



## Evidence-Based Series 5-9 Version 2

### A Quality Initiative of the Program in Evidence-Based Care (PEBC), Cancer Care Ontario (CCO)

## Routine HPV Testing in Head and Neck Squamous Cell Carcinoma

*The Expert Panel on HPV Testing in Head and Neck Squamous Cell Carcinoma*

January 13, 2020

An assessment conducted in November 2025 deferred review of Evidence-Based Series 5-9 Version 2. This means that the document remains current until it is assessed again next year. The PEBC has a formal and standardized process to ensure the currency of each document ([PEBC Assessment & Review Protocol](#))

EBS 5-9v2 is comprised of 4 sections. You can access the summary and full report here:

<https://www.cancercareontario.ca/en/guidelines-advice/types-of-cancer/581>

Section 1:	Guideline Recommendations (ENDORSED)
Section 2:	Evidentiary Base
Section 3:	EBS Development Methods and External Review Process
Section 4:	Document Assessment and Review

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## Evidence-Based Series 5-9

**A Quality Initiative of the  
Program in Evidence-Based Care (PEBC), Cancer Care Ontario (CCO)**

**Routine HPV Testing in Head and Neck Squamous Cell Carcinoma**

## Table of Contents

Section 1: Guideline Recommendations .....	3
Section 2: Evidentiary Base .....	10
Section 3: EBS Development Methods and External Review Process .....	47
Section 4: Document Assessment and Review .....	59

## Guideline Report History

GUIDELINE VERSION	SYSTEMATIC REVIEW		PUBLICATIONS	NOTES and KEY CHANGES
	Search Dates	Data		
Original May 13, 2013	1996 to Apr 2013	Full Report	Web publication	N.A.
Version 2 January 13, 2020	2013 to Feb 2019	New data found in <a href="#">Section 4:</a> Document Assessment and Review	Updated web publication	2013 recommendations are ENDORSED

## Evidence-Based Series 5-9: Section 1

### A Quality Initiative of the Program in Evidence-Based Care (PEBC), Cancer Care Ontario (CCO)

## Routine HPV Testing in Head and Neck Squamous Cell Carcinoma: Guideline Recommendations

The 2013 guideline recommendations have been ENDORSED, which means that the recommendations are still current and relevant for decision making. Please see Section 4: Document Assessment and Review for a summary of updated evidence published between 2013 and 2019, and for details on how this guideline was ENDORSED.

#### GUIDELINE OBJECTIVES

To evaluate the appropriateness of, and make recommendations on, routine testing for human papillomavirus (HPV) status in adult patients with primary, or neck nodal metastatic, squamous cell carcinoma (SCC) of the head and neck.

#### TARGET POPULATION

Adult patients with squamous cell carcinomas arising in oropharynx, larynx, hypopharynx, nasopharynx, sinonasal tract, or oral cavity subsites or an unknown primary head and neck site.

#### INTENDED USERS

This guideline is targeted for:

1. Clinicians involved in the delivery of care of adult patients with head and neck squamous cell carcinoma (HNSCC).
2. Pathologists involved in the evaluation of HNSCCs.

#### RECOMMENDATIONS, KEY EVIDENCE, AND JUSTIFICATION

##### RECOMMENDATION 1

The tumours of all adult patients presenting with oropharyngeal squamous cell carcinomas should be routinely tested for HPV status.

##### *Summary of Key Evidence for Recommendation 1*

- A meta-analysis showed a definite survival benefit for HPV-positive patients compared to those whose tumour was HPV negative in terms of overall survival (OS) (HR: 0.43 (95%CI: 0.32-0.58), progression-free survival (PFS) (HR: 0.40, 95%CI: 0.28-0.56), and disease-specific survival (DSS) (HR: 0.45 (95%CI: 0.27-0.76)).
- A published data meta-analysis by Ragin and Taioli (1) demonstrated that patients with HPV-positive oropharyngeal tumours had a 28% reduced risk of death compared to patients with HPV-negative oropharyngeal tumours (HR: 0.72, 95%CI: 0.5-1.0). Similar

results were calculated for disease-specific survival (DSS) (HR: 0.51, 95%CI: 0.4-0.7). However, no benefit in overall survival (OS) or DSS was seen in HPV-positive versus negative patients with non-oro-pharyngeal tumours.

#### *Justification for Recommendation 1*

There is evidence from a meta-analysis of randomized trials that HPV-positivity is a strong predictor of prognosis in patients with oropharyngeal squamous cell carcinoma. In addition, it is likely that HPV status will influence management decisions in the near future and is now regarded as a mandatory stratification factor for clinical trials. Therefore, even though at this time no recommendation can be made to base clinical management decisions on HPV status, the valuable prognostic benefits of HPV testing are sufficient to warrant routine testing.

#### *Qualifying Statements for Recommendation 1*

- The above recommendation only applies to patients with squamous cell carcinoma of the oropharynx, which includes tonsil, base of tongue, soft palate and associated pharyngeal walls. The data and recommendation do not apply to patients with non-oro-pharyngeal cancers.
- Altering management decisions based on results from HPV testing is not recommended beyond the context of a clinical trial at this time.

### **RECOMMENDATION 2**

It is recommended that the neck nodal tissue of patients with metastatic squamous cell carcinoma to neck nodes from an unknown head and neck primary be routinely tested for HPV status.

#### *Summary of Key Evidence for Recommendation 2*

- Twelve studies (2-13) found the prevalence of HPV-positive lymph node metastases ranged from 0%-19% in patients with non-oro-pharyngeal primary sites compared to 66%-87% in those whose primary tumour originated in the oropharynx.

#### *Justification for Recommendation 2*

The evidence indicates that there is relationship between HPV positivity and whether the initial cancer arises in the oropharynx or not. As detection of the primary tumour offers a reduction in morbidity due to the benefits of localized treatment, the additional diagnostic information provided by HPV status is sufficient to warrant routine testing of these tissues.

#### *Qualifying Statements for Recommendation 2*

Currently, there are no standardized protocols or extensive published experience regarding the performance of p16 immunohistochemical (IHC) or HPV in situ hybridization (ISH) in fine-needle aspiration (FNA) or cytology material from metastatic squamous cell carcinoma to cervical lymph nodes.

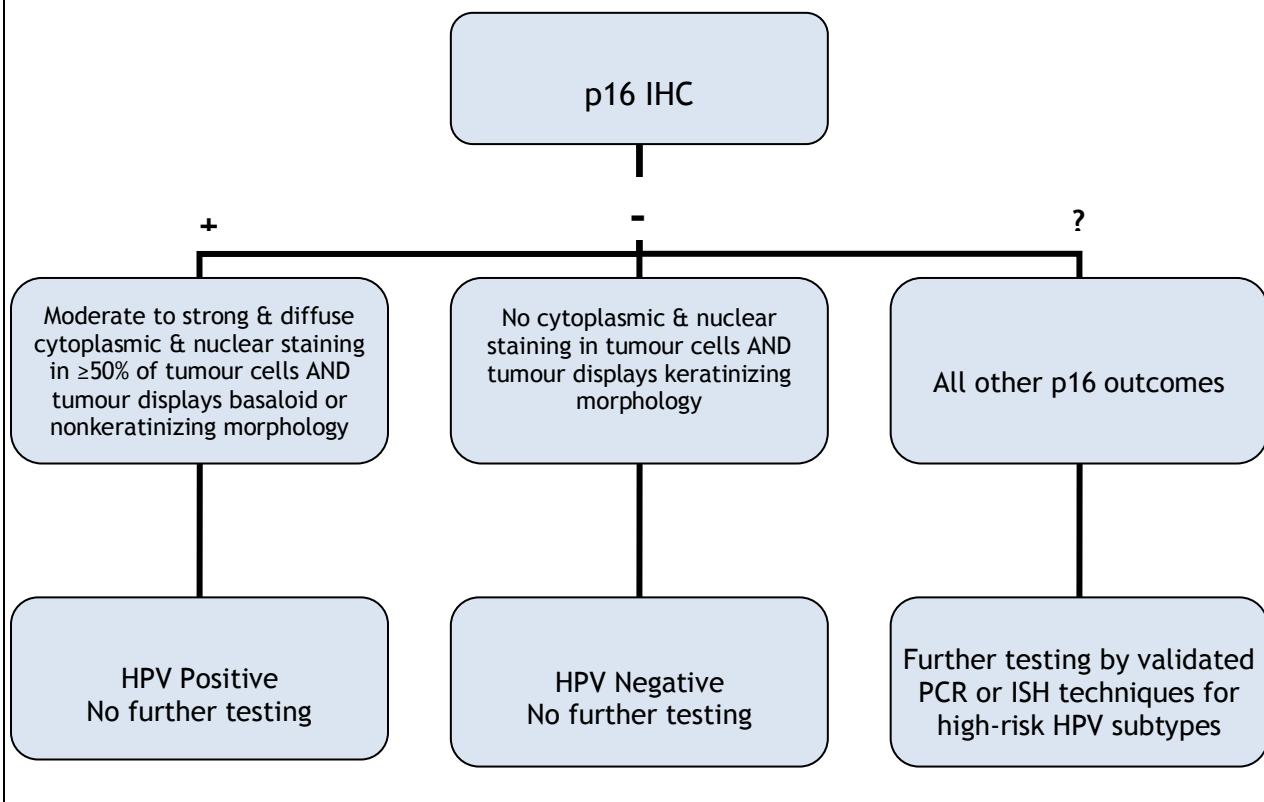
**RECOMMENDATION 3**

It is recommended that HPV status in oropharyngeal SCC be initially determined using immunohistochemical (IHC) staining for p16.

IHC staining for p16 can be considered positive when the following three criteria are met:

- cytoplasmic and nuclear staining
- staining is moderate to strong and diffuse
- staining is present in at least 70%\* of tumour cells (\*See [Section 4](#) for explanation)

A validated polymerase chain reaction (PCR) or in situ hybridization (ISH) technique for high-risk HPV subtypes may be necessary to confirm p16 results in selected cases according to the following algorithm:

**Summary of Key Evidence for Recommendation 3**

- The above recommendations are based on a comparison of HPV diagnostic testing methods published in the literature. Thirteen retrospective cohort studies (14-26) were included in this guideline. The evidence suggests that, in patients with OPSCC, the performance of the three main techniques - PCR-based amplification, DNA ISH, and p16 IHC - is comparable.
  - PCR amplification of HPV DNA showed a sensitivity of 97% and specificity of 87%
  - DNA ISH showed a sensitivity that ranged from 83% to 93% and a specificity that ranged from 88% to 100%
  - IHC staining for p16 showed a sensitivity and specificity that ranged from 89% to 100% and 38% to 94%, respectively

*Technical Considerations for Recommendation 3*

While it is not possible to make evidence-based recommendations regarding the minimum set of criteria requiring adherence in a pathology laboratory with respect to HPV testing at this time, the following guidance is offered based on expert opinion and a consensus process by members of the Head and Neck DSG:

- Analysis should be performed on sections from paraffin blocks or unstained slides cut at 4 microns
- In cases of metastatic disease, where a core biopsy may not be a possibility, all efforts should be made to obtain enough tissue with FNA to prepare cell blocks.

*Justification for Recommendation 3*

The current evidence suggests that PCR, DNA ISH, and IHC staining are all comparable. With no unequivocal evidence exclusively supporting any particular scheme, the Head & Neck Disease Site Group believes this scheme is practical and simple, and it minimizes the impact of testing on available pathology resources and is appropriate until such time as further evidence becomes available. The Head & Neck DSG acknowledges that the algorithm may be considered controversial by some, but it is believed to address the proficiencies that are most readily available in laboratories across the province.

*Qualifying Statements for Recommendation 3*

- The Head & Neck DSG considers quality assurance and quality control in HPV-status testing to be paramount. As such, all testing should be carried out in licensed and accredited laboratories, and test results should be interpreted by experienced pathologists/scientists. Laboratories need to follow proper quality control and participate in external proficiency testing to ensure test accuracy. Further discussion of specific quality and proficiency parameters necessary for individual laboratories performing HPV-status testing is beyond the scope of this guideline.
- Qualitative HPV PCR assay detection alone should be avoided
- The above recommendations do not apply to samples from dental procedures.

**FUTURE RESEARCH**

Insufficient data currently exist to assess the prognostic benefit of HPV positivity in SCC of the larynx, hypopharynx, nasopharynx, sinonasal tract and oral cavity. There is evidence in the literature to suggest that the prevalence of HPV in these subsites may be higher than originally believed. Meta-analyses (1,27,28) report a pooled prevalence in the oral cavity and the larynx as high as 40% and 24%, respectively. Lip and oral cavity, pharynx, larynx, nasopharynx and lymph nodes combined have a reported pooled HPV prevalence of 32%. Such values warrant further prospective local data collection via the creation of a provincial patient registry to establish the prevalence of HPV-associated SCC and to clarify the prognosis associated with HPV positivity in these patients. This will ensure the acquisition and availability of evidence upon which future clinical decisions can be based.

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*Updating*

All PEBC documents are maintained and updated as described in the PEBC Document Assessment and Review Protocol.

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## **Evidence-Based Series 5-9: Section 2**

### **A Quality Initiative of the Program in Evidence-Based Care (PEBC), Cancer Care Ontario (CCO)**

#### **Routine HPV Testing in Head and Neck Squamous Cell Carcinoma: Evidentiary Base**

The 2013 guideline recommendations have been ENDORSED, which means that the recommendations are still current and relevant for decision making. Please see Section 4: Document Assessment and Review for a summary of updated evidence published between 2013 and 2019, and for details on how this guideline was ENDORSED.

#### **INTRODUCTION**

The estimated incidence of head and neck squamous cell carcinoma (HNSCC) in Canada in 2009 was 4550 cases with 1660 deaths (1,2). Despite a decline in known risk factors for the disease, namely smoking and alcohol consumption, some head and neck carcinomas are on the rise (3,4). Today, many newly diagnosed patients tend to be young (40-55 years old), male, and white, and with little or no history of tobacco or alcohol use (5,6). Despite efforts in screening and early diagnosis, the 5-year disease-free survival for patients with HNSCC still remains poor (7).

The identification of human papillomavirus (HPV) as the etiological agent of cervical cancer has led to its recognition in other types of cancers (8). Involvement of HPV in oral and oropharyngeal carcinogenesis was first proposed by Syrjanen et al. (9) in 1983 and has been supported over recent years by both epidemiological and experimental evidence (7,10-12). The majority of HPV-related cancers contain high-risk HPV DNA integrated into the host cell genome. The viral oncoproteins E6 and E7 become expressed early in the infection and can inactivate the tumour suppressors p53 and retinoblastoma (Rb) (5,13,14). pRb inactivation results in a reciprocal overexpression of the cyclin-dependent kinase inhibitor, p16, which inhibits normal cell-cycle progression (15-17). As such, p16 has been used as a replacement assay for HPV positivity and an independent HNSCC tumour marker (15,18).

The overall prevalence of HPV-associated HNSCC reported in the literature varies greatly. This variability is due to differences in tumour site, tumour types, specimens and the method used for analysis (19). Currently, no universal method for HPV detection exists. Although generally regarded as the gold standard, polymerase-chain reaction (PCR)-based detection of HPV E6 oncogene expression may not be the most appropriate detection method for use in the clinical setting. The decision may ultimately come down to not only the test's diagnostic properties, namely sensitivity and specificity, but also to technical challenges, feasibility, reproducibility and cost (5).

The objective of this evidence series is to review the existing literature on the relationship between HPV positivity and survival, to establish the prevalence of HPV-associated SCC and outline when the prevalence is high enough to warrant routine testing, to examine the

value of HPV testing in cancers of unknown primaries, and to determine the optimal HPV detection method for clinical use.

In order to make recommendations as part of a clinical practice, the working group and the Head and Neck Cancer DSG developed this evidentiary base upon which those recommendations are based. Based on the objectives of the guideline, the working group derived the research questions outlined below.

## RESEARCH QUESTIONS

1. What is the relationship between HPV positivity and outcome in head and neck squamous cell carcinomas (HNSCC)?
2. In which head and neck subsites is the prevalence of HPV-associated squamous cell carcinoma high enough to justify routine testing of HPV positivity?
3. What is the diagnostic and prognostic value of routine testing of HPV status in patients with neck nodal metastatic squamous cell carcinoma from an unknown head and neck primary?
4. What is the optimal testing method for the identification of HPV positivity in head and neck squamous cell carcinomas (HNSCC)?

