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TOPIC HIGHLIGHT

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

Jeffrey M Friedman, Mark J Stavas, Anthony J Cmelak

Jeffrey M Friedman, Mark J Stavas, Anthony J Cmelak, Department of Radiation Oncology, Vanderbilt University Medical Center, Nashville, TN 37232, United States
Author contributions: All authors contributed to this work.
Correspondence to: Anthony J Cmelak, MD, Professor of Medicine, Department of Radiation Oncology, Vanderbilt University Medical Center, Preston Research Building, B-1003, Nashville, TN 37232-5671, United States. anthony.cmelak@vanderbilt.edu
Telephone: +1-615-3222555 Fax: +1-615-3436589
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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, disease specific survival and overall survival. 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.

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]. 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]. 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].

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]. 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]. 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].

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]. There have been a number of reviews that have addressed HPV in HNSCC[9,10]. 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]. 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]. 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]. 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]. 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].

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]. In particular, a French study showed the lifetime prevalence of oral sex increased from roughly 50% in 1970 to 90% in 2006[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[21,22]. 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[23,24]. Finally, a case-control study by D'Souza *et al*[25] revealed that the number of vaginal and oral sexual partners [odds ratios (OR), 3.1 and

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.

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].

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]. 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]. 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]. 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]. 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]. 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].

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]. 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]. 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]. 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]. 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]. 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]. 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]. The trend of better post-DM OS was not seen when analyzing data from the SPECTRUM trial of metastatic or recurrent HNSCC.

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 time[c] ([1]median, [2]mean, [3]range)	Significantly improved prognosis for HPV-positive tumor status? (Yes/No)	Prognosis for HPV-positive *vs* HPV-negative disease[d]	Factors adjusted for
Andl *et al*[31], 1998	Germany	31	Tonsil	PCR, p16	48%	28 mo[2]	Yes	OS: improved (P = 0.0071) DFS: median 61.1 mo *vs* 25.8 mo (P = 0.028)	Overall stage
Gillison *et al*[47], 2000	United States	259	HNSCC	PCR, ISH	25% overall; 57% OP	31 mo[2]	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 mo[1]	Yes	OS: 5-yr 53.5% *vs* 31.5%; RFS: OR 19.6 (P = 0.014); 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 mo[2]	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 mo[2]	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 yr[1]	Yes	OS: 5-yr 79% *vs* 46% (P = 0.0018), improved when adjusted for stage	Overall stage
Reimers *et al*[72], 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 mo[1]	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 mo[2]	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 mo[1]	Yes	OS: improved (P = 0.008) DSS: improved (P = 0.004)	gender, smoking, T, N, age, site
Smith *et al*[28], 2009	Germany	60	OP	PCR, ISH, p16	47%	27.5 mo[1]	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	63.80%	4.8 yr[1]	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 mo[2]	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 mo[1]	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 mo[1]	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)	

[a]PCR 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; [b]HPV tumor status as defined by individual study; [c]Follow-up time included when reported ([1]Median, [2]Mean, [3]Range); [d]Includes prognos

tic 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.

Of 443 patients, 99 were p16-positive, and no significant difference was observed in OS for these patients when compared to p16-negative patients[43]. Additionally, when Rischin *et al*[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]. 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]. 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]. 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]. 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]. 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[6,47]. 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]. 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].

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]. E6 likely promotes immortalization through other mechanisms including increasing telomerase activity[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]. The CDK inhibitor p16 is normally suppressed by pRb and E7-mediated pRb degradation causes p16 upregulation[59]. However, E7 stimulates the S-phase cyclins E and A, which bypass the normal p16 cell cycle inhibitory effects[60]. E7 also binds to another DNA damage checkpoint protein p21, a p53-induced CDK inhibitor, driving the cell cycle into S phase[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]. 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]. 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].

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]. 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]. 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]. 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]. 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].

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]. 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]. 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 ($P = 0.001$)[76,77]. 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]. 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].

One explanation for the improved LRC seen in HPV-driven HNSCC is an increased sensitivity to chemoradiation[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*[80,81]. Interestingly, p53 overexpression has been found in HPV-positive tumors, which indicates that another mechanism unrelated to E6 may be involved[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].

