gillison *et al*[46] 2000 | United States | 259 | HNSCC | PCR, ISH | 25%  overall; 57% OP | 31 mo2 | Yes | **OS:** 91 mo *vs* 76 mo,  HR 0.6 (0.35-1.0, *P* = 0.07); **DSS:** HR 0.41 (0.20-0.88, *P* = 0.02) | Age, LN disease, alcohol |
| Mellin *et al*[32] 2000 | Sweden | 60 | Tonsil | PCR | 43% | 59 mo1 | Yes | **OS:** 5-yr 53.5% *vs* 31.5%;  **RFS:** OR 19.6 (p=.014); **DSS:** improved (p=0.047) | RFS: Overall stage DSS: Overall stage, LN metastases, age, gender |
| Lindel *et al*[33] 2001 | Switzerland | 99 | OP | PCR | 14% |  | Yes | **LFFS:** RR 0.31 (0.09-0.99, *P* = 0.048) | T, alcohol, intratumoral microvessel density |
| Weinberger *et al*[78] 2006 | United States | 123 | OP | p16 | 13% | 33 mo2 | Yes | **OS:** 5-yr 60% *vs* 21%, HR 0.422 (0.2-0.9, p=0.021) **DFS:** 5-yr 62% *vs* 19%, HR 0.359 (0.2-0.7, *P* = 0.006) | tumor type (primary vs recurrent), overall stage, grade, treatment |
| Weinberger *et al*[78] 2006 | United States | 79 | OP | PCR, p16 | 61% | 22 mo2 | Yes | **OS:** 5-yr 79% *vs* 18-20%, HR 0.19 (0.1-0.7, p=0.13) **DFS:** 5-yr 75% *vs* 13-15%, HR 0.20 (0.1-0.6), p=0.005) | primary vs recurrence, treatment, overall stage, grade |
| Licitra *et al*[82] 2006 | Italy | 90 | OP | PCR, p16 | 19% | 5.8 yr1 | Yes | **OS:** 5-yr 79% *vs* 46% (*P* = 0.0018), improved when adjusted for stage | Overall stage |
| Reimers *et al*[77] 2007 | Germany | 106 | OP | PCR, p16 | 30% |  | Yes | **DFS:** 85% *vs* 49% (*P* = 0.009),  HR for HPV-negative tumors 7.5 (1.22-46.19), *P* = 0.030 | EGFR expression status, overall stage |
| Kumar *et al*[73] 2008 | United States | 66 | OP | PCR, p16 |  |  | Yes | **OS:** improved (*P* = 0.006)  **DSS:** improved (*P* = 0.02) | Smoking, gender |
| Fakhry *et al*[6] 2008 | United States | 96 | OP, larynx | PCR, ISH | 63% OP, 0% larynx | 39.1 mo1 | Yes | **OS:** 2-yr 95% *vs* 62% (*P* = 0.005),  HR 0.36, (0.15-0.85) **PFS:** HR 0.27 (0.10-0.75) | age, overall stage, ECOG performance status |
| Hafkamp *et al*[71]2008 | Netherlands | 81 | tonsil | ISH, p16 | 41% | 30 mo2 | Yes | **DSS:** 5-yr 55% *vs* 29%, unadjusted HR 2.3 (1.1-4.5) |  |
| Worden *et al*[37]2008 | United States | 66 | OP | PCR | 64% | 64 mo1 | Yes | **OS:** improved (*P* = 0.008)  **DSS:** improved (*P* = 0.004) | gender, smoking, T, N, age, site |
| Klussmann *et al*[28] 2009 | Germany | 60 | OP | PCR, ISH, p16 | 47% | 27.5 mo1 | Yes | **DFS:** 5-yr 71% *vs* 46%( p=0.02) |  |
| Lassen *et al*[36] 2009 | Denmark | 156 | Supraglottic larynx, pharynx | p16 | 22% | > 5 yr | Yes | **OS:** 5-yr 62% *vs* 26% (*P* = 0.0003), HR 0.44 (0.28-0.68)  **DSS:** 5-yr 72% *vs* 34% (*P* = 0.0006),  HR 0.36 (0.20-0.64) | T, N |
| Ang *et al*[7] 2010 | United States | 323 | OP | ISH, p16 | 63.8% | 4.8 yr1 | Yes | **OS:** 3-yr 82.4% *vs* 57.1% (*P* < 0.001), HR 0.42 (0.27-0.66) **PFS:** 3-yr 73.7% *vs* 43.4%  (*P* < 0.001), HR 0.49 (0.33-0.74) | age, race, T, N, tobacco exposure, treatment assignment |
| Rischin *et al*[44] 2010 | United States, Canada, Australia, New Zealand, Europe | 184 | OP | PCR, ISH, p16 | 57% | 29 mo2 | Yes | **OS:** 2-yr 91% *vs* 74% (*P* = 0.004), HR 0.43 (0.20-0.93), *P* = 0.031 | ECOG performance status, hemoglobin, T, N |
| Chaturvedi *et al*[14] 2011 | United States | 271 | OP | PCR , ISH | 44% | 112 mo1 | Yes | **OS:** median 131 *vs* 20 mo (*P* < 0.001),  HR 0.31 (0.21-0.46) | age, calendar period of diagnosis, overall stage, treatment |
| Posner *et al*[35] 2011 | United States | 111 | OP | PCR | 50% | 82-83 mo1 | Yes | **OS:** improved, unadjusted HR 0.2 (0.10-0.38, *P* < 0.0001)  **PFS:** 73% *vs* 29% (*P* < 0.0001) **LRF:** 13% *vs* 42% (*P* = 0.0006) |  |
| Liang[36] 2012 | United States | 488 | HNSCC | PCR, p16 | 62% |  | No | **DSS** (OP)**:** HR 0.1 (0.02-0.4) |  |

aPCR is polymerase chain reaction to assess for HPV-related DNA; p16 refers to immunohistochemistry for HPV oncoprotein p16; ISH is in-site hybridization for HPV;  bHPV tumor status as defined by individual study;  cFollow-up time included when reported (1median, 2mean, 3range);  dIncludes prognostic data when available and as reported.  HR are adjusted and for HPV-positive tumors unless indicated otherwise.  95% confidence intervals are included in parentheses.PCR: Polymerase chain reaction to assess for HPV-related DNA; ISH: In-situ hybridization; P16: Immunohistochemical assay for p16 expression; OS: Overall survival; DSS: Disease-specific survival; RFS: Recurrence-free survival; EFS: Event-free survival; LR: Local recurrence; LFFS: Local failure free survival; FFS: Failure-free survival; LRC: Locoregional control; LRR: Locoregional recurrence; HR: Hazard ratio; OR: Odds ratio; RR: Relative risk; LN: Lymph node; OC: Oral cavity; OP: Oropharynx; BOT: Base of tongue; DSS: Disease-specific survival; FOM: Floor of mouth; T: Tumor stage; N: Nodal stage.