## METHODS

This evidentiary base was developed using a planned two-stage method, summarized here and described in more detail below.

- 1) Search and evaluation of existing systematic reviews: If one or more existing systematic reviews are identified that address the research questions and are of reasonable quality, then those systematic reviews would form the core of the evidentiary base.
- 2) Systematic review of the primary literature: This review would focus on those areas not covered by existing reviews if any are located and accepted.

The PEBC is supported by the Ontario Ministry of Health and Long-Term Care. All work produced by the PEBC is editorially independent from the Ministry.

### *Literature Search*

The literature was searched using MEDLINE (OVID: 1996 through March Week 4, 2013), Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations (April 09, 2013), EMBASE (OVID: 1996 through 2013, Week 14), and the Cochrane Library (OVID: 1st Quarter 2013). In addition, the proceedings of the meetings of the American Society of Clinical Oncology (ASCO), the American Society of Therapeutic Radiology and Oncology (ASTRO), and the European Society for Radiotherapy and Oncology (ESTRO) were all searched for relevant abstracts from 2007 to 2010. Reference lists of studies deemed eligible for inclusion were scanned for additional citations.

The literature search of the electronic databases combined disease-specific terms (squamous cell carcinoma, cancer, malignancy, neoplasm, tumour) along with site-specific terms (oropharynx, larynx, hypopharynx, oral cavity) and testing-specific terms (HPV, p16, immunohistochemistry, polymerase chain reaction, in situ hybridization) for all study designs (Appendix 1). After this initial literature search was completed, the Working Group recognized the need to include an additional question on HPV and cancers of unknown primaries (CUPs). That systematic search was conducted in June 2012 and updated in April 2013 in MEDLINE and EMBASE for all study designs (Appendix 2).

*A priori* decision rules were established that specified only comprehensive systematic reviews with relevance to at least one of the three original questions posed would receive formal quality assessments. Identified systematic reviews that required further consideration based on the criteria above were assessed using the AMSTAR tool (20). The results of the

AMSTAR assessment were used to determine whether or not an existing review could be incorporated as part of the evidentiary base. Any identified reviews that did not meet the criteria above, whose AMSTAR assessment indicated important deficiencies in quality, or that were otherwise not incorporated as part of the evidence base would be reported in the reference list, but not further described or discussed.

Further to the searches of the electronic databases, an internet search of Canadian and international health organizations and the National Guidelines Clearinghouse was conducted for existing guidelines and systematic reviews relevant to our research questions. Guidelines were included if they were published since 2008 in English. This environmental scan yielded one practice guideline (21). The working group decided that proceeding with a new systematic review that includes the latest research was warranted given the lack of reporting of the literature included in this practice guideline.

### ***Study Selection Criteria and Protocol***

#### **Inclusion Criteria**

Articles were eligible for inclusion in this systematic review of the evidence if they met the following criteria:

#### ***HPV Positivity***

- Full reports or abstracts of phase III randomized controlled trials that evaluated tumour HPV status and clinical outcome.
- Studies that included adult patients with squamous cell carcinomas arising in the oropharynx, larynx, hypopharynx, nasopharynx, sinonasal tract, or oral cavity.
- Results were reported for one or more of the following outcomes: overall survival, disease-free survival, disease-specific survival or progression-free survival.

#### ***Prevalence***

- Studies that included a minimum of 50 cases of HNSCC.
- Testing that included a clearly described detection method of interest.
- Prevalence of HPV-associated tumours for any of the following subsites is reported: oropharynx, larynx, hypopharynx, nasopharynx, sinonasal tract or oral cavity.

#### ***Unknown Primaries***

- Studies that included a minimum of 20 cases of nodal metastatic squamous cell carcinoma from an unknown head and neck primary.
- Testing that included a clearly described detection method of interest.
- Results were reported for one or more of the following outcomes: prevalence of HPV-associated metastatic squamous cell carcinoma, correlation between HPV positivity and later detection of the primary tumour, or the sensitivity and specificity of a test for a diagnosis of an oropharyngeal tumour.

#### ***Testing***

- Comparative studies that evaluated the following HPV detection methods: p16 immunohistochemistry (IHC), polymerase chain reaction (PCR), or in situ hybridization (ISH).
- Concordance between detection methods or sensitivity and specificity of the detection method are reported or enough information is provided to allow for the calculation of these outcomes, using PCR for high-risk HPV as the gold standard comparator.

#### **Exclusion Criteria**

Articles published in languages other than English were excluded because of limited translation resources.

A review of the titles and abstracts that resulted from the search was done by one reviewer (CL). For those items that warranted full-text review, one reviewer (CL) reviewed each item with collaboration from a second reviewer (JW or BPO) if uncertainty existed.

### ***Data Extraction and Assessment of Study Quality and Potential for Bias***

All eligible studies underwent data extraction independently by a research methodologist (CL), with all extracted data and information subsequently audited by an independent auditor. The following data were among the items recorded for each study: (a) author and year of publication, (b) patient population, HPV status and sample size, (c) tumour site and (d) outcomes of interest. Ratios, including hazard ratios (HR), were expressed such that a ratio <1.0 indicates a survival benefit favouring HPV-positive patients; conversely, a survival benefit that favours HPV-negative patients is expressed by a HR >1.0.

An assessment of study quality was performed for all the included evidence by one methodologist (CL). Systematic reviews and meta-analyses were assessed for quality using the AMSTAR tool (20). For studies that re-analyzed results of completed randomized clinical trials (RCTs), no specific instrument was used, but items such as pre-specified versus post hoc analyses, differences in baseline characteristics between patients whose HPV status was assessed and those in which it was not, and power calculations for subgroups analyses were reported on. Methodological criteria assessed for other study designs were informed by the Newcastle-Ottawa Quality Assessment Scale (22) and included study design, type of data collection, sampling method, and blinding in outcome assessment. Blinding of the quality assessor to the author, institution or journal was not considered necessary.

### ***Synthesizing the Evidence***

When clinically homogenous results from two or more trials were available, the data was pooled using the Review Manager software (RevMan 5.1) provided by the Cochrane Collaboration (23). Since hazard ratios (HR), rather than the number of events at a certain time point, are the preferred statistic for pooling time-to-event outcomes (24), those were extracted directly from the most recently reported trial results. The variances of the hazard ratio estimates were calculated from the reported confidence intervals (CI) using the methods described by Parmar et al (24). A random effects model was used for all pooling.

Statistical heterogeneity was calculated using the  $X^2$  test for heterogeneity and the  $I^2$  percentage. A probability level for the  $X^2$  statistic less than or equal to 10% ( $p \leq 0.10$ ) and/or an  $I^2$  greater than 50% were considered indicative of statistical heterogeneity. Results are expressed as hazard ratios with 95%CI.

## **RESULTS**

### ***Literature Search Results***

A total of 553 unique citations were identified from the MEDLINE, EMBASE, and Cochrane Library databases for the first search of the literature. From those citations, 213 were pulled for full-text review (see Appendix 3 for flow diagram of search results). From the second literature search on HPV and CUPs, a total of 142 citations were found, of which 16 underwent full-text review.

- In the relationship between HPV positivity and outcome, six unique randomized controlled trials examining the association between tumour HPV status and survival were identified and included.

- In outlining the prevalence of HPV-associated squamous cell carcinoma (SCC), the literature search yielded a large number of fully published reports. Due to the volume of studies and the availability of five systematic reviews, four with meta-analyses, the Head and Neck Cancer DSG agreed to limit the reporting to only the findings of these previously published systematic reviews.
- In examining the value of testing for HPV in CUPs, 12 comparative studies were included.
- In determining the optimal testing method for HPV positivity, 13 comparative studies were identified and included.

**Table 1. Studies eligible for inclusion in this report.**

Question	Number of studies	Reference numbers	Summary of results
Q1. HPV Positivity	6 RCTs	31-36	Table 3 & Figures 2-4
Q2. Prevalence	6 systematic reviews	25-30	Table 4
Q3. Unknown Primaries	12 comparative studies	37-42,47,49-53	Table 5
Q4. Testing	13 comparative studies	18,43-46,48,54-60	Table 6

### Study Methodological Quality

Six systematic reviews (25-30), five with meta-analyses, were assessed for methodological quality using the AMSTAR tool (20). One review (30) was assessed to be of high quality and four others (25-27,29) received an overall quality rating of moderate, each showing some deficiencies in the literature search and assessment of included studies. The final meta-analysis (28) was rated as poor with several methodological shortcomings apparent, including literature search deficits, lack of quality and publication bias assessments, and no statement regarding conflict of interest. As such, it will not be discussed further.

The quality assessment of subgroup analyses from RCTs is summarized in Table 2. Only one trial (31) had a pre-specified subgroup analysis, with the remaining five trials having no such analyses planned in their study protocols. Two studies (32,33) reported that no significant differences were observed in baseline characteristics between patients who underwent testing for HPV status and those who did not. Conversely, two studies (34,35) did report that differences were seen, with tested patients more likely to have operable tumours, better performance status, lower T categories, and less likely to be current smokers. The remaining two trials (31,36) made no mention of baseline differences. No trial adequately reported on separate power calculations being made for the subgroup analysis.

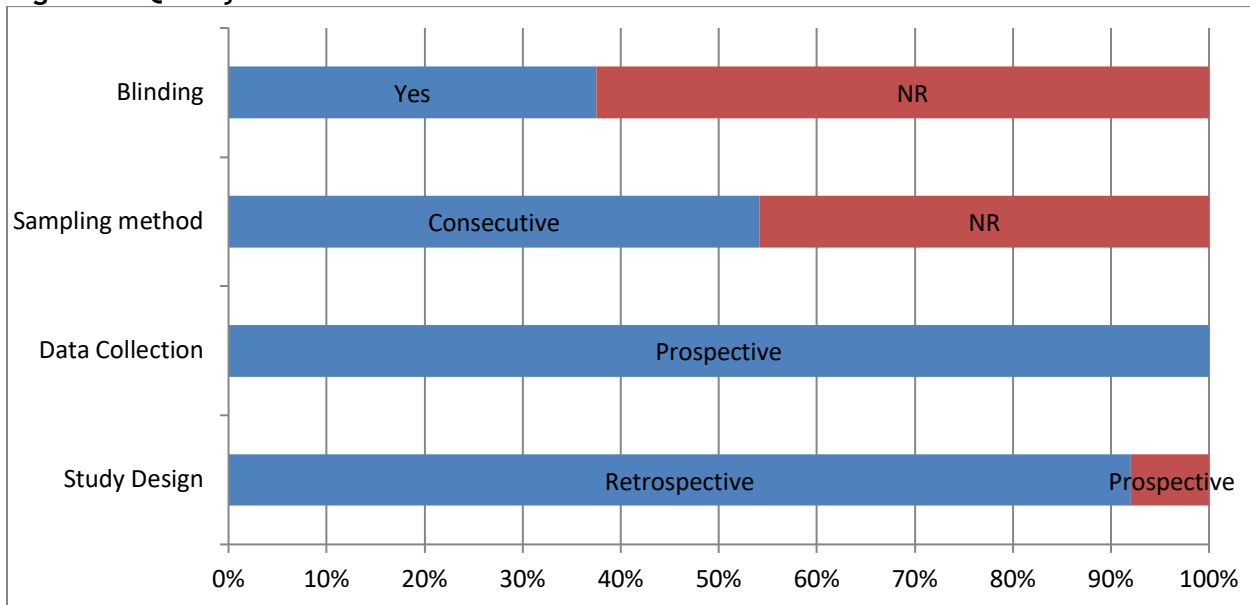
**Table 2. Summary of methodological quality of subgroup analyses from RCTs.**

	Pre-specified or post hoc analysis	Differences in baseline characteristics between patients with know HPV status and those with no HPV status testing	Power calculations for subgroup
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TAX 324 (Posner 2011) (35)	Post hoc	Yes, no-HPV status patients more likely to have unresectable and low-curability tumours	No
DAHANCA 6&7 (Lassen 2011) (33)	Post hoc	No	NR
RTOG 0129 (Ang 2010) (32)	Post hoc	No	No
TROG 02.02 (Rischin 2010) (34)	Post hoc	Yes, know HPV status patients had better PS, lower T category, higher haemoglobin, and were less likely to be current smokers	NR
DAHANCA 5 (Lassen 2009) (36)	Post hoc	NR	No
ECOG 2399 (Fakhry 2008) (31)	Pre-specified	NR	Unclear

Figure 1 provides a summary of methodological quality of other comparative studies included in this review. While the vast majority of included studies were retrospective cohorts, and the inherent limitations of retrospective designs should be taken into consideration, the collection of data did occur prospectively in all studies. The study population in just over half the included papers (18,37-48) was comprised of patients selected in a consecutive fashion. The remaining papers did not report the sampling method (49-60). Outcome assessors were reported to be blinded to HPV status in 38% of studies (37-39,44,45, 47,49,56,59), with the remaining 62% of studies not describing any such blinding.

**Figure 1: Quality characteristics of included studies.**



## Outcomes

### *Question #1: What is the relationship between HPV positivity and outcome?*

Six trials considered various treatment regimens for patients with predominately locally advanced squamous cell carcinoma (SCC) of the head and neck. Subgroup analyses were conducted to examine the association between tumour HPV status and survival. All six studies

found a statistically significant improved outcome for patients whose tumour was HPV positive over those whose tumour was HPV negative. Results are summarized in Table 3.

The Radiation Therapy Oncology Group (RTOG) 0129 trial (32) compared accelerated-fractionation radiotherapy to standard-fractionation radiotherapy when both regimens were combined with concurrent cisplatin therapy. Restricting the post hoc subgroup analysis to patients with oropharyngeal SCC, Ang et al. found patients with HPV-positive cancer had a 58% reduction in the risk of death as compared to patients with HPV-negative tumours (HR:0.42, 95%CI: 0.27-0.66) (32). Three-year rates for overall survival were 82.4% (95%CI: 77.2-87.6) in the HPV-positive patients and 57.1% (95%CI: 48.1-66.1) in the HPV-negative patients. Similarly, 3-year progression-free survival rates were 73.7% (95%CI: 67.7-79.8) in the HPV-positive subgroup and 43.4% (95%CI: 34.4-52.4) in the HPV-negative subgroup.

The TAX 324 international trial (35) investigated sequential therapy (ST) with docetaxel, cisplatin, and 5-fluorouracil (TPF) versus ST with cisplatin and 5-fluorouracil in patients with locally advanced HNSCC. In a post hoc subgroup analysis of the oropharynx cancer patients, HPV16 status and survival was evaluated, with both overall survival and progression-free survival showing significant superiority in the HPV-positive patients (OS, HR:0.20, 95%CI: 0.10-0.38,  $p<0.0001$ ). At 5 years, the overall survival rate in HPV-positive patients was 82% (95%CI: 69-90) versus 35% (95%CI: 23-48) in those HPV-negative ( $p<0.0001$ ). Progression-free survival was 78% (95%CI: 64-87) versus 28% (95%CI: 17-40) ( $p<0.0001$ ), respectively.

Retrospective analyses of survival and HPV status were also conducted in the TROG 02.02 phase III trial of concurrent radiotherapy and cisplatin with or without tirapazamine (34). At 2 years, survival rates of 91% and 74% were reported for the p16-positive group and p16-negative group, respectively (HR: 0.36, 95%CI: 0.17-0.74,  $p=0.004$ ). Failure-free survival was also better at 2 years (87% versus 72%,  $p=0.003$ ) for p16-positive versus negative patients.

The Eastern Cooperative Oncology Group (ECOG) 2399 phase II trial of chemoradiation in patients with locally advanced oropharyngeal or laryngeal SCC prospectively evaluated HPV status on survival (31). Overall survival for patients with HPV-positive tumours was significantly improved compared to that of patients with HPV-negative tumours ( $p=0.005$ ). Two-year overall survival rates were 97% (95%CI: 87-100) versus 62% (95%CI: 49-74) for HPV-positive patients versus those who were HPV negative.

The Danish Head and Neck Cancer Group's DAHANCA 5 trial investigated nimorazole as a hypoxic radiosensitizer of primary radiotherapy in supraglottic larynx and pharynx carcinoma (36). An analysis of the patients enrolled in the placebo arm only found HPV-positive patients had a significantly better prognosis compared with patients with virus-negative tumours. Both overall ( $p=0.0003$ ) and disease-specific survival ( $p=0.0006$ ) were significantly improved for patients with p16-positive tumours compared to those whose tumour was p16 negative. In multivariate analyses, p16 remained a strong independent prognostic factor for both overall death (HR: 0.44, 95%CI: 0.28-0.68) and disease-specific death (HR: 0.36, 95%CI: 0.20-0.64).