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[83,84]. 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]. 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].

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]. 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].

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 preclinical 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.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; **63**: 11-30 [PMID: 23335087 DOI: 10.3322/caac.21166]

3 **Cmelak AJ**, Arneson K, Chau NG, Gilbert RW, Haddad RI. Locally advanced head and neck cancer. *Am Soc Clin Oncol Educ Book* 2013: 237-244 [PMID: 23714512 DOI: 10.1200/EdBook_AM.2013.33.237]

4 **Mashberg A**, Boffetta P, Winkelman R, Garfinkel L. Tobacco smoking, alcohol drinking, and cancer of the oral cavity and oropharynx among U.S. veterans. *Cancer* 1993; **72**: 1369-1375 [PMID: 8339227]

5 **Syrjänen KJ**, Pyrhönen S, Syrjänen SM, Lamberg MA. Immunohistochemical demonstration of human papilloma

virus (HPV) antigens in oral squamous cell lesions. *Br J Oral Surg* 1983; **21**: 147-153 [PMID: 6307342]
6 **Fakhry C**, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A, Gillison ML. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst* 2008; **100**: 261-269 [PMID: 18270337 DOI: 10.1093/jnci/djn011]
7 **Ang KK**, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010; **363**: 24-35 [PMID: 20530316 DOI: 10.1056/NEJMoa0912217]
8 **Cmelak AJ**. Current issues in combined modality therapy in locally advanced head and neck cancer. *Crit Rev Oncol Hematol* 2012; **84**: 261-273 [PMID: 22595517 DOI: 10.1016/j.critrevonc.2012.04.004]
9 **Marur S**, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol* 2010; **11**: 781-789 [PMID: 20451455 DOI: 10.1016/S1470-2045(10)70017-6]
10 **Mehra R**, Ang KK, Burtness B. Management of human papillomavirus-positive and human papillomavirus-negative head and neck cancer. *Semin Radiat Oncol* 2012; **22**: 194-197 [PMID: 22687943 DOI: 10.1016/j.semradonc.2012.03.003]
11 **Kreimer AR**, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 467-475 [PMID: 15734974 DOI: 10.1158/1055-9965.EPI-04-0551]
12 **Chaturvedi AK**, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. *J Clin Oncol* 2008; **26**: 612-619 [PMID: 18235120 DOI: 10.1200/JCO.2007.14.1713]
13 **Sturgis EM**, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? *Cancer* 2007; **110**: 1429-1435 [PMID: 17724670 DOI: 10.1002/cncr.22963]
14 **Chaturvedi AK**, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, Jiang B, Goodman MT, Sibug-Saber M, Cozen W, Liu L, Lynch CF, Wentzensen N, Jordan RC, Altekruse S, Anderson WF, Rosenberg PS, Gillison ML. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol* 2011; **29**: 4294-4301 [PMID: 21969503 DOI: 10.1200/JCO.2011.36.4596]
15 **Auluck A**, Hislop G, Bajdik C, Poh C, Zhang L, Rosin M. Trends in oropharyngeal and oral cavity cancer incidence of human papillomavirus (HPV)-related and HPV-unrelated sites in a multicultural population: the British Columbia experience. *Cancer* 2010; **116**: 2635-2644 [PMID: 20336792 DOI: 10.1002/cncr.25087]
16 **Hocking JS**, Stein A, Conway EL, Regan D, Grulich A, Law M, Brotherton JM. Head and neck cancer in Australia between 1982 and 2005 show increasing incidence of potentially HPV-associated oropharyngeal cancers. *Br J Cancer* 2011; **104**: 886-891 [PMID: 21285981 DOI: 10.1038/sj.bjc.6606091]
17 **Näsman A**, Attner P, Hammarstedt L, Du J, Eriksson M, Giraud G, Ahrlund-Richter S, Marklund L, Romanitan M, Lindquist D, Ramqvist T, Lindholm J, Sparén P, Ye W, Dahlstrand H, Munck-Wikland E, Dalianis T. Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? *Int J Cancer* 2009; **125**: 362-366 [PMID: 19330833 DOI: 10.1002/ijc.24339]