Another trial by the Danish Head and Neck Cancer Group, DAHANCA 6&7, compared the use of five fractions per week to six weekly radiotherapy fractions, thereby shortening overall treatment time, but preserving the same total dose and fraction number (33). When HPV-associated p16 expression was used as a retrospective stratification parameter, both overall survival (62% vs 47%,  $p<0.0001$ ) and 5-year disease-specific survival (78% vs 64%,  $p=0.001$ ) were improved for those p16 positive compared to those who were p16 negative. This translated into a 38% reduction in the overall risk of death for p16-positive patients compared to p16-negative patients (HR: 0.62, 95%CI: 0.49-0.78).

### *Meta-Analysis*

To estimate the overall effect of HPV status on prognosis, a meta-analysis was conducted on overall, progression-free, and disease-specific survival. All six studies provided



sufficient information to derive a log-hazard ratio and its standard error for overall survival. Three studies provided sufficient information for progression-free survival, while the log-hazard ratio and its standard error were only obtainable from two studies that considered disease-specific survival. The results of these analyses are shown in Figures 2, 3 and 4. A definite survival benefit for HPV-positive patients is seen for all three outcome measures (overall survival HR: 0.43 (95%CI: 0.32-0.58), progression-free survival (HR: 0.40, 95%CI: 0.28-0.56) and disease-specific survival HR: 0.45 (95%CI: 0.27-0.76).

While there was no statistical heterogeneity for the meta-analysis of PFS, considerable statistical heterogeneity was introduced by the Lassen et al. trial (33) for the analysis of OS ( $I^2=52\%$ ) and DSS ( $I^2=74\%$ ). When this trial was excluded from the OS analysis (forest plot not shown), the difference in OS in favour of HPV-positive patients remained statistically significant (HR, 0.38; 95%CI: 0.30-0.50;  $p<0.00001$ ), but with no statistical heterogeneity ( $I^2=0\%$ ).

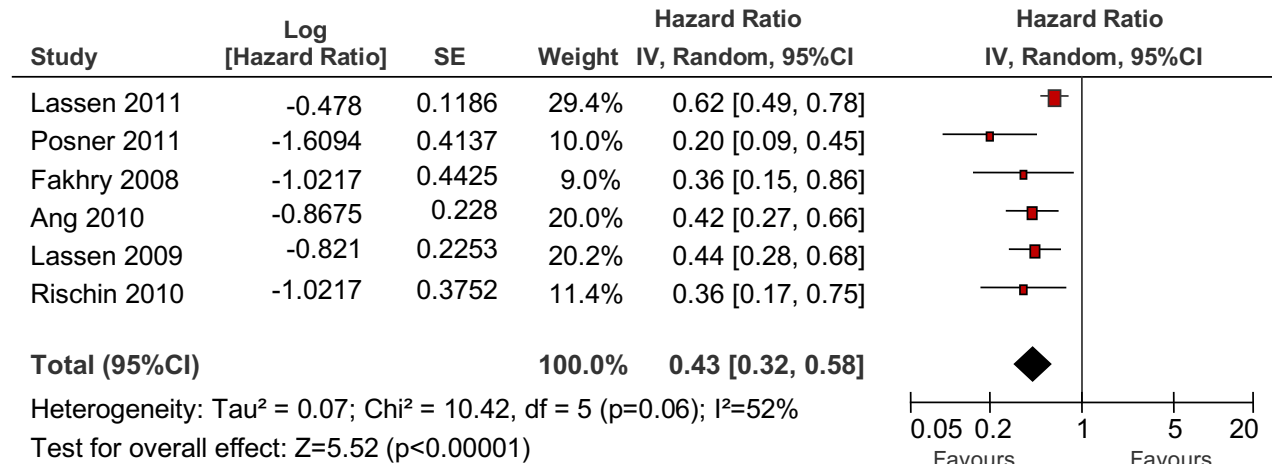
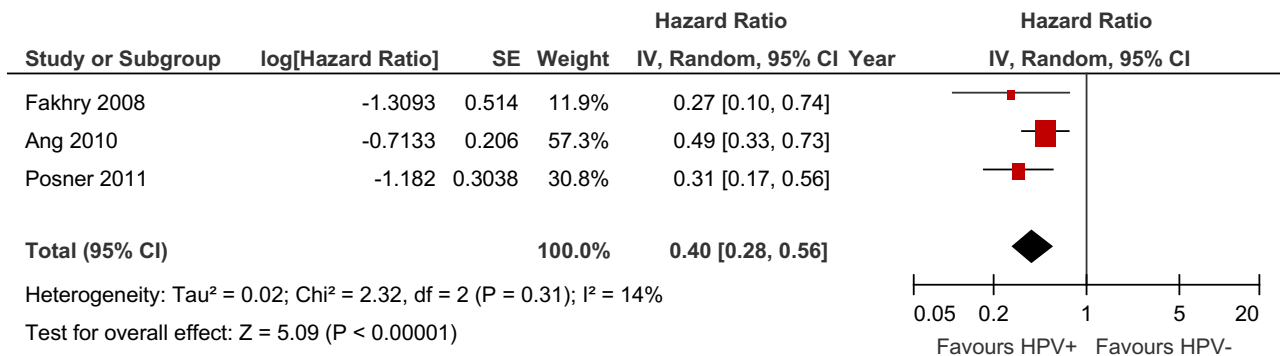
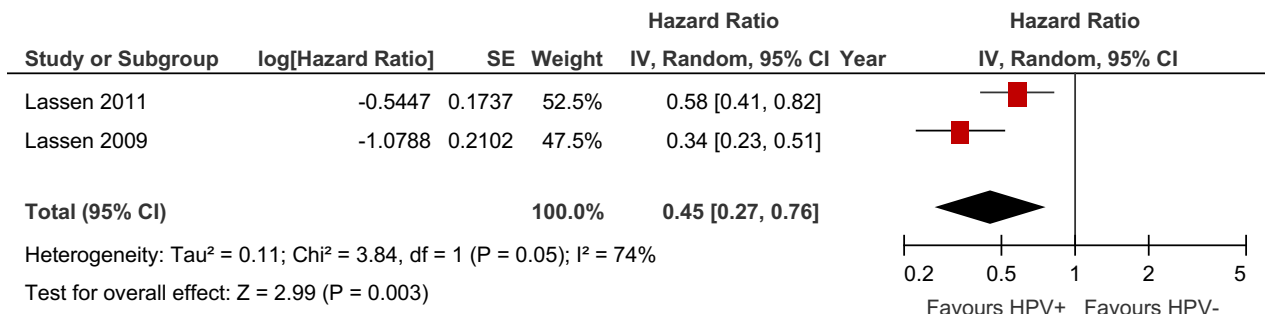
#### *Meta-Analysis identified in the Search of the Literature*

Ragin and Taioli (26) conducted a published data meta-analysis of the relationship between HPV infection and OS and DFS. Analyses were performed separately for patients with oropharyngeal and non-oropharyngeal tumours to evaluate site-specific differences in outcomes. Patients with HPV-positive oropharyngeal tumours had a 28% reduced risk of death compared to patients with HPV-negative oropharyngeal tumours (HR: 0.72, 95%CI: 0.5-1.0). Similar results were calculated for DFS (HR: 0.51, 95%CI: 0.4-0.7). However, no benefit in OS or DFS was seen in HPV-positive versus negative patients with non-oropharyngeal tumours.

Table 3: HPV status and clinical outcome from RCTs.

Trial	Tumour site	HPV status (n)	Overall Survival			Progression-free Survival			Disease-specific Survival		
			Rate (95%CI)	HR for death (95%CI)	p-value	Rate (95%CI)	HR for death (95%CI)	p-value	Rate	HR for death (95%CI)	p-value
Posner et al, 2011 (TAX 324) (35)	Oropharynx	HPV+ = 56	82% (69-90)	0.20	0.0001	78% (64-87)	NR	0.0001	NR	NR	NR
		HPV- = 55	35% (23-48)			28% (17-40)					
Lassen et al, 2011 (DAHANCA 6&7) (33)	Pharynx, supraglottic larynx, and oral cavity	HPV+ = 179	62%	0.62 (0.49-0.78)	NR	NR	NR	NR	78%	0.58 (0.41-0.81)	NR
		HPV- = 615	47%						64%		
Ang et al, 2010 (RTOG 0129) (32)	Oropharynx	HPV+ = 206	82.4% (77.2-87.6)	0.42 (0.27-0.66)	0.001	73.7% (67.7-79.8)	0.49 (0.33-0.74)	0.001	NR	NR	NR
		HPV- = 117	57.1% (48.1-66.1)			43.4% (34.4-52.4)					
Rischin et al, 2010 (TROG 02.02) (34)	Oropharynx	HPV+ = 106	91%	0.36 (0.17-0.74)	0.004	NR	NR	NR	NR	NR	NR
		HPV- = 79	74%								
Lassen et al, 2009 (DAHANCA 5) (36)	Pharynx and supraglottic larynx	HPV+ = 35	62%	0.44 (0.28-0.68)	NR	NR	NR	NR	72%	0.36 (0.20-0.64)	NR
		HPV- = 121	26%						34%		
Fakhry et al, 2008 (ECOG 2399) (31)	Oropharynx and larynx	HPV+ = 38	95% (87-100)	0.36 (0.15-0.85)	0.02	86% (74-99)	0.27 (0.10-0.75)	0.01	NR	NR	NR
		HPV- = 58	62% (49-74)			53% (36-67)					

HPV = human papillomavirus; HR = hazard ratio; CI = confidence interval; n = number of patients; NR = not reported.

**Figure 2. Meta-analysis of overall survival hazard ratios (HR) in trials comparing outcome by HPV status.****Figure 3. Meta-analysis of progression-free survival hazard ratios (HR) in trials comparing outcome by HPV status.****Figure 4. Meta-analysis of disease-specific survival hazard ratios (HR) in trials comparing outcome by HPV status.**

***Question #2: In which head and neck subsites is the prevalence of HPV-associated squamous cell carcinoma high enough to justify routine testing of HPV positivity?***

Table 4 summarizes the results of four systematic reviews on prevalence of HPV in head and neck squamous cell carcinoma (HNSCC). An additional meta-analysis (61) was also identified and tested the association between HPV16 and cancer of various anatomical sites. Overall prevalence of HPV in the included studies ranged from 20.8% to 46.5%. Prevalence rates tended to be lower when reported on non-site-specific HNSCC versus rates stratified by cancer site.

The recent systematic review by Li et al. (30) considered the prevalence of HPV in laryngeal cancer. Included in this review were 53 articles; however, only 38 studies considered squamous cell carcinoma. Restricting the analysis to this histological type, the prevalence of HPV among laryngeal SCC was 27.8% (95%CI: 22.8-33.4%). Dayyani et al. (25) included 5681 patients from 33 international and 1 Canadian study. Only studies that solely or separately reported on oropharyngeal cancer were included. The authors established a prevalence of HPV among all HNSCC patients of 22% (95%CI: 21-23%) and, in the subgroup of oropharyngeal cancers, prevalence of HPV was 41% (95%CI: 38-44%). Termine et al. (27) estimated the pooled prevalence of HPV DNA in HNSCC using a meta-analytical method. The pooled prevalence in 3238 oral squamous cell carcinoma (OSCC) samples was calculated to be 38.1% (95%CI: 30.0-46.2%). When the analysis was restricted to only studies that used PCR as the detection method, the prevalence increased to 39.9% (95%CI: 30.2-49.8%).

The systematic review by Ragin and Taioli (26) compared overall and site-specific prevalence for three outcomes categories: studies that reported an improved prognosis in HPV positive patients, studies that report worse prognosis, and studies that reported no such differences. In the studies that reported an improved prognosis, HPV subsite-specific prevalence was 38.2% (95%CI: 35.1-41.5%) in oropharyngeal SCC and 25.1% (95%CI: 18.8-32.4%) in laryngeal SCC. Considering the prevalence in the three studies that reported worse prognosis in HPV positive patients, the prevalence was 44.8% (95%CI: 26.4-64.3%) in the pharynx and 40.7% (95%CI: 28.1-54.2%) in the larynx. Similar prevalence rates were observed in the nine studies that reported no difference in prognosis by HPV status, with 40.9% (95%CI: 33.6-48.6%) and 39.6% (95%CI: 33.2-46.4%) of pharyngeal and laryngeal SCC patients, respectively, testing positive.

A comprehensive systematic review published in 2005 (29) explored the prevalence and type distribution of HPV-associated HNSCC worldwide. With literature as recent as 2004, 60 eligible studies from 26 countries with a total of 5046 cases were identified. Stratification of cases was made by the following cancer sites: oral cavity, oropharynx and larynx. Overall, 26% of all HNSCC biopsy specimens were positive for HPV. The site-specific prevalence, however, varied by site. The overall HPV prevalence in oral cavity SCC was calculated to be 23.5% (95%CI: 21.9-25.1%). Similarly, the prevalence of laryngeal SCC, which also included some cases of hypopharynx, was 24.0% (95%CI: 21.8-26.3%). Oropharyngeal SCC was significantly higher than either of these sites at 35.6% (95%CI: 32.6-38.7%). When the data were analysed by geographical location, HPV prevalence in oral SCC was similar in both North America (NA) (16.1%; 95%CI: 13.2-19.4%) and Europe (16.0%; 95%CI: 13.4-18.8%).

Prevalence of HPV was slightly lower for laryngeal SCC in each continent, with NA reporting a prevalence of 10.1% (95%CI: 7.0-14.1%) and Europe a prevalence of 13.8% (11.5-16.4). In contrast, HPV prevalence was significantly higher in North American populations (47.0%; 95%CI: 41.1-53.0%) than in Europeans (28.2%; 95%CI: 24.4-32.2%) for oropharyngeal SCC.

The meta-analysis by Hobbs et al. (61) included 17 studies and found that the association between HPV and cancer is strongest for the tonsil (OR: 15.1; 95%CI: 6.8-33.7%), intermediate for oropharynx (OR: 4.3; 95%CI: 2.1-8.9%), and weakest for oral (OR: 2.0; 95%CI: 1.2-3.4%) and larynx (OR: 2.0; 95%CI: 1.0-4.2%).

Table 4: Prevalence of HPV in HNSCC and subsites.

Trial	Number of studies included	Continents or countries included (n) <sup>a</sup>	Tumour site	Total number of cases	No. of HPV positive	HPV detection method	Prevalence	95% Confidence Interval (%)
Li et al, 2013 (30)	38	North America, Central and South America, Europe, and Asia	Larynx	NR	NR	PCR, ISH or IHC	27.8.0%	22.8-33.4
Dayyani et al, 2010 (25)	34	USA (14), Canada (1), Puerto Rico (1), France (2), Germany (4), Netherlands (2), Italy (3), Switzerland (1), Norway- Finland-Sweden (4), Japan (1), International (1)	Not site-specific HNSCC	5681	1247	PCR (33 studies) FISH (1 study)	21.95%	21-23
	NR		Oropharynx	925	379		41%	38-44
Termine et al, 2008 (27)	62	North America, Europe, Asia	All HNSCC sites combined	4852	NR	PCR or ISH	34.5%*	28.4-40.6
	15		Not site-specific HNSCC	1269	272	PCR or ISH	24.1%*	16.8-31.4
	47		Oral cavity	3238	1089	PCR or ISH	38.1%*	30.0-46.2
	13		Not site-specific HNSCC	NR	NR	PCR only	20.8%*	13.5-28.1
	36		Oral cavity	NR	NR	PCR only	39.9%*	30.2-49.8
Ragin & Taioli, 2007 (26)	33	NR	Lip and oral cavity, pharynx, larynx, nasopharynx, lymph nodes	2538	815	NR	32.1%	30.3 -34.0

Kreimer et al, 2005 (29)	35	Europe (15), North America (8), Asia (13), Other (2)	Oral cavity	2642	NR	PCR	23.5%	21.9-25.1
	27	Europe (17), North America (7), Asia (4), Other (2)	Oropharynx	969	NR	PCR	35.6%	32.6-38.7
	35	Europe (19), North America (7), Asia (8), Other (1)	Larynx	1435	NR	PCR	24.0%	21.8-26.3
	60	As listed above	Overall	5046	NR	PCR	25.9%	24.7-27.2

<sup>a</sup>May not sum to total number of studies in cases where multiple subsites were investigated.

<sup>\*</sup>Pooled prevalence estimates from random-effects model.

Abbreviations: HPV = human papillomavirus; PCR = polymerase chain reaction; ISH = in situ hybridization; FISH = fluorescence in situ hybridization; NR = not reported.

***Question 3: What is the diagnostic and prognostic value of routine testing of HPV status in patients with neck nodal metastatic squamous cell carcinoma from an unknown head and neck primary?***

There are 12 studies that considered HPV testing as a way to discern tumour origin in patients with SCC and lymph node metastases. Results of these studies are summarized in Table 5.

While many studies have examined the HPV status of lymph node neck metastases in correlation with a known primary, only five have considered and reported on true unknown primaries (38,39,47,50,52). Unfortunately, the sample size of unknown primaries in these studies has been extremely small, ranging from 3 to 58 patients. As such, caution should be practiced when interpreting these results. Park et al. (47) reported that, out of 58 patients with CUP, 50% were positive for p16. Similarly, Compton et al. (50) found that 44% (11/25) of metastatic lymph nodes from unknown primary tumours were p16 positive. Begum and Westra (38) reported 3 of 10 were positive. The two remaining studies (39,52) both found approximately 66% (4/6 and 2/3, respectively) of these lymph nodes turned out to be positive for HPV.

The remaining studies considered the correlation between HPV positivity and later detection of the primary tumour in the oropharynx. Begum et al. (49) found that 77% of surgically excised metastatic nodes from the oropharynx overexpressed p16 compared to only 3% of those from nonoropharyngeal primary sites ( $p<0.001$ ). HPV detection in fine-needle aspirates (FNAs) of patients with metastatic HNSCC was investigated in a later study by Begum et al. (37). Oropharyngeal metastases were p16 positive in 68% of the cases compared to 2% in nonoropharyngeal metastases ( $p<0.0001$ ). Similarly, Zhang et al. (42) found the identification of HPV by ISH in cervical lymph node metastases was highly predictable of an oropharyngeal primary (69% in OP vs. 6% in non-OP,  $p<0.0004$ ). HPV was identified by ISH in 25% of metastatic lymph nodes in a study by El-Mofty et al. (51). In 95.6% of these HPV-positive lymph nodes, the tumour originated in the oropharynx ( $p<0.0001$ ). Using a histochemical diagnostic panel in metastatic cervical lymph nodes, Park et al. determined that p16 was the single best predictor of occult HNSCCs arising in the oropharynx (41). They found that 78.1% of p16-positive cervical metastatic SCC arose from the oropharynx, whereas only 21.9% were non-oropharyngeal in origin.

The diagnostic performance of p16 IHC or ISH was also considered in five studies (38,40,49,51,53). Begum et al. (49) reported that the sensitivity of a positive p16 IHC stain for a diagnosis of an oropharyngeal tumour was 77%. The specificity of a negative p16 stain for a diagnosis of a nonoropharyngeal tumour was found to be 97%. Considerably higher diagnostic parameters were calculated from the data reported by Weiss et al. (53). The sensitivity and specificity of p16 IHC was calculated to be 92% and 100%, respectively. The overall sensitivity and specificity of p16 overexpression as a marker of HPV16 was 100% and 76%, respectively, as reported by Begum and Westra (38). El-Mofty et al. (51) found that the identification of HPV by ISH resulted in a sensitivity of 96% and specificity of 86%. Jannapureddy et al. (40) reported p16 overexpression in FNA material of cervical lymph nodes with metastatic SCC corresponded to a sensitivity of 82% and specificity of 76%.

Table 5: HPV in neck nodal tissue of patients with metastatic SCC.

Trial	Patient population	Specimens	No. of cases or tissue specimens	Primary tumour site	Prevalence of HPV+ in lymph node mets	HPV detection method*	Sensitivity	Specificity	Notes
Park et al, 2012 (47)	Pts with unknown primary SCC and diagnosed with SCC of metastatic lymph nodes	FFPE tissue blocks from biopsies	58	oropharynx: 20 non-oropharynx: 2 unknown: 36	53.4%	ISH	90.0%	65.8%	
					50.0%	IHC	80.0%	65.8%	
Compton et al, 2011 (50)	Pts with unknown primary SCC who underwent neck dissection or excisional biopsy	FFPE tissue blocks from neck dissections or cervical LN biopsy	25	NR	44%	IHC	NR	NR	
Weiss et al, 2011 (53)	Pts presenting with cervical lymph node mets and an unknown primary	FFPE tissue blocks from biopsies	13	tonsil: 7 tongue base: 5 unknown: 1	84.6% 92.3%	IHC PCR	91.7%	100%	20 cervical LN mets of HPV16+ pts found 11 primary tumours were in tongue base and 9 in tonsils
Jannapureddy et al, 2010 (40)	Pts with a cell block cytologic diagnosis of metastatic SCC in cervical lymph nodes	Cell blocks from FNA material	40	OP: 11 other H&N: 15 other non-H&N: 5 unknown: 9	40%	IHC	81.8%	75.8%	
Park et al, 2010 (41)	Pts treated for cervical lymph node metastases from HNSCC	FFPE tissue blocks from neck dissections	101	OP: 38 oral cavity: 16 hypopharynx: 26 larynx: 21	65.8% 18.8% 11.5% 4.8% (p<0.001)	IHC	NR	NR	78% of p16+ cervical mets arose from OP and 22% were nonOP



Desai et al, 2009 (39)	Pts with metastatic SCC of any origin with excised neck lymph nodes	FFPE tissue blocks from excised cervical LN	41	oral cavity: 7 OP: 6 laryngeal: 4 tonsillar: 3 other: 21	36.6%	IHC	NR	NR	
Begum and Westra, 2008 (38)	Pts with biopsied or resected BSCC of the H&N	FFPE tissue blocks from resections or biopsies	53	OP: 21 nonOP: 32	86% 28%	IHC	100% (95%CI: 79.1-100%)	76% (95%CI: 58.4-88.6%)	
El-Mofty et al, 2008 (51)	Pts with SCC of the head and neck and lymph node metastases	FFPE tissue blocks from neck dissections	93	OP: 32 oral cavity: 35 larynx/ hypopharynx: 26	68.7% 0 3.8% (p< 0.0001)	ISH & IHC	95.7%	85.7%	
Zhang et al, 2008 (42)	Pts with HNSCC metastatic to cervical lymph nodes with available FNA biopsies	Ethanol-fixed smears obtained from FNA	30	OP: 13 oral cavity: 13 Hypopharynx/ Larynx: 4	69% in OP 6% in non-OP p<0.0004  33% of all lymph node aspirates	ISH	NR	NR	90% of HPV+ tumours were OP
Begum et al, 2007 (37)	Pts diagnosed with metastatic SCC based on FNA of a neck mass	FFPE tissue blocks from aspirated material	77	OP: 19 nonOP: 48 unknown: 10	68% 2% (p<0.0001) 30%	IHC	NR	NR	92.3% HPV+ tumours overexpressed p16 whereas only 6% of HPV- tumours did (p<0.0001)

Hoffman et al, 2005 (52)	Pts with SCCHN	Fresh-frozen tissue samples from surgical specimens	35	OP: 3 oral cavity: 5 larynx: 7 unknown: 3 hypopharynx: 8 tonsillar: 9	55.6% (30/54 samples)	PCR	NR	NR	Of 18 pts with both PT & N samples, 39% were HPV+ in both, 39% HPV- in both, and 22% of samples were discordant
Begum et al, 2003 (49)	Pts with HNSCC who underwent neck dissection for carcinoma metastatic to regional lymph nodes	FFPE tissue blocks from neck dissections	68	OP: 31 nonOP: 37	77% 3%  p<0.001	IHC	77.4% (95%CI: 58.9-90.4%)	97.4% (95%CI: 86.2-99.9%)	95.5% HPV+ tumours overexpressed p16 whereas only 2.2% of HPV- tumours did (p<0.001)

\*More than one detection method may have been used in the studies, but numbers reflect those specific to the method listed.

Abbreviations: HPV = human papillomavirus; Pt = patients; HNSCC = head and neck squamous cell carcinoma; BSCC = basaloid squamous cell carcinoma; FNA = fine-needle aspirate; FFPE = formalin fixed paraffin embedded; OP = oropharynx; IHC = immunohistochemistry; PCR = polymerase chain reaction; ISH = in situ hybridization; NR = not reported.

***Question 4: What is the optimal testing method for the identification of HPV positivity in head and neck squamous cell carcinomas (HNSCC)?***

Thirteen recent studies evaluated and compared a variety of HPV diagnostic testing methods in patients with HNSCC. Nine of these studies reported the sensitivity and specificity of the testing methods, and nine reported concordance or correlation between tests. Table 6 summarizes the results.

Tissue microarrays containing 282 HNSCC were tested for the presence of HPV using p16 IHC, HPV DNA ISH, and an RNA ISH assay targeting high-risk HPV E6/E7 mRNA transcripts in a study by Bishop et al. (58). A high rate of concordance (99%) between the E6/E7 mRNA method and HPV DNA ISH was observed. Furthermore, 94% of HPV-positive tumours exhibited high p16 expression, compared to 9% of HPV-negative tumours ( $p < 0.0001$ ), demonstrating a strong association between p16 expression and the presence of HPV E6/E7 mRNA. Similarly, Hoffmann and colleagues (59) found p16 to be strongly correlated with HPV DNA status in combination with E6\*I expression in 78 patients with histologically confirmed HNSCC ( $p < 0.0001$ ).

A recently published study, validating the methods for testing HPV status used in the US Cooperative Group trials (46), evaluated assay performance in comparison with the gold standard test for high-risk (HR)-HPV E6/7 oncogene expression. The evaluation included testing for both type 16 alone and for all HR types. In 232 formalin-fixed paraffin-embedded (FFPE) biopsies, type-16-specific p16 IHC showed a sensitivity of 97% and specificity of 72%. While sensitivity remained the same, the specificity was increased to 84% with HR-HPV p16 IHC. The sensitivity of ISH was not as high for either type-16-specific (93%) or HR-HPV-specific (88%), as was observed with p16 IHC. However, the specificity of ISH improved to 92% for type-16-specific and 95% for HR-HPV types. When p16 IHC and HPV16 ISH tests were evaluated in combination, the combination of HPV-16 ISH-positive and p16 IHC-positive had the highest specificity in comparison with the gold standard test, with a false-positive rate of approximately 3%. By contrast, use of a combination of either p16 IHC-positive or HPV16 ISH-positive will result in the highest sensitivity, but is expected to result in a false-positive rate of approximately 19%.

Another evaluation of HPV diagnostic testing methods was conducted by Schache and colleagues (43) on fixed and fresh-frozen tissue from 108 OPSCC cases subjected to eight possible assay combinations. Using RNA qPCR as the gold standard, the sensitivity of the seven tests ranged from 88% for HR-HPV ISH to 97% for a combined p16/DNA qPCR. Specificity ranged from 82% for p16 IHC to 100% for both combined p16/RNA qPCR and combined DNA qPCR/RNA qPCR. The authors concluded that neither p16 IHC, HR-HPV ISH, nor DNA qPCR was sufficiently specific to recommend in isolation.

Agoston et al. (54) evaluated three approaches to detecting HPV in oropharyngeal tissue samples: PCR with generic L1 primers, PCR with early (E7) HPV-16-specific primers, and DNA-DNA ISH. These three were compared with p16 IHC in a subset of patients. Considering the Maximum Positive Rate (MPR), defined as positivity by either the L1 or E7 primers or both, the sensitivity of the E7 PCR and the L1 PCR were 72.5% and 90.2%, respectively. An improvement in sensitivity was seen in the subset of 97 tissue samples that underwent p16 IHC staining. Sensitivity was increased to 100%; however, specificity was poor at 38%.

A comparison of HPV ISH and p16 IHC in the detection of the virus as part of clinical care was conducted by Singhi et al. (18) in 256 HNSCCs. The authors found that the overall sensitivity of ISH was 81%. Specificity, however, was not reported nor could it be calculated from the included results. Perfect overall sensitivity was observed when p16 expression was used as a surrogate marker for HPV infection. Specificity was lower, at 85%. The authors reported a 93% correlation rate between HPV-16 status as determined by ISH and p16 IHC.

Smeets and colleagues (56) analyzed 48 frozen HNSCC specimens for the presence of HPV DNA and E6/E7 mRNA. The presence of HPV-16 E6/E7 mRNA in the frozen specimens was regarded as the gold standard and used as the selection criteria for the case group. Samples

were classified into three groups: those positive for both HPV DNA and HPV RNA (D+/R+, HPV positive), those positive for HPV DNA but RNA negative (D+/R-, HPV negative) and those with no evidence of HPV DNA or RNA (D-/R-, HPV negative). A series of diagnostic tests were then implemented and their ability to correctly classify the specimens was assessed. Perfect specificity was observed for p16 IHC, GP5+/6+ PCR, and E6\*I mRNA PCR. The specificity for these tests was 79%, 89%, and 100%, respectively. Other testing methods considered included quantification of viral load and FISH. Sensitivity for these two tests was 92% and 83%, respectively, with specificity higher at 97% and 100%, respectively. The authors concluded that, with each single method showing limitations in their diagnostic abilities, and E6 mRNA PCR not available for HPV types other than 16, a combination of methods should be considered. They recommend a two-tiered approach, with p16 IHC that is followed by GP5+/6+ PCR on the p16-positive cases, thereby reaching 100% sensitivity and specificity.

IHC staining was performed on paraffin-embedded samples of 34 patients with newly diagnosed and histologically confirmed tonsillar SCC enrolled in a study by Klussmann et al. (57). Using HPV typing by nested PCR protocols as the gold standard, the sensitivity and specificity of IHC was 89% and 94%, respectively. Similarly, in 30 patients with tonsillar SCC, Evans et al. (48) found p16 IHC to have a sensitivity of 91%, but a specificity of only 50%. Pannone et al. (60) reported a sensitivity rate of 100% for p16 IHC in their study of 22 patients with OPSCC. The specificity was found to be 93.5%.

In 239 cases of oropharyngeal SCC, HPV status was assessed by both p16 IHC and by ISH for high-risk HPV in a study by Lewis et al. (44). Considering all cases, 187 (78%) were positive for p16. Of these, 139 (74%) were positive for HPV by ISH, resulting in a concordance rate of 78%. When the authors considered survival outcomes, they concluded that no significant difference exists between p16-positive/HPV-negative tumours and p16-positive/HPV-positive tumours, suggesting that p16 IHC alone is the best test for use in risk stratification in OPSCC.

HPV positivity was assessed in a sample of 111 oropharyngeal SCCs by qRT-PCR for E6 mRNA, ISH for DNA and p16 IHC in a recent Canadian study by Shi et al. (45). Considering concordance between the tests, the authors reported an 86% concordance rate between HPV-16 DNA ISH and HPV-16 E6 mRNA. Concordance was improved to 92% between p16 IHC and HPV-16 ISH, but remained the same at 86% between p16 IHC and E6 mRNA.

Kuo et al. (55) assessed the presence of HPV in 92 Taiwanese patients with primary tonsillar SCC. ISH, p16 IHC, and HPV-PCR were each employed. Among the 58 cases of HPV 16 genotype, there was a 91% concordance rate between p16 IHC and ISH. Comparing this double testing with real-time PCR, a concordance rate of 95% was observed.

Table 6. Studies that compared HPV detection methods.