18 **Turner CF**, Danella RD, Rogers SM. Sexual behavior in the United States 1930-1990: trends and methodological problems. *Sex Transm Dis* 1995; **22**: 173-190 [PMID: 7652662]
19 **Bajos N**, Bozon M, Beltzer N, Laborde C, Andro A, Ferrand M, Goulet V, Laporte A, Le Van C, Leridon H, Levinson S, Razafindratsima N, Toulemon L, Warszawski J, Wellings K. Changes in sexual behaviours: from secular trends to public health policies. *AIDS* 2010; **24**: 1185-1191 [PMID: 20299962 DOI: 10.1097/QAD.0b013e328336ad52]
20 **Herbenick D**, Reece M, Schick V, Sanders SA, Dodge B, Fortenberry JD. Sexual behavior in the United States: results from a national probability sample of men and women ages 14-94. *J Sex Med* 2010; **7** Suppl 5: 255-265 [PMID: 21029383 DOI: 10.1111/j.1743-6109.2010.02012.x]
21 **Gillison ML**, Broutian T, Pickard RK, Tong ZY, Xiao W, Kahle L, Graubard BI, Chaturvedi AK. Prevalence of oral HPV infection in the United States, 2009-2010. *JAMA* 2012; **307**: 693-703 [PMID: 22282321 DOI: 10.1001/jama.2012.101]
22 **D'Souza G**, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral sexual behaviors associated with prevalent oral human papillomavirus infection. *J Infect Dis* 2009; **199**: 1263-1269 [PMID: 19320589 DOI: 10.1086/597755]
23 **Gillison ML**, Koch WM, Shah KV. Human papillomavirus in head and neck squamous cell carcinoma: are some head and neck cancers a sexually transmitted disease? *Curr Opin Oncol* 1999; **11**: 191-199 [PMID: 10328594]
24 **Mork J**, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P, Møller B, Pukkala E, Schiller JT, Youngman L, Lehtinen M, Dillner J. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2001; **344**: 1125-1131 [PMID: 11297703 DOI: 10.1056/NEJM200104123441503]
25 **D'Souza G**, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, Westra WH, Gillison ML. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007; **356**: 1944-1956 [PMID: 17494927 DOI: 10.1056/NEJMoa065497]
26 **Gillison ML**, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, Viscidi R. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst* 2008; **100**: 407-420 [PMID: 18334711 DOI: 10.1093/jnci/djn025]
27 **Schwartz SM**, Daling JR, Doody DR, Wipf GC, Carter JJ, Madeleine MM, Mao EJ, Fitzgibbons ED, Huang S, Beckmann AM, McDougall JK, Galloway DA. Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. *J Natl Cancer Inst* 1998; **90**: 1626-1636 [PMID: 9811312]
28 **Smith EM**, Ritchie JM, Summersgill KF, Klussmann JP, Lee JH, Wang D, Haugen TH, Turek LP. Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. *Int J Cancer* 2004; **108**: 766-772 [PMID: 14696105 DOI: 10.1002/ijc.11633]
29 **Goon PK**, Stanley MA, Ebmeyer J, Steinsträsser L, Upile T, Jerjes W, Bernal-Sprekelsen M, Görner M, Sudhoff HH. HPV & amp; head and neck cancer: a descriptive update. *Head Neck Oncol* 2009; **1**: 36 [PMID: 19828033 DOI: 10.1186/1758-3284-1-36]
30 **Portugal LG**, Goldenberg JD, Wenig BL, Ferrer KT, Nodzenski E, Sabnani JB, Javier C, Weichselbaum RR, Vokes EE. Human papillomavirus expression and p53 gene mutations in squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg* 1997; **123**: 1230-1234 [PMID: 9366703]
31 **Andl T**, Kahn T, Pfuhl A, Nicola T, Erber R, Conradt C, Klein W, Helbig M, Dietz A, Weidauer H, Bosch FX. Etiological involvement of oncogenic human papillomavirus in tonsillar squamous cell carcinomas lacking retinoblastoma cell cycle control. *Cancer Res* 1998; **58**: 5-13 [PMID: 9426048]
32 **Mellin H**, Friesland S, Lewensohn R, Dalianis T, Munck-Wikland E. Human papillomavirus (HPV) DNA in tonsillar cancer: clinical correlates, risk of relapse, and survival. *Int J Cancer* 2000; **89**: 300-304 [PMID: 10861508]