Author	No. of Cases	Patient population	Tissue samples	Testing method (definition of positive result)	Sensitivity	Specificity	Concordance or correlation
Bishop et al, 2012 (58)	282	HNSCC	FFPE	1) p16 IHC (diffuse staining in >50% of tumour cells) 2) HPV E6/E7 mRNA ISH 3) HPV ISH (punctate hybridization signals localized to the tumour cell nuclei)	NR	NR	E6/E7 mRNA & HPV ISH = 99%  p16 was strongly associated with HPV E6/E7 mRNA (p<0.0001)
Hoffmann et al, 2012 (59)	78	HNSCC	FFPE Fresh frozen	1) p16 IHC (strong nuclear & cytoplasmic staining)) 2) HPV E6*I mRNA (cutoff of 5 net MFI for positivity)	82% NR	52% NR	p16 was strongly correlated with HVP DNA status in combination with E6*I expression status (p<0.0001)
Jordan et al, 2012 (46)	233	OPSCC	FFPE	<i>HPV-16-type specific:</i> 1) p16 IHC 2) HPV16 ISH 3) IHC/ISH combined with both positive  <i>HR-HPV-type specific:</i> 1) p16 IHC 2) HPV-16 ISH 3) IHC/ISH combined with both positive	96.6% 93.2% 91.1%  96.8% 88.0% 86.1%	72.1% 92.0% 94.2%  83.8% 94.7% 97.3%	p16 IHC & HPV16 ISH = 88.6%
Pannone et al, 2012 (60)	64	OPSCC	FFPE	1) p16 IHC (high and diffuse levels of staining) 2) HPV DNA PCR (Consensus PCR) 3) HPV ISH	100% NR NR	93.5% NR NR	The concordance between ISH and consensus PCR was 73.7%

Author	No. of Cases	Patient population	Tissue samples	Testing method (definition of positive result)	Sensitivity	Specificity	Concordance or correlation
Evans et al, 2011 (48)	26	Tonsillar SCC	FFPE	1) GP5+/6+ PCR 2) Chromogenic ISH (CISH) (diffuse, punctate or mixed) 3) p16 IHC	NR NR 90.9%	NR NR 50%	NR
Schache et al, 2011 (43)	108	OPSCC	Fresh-frozen and FFPE	1) p16 IHC (strong and diffuse nuclear & cytoplasmic staining in >70% of tumour cells) 2) HR HPV ISH (any blue reaction product colocalized with the nuclei of tumour cells) 3) Combined p16/HR HPV ISH 4) DNA qPCR ( $\geq 1$ E6 gene copy/diploid genome) 5) Combined p16/DNA qPCR 6) Combined p16/RNA qPCR 7) Combined DNA qPCR/RNA qPCR	94% 88% 88% 97% 97% 94% 94%	82% 88% 90% 87% 94% 100% 100%	NR
Agoston et al, 2010 (54)	141	OPSCC	FFPE	1) PCR with generic L1 primers 2) PCR with early (E7) HPV-16-specific primers (E7PCR) 3) DNA-DNA ISH 4) p16 IHC (strong staining involving >50% of tumour cells)	90.2% 72.5% NR 100%	NR NR NR 38%	PCR & ISH = 82%
Lewis et al, 2010 (44)	239	OPSCC	FFPE	1) p16 IHC (nuclear & cytoplasmic staining) 2) HPV ISH (any definitive nuclear staining in tumour cell)	NR	NR	78%
Singhi et al, 2010 (18)	256	HNSCC	FFPE	1) p16 IHC (strong and diffuse nuclear & cytoplasmic staining present in $\geq 70\%$ of tumour specimen) 2) HPV ISH (punctuated hybridization signals localized to tumour cell nuclei)	100% NR	85% NR	93% correlation rate between HPV-16 status and p16 IHC
Shi et al, 2009 (45)	111	OPSCC	FFPE	1) p16 (strong signals detected in both tumour nuclei & cytoplasm) 2) HPV E6 mRNA (NR) 3) HPV ISH (punctate signal specific to tumor cell nuclei present)	NR	NR	ISH & E6 mRNA = 86% p16 & ISH = 92% p16 & E6 mRNA = 86%

Author	No. of Cases	Patient population	Tissue samples	Testing method (definition of positive result)	Sensitivity	Specificity	Concordance or correlation
Kuo et al, 2008 (55)	92	Primary tonsillar SCC	FFPE	1) Real-time PCR ( $\geq 10^2$ viral copies) 2) IHC (>50% of tumour cells showing strong nuclear staining with/without cytoplasmic staining) 3) ISH (>10% of tumour cells containing the integrated form (nuclear dots) of HPV)	NR	NR	p16 & ISH = 91% p16 & PCR = 94.8%
Smeets et al, 2007 (56)	48	HNSCC	FFPE	1) p16 IHC (staining intensity greater than the background) 2) GP5+/6+ 3) <i>E6*I mRNA - Gold Standard</i> 4) Viral load (>0.5 copies per cell) 5) FISH (strong staining with punctuated and/or diffuse signals throughout the nucleus)	100% 100% 100% 92% 83%	79% 89% 100% 97% 100%	NR
Klussmann et al, 2003 (57)	34	Tonsillar SCC	FFPE	1) p16 IHC (>25% immunoreactivity) 2) <i>HPV-DNA load by RT-PCR - Gold Standard</i>	88.9% 100%	93.8% 100%	NR

HPV = human papillomavirus; HNSCC = head and neck squamous cell carcinoma; OPSCC = oropharyngeal squamous cell carcinoma; FFPE = formalin-fixed paraffin-embedded; HR = high risk; q = quantitative; RT = real time; IHC = immunohistochemistry; PCR = polymerase chain reaction; ISH = in situ hybridization; NR = not reported.

**ONGOING TRIALS**

The US National Institutes of Health's clinical trial registry (<http://www.clinicaltrials.gov>) was searched on May 8, 2013. While this guideline does not make recommendations on treatment, many such studies are currently underway. It is hypothesized that a reduction in the intensity of therapy for HPV positive oropharyngeal SCC patients will reduce treatment sequelae, without affecting cure rates. Such ongoing trials are listed and described in Table 7.

**Table 7. Ongoing trials of HPV and HNSCC.**

<b><i>Phase II Trials:</i></b>	
<a href="#">De-intensification of Radiation and Chemotherapy for Low-Risk Human Papillomavirus-related Oropharyngeal Squamous Cell Carcinoma</a>	
Conditions:	Carcinoma, Squamous Cell; Head and Neck Neoplasms; Oropharyngeal Neoplasms
Interventions:	Radiation: Intensity Modulated Radiotherapy (IMRT); Drug: Cisplatin; Procedure: Limited surgical evaluation
Sponsor/Collaborators:	UNC Lineberger Comprehensive Cancer Center; University of Florida
Funded By:	Other
Study Type:	Interventional
Study Design:	Endpoint Classification: Efficacy Study; Intervention Model: Single Group Assignment; Masking: Open Label
Comparison:	Single Group Assignment
NCT Number:	NCT01530997
Outcome Measures:	Complete pathological response rate after de-escalated CRT in HPV-positive and/or p16-positive OPSCC.; Local control rate; Regional control rate; Local-regional control rate; Cause-specific survival rate; Overall survival rate; Head and neck quality of life assessments; Speech and swallowing function
<a href="#">Reduced-intensity Therapy for Advanced Oropharyngeal Cancer in Non-smoking Human Papilloma Virus (HPV)-16 Positive Patients</a>	
Condition:	Oropharyngeal Cancer
Intervention:	Radiation: Chemotherapy plus Radiation therapy
Sponsor:	University of Michigan Cancer Center
Funded By:	Other
Study Type:	Interventional
Study Allocation:	Non-randomized
Design:	Randomized; Endpoint Classification: Safety/Efficacy Study; Intervention Model: Single Group Assignment; Masking: Open Label
Comparison:	Single Group Assignment
NCT Number:	NCT01649414
Outcome Measures:	Number of Patients with Tumor Reoccurrence; Rate of Toxicity in Patients
<a href="#">Study of Chemotherapy Prior to Radiotherapy and Chemotherapy in Patients With HPV Associated Cancer of the Oral Cavity</a>	
Condition:	Oropharyngeal Neoplasms
Interventions:	Drug: Docetaxel; Drug: Cisplatin; Drug: Fluorouracil; Radiation: External beam radiation therapy/ Intensity Modulated Radiotherapy (IMRT); Drug: Carboplatin
Sponsor/Collaborators:	North Shore Long Island Jewish Health System; Bhoomi Mehrotra
Funded By:	Other
Study Type:	Interventional
Study Design:	Endpoint Classification: Efficacy Study; Intervention Model: Single Group Assignment; Masking: Open Label
Comparison:	Single Group Assignment
NCT Number:	NCT01525927
Outcome Measures:	Response (CR+PR) status at 3 months post-therapy; To define objective tumour response rates to include subsequent radiation-based treatment, per RESIST version 1.1 criteria.; To assess progression-free survival at 2 years; To assess overall survival at 2 years.; To assess locoregional disease control at 2 years; To assess distant disease control at 2 years; Assessment of quality-of-life outcomes; To identify additional prognostic factors
<b><i>Phase III Trials:</i></b>	
<a href="#">The Quarterback Trial: A Randomized Phase III Clinical Trial Comparing Reduced and Standard Radiation Therapy Doses for Locally Advanced Oropharyngeal Cancer</a>	



Condition:	Squamous Cell Carcinoma of Oropharynx
Interventions:	Radiation: Reduced Dose Radiation; Radiation: Standard Dose Radiation
Sponsor/Collaborators:	Mount Sinai School of Medicine; The Biodesign Institute; Arizona State University
Funded By:	NR
Study Type:	Interventional
Study Design:	Endpoint Classification: Safety/Efficacy Study; Intervention Model: Parallel Assignment Masking: Single Blind (Outcomes Assessor); Primary Purpose: Treatment
Comparison:	Reduced Dose Radiation versus Standard Dose Radiation
NCT Number:	NCT01706939
Outcome Measures:	Progression Free Survival (PFS) at 3 years; Rate of local-regional control; Overall survival; Acute toxicity
<a href="#"><u>Post Operative Adjuvant Therapy De-intensification Trial for Human Papillomavirus-related, p16+ Oropharynx Cancer</u></a>	
Condition:	Oropharyngeal Neoplasms
Interventions:	Radiation: Intensity-modulated radiation therapy (IMRT); Drug: Cisplatin
Sponsor/Collaborators:	Washington University School of Medicine
Funded By:	NR
Study Type:	Interventional
Study Design:	Endpoint Classification: Efficacy Study; Intervention Model: Parallel Assignment; Masking: Open Label; Primary Purpose: Treatment
Comparison:	Experimental: Radiotherapy: Patients undergo postoperative IMRT once daily, 5 days a week, for 6 weeks Active Comparator: Radiotherapy, cisplatin: Patients undergo postoperative IMRT as in Arm I. Patients and 36 of RT.
NCT Number:	NCT01687413
Outcome Measures:	Disease-free survival; Locoregional control; Distant metastasis rate; Disease-specific survival; Cumulative Function and QOL
<a href="#"><u>Paclitaxel, Cisplatin, and Cetuximab Followed By Cetuximab and Intensity-Modulated Radiation Therapy in Treating Patients With Oropharynx That Can Be Removed By Surgery</u></a>	
Conditions:	Head and Neck Cancer; Precancerous Condition
Interventions:	Biological: cetuximab; Radiation: intensity-modulated radiation therapy
Sponsor/Collaborators:	National Cancer Institute (NCI); Eastern Cooperative Oncology Group
Funded By:	Other / NIH
Study Type:	Interventional
Study Design:	Masking: Open Label; Primary Purpose: Treatment
Comparison:	Arm 1: low-dose intensity-modulated radiotherapy (IMRT) 5 days per week for approximately 5 weeks (27 fractions). Patients also receive cetuximab IV over 1-2 hrs once weekly for 6 weeks.  Arm 2: standard-dose IMRT 5 days per week for approximately 6 weeks (33 fractions). Patients also receive cetuximab IV over 1-2 hrs once weekly for 7 weeks.
NCT Number:	NCT01084083
Outcome Measures:	2-year progression- free survival; Toxicity; Overall survival; Objective response; Quality of life as assessed at baseline and at 1, 6, 12, and 24 months after completion of study treatment; Correlative biomarker studies
<a href="#"><u>Radiation Therapy With Cisplatin or Cetuximab in Treating Patients With Oropharyngeal Cancer</u></a>	
Conditions:	Head and Neck Cancer; Precancerous Condition
Interventions:	Biological: cetuximab; Drug: cisplatin
Sponsor/Collaborators:	National Cancer Institute (NCI); Radiation Therapy Oncology Group
Funded By:	Other / NIH
Study Type:	Interventional
Study Design:	Allocation: Randomized; Masking: Open Label; Primary Purpose: Treatment
Comparison:	Arm I: Patients undergo image-guided intensity-modulated radiation therapy (IMRT) once daily on days 1-4 and twice daily on day 5 weekly for 6 weeks. Patients also receive high-dose cisplatin IV over 1-2 hours on days 1 and 22.

NCT Number:	Arm 2: Beginning 1 week prior to IMRT, patients receive cetuximab IV over 2 hours. Patients then receive cetuximab IV over 1 hour once weekly for 7 weeks. Patients undergo IMRT as in arm I.
Outcome Measures:	NCT01302834 5-year overall survival; Progression-free survival; Local-regional failure; Distant metastasis; Acute toxicities (CTCAE v. 4) and overall toxicity burden at end of treatment and at 1, 3, and 6 months after completion of treatment; Late toxicities (CTCAE v. 4) at 1, 2, and 5 years

## DISCUSSION

Over the past several years, there has been an increase in the annual incidence of HPV-related HNSCC in North America and Europe (62). Numerous studies have investigated the prevalence of HPV in tumour specimens of patients with HNSCC, with a wide range in reported estimates. The evidentiary base for establishing prevalence in this review was comprised exclusively of systematic reviews, most with meta-analyses. This evidence demonstrated that both the prevalence and association with HPV-16 is highest in the oropharynx.

Recent randomized trials have established HPV-related oropharyngeal carcinoma as a distinct disease entity. Significant improvements in overall survival in HPV-positive patients are unequivocally confirmed in the reported trials and when the data from these trials were pooled in this meta-analysis. The reported survival benefit experienced by HPV-positive patients does not appear to be dependent on treatment strategy. Studies have demonstrated improved survival in these patients with surgery (63), radiation therapy (36,64), concurrent chemoradiation therapy (32), and induction chemotherapy followed by concurrent chemoradiation therapy (31). While the reason for the improved survival is not fully understood, it can be explained in part by improved loco-regional control (32,34,35). Death from second primaries and non-cancer-related causes was also reduced in HPV-positive patients and accounted for a 30%-50% improvement in survival in these patients (35).

The reconsideration of therapeutic attitudes in HPV-positive patients has now become a highly relevant clinical question (32) and the focus of several new trials. While current clinical guidelines do not consider HPV status in treatment planning (17), it is possible that these patients do not require the same intensive, multimodality treatment protocols. De-intensification strategies are currently being investigated, based on high treatment response rates in HPV-associated tumours, as a way to minimize treatment-associated morbidity and toxicities (13,17).

Given the distinctiveness of HPV-related carcinoma as a biological and clinical variant of HNSCC, the need for standard HPV testing of oropharyngeal carcinomas is urgent and compelling (18). The need for a highly accurate, reproducible, and practical testing method is pressing, yet the best method for HPV detection is not yet established (5). The evidence suggests that, in patients with OPSCC, the performance of the three main techniques - PCR, ISH, and p16 IHC - is comparable. Other factors, namely practicality, availability, simplicity, and cost, thus become more important in the selection of the paramount HPV testing method. p16IHC was first described as a surrogate for HPV status by Klussmann et al. (57) and later used in the DAHANCA 5 trial (36). Concordance rates between p16 IHC and HPV-16 ISH and E6 mRNA are reported to be 92% and 86%, respectively (45). Discordant cases reported in the literature are often due to cases that are not HPV type-16 related (45). Thus, with p16 overexpressed regardless of HPV type, IHC testing offers another advantage in that it is not type specific. While further testing may be required in selected patients, the evidence compiled suggests p16 IHC alone is sufficient to classify tumours according to their association with HPV.

The Head and Neck DSG acknowledges the importance of the cost implications associated with routine testing. While a formal cost analysis is beyond the scope of this clinical practice guideline, the DSG did take into account practicality, availability, simplicity, and cost of the HPV testing method when making the recommendations. Implementation issues are outside the scope of this document, and will need to be considered by Cancer Care Ontario when and if this guideline becomes the basis for practice in Ontario.

Several limitations of this systematic review should be noted. The quality assessment of the included literature revealed several shortcomings, especially in study design and reporting. Blinding is a crucial issue in prognostic studies, as it is necessary to prevent information bias. The majority of studies did not report such blinding. Moreover, retrospective study designs are inherently more prone to bias than are prospective studies and can be more difficult to interpret, especially if the sampling did not include consecutive patients. The reporting of consecutive patient sampling occurred in only half of the included studies.

Despite these limitations, the best available evidence with respect to the questions posed was collected and included. A rigorous systematic review and meta-analysis, planned *a priori*, provided an abundant evidentiary base and the context and direction for the development of recommendations.