33 **Lindel K**, Beer KT, Laissue J, Greiner RH, Aebersold DM. Human papillomavirus positive squamous cell carcinoma of the oropharynx: a radiosensitive subgroup of head and neck carcinoma. *Cancer* 2001; **92**: 805-813 [PMID: 11550151]

34 **Ragin CC**, Taioli E. Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: review and meta-analysis. *Int J Cancer* 2007; **121**: 1813-1820 [PMID: 17546592 DOI: 10.1002/ijc.22851]

35 **Posner MR**, Lorch JH, Goloubeva O, Tan M, Schumaker LM, Sarlis NJ, Haddad RI, Cullen KJ. Survival and human papillomavirus in oropharynx cancer in TAX 324: a subset analysis from an international phase III trial. *Ann Oncol* 2011; **22**: 1071-1077 [PMID: 21317223 DOI: 10.1093/annonc/mdr006]

36 **Lassen P**, Eriksen JG, Hamilton-Dutoit S, Tramm T, Alsner J, Overgaard J. Effect of HPV-associated p16INK4A expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. *J Clin Oncol* 2009; **27**: 1992-1998 [PMID: 19289615 DOI: 10.1200/JCO.2008.20.2853]

37 **Worden FP**, Kumar B, Lee JS, Wolf GT, Cordell KG, Taylor JM, Urba SG, Eisbruch A, Teknos TN, Chepeha DB, Prince ME, Tsien CI, D'Silva NJ, Yang K, Kurnit DM, Mason HL, Miller TH, Wallace NE, Bradford CR, Carey TE. Chemoselection as a strategy for organ preservation in advanced oropharynx cancer: response and survival positively associated with HPV16 copy number. *J Clin Oncol* 2008; **26**: 3138-3146 [PMID: 18474879 DOI: 10.1200/JCO.2007.12.7597]

38 **Huang SH**, Perez-Ordonez B, Weinreb I, Hope A, Massey C, Waldron JN, Kim J, Bayley AJ, Cummings B, Cho BC, Ringash J, Dawson LA, Siu LL, Chen E, Irish J, Gullane P, Hui A, Liu FF, Shen X, Xu W, O'Sullivan B. Natural course of distant metastases following radiotherapy or chemoradiotherapy in HPV-related oropharyngeal cancer. *Oral Oncol* 2013; **49**: 79-85 [PMID: 22917550 DOI: 10.1016/j.oraloncology.2012.07.015]

39 **Huang SH**, Perez-Ordonez B, Liu FF, Waldron J, Ringash J, Irish J, Cummings B, Siu LL, Kim J, Weinreb I, Hope A, Gullane P, Brown D, Shi W, O'Sullivan B. Atypical clinical behavior of p16-confirmed HPV-related oropharyngeal squamous cell carcinoma treated with radical radiotherapy. *Int J Radiat Oncol Biol Phys* 2012; **82**: 276-283 [PMID: 20950953 DOI: 10.1016/j.ijrobp.2010.08.031]

40 **O'Sullivan B**, Huang SH, Perez-Ordonez B, Massey C, Siu LL, Weinreb I, Hope A, Kim J, Bayley AJ, Cummings B, Ringash J, Dawson LA, Cho BC, Chen E, Irish J, Gilbert RW, Hui A, Liu FF, Zhao H, Waldron JN, Xu W. Outcomes of HPV-related oropharyngeal cancer patients treated by radiotherapy alone using altered fractionation. *Radiother Oncol* 2012; **103**: 49-56 [PMID: 22410204 DOI: 10.1016/j.radonc.2012.02.009]

41 **Ruzevick J**, Olivi A, Westra WH. Metastatic squamous cell carcinoma to the brain: an unrecognized pattern of distant spread in patients with HPV-related head and neck cancer. *J Neurooncol* 2013; **112**: 449-454 [PMID: 23408186 DOI: 10.1007/s11060-013-1075-9]