## CONCLUSIONS

HPV is now emerging as a valid diagnostic, prognostic and predictive biomarker for discerning the presence and progress of disease (5). The comprehensive evidentiary base compiled suggests that routine testing of patients with oropharyngeal SCC and patients with metastatic squamous cell carcinoma to neck nodes from an unknown primary is both compelling and necessary. It is extremely likely that HPV status will influence management decisions in the near future and is now regarded as a mandatory stratification factor for clinical trials. Testing should initially be performed by IHC staining for p16. Subsequent validated tests may be necessary to confirm p16 results in selected cases. Future research should focus on establishing the prevalence of HPV-associated SCC in other head and neck subsites and on clarifying the prognosis associated with HPV positivity in these patients.

## CONFLICT OF INTEREST

In accordance with the PEBC Conflict of Interest (COI) Policy, the guideline authors, Head and Neck Cancer DSG members, and internal and external reviewers were asked to disclose potential conflicts of interest. The authors, members, and reviewers reported that they had no conflicts of interest.

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A complete list of the members of the Head and Neck Cancer DSG and the Working Group, with their affiliations, is provided in Section 3 Appendix 4.



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## Appendix 1. Literature search strategy.

Database: Ovid MEDLINE(R) <1996 to March Week 4 2013>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations <April 09, 2013>, and the Cochrane Library (OVID: 1st Quarter 2013).

```

-----
1  exp "head and neck neoplasms"/
2  exp *carcinoma, squamous cell/
3  HNSCC.ab,mp,tw.
4  (oropharyngeal adj2 (cancer? or carcinoma? or neoplasm? or tumor? or
   malignan$)).mp,tw.
5  (laryngeal adj2 (cancer? or carcinoma? or neoplasm? or tumor? or malignan$)).mp,tw.
6  (hypopharyngeal adj2 (cancer? or carcinoma? or neoplasm? or tumor? or
   malignan$)).mp,tw.
7  exp Oropharynx/
8  exp larynx/
9  exp hypopharynx/
10 exp oral cavity/
11 1 and 2
12 or/4-6
13 (or/7-10) and 11
14 3 or 12 or 13
15 (P16 adj2 protein).mp,tw.
16 immunohistochemistry.mp,tw.
17 PCR.mp,tw.
18 polymerase chain reaction.mp,tw.
19 (polymerase adj2 chain adj2 reaction).mp,tw.
20 *In Situ Hybridization/
21 (in adj2 situ adj2 hybridization).mp,tw.
22 $ISH.mp,tw.
23 or/15-22
24 HPV.mp,tw.
25 human papillomavirus.mp,tw.
26 papillomavirus.mp,tw.
27 or/24-26
28 "sensitivity and specificity"/
29 14 and 23 and 27 and 28
30 14 and 27
31 or/29-30
32 meta-analysis.pt,sh,tw.
33 (meta-analy$ or meta analy$ or metaanaly$).tw.
34 32 or 33
35 31 and 34
36 guideline$.pt,sh,tw.
37 31 and 36
38 exp randomized controlled trials/
39 random$.pt,sh,tw.
40 38 or 39

```

- 41 31 and 40
- 42 35 or 37 or 41
- 43 exp clinical trials/
- 44 exp longitudinal studies/
- 45 retrospective studies.mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]
- 46 exp cohort studies/
- 47 43 or 44 or 45 or 46
- 48 31 and 47
- 49 42 or 48
- 50 (case report\$ or editorial\$ or comment\$ or letter\$ or news).pt.
- 51 49 not 50
- 52 limit 51 to (English language and humans)

.....

**Database: EMBASE <1996 to 2013 Week 14>**

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- 1 exp "head and neck cancer"/
- 2 exp "squamous cell carcinoma"/
- 3 HNSCC.ab,mp,tw.
- 4 (oropharyngeal adj2 (cancer: or carcinoma: or neoplasm: or tumor?: or malignan:)).mp,tw.
- 5 (laryngeal adj2 (cancer: or carcinoma: or neoplasm: or tumor?: or malignan:)).mp,tw.
- 6 (hypopharyngeal adj2 (cancer: or carcinoma: or neoplasm: or tumor?: or malignan:)).mp,tw.
- 7 exp oropharynx/
- 8 exp larynx/
- 9 exp hypopharynx/
- 10 exp oral cavity/
- 11 1 and 2
- 12 or/4-6
- 13 (or/7-10) and 11
- 14 3 or 12 or 13
- 15 (p16 adj2 protein).mp,tw.
- 16 immunohistochemistry.mp,tw.
- 17 polymerase chain reaction.mp,tw.
- 18 PCR.mp,tw.
- 19 \*in situ hybridization/
- 20 (in adj2 situ adj2 hybridization).mp,tw.
- 21 :ISH.tw.
- 22 or/15-21
- 23 HPV.mp,tw.
- 24 human papillomavirus.mp,tw.
- 25 papillomavirus.mp,tw.
- 26 or/23-25
- 27 "sensitivity and specificity"/
- 28 14 and 22 and 26 and 27
- 29 14 and 26
- 30 or/28-29

31 meta-analysis.ti,tw.  
32 (meta-analy: or meta analy: or metaanaly:).ti,tw.  
33 31 or 32  
34 30 and 33  
35 guideline:.ti,tw.  
36 30 and 35  
37 exp Randomized Controlled Trial/  
38 random:.ti,tw.  
39 37 or 38  
40 30 and 39  
41 34 or 36 or 40  
42 exp controlled Study/  
43 exp logitudinal studies/  
44 retrospective studies.ti,tw.  
45 exp Cohort Studies/  
46 42 or 43 or 44 or 45  
47 30 and 46  
48 41 or 47  
49 (case report: or editorial: or comment: or letter: or news).ti,tw.  
50 48 not 49  
51 limit 50 to (English language and humans)

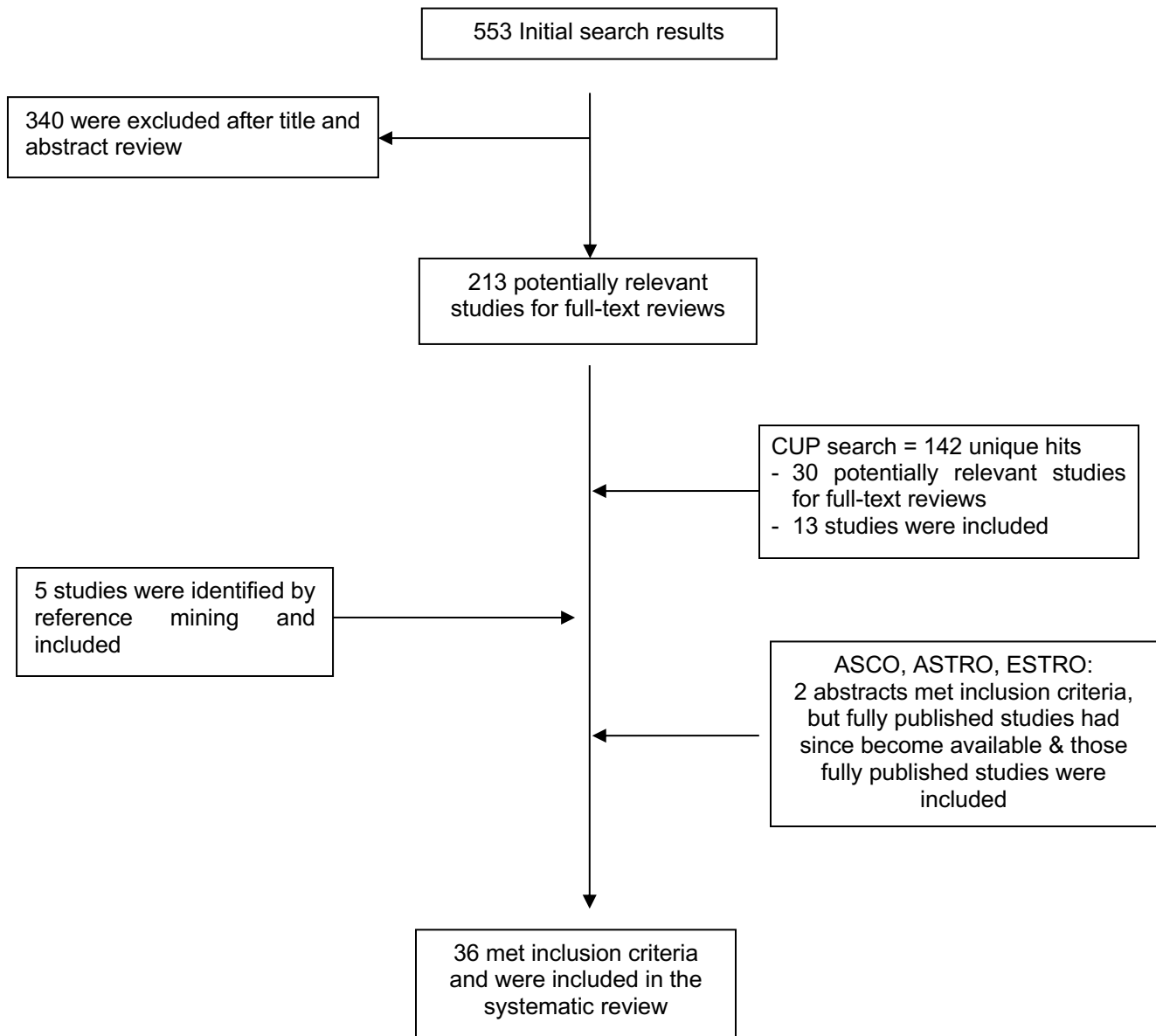
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## Appendix 2. Additional literature search strategy on CUPs.

Ovid MEDLINE(R) without Revisions 1996 March Week 4 2013, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations April 09, 2013, EMBASE 1996 to 2013 Week 14

1. exp "head and neck neoplasms"/
2. exp \*carcinoma, squamous cell/
3. neoplasm metastasis.mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
4. or/1-3
5. (unknown adj2 primary).mp,tw.
6. (occult adj2 tumor?).mp,tw.
7. (unknown adj2 origin?).mp,tw.
8. or/5-7
9. HPV.mp,tw.
10. human papillomavirus.mp,tw.
11. papillomavirus infection/ge
12. papillomavirus infection/pa
13. papillomavirus infection/vi
14. p16.mp,tw.
15. or/9-14
16. lymph nodes/pa
17. lymph nodes/vi
18. or/16-17
19. 4 and 8 and 15
20. 8 and 15
21. 15 and 18
22. 4 and 8 and 15 and 18
23. 19 or 20 or 21 or 22

**Appendix 3. Flow of studies considered for this systematic review.**



#### **Appendix 4.**

##### **Head and Neck Cancer Disease Site Group (DSG) members.**

- Dr. John Yoo, Co-Chair, Otolaryngology, London Health Sciences Centre
- Dr. Eric Winkvist, Co-Chair, Medical Oncology, London Health Sciences Centre
- Dr. Adam Andronowski, Radiation Oncology, Integrated Cancer Program, Sudbury Regional Hospital
- Dr. Margaret Anthes, Radiation Oncology, Thunder Bay Regional Health Sciences Centre
- Dr. Stuart Archibald, Surgery, St. Joseph's Hospital, Hamilton
- Dr. Christine Cripps, Medical Oncology, The Ottawa Hospital Regional Cancer Centre
- Dr. Ralph Gilbert, Otolaryngology, Princess Margaret Hospital, Toronto
- Dr. Laval Grimard, Radiation Oncology, The Ottawa Hospital Regional Cancer Centre
- Dr. Steven Hall, Surgery, Cancer Centre of Southeastern Ontario, Kingston General Hospital
- Dr. Alex Hammond, Radiation Oncology, London Regional Cancer Program
- Dr. Ian Hodson, Radiation Oncology, Juravinski Cancer Centre, Hamilton
- Ms. Christina Lacchetti, Health Research Methodology, Program in Evidence-Based Care / Cancer Care Ontario
- Dr. Aamer Mahmud, Radiation Oncology, Kingston Regional Cancer Centre
- Dr. Fidel Ishak, Surgery, Northeastern Ontario Regional Cancer Centre, Sudbury Regional Hospital
- Dr. Ian Poon, Radiation Oncology, Odette Cancer Centre, Toronto
- Dr. Ken Schneider, Radiation Oncology, Windsor Regional Cancer Centre
- Dr. Sarwat Shehata, Radiation Oncology, Northeastern Ontario Regional Cancer Centre, Sudbury Regional Hospital
- Dr. John Waldron, Radiation Oncology, Princess Margaret Hospital, Toronto

##### **Guest members:**

- Dr. Bayardo Perez-Ordóñez, Director of Surgical Pathology, University Health Network, Toronto
- Dr. Suzanne Kamel-Reid, Director of Molecular Diagnostics, University Health Network, Toronto

## Evidence-Based Series 5-9: Section 3

### A Quality Initiative of the Program in Evidence-Based Care (PEBC), Cancer Care Ontario (CCO)

#### Routine HPV Testing in Head and Neck Squamous Cell Carcinoma: Development Methods, Recommendations Development and External Review Process

The 2013 guideline recommendations have been ENDORSED, which means that the recommendations are still current and relevant for decision making. Please see Section 4: Document Assessment and Review for a summary of updated evidence published between 2013 and 2019, and for details on how this guideline was ENDORSED.

#### THE PROGRAM IN EVIDENCE-BASED CARE

The Program in Evidence-Based Care (PEBC) is an initiative of the Ontario provincial cancer system, Cancer Care Ontario (CCO). The PEBC mandate is to improve the lives of Ontarians affected by cancer through the development, dissemination, and evaluation of evidence-based products designed to facilitate clinical, planning, and policy decisions about cancer care.

The PEBC supports a network of disease-specific panels, termed Disease Site Groups (DSGs), as well as other groups or panels called together for a specific topic, all mandated to develop the PEBC products. These panels are comprised of clinicians, other health care providers and decision makers, methodologists, and community representatives from across the province.

The PEBC produces evidence-based and evidence-informed guidelines, known as Evidence-Based Series (EBS) reports, using the methods of the Practice Guidelines Development Cycle (1,2). The EBS report consists of an evidentiary base (typically a systematic review), an interpretation of and consensus agreement on that evidence by our Groups or Panels, the resulting recommendations, and an external review by Ontario clinicians and other stakeholders in the province for whom the topic is relevant. The PEBC has a formal standardized process to ensure the currency of each document through the periodic review and evaluation of the scientific literature and, where appropriate, the integration of that literature with the original guideline information.

This EBS is comprised of the following sections:

- *Section 1: Guideline Recommendations.* Contains the clinical recommendations derived from a systematic review of the clinical and scientific literature and its interpretation by the Group or Panel involved and a formalized external review in Ontario by review participants.

- *Section 2: Evidentiary Base.* Presents the comprehensive evidentiary/systematic review of the clinical and scientific research on the topic and the conclusions reached by the Group or Panel.
- *Section 3: Development Methods, Recommendations Development, and External Review Process.* Summarizes the EBS development process, the recommendations development process and the results of the formal external review of the draft version of the EBS.

## FORMATION OF GUIDELINE WORKING GROUP

The Head and Neck Disease Site Group (DSG) asked the PEBC to develop a guideline on routine testing of HPV in head and neck squamous cell carcinomas. In consultation with the Head and Neck DSG, a Working Group was identified from the DSG membership. Additionally, two experts in the field of pathology and laboratory medicine were invited to join the working group. This Working Group consisted of one radiation oncologist, one medical oncologist, one head and neck surgeon, one pathologist, one laboratory medicine specialist and one methodologist. The Working Group and DSG also formed the Routine HPV Testing in Head & Neck SCC GDG. This group would take responsibility for providing feedback on the guideline as it was being developed and acted as the Expert Panel for the document at Internal Review, reviewing the document and requiring changes as necessary before approving it.

## OBJECTIVES AND RESEARCH QUESTIONS

This Working Group developed the following objective for this guideline in consultation with the Head and Neck DSG:

- To evaluate the appropriateness of, and make recommendations on, routine testing for human papillomavirus (HPV) status in adult patients with primary, or neck nodal metastatic, squamous cell carcinoma (SCC) of the head and neck.