42 **McBride SM**, Busse PM, Clark JR, Wirth LJ, Ancukiewicz M, Chan AW. Long-term survival after distant metastasis in patients with oropharyngeal cancer. *Oral Oncol* 2014; **50**: 208-212 [PMID: 24387975 DOI: 10.1016/j.oraloncology.2013.10.020]

43 **Vermorken JB**, Stöhlmacher-Williams J, Davidenko I, Licitra L, Winquist E, Villanueva C, Foa P, Rottey S, Skladowski K, Tahara M, Pai VR, Faivre S, Blajman CR, Forastiere AA, Stein BN, Oliner KS, Pan Z, Bach BA. Cisplatin and fluorouracil with or without panitumumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck (SPECTRUM): an open-label phase 3 randomised trial. *Lancet Oncol* 2013; **14**: 697-710 [PMID: 23746666 DOI: 10.1016/S1470-2045(13)70181-5]

44 **Rischin D**, Young RJ, Fisher R, Fox SB, Le QT, Peters LJ, Solomon B, Choi J, O'Sullivan B, Kenny LM, McArthur GA. Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. *J Clin Oncol* 2010; **28**: 4142-4148 [PMID: 20697079 DOI: 10.1200/JCO.2010.29.2904]

45 **Goldenberg D**, Begum S, Westra WH, Khan Z, Sciubba J, Pai SI, Califano JA, Tufano RP, Koch WM. Cystic lymph node metastasis in patients with head and neck cancer: An HPV-associated phenomenon. *Head Neck* 2008; **30**: 898-903 [PMID: 18383529 DOI: 10.1002/hed.20796]

46 **Huang SH**, O'Sullivan B, Xu W, Zhao H, Chen DD, Ringash J, Hope A, Razak A, Gilbert R, Irish J, Kim J, Dawson LA, Bayley A, Cho BC, Goldstein D, Gullane P, Yu E, Perez-Ordonez B, Weinreb I, Waldron J. Temporal nodal regression and regional control after primary radiation therapy for N2-N3 head-and-neck cancer stratified by HPV status. *Int J Radiat Oncol Biol Phys* 2013; **87**: 1078-1085 [PMID: 24210079 DOI: 10.1016/j.ijrobp.2013.08.049]

47 **Gillison ML**, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 2000; **92**: 709-720 [PMID: 10793107]

48 **Tabor MP**, Brakenhoff RH, van Houten VM, Kummer JA, Snel MH, Snijders PJ, Snow GB, Leemans CR, Braakhuis BJ. Persistence of genetically altered fields in head and neck cancer patients: biological and clinical implications. *Clin Cancer Res* 2001; **7**: 1523-1532 [PMID: 11410486]

49 **Brennan JA**, Mao L, Hruban RH, Boyle JO, Eby YJ, Koch WM, Goodman SN, Sidransky D. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. *N Engl J Med* 1995; **332**: 429-435 [PMID: 7619114 DOI: 10.1056/NEJM199502163320704]

50 **Califano J**, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, Corio R, Lee D, Greenberg B, Koch W, Sidransky D. Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res* 1996; **56**: 2488-2492 [PMID: 8653682]

51 **Klussmann JP**, Weissenborn SJ, Wieland U, Dries V, Kolligs J, Jungehuelsing M, Eckel HE, Dienes HP, Pfister HJ, Fuchs PG. Prevalence, distribution, and viral load of human papillomavirus 16 DNA in tonsillar carcinomas. *Cancer* 2001; **92**: 2875-2884 [PMID: 11753961 DOI: 10.1002/1097-0142(20011201)92: 11<2875: : AID-CNCR10130>3.0.CO; 2-7]

52 **Scheffner M**, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell* 1993; **75**: 495-505 [PMID: 8221889 DOI: 10.1016/0092-8674(93)90384-3]

53 **Kessis TD**, Slebos RJ, Nelson WG, Kastan MB, Plunkett BS, Han SM, Lorincz AT, Hedrick L, Cho KR. Human papillomavirus 16 E6 expression disrupts the p53-mediated cellular response to DNA damage. *Proc Natl Acad Sci USA* 1993; **90**: 3988-3992 [PMID: 8387205 DOI: 10.1073/pnas.90.9.3988]

54 **Scheffner M**, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 1990; **63**: 1129-1136 [PMID: 2175676 DOI: 10.1016/0092-8674(90)90409-8]

55 **Klingelhutz AJ**, Foster SA, McDougall JK. Telomerase activation by the E6 gene product of human papillomavirus type 16. *Nature* 1996; **380**: 79-82 [PMID: 8598912 DOI: 10.1038/380079a0]

56 **Dyson N**, Howley PM, Münger K, Harlow E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 1989; **243**: 934-937 [PMID: 2537532 DOI: 10.1126/science.2537532]

57 **Demers GW**, Foster SA, Halbert CL, Galloway DA. Growth arrest by induction of p53 in DNA damaged keratinocytes is bypassed by human papillomavirus 16 E7. *Proc Natl Acad*

Sci USA 1994; **91**: 4382-4386 [PMID: 8183918 DOI: 10.1073/pnas.91.10.4382]
58 **Slebos RJ**, Lee MH, Plunkett BS, Kessis TD, Williams BO, Jacks T, Hedrick L, Kastan MB, Cho KR. p53-dependent G1 arrest involves pRB-related proteins and is disrupted by the human papillomavirus 16 E7 oncoprotein. *Proc Natl Acad Sci USA* 1994; **91**: 5320-5324 [PMID: 8202487 DOI: 10.1073/pnas.91.12.5320]
59 **Kiyono T**, Foster SA, Koop JI, McDougall JK, Galloway DA, Klingelhutz AJ. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature* 1998; **396**: 84-88 [PMID: 9817205 DOI: 10.1038/23962]
60 **zur Hausen H**. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2002; **2**: 342-350 [PMID: 12044010 DOI: 10.1038/nrc798]
61 **Funk JO**, Waga S, Harry JB, Espling E, Stillman B, Galloway DA. Inhibition of CDK activity and PCNA-dependent DNA replication by p21 is blocked by interaction with the HPV-16 E7 oncoprotein. *Genes Dev* 1997; **11**: 2090-2100 [PMID: 9284048 DOI: 10.1101/gad.11.16.2090]
62 **Sidransky D**. Molecular genetics of head and neck cancer. *Curr Opin Oncol* 1995; **7**: 229-233 [PMID: 7654824 DOI: 10.1097/00001622-199505000-00007]
63 **Liggett WH**, Sidransky D. Role of the p16 tumor suppressor gene in cancer. *J Clin Oncol* 1998; **16**: 1197-1206 [PMID: 9508208]
64 **Boyle JO**, Hakim J, Koch W, van der Riet P, Hruban RH, Roa RA, Correo R, Eby YJ, Ruppert JM, Sidransky D. The incidence of p53 mutations increases with progression of head and neck cancer. *Cancer Res* 1993; **53**: 4477-4480 [PMID: 8402617]
65 **Reed AL**, Califano J, Cairns P, Westra WH, Jones RM, Koch W, Ahrendt S, Eby Y, Sewell D, Nawroz H, Bartek J, Sidransky D. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. *Cancer Res* 1996; **56**: 3630-3633 [PMID: 8705996]
66 **Poeta ML**, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, Ridge JA, Goodwin J, Kenady D, Saunders J, Westra W, Sidransky D, Koch WM. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med* 2007; **357**: 2552-2561 [PMID: 18094376 DOI: 10.1056/NEJMoa073770]
67 **Ciardiello F**, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res* 2001; **7**: 2958-2970 [PMID: 11595683]
68 **Mendelsohn J**. Targeting the epidermal growth factor receptor for cancer therapy. *J Clin Oncol* 2002; **20**: 1S-13S [PMID: 12235219]
69 **Temam S**, Kawaguchi H, El-Naggar AK, Jelinek J, Tang H, Liu DD, Lang W, Issa JP, Lee JJ, Mao L. Epidermal growth factor receptor copy number alterations correlate with poor clinical outcome in patients with head and neck squamous cancer. *J Clin Oncol* 2007; **25**: 2164-2170 [PMID: 17538160 DOI: 10.1200/JCO.2006.06.6605]
70 **Ang KK**, Berkey BA, Tu X, Zhang HZ, Katz R, Hammond EH, Fu KK, Milas L. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. *Cancer Res* 2002; **62**: 7350-7356 [PMID: 12499279]
71 **Hafkamp HC**, Speel EJ, Haesevoets A, Bot FJ, Dinjens WN, Ramaekers FC, Hopman AH, Manni JJ. A subset of head and neck squamous cell carcinomas exhibits integration of HPV 16/18 DNA and overexpression of p16INK4A and p53 in the absence of mutations in p53 exons 5-8. *Int J Cancer* 2003; **107**: 394-400 [PMID: 14506739 DOI: 10.1002/ijc.11389]