From this objective, the following research questions were derived to direct the search for available evidence to inform recommendations to meet the objectives:

1. What is the relationship between HPV positivity and outcome in head and neck squamous cell carcinomas (HNSCC)?
2. In which head and neck subsites is the prevalence of HPV-associated squamous cell carcinoma high enough to justify routine testing of HPV positivity?
3. What is the diagnostic and prognostic value of routine testing of HPV status in patients with neck nodal metastatic squamous cell carcinoma from an unknown head and neck primary?
4. What is the optimal testing method for the identification of HPV positivity in head and neck squamous cell carcinomas (HNSCC)?

## GUIDELINE REVIEW

Almost all PEBC document projects begin with a search for existing guidelines that may be suitable for adaptation. The PEBC defines adaptation, in accordance with the ADAPTE Collaboration, as “the use and/or modification of (a) guideline(s) produced in one cultural and organizational setting for application in a different context” (3). This includes a wide spectrum of potential activities from the simple endorsement, with little or no change, of an existing guideline, to the use of the evidence base of an existing guideline with *de novo* recommendations development.

For this document, a search was conducted of the Inventory of Cancer Guidelines (<http://www.cancerguidelines.ca/guidelines/inventory/search.php>), the National Guidelines Clearinghouse (<http://guideline.gov/>), and CMA Infobase ([http://www.cma.ca/index.php/ci\\_id/54316/la\\_id/1.htm](http://www.cma.ca/index.php/ci_id/54316/la_id/1.htm)). In addition, the websites of several known high-quality guideline developers, including NICE, SIGN, ASCO and NCCN were



searched. Only guidelines published in English after 2008 were considered. Guidelines that were considered relevant to the objectives and the research questions were then evaluated for quality using the AGREE II instrument.

This search yielded one practice guideline (4). The working group decided that proceeding with a new systematic review that includes the latest research was warranted given the lack of reporting of the literature included in this practice guideline.

## **EVIDENTIARY BASE DEVELOPMENT**

Using the research questions described above, a search for existing systematic reviews and systematic review of the primary literature was conducted, as described in Section 2 of this EBS.

## **INITIAL RECOMMENDATIONS**

Using the evidentiary base in Section 2, the Working Group developed a set of initial recommendations. These initial recommendations were developed through a consideration of the aggregate-evidence quality and the potential for bias in the evidence and the likely benefits and harms of routine HPV testing. The Working Group considered the values they used in weighing benefits compared to harms, and then made a considered judgement. This process is described in detail for each topic area described below.

### ***Oropharyngeal squamous cell carcinomas:***

#### ***Key Evidence for Benefits and Harms***

A meta-analysis showed a definite survival benefit for HPV-positive patients compared to those whose tumour was HPV negative in terms of overall survival (OS) (HR: 0.43 (95%CI: 0.32-0.58%), progression-free survival (PFS) (HR: 0.40, 95%CI: 0.28-0.56%), and disease-specific survival (DSS) (HR: 0.45 (95%CI: 0.27-0.76%).

A published data meta-analysis by Ragin and Taioli (5) demonstrated that patients with HPV-positive oropharyngeal tumours had a 28% reduced risk of death compared to patients with HPV-negative oropharyngeal tumours (HR: 0.72, 95%CI: 0.5-1.0%). Similar results were calculated for disease-specific survival (DSS) (HR: 0.51, 95%CI: 0.4-0.7%). However, no benefit in overall survival (OS) or DSS was seen in HPV-positive versus negative patients with non-oropharyngeal tumours.

#### ***Aggregate-Evidence Quality and Potential for Bias***

Only the ECOG 2399 trial (6) had a pre-specified subgroup analysis, while the remaining five trials had no such analyses planned in their study protocols. Two studies (7,8) reported that no significant differences were observed in baseline characteristics between patients who underwent testing for HPV status and those who did not. Conversely, two studies (9,10) did report that differences were seen, with tested patients more likely to have operable tumours, better performance status, lower T categories, and less likely to be current smokers. The remaining two trials (6,11) made no mention of baseline differences. No trial adequately reported on separate power calculations being made for the subgroup analysis.

#### ***Values of the Working Group***

A high value was ascribed to the additional prognostic information made available by HPV testing.

HPV status information is now required for entrance into trials.

*Considered Judgement*

There is evidence from a meta-analysis of randomized trials that HPV positivity is a strong predictor of prognosis in patients with oropharyngeal squamous cell carcinoma. In addition, it is likely that HPV status will influence management decisions in the near future and is now regarded as a mandatory stratification factor for clinical trials. Therefore, even though at this time no recommendation can be made to base clinical management decisions on HPV status, the valuable prognostic benefits of HPV testing are sufficient to warrant routine testing.

**Initial (DRAFT) Recommendation 1**

**The tumours of all adult patients presenting with oropharyngeal squamous cell carcinomas should be routinely tested for HPV status.**

*Neck nodal tissue of patients with metastatic SCC from an unknown primary:**Key Evidence for Benefits and Harms*

Eleven studies found the prevalence of HPV-positive lymph nodes metastases ranged from 0%-19% in patients with non-oropharyngeal primary sites compared to 66%-87% in those whose primary tumour originated in the oropharynx.

*Aggregate-Evidence Quality and Potential for Bias*

While many studies have examined the HPV status of lymph node neck metastases in correlation with a known primary, only four have considered and reported on true unknown primaries. Unfortunately, the sample size of unknown primaries in these studies has been extremely small, ranging from 3 to 25 patients. As such, caution should be practiced when interpreting these results.

*Values of the Working Group*

A high value was ascribed to the detection of the primary tumour and the resultant reduction of morbidity that a localized treatment would offer.

*Considered Judgement*

The evidence indicates that there is a relationship between HPV positivity and whether the initial cancer arises in the oropharynx or not. As detection of the primary tumour offers a reduction of mortality due to the benefits of localized treatment, the additional diagnostic information provided by HPV status is sufficient to warrant routine testing of these tissues.

**Initial (DRAFT) Recommendation 2**

**It is recommended that the neck nodal tissue of patients with metastatic squamous cell carcinoma to neck nodes from an unknown head and neck primary be routinely tested for HPV status.**

*Optimal testing method:**Key Evidence for Benefits and Harms*

Recommendation 3 is based on a comparison of HPV diagnostic testing methods published in the literature. Nine retrospective cohort studies were included in this guideline. The evidence suggests that, in patients with OPSCC, the performance of the three main techniques - PCR-based amplification, DNA in ISH, and p16 IHC - is comparable.

- PCR amplification of HPV DNA showed a sensitivity of 97% and specificity of 87%
- DNA ISH showed a sensitivity that ranged from 83% to 88% and a specificity that ranged from 88% to 100%
- IHC staining for p16 showed a sensitivity and specificity that ranged from 89% to 100% and 38% to 94%, respectively

#### *Aggregate-Evidence Quality and Potential for Bias*

While the majority of included studies were retrospective cohorts, and the inherent limitations of retrospective designs should be taken into consideration, the collection of data did occur prospectively in all studies. The study population in just over half the included papers was comprised of patients selected in a consecutive fashion. The remaining papers did not report the sampling method. Outcome assessors were reported to be blinded to HPV status in 33% of studies, with the remaining 67% of studies not describing any such blinding.

#### *Values of the Working Group*

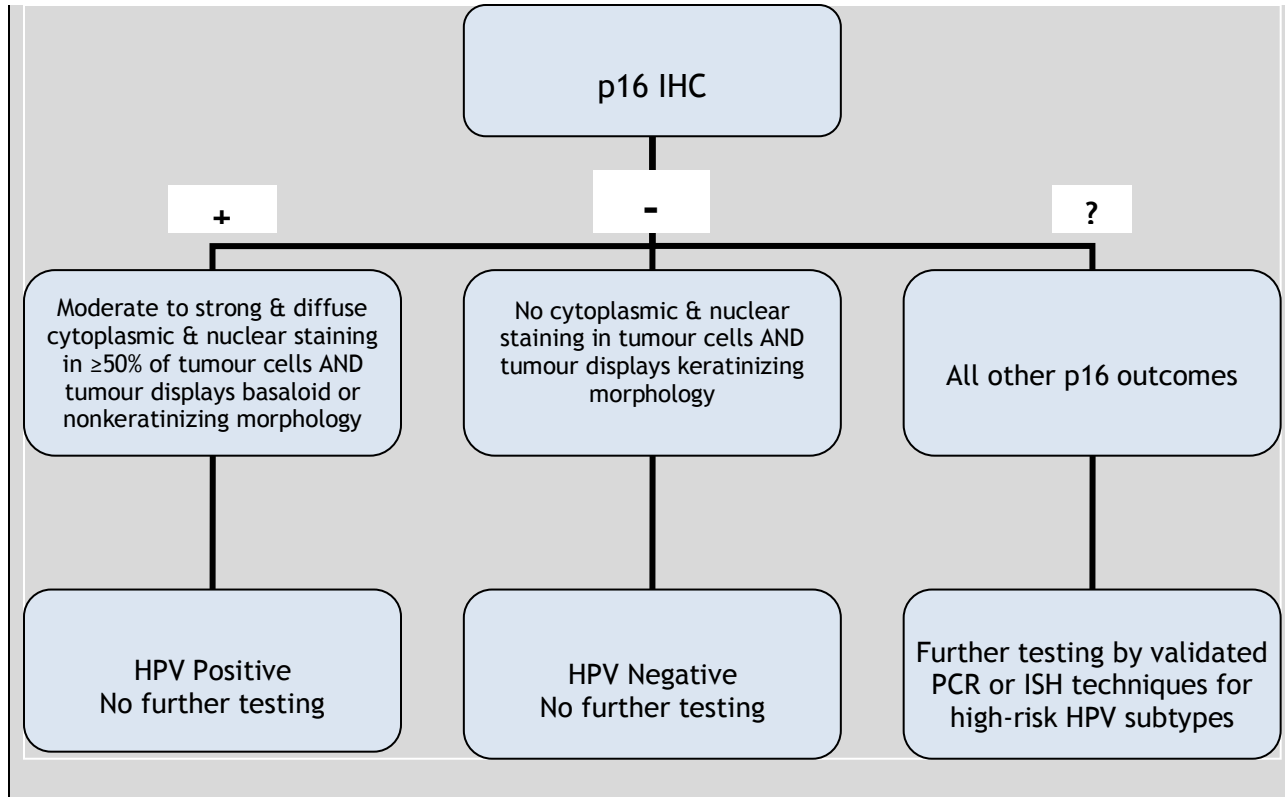
A high value was ascribed to practicality, availability, simplicity, and cost of the HPV testing method.

#### *Considered Judgement*

The current evidence suggests that PCR, DNA ISH, and IHC staining are all comparable. With no unequivocal evidence exclusively supporting any particular scheme, the Head & Neck Disease Site Group believes this scheme is practical, simple, and minimizes the impact of testing on available pathology resources and is appropriate until such time as further evidence becomes available.

#### **Initial (DRAFT) Recommendation 3**

- **It is recommended that HPV status in oropharyngeal SCC be initially determined using immunohistochemical (IHC) staining for p16.**
  - **IHC staining for p16 can be considered positive when the following three criteria are met:**
    - **cytoplasmic and nuclear staining**
    - **staining is moderate to strong and diffuse**
    - **staining is present in at least 50% of tumour cells**
- **A validated polymerase chain reaction (PCR) or in situ hybridization (ISH) technique for high-risk HPV subtypes may be necessary to confirm p16 results in selected cases according to the following algorithm:**



### INTERNAL REVIEW

Almost all PEBC documents undergo internal review. This review is conducted by the Expert Panel and the Report Approval Panel. The Working Group was responsible for incorporating the feedback and required changes of both of these panels, and both panels had to approve the document before it could be sent to External Review.

### Expert Panel Review and Approval

The Head and Neck Disease Site Group (DSG) acted as the Expert Panel for this document. The members of this group were required to submit conflict of interest declarations prior to reviewing the document. These declarations are described at the end of Section 2. The document must be approved by a formal vote. In order to be approved, 75% of the Head and Neck DSG membership must cast a vote or abstain, and of those that vote, 75% must approve the document. At the time of the voting, the Head and Neck DSG members could suggest changes to the document, and possibly make their approval conditional on those changes. In those cases, the Working Group was responsible for considering the changes, and if those changes could be made without substantially altering the recommendations, the altered draft would not need to be resubmitted for approval.

The Head and Neck DSG reviewed the document during the fall of 2012. During this review, the Head and Neck DSG unanimously approved the document and no changes were requested nor made.

On January 6, 2013, by email, the Head and Neck DSG formally approved the document by vote. Of the 14 members of the Head and Neck DSG (who were not part of the working group), 11 members cast votes, for a total of 79% response. Of those who cast votes, all 11 approved the document (100%).

### **Report Approval Panel Review and Approval**

The purpose of the Report Approval Panel (RAP) review is to ensure the methodological rigour and quality of PEBC documents. The RAP consists of nine clinicians with broad experience in clinical research and guideline development, and the Director of the PEBC. For each document, three RAP members review the document: the Director and two others. RAP members must not have had any involvement in the development of the guideline prior to Internal Review. All three RAP members must approve the document, although they may do so conditionally. If there is a conditional approval, the Working Group is responsible for ensuring the necessary changes are made, with the Assistant Director of Quality and Methods, PEBC, making a final determination that the RAP's concerns have been addressed.

In December 2012, the Report Approval Panel (RAP) reviewed and approved this document. Key issues raised by the RAP included the following:

1. The guideline development group would have benefitted from having a pathologist on board.
2. Methods for formulating the recommendations are not extensively described.
3. No procedure for updating the guideline is provided.
4. The potential resource implications of applying the recommendations have not been considered.
5. No advice and/or tools on how the recommendations can be put in place are included and should be in the discussion.

The Working Group made the following changes in response to the RAP review:

1. A staff pathologist (BPO) and medical director of surgical pathology at the University Health Network (UHN) who specializes in head and neck cancer and the director of molecular diagnostics (SKR) at the UHN were both included on the working group. Their names and affiliations now appear in Appendix 4.
2. The methods for formulating recommendations are described in Section 3.
3. The following statement has been added to the end of Sections 1 and 2: "All PEBC documents are maintained and updated as described in the PEBC Document Assessment and Review Protocol."
4. A formal cost analysis falls outside of the scope of this review. The DSG did, however, consider the cost implications when formulating the recommendations. This has now been explained in the discussion.
5. Implementation of the guideline is not part of the DSG mandate. Cancer Care Ontario assumes this role. This explanation has now been added to the discussion.

### **External Review by Ontario Clinicians and Other Experts**

The PEBC external review process is two-pronged and includes a targeted peer review that is intended to obtain direct feedback on the draft report from a small number of specified content experts and a professional consultation that is intended to facilitate dissemination of the final guidance report to Ontario practitioners.

Following approval of the document at Internal Review, the Head and Neck DSG circulated the draft document with recommendations modified as noted under Internal Review, above, to external review participants for review and feedback.

### ***Methods***

**Targeted Peer Review:** During the guideline development process, five targeted peer reviewers from Ontario, Canada and across the United States considered to be clinical and/or methodological experts on the topic were identified by the working group. Several weeks prior to completion of the draft report, the nominees were contacted by email and asked to serve as reviewers. Five reviewers agreed and the draft report and a questionnaire were sent via email for their review. The questionnaire consisted of items evaluating the methods, results, and interpretive summary used to inform the draft recommendations and whether the draft recommendations should be approved as a guideline. Written comments were invited. The questionnaire and draft document were sent out on February 5, 2013. Follow-up reminders were sent at two weeks (email) and at four weeks (telephone call). The Working Group reviewed the results of the survey.

**Professional Consultation:** Feedback was obtained through a brief online survey of health care professionals who are the intended users of the guideline. All clinicians in Ontario in the PEBC database whose discipline was categorized as pathology and laboratory medicine or head and neck were contacted by email to inform them of the survey. Participants were asked to rate the overall quality of the guideline (Section 1) and whether they would use and/or recommend it. Written comments were invited. Participants were contacted by email and directed to the survey website where they were provided with access to the survey, the guideline recommendations (Section 1) and the evidentiary base (Section 2). The notification email was sent on February 5, 2013. The consultation period ended on March 19, 2013. The Working Group reviewed the results of the survey. During the professional consultation phase, the PEBC was contacted by the College of American Pathologists (CAP) for an opportunity to also review the draft report. The draft report was provided to four CAP chairs, three of which provided written feedback.

### Results

**Targeted Peer Review:** Four responses were received from five reviewers. Key results of the feedback survey are summarized in Table 1.