72 **Reimers N**, Kasper HU, Weissenborn SJ, Stützer H, Preuss SF, Hoffmann TK, Speel EJ, Dienes HP, Pfister HJ, Guntinas-Lichius O, Klussmann JP. Combined analysis of HPV-DNA, p16 and EGFR expression to predict prognosis in oropharyngeal cancer. *Int J Cancer* 2007; **120**: 1731-1738 [PMID: 17236202 DOI: 10.1002/ijc.22355]
73 **Kumar B**, Cordell KG, Lee JS, Worden FP, Prince ME, Tran HH, Wolf GT, Urba SG, Chepeha DB, Teknos TN, Eisbruch A, Tsien CI, Taylor JM, D'Silva NJ, Yang K, Kurnit DM, Bauer JA, Bradford CR, Carey TE. EGFR, p16, HPV Titer, Bcl-xL and p53, sex, and smoking as indicators of response to therapy and survival in oropharyngeal cancer. *J Clin Oncol* 2008; **26**: 3128-3137 [PMID: 18474878 DOI: 10.1200/JCO.2007.12.7662]
74 **Roepman P**, Wessels LF, Kettelarij N, Kemmeren P, Miles AJ, Lijnzaad P, Tilanus MG, Koole R, Hordijk GJ, van der Vliet PC, Reinders MJ, Slootweg PJ, Holstege FC. An expression profile for diagnosis of lymph node metastases from primary head and neck squamous cell carcinomas. *Nat Genet* 2005; **37**: 182-186 [PMID: 15640797 DOI: 10.1038/ng1502]
75 **Slebos RJ**, Yi Y, Ely K, Carter J, Evjen A, Zhang X, Shyr Y, Murphy BM, Cmelak AJ, Burkey BB, Netterville JL, Levy S, Yarbrough WG, Chung CH. Gene expression differences associated with human papillomavirus status in head and neck squamous cell carcinoma. *Clin Cancer Res* 2006; **12**: 701-709 [PMID: 16467079 DOI: 10.1158/1078-0432.CCR-05-2017]
76 **Agrawal N**, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, Fakhry C, Xie TX, Zhang J, Wang J, Zhang N, El-Naggar AK, Jasser SA, Weinstein JN, Treviño L, Drummond JA, Muzny DM, Wu Y, Wood LD, Hruban RH, Westra WH, Koch WM, Califano JA, Gibbs RA, Sidransky D, Vogelstein B, Velculescu VE, Papadopoulos N, Wheeler DA, Kinzler KW, Myers JN. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science* 2011; **333**: 1154-1157 [PMID: 21798897 DOI: 10.1126/science.1206923]
77 **Stransky N**, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, Kryukov GV, Lawrence MS, Sougnez C, McKenna A, Shefler E, Ramos AH, Stojanov P, Carter SL, Voet D, Cortés ML, Auclair D, Berger MF, Saksena G, Guiducci C, Onofrio RC, Parkin M, Romkes M, Weissfeld JL, Seethala RR, Wang L, Rangel-Escareño C, Fernandez-Lopez JC, Hidalgo-Miranda A, Melendez-Zajgla J, Winckler W, Ardlie K, Gabriel SB, Meyerson M, Lander ES, Getz G, Golub TR, Garraway LA, Grandis JR. The mutational landscape of head and neck squamous cell carcinoma. *Science* 2011; **333**: 1157-1160 [PMID: 21798893 DOI: 10.1126/science.1208130]
78 **Weinberger PM**, Yu Z, Haffty BG, Kowalski D, Harigopal M, Brandsma J, Sasaki C, Joe J, Camp RL, Rimm DL, Psyrri A. Molecular classification identifies a subset of human papillomavirus--associated oropharyngeal cancers with favorable prognosis. *J Clin Oncol* 2006; **24**: 736-747 [PMID: 16401683 DOI: 10.1200/JCO.2004.00.3335]
79 **Fakhry C**, Gillison ML. Clinical implications of human papillomavirus in head and neck cancers. *J Clin Oncol* 2006; **24**: 2606-2611 [PMID: 16763272 DOI: 10.1200/JCO.2006.06.1291]
80 **Huang H**, Li CY, Little JB. Abrogation of P53 function by transfection of HPV16 E6 gene does not enhance resistance of human tumour cells to ionizing radiation. *Int J Radiat Biol* 1996; **70**: 151-160 [PMID: 8794844]
81 **DeWeese TL**, Walsh JC, Dillehay LE, Kessis TD, Hedrick L, Cho KR, Nelson WG. Human papillomavirus E6 and E7 oncoproteins alter cell cycle progression but not radiosensitivity of carcinoma cells treated with low-dose-rate radiation. *Int J Radiat Oncol Biol Phys* 1997; **37**: 145-154 [PMID: 9054890]
82 **Licitra L**, Perrone F, Bossi P, Suardi S, Mariani L, Artusi R, Oggionni M, Rossini C, Cantù G, Squadrelli M, Quattrone P, Locati LD, Bergamini C, Olmi P, Pierotti MA, Pilotti S. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. *J Clin Oncol* 2006; **24**: 5630-5636 [PMID: 17179101 DOI: 10.1200/JCO.2005.04.6136]
83 **Apetoh L**, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Cri-

ollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P, Yang H, Amigorena S, Ryffel B, Barrat FJ, Saftig P, Levi F, Lidereau R, Nogues C, Mira JP, Chompret A, Joulin V, Clavel-Chapelon F, Bourhis J, André F, Delaloge S, Tursz T, Kroemer G, Zitvogel L. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med* 2007; **13**: 1050-1059 [PMID: 17704786 DOI: 10.1038/nm1622]

84 **Demaria S**, Formenti SC. Sensors of ionizing radiation effects on the immunological microenvironment of cancer. *Int J Radiat Biol* 2007; **83**: 819-825 [PMID: 17852561 DOI: 10.1080/09553000701481816]

85 **Wansom D**, Light E, Worden F, Prince M, Urba S, Chepeha DB, Cordell K, Eisbruch A, Taylor J, D'Silva N, Moyer J, Bradford CR, Kurnit D, Kumar B, Carey TE, Wolf GT. Correlation of cellular immunity with human papillomavirus 16 status and outcome in patients with advanced oropharyngeal cancer. *Arch Otolaryngol Head Neck Surg* 2010; **136**: 1267-1273 [PMID: 21173378 DOI: 10.1001/archoto.2010.211]

86 **Spanos WC**, Nowicki P, Lee DW, Hoover A, Hostager B, Gupta A, Anderson ME, Lee JH. Immune response during therapy with cisplatin or radiation for human papillomavirus-related head and neck cancer. *Arch Otolaryngol Head Neck Surg* 2009; **135**: 1137-1146 [PMID: 19917928 DOI: 10.1001/archoto.2009.159]

87 **Marur SC.** E 1308: A phase ii trial of induction chemotherapy (ic) followed by cetuximab with low dose versus standard dose imrt in patients with human papilloma virus (hpv)-associated resectable squamous cell carcinoma of the oropharynx (opscc). *J Clin Oncol* 2013; **31**: 364s

88 **Kimple RJ**, Harari PM. Is radiation dose reduction the right answer for HPV-positive head and neck cancer? *Oral Oncol* 2014; **50**: 560-564 [PMID: 24134946 DOI: 10.1016/j.oraloncology.2013.09.015]

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