**Table 1. Responses to nine items on the targeted peer reviewer questionnaire.**

	Reviewer Ratings (N=4)				
Question	Lowest Quality (1)	(2)	(3)	(4)	Highest Quality (5)
1. Rate the guideline development methods.			1	1	2
2. Rate the guideline presentation.*				1	2
3. Rate the guideline recommendations.			1	2	1
4. Rate the completeness of reporting.				2	2
5. Does this document provide sufficient information to inform your decisions? If not, what areas are missing?			1	2	1
6. Rate the overall quality of the guideline report.				3	1
	Strongly Disagree (1)	(2)	Neutral (3)	(4)	Strongly Agree (5)
7. I would make use of this guideline in my professional decisions.*			1	2	

8. I would recommend this guideline for use in practice.				4	
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\* Note: One response for both questions 2 and 7 were missing and, as such, totals in these row only total 3

#### 9. What are the barriers or enablers to the implementation of this guideline report?

Two reviewers suggested that HPV testing following p16 IHC could be potentially problematic for some centres in Canada and the USA. Specifically, the reviewers indicated that PCR can be tricky to perform and it lacks specificity. Furthermore, ISH may be challenging because the availability of HPV-16 specific probes is not assured.

#### *Summary of Written Comments*

The written comments received from the reviewers were predominantly favourable and included positive feedback on the quality of the report, the appropriateness of the recommendations, and the thoroughness of the analysis. The main points for consideration contained in the written comments were:

1. The need for a clarification that the recommendations do not apply to patients with non-oro-pharyngeal cancers.
2. Questioning of the 50% cutoff point for interpretation of p16 IHC expression in tumour.
3. No distinction made between biopsy and resection specimens in Recommendation 3.
4. There is a preference for concurrent rather than sequential p16 ISH testing because of the difficulties with secondary testing methods.
5. A note pointing out that many readers may not know the difference between HPV DNA PCR and quantitative real-time PCR, the latter of which significantly improves specificity. What should be avoided is qualitative HPV PCR assay detection alone.
6. No mention of commercial assays.
7. A suggestion that a specific recommendation be made that, specimens from the oropharynx be accompanied by a clear indication on the requisition for p16 testing.

#### *Modifications/Actions*

1. A statement was added clarifying that the recommendations only apply to patients with oropharyngeal cancers, which include cancers of the tonsil, base of tongue, soft palate, and associated pharyngeal walls.
2. No validated cutoff number currently exists, and the Working Group will continue to recommend a cutoff of  $\geq 50\%$  positive cells. As long as there is moderate to strong & diffuse cytoplasmic & nuclear staining in at least 50% of tumour cells, there is good positive predictive value with the presence of HPV. While the often used 70% cutoff is highly correlated with the presence of HPV in the tumor, the number is felt to be too restrictive and not supported by any existing data. As such, the Working Group did not make any modifications.
3. Recommendation 3 is applicable to biopsy or surgical resection specimens.
4. The recommended algorithm is believed to be both practical and simple, and it minimizes the impact of testing on available pathology resources. It also addresses the proficiencies that are most readily available in laboratories across the province. As such, no modifications were made.
5. A note specifying that qualitative HPV PCR assay detection alone should be avoided has now been added to the Qualifying Statement.
6. The Working Group prefers not to endorse any specific commercial assays.
7. The guideline recommends the testing be routinely performed in oropharyngeal specimens and, as such, specific requests are unnecessary.

*Professional Consultation:* Sixteen responses were received. Key results of the feedback survey are summarized in Table 2.

**Table 2. Responses to four items on the professional consultation survey.**

General Questions: Overall Guideline Assessment	Number (%)				
	Lowest Quality (1)	(2)	(3)	(4)	Highest Quality (5)
1. Rate the overall quality of the guideline report.	0	0	2 (12.5)	10 (62.5)	4 (25)
	Strongly Disagree (1)	(2)	(3)	(4)	Strongly Agree (5)
2. I would make use of this guideline in my professional decisions.	1 (6.3)	1 (6.3)	2 (12.5)	7 (43.8)	5 (31.3)
3. I would recommend this guideline for use in practice.	1 (6.3)	1 (6.3)	3 (18.8)	4 (25)	7 (43.8)

**What are the barriers or enablers to the implementation of this guideline report?**

The main barriers mentioned by the respondents were:

- Cost, available funding and resources for testing may vary by cancer centre
- Sufficient samples for testing may not be available for all patients
- Possible delays to therapy if re-biopsies are needed for testing

### ***Summary of Written Comments***

The written comments summarized below include responses from the Professional Consultation survey and the feedback received from the CAP chairs review. The main points contained in the written comments were:

1. Given limited funding, it may be more prudent to wait until there are actual treatment differences.
2. Clarification is needed as to whether the p16 IHC is to be performed on the initial biopsy or the excisional specimen. If at the initial biopsy, then community hospital pathologists will need guidance on how big a biopsy is required to minimize sampling issues. How many cells are considered an adequate biopsy to ensure appropriate representation of the entire tumour and a prediction of p16+ is not clear.
3. Recommendation 1 and 3 should be combined into one.
4. It is not clear that p16 positivity always equates (100%) with p16 positivity in the lymph node mets. It might be worthwhile to state what percentage of lymph node mets are p16 positive when the primary is p16 positive.
5. Clarification on the role of cytology for p16/ISH testing is needed. If cytology is to be used, then guidance on an appropriate protocol is required.
6. There should be a mention of what validated PCR/ISH techniques are recommended.
7. It would be helpful to define oropharynx in the document.
8. p16 is an extremely valuable prognostic marker, but is not highly specific for HPV infection in this context. A brief explanation of the fact that not all p16-positive squamous cell carcinomas of the H&N are HPV-driven should be given.
9. The guideline does not address any quality assurance issues that could affect the accuracy of the results.



***Modifications/Actions***

1. As stated in our Justification for Recommendation 1, even though at this time no recommendation can be made to base clinical management decisions on HPV status, the Head and Neck DSG felt that the prognostic benefits of HPV testing are valuable and sufficient to warrant routine testing. As such, no modification was made.
2. Any recommendation regarding biopsy size would not be evidence based. The potential for sampling bias is always present in biopsies. For this reason biopsies should be read by pathologists with experience with the tests. Again what constitutes a pathologist with experience with p16 is unclear.
3. Recommendation 1 and 3 were derived from two separate research questions. As such, no modifications were made.
4. The literature suggests that overexpression of p16 in metastatic sites can be a reliable surrogate for the identification of hidden oropharyngeal primary tumours in patients with an unknown primary.
5. The performance of p16 and ISH should be limited to cytology samples in which cell blocks are available and should be performed with protocols similar to biopsies.
6. The Working Group prefers not to endorse any specific PCR/ISH techniques. Any testing, however, should be conducted under strict QA/QC to ensure test accuracy.
7. As mentioned above, a statement was added clarifying that the recommendations only apply to patients with oropharyngeal cancers, which include cancers of the tonsil, base of tongue, soft palate, and associated pharyngeal walls.
8. The recommendation only applies to p16 testing in oropharyngeal SCC, not all HNSCC.
9. The following has now been added to the Qualifying Statement for Recommendation 3: “The Head & Neck DSG considers quality assurance and quality control in HPV-status testing to be paramount. As such, all testing should be carried out in licensed and accredited laboratories, and test results should be interpreted by experienced pathologists/scientists. Laboratories need to follow proper quality control and participate in external proficiency testing to ensure test accuracy. Further discussion of specific quality and proficiency parameters necessary for individual laboratories performing HPV-status testing is beyond the scope of this guideline.”

**Conclusion**

This EBS report reflects the integration of feedback obtained through the external review process with final approval given by the Head and Neck DSG and the Report Approval Panel of the PEBC. Updates of the report will be conducted in accordance with the PEBC Document Assessment and Review Protocol.

**Conflict of Interest**

In accordance with the PEBC Conflict of Interest (COI) Policy, the guideline authors, Head and Neck DSG members, and internal and external reviewers were asked to disclose potential conflicts of interest. The authors, members, and reviewers reported that they had no conflicts of interest.

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Evidence-Based Series 5-9 Version 2: Section 4

**Routine HPV Testing in Head and Neck Squamous Cell Carcinoma**

**Document Review Summary**

*B. Perez-Ordenez, R. Poon, and Members of the Expert Panel on HPV Testing in Head and Neck Squamous Cell Carcinoma*

January 13, 2020

*The 2013 guideline recommendations are*

***ENDORSED***

*This means that the recommendations are still current and relevant for decision making*

**OVERVIEW**

The original version of this guidance document was released by Cancer Care Ontario's Program in Evidence-based Care in 2013.

In December 2017, this document was assessed in accordance with the PEBC Document Assessment and Review Protocol and was determined to require a review. As part of the review, a PEBC methodologist (RP) conducted an updated search of the literature. A clinical expert (B. P-O) reviewed and interpreted the new eligible evidence and proposed the existing recommendations could be endorsed with a minor revision. The Expert Panel on HPV Testing in Head and Neck Squamous Cell Carcinoma (See Appendix 1 for membership) endorsed the recommendations found in Section 1 (Clinical Practice Guideline) on January 13, 2020.

**DOCUMENT ASSESSMENT AND REVIEW RESULTS**

**Questions Considered**

1. What is the relationship between HPV positivity and outcome in head and neck squamous cell carcinomas (HNSCC)?
2. In which head and neck subsites is the prevalence of HPV-associated squamous cell carcinoma high enough to justify routine testing of HPV positivity?

3. What is the diagnostic and prognostic value of routine testing of HPV status in patients with neck nodal metastatic squamous cell carcinoma from an unknown head and neck primary?
4. What is the optimal testing method for the identification of HPV positivity in head and neck squamous cell carcinomas (HNSCC)?

### Literature Search and New Evidence

The new search (April 2013 to February 2019) yielded 5 RCTs, and 26 non-randomized studies. See Appendix 2 for the search strategy. An additional search for ongoing studies on clinicaltrials.gov yielded 3 potentially relevant ongoing trials. Brief results of these publications are shown in the Document Summary and Review Tool.

### Impact on the Guideline and Its Recommendations

The new data supports existing recommendations. However, a small modification to the recommendation on immunohistochemical (IHC) staining for p16 to determine HPV status in oropharyngeal squamous cell carcinomas was suggested by the clinical expert based on most current evidence using  $\geq 70\%$  staining in the tumour cells as the cutoff value for p16 positive. The College of American Pathologists [32] and the American Society of Clinical Oncology [33] have also recommended  $\geq 70\%$  cutoff for p16 IHC. The Expert Panel therefore has changed the tumour cell threshold for staining being present from “at least 50%” to “at least 70%.”

### Recommendation 3:

It is recommended that HPV status in oropharyngeal SCC be initially determined using immunohistochemical (IHC) staining for p16.

IHC staining for p16 can be considered positive when the following three criteria are met:

- cytoplasmic and nuclear staining
- staining is moderate to strong and diffuse
- staining is present in at least 70% of tumour cells

With this modification, the Expert Panel on HPV Testing in Head and Neck Squamous Cell Carcinoma **ENDORSED** the 2013 recommendations on routine HPV testing in head and neck squamous cell carcinoma.

### Document Summary and Review Tool

Number and Title of Document under Review	5-9 Routine HPV Testing in Head and Neck Squamous Cell Carcinoma
Current Report Date	May 13, 2013
Date Assessed (by DSG or Clinical Program Chairs)	December 1, 2017
Health Research Methodologist	Raymond Poon
Clinical Expert	Dr. Bayardo Perez-Ordóñez
Approval Date and Review Outcome (once completed)	<b>ENDORSE</b>
Original Question(s):	
<ol style="list-style-type: none"> <li>1. What is the relationship between HPV positivity and outcome in head and neck squamous cell carcinomas (HNSCC)?</li> <li>2. In which head and neck subsites is the prevalence of HPV-associated squamous cell carcinoma high enough to justify routine testing of HPV positivity?</li> </ol>	

3. What is the diagnostic and prognostic value of routine testing of HPV status in patients with neck nodal metastatic squamous cell carcinoma from an unknown head and neck primary?
4. What is the optimal testing method for the identification of HPV positivity in head and neck squamous cell carcinomas (HNSCC)?

Target Population:

Adult patients with squamous cell carcinomas arising in oropharynx, larynx, hypopharynx, nasopharynx, sinonasal tract, or oral cavity subsites or an unknown primary head and neck site.

Study Selection Criteria:

**Inclusion Criteria**

Articles were eligible for inclusion in this systematic review of the evidence if they met the following criteria:

*HPV Positivity*

- Full reports or abstracts of phase III randomized controlled trials that evaluated tumour HPV status and clinical outcome.
- Studies that included adult patients with squamous cell carcinomas arising in the oropharynx, larynx, hypopharynx, nasopharynx, sinonasal tract, or oral cavity.
- Results were reported for one or more of the following outcomes: overall survival, disease-free survival, disease-specific survival or progression-free survival.

*Prevalence*

- Studies that included a minimum of 50 cases of HNSCC.
- Testing that included a clearly described detection method of interest.
- Prevalence of HPV-associated tumours for any of the following subsites is reported: oropharynx, larynx, hypopharynx, nasopharynx, sinonasal tract or oral cavity.

*Unknown Primaries*

- Studies that included a minimum of 20 cases of nodal metastatic squamous cell carcinoma from an unknown head and neck primary.
- Testing that included a clearly described detection method of interest.
- Results were reported for one or more of the following outcomes: prevalence of HPV-associated metastatic squamous cell carcinoma, correlation between HPV positivity and later detection of the primary tumour, or the sensitivity and specificity of a test for a diagnosis of an oropharyngeal tumour.

*Testing*

- Comparative studies that evaluated the following HPV detection methods: p16 immunohistochemistry (IHC), polymerase chain reaction (PCR), or in situ hybridization (ISH).
- Concordance between detection methods or sensitivity and specificity of the detection method are reported or enough information is provided to allow for the calculation of these outcomes, using PCR for high-risk HPV as the gold standard comparator.

**Exclusion Criteria**

Articles published in languages other than English were excluded because of limited translation resources.

Search Details:

- April 2013 to February 28, 2019 (MEDLINE, EMBASE, Cochrane Database of Systematic Reviews)
- 2010 to 2019 (the proceedings of the meetings of ASCO and ESTRO)

Summary of new evidence:

Of the 1252 total hits from MEDLINE, EMBASE and Cochrane Database of Systematic Reviews + 41 hits from ASCO + 12 hits from ESTRO, 31 references were identified. An additional search for ongoing studies on clinicaltrials.gov yielded 3 potentially relevant ongoing trials.

Clinical Expert Interest Declaration:

Dr. Perez-Ordóñez declared no conflict of interest.

1. Does any of the newly identified evidence contradict the current recommendations? (i.e., the current recommendations may cause harm or lead to unnecessary or improper treatment if followed)	No
2. Does the newly identified evidence support the existing recommendations?	Yes
3. Do the current recommendations cover all relevant subjects addressed by the evidence? (i.e., no new recommendations are necessary)	Yes
<b>Review Outcome as recommended by the Clinical Expert</b>	ENDORSE
<i>If the outcome is UPDATE, are you aware of trials now underway (not yet published) that could affect the recommendations?</i>	NA
<b>DSG/GDG Commentary</b>	

**Evidence Tables****HPV status and clinical outcome from RCTs**

Study	Tumour site	HPV status (n)	Outcomes
Zackrisson et al, 2015 [1] (ARTSCAN)	Oropharynx, larynx, oral cavity, and hypopharynx	HPV+=153 vs. HPV-=53	<ul style="list-style-type: none"> <li>Cancer-specific survival at 5 years               <ul style="list-style-type: none"> <li>80.4% vs. 51.2%; HR=0.36; 95% CI: 0.21 to 0.61; p&lt;0.0001</li> </ul> </li> <li>Overall survival at 5 years               <ul style="list-style-type: none"> <li>75.8% vs. 34.0%; HR=0.32; 95% CI: 0.21 to 0.49; p&lt;0.0001</li> </ul> </li> </ul>
Seiwert et al, 2016 [2]	Hypopharynx, larynx, nasal cavity, nasopharynx, oral cavity, oropharynx, and unknown	HPV+=47 vs. HPV-=56	<ul style="list-style-type: none"> <li>Overall survival at 5 years               <ul style="list-style-type: none"> <li>91.3% vs. 72.5%</li> </ul> </li> <li>Progression-free survival at 5 years               <ul style="list-style-type: none"> <li>84.4% vs. 65.9%</li> </ul> </li> </ul>
Rosenthal et al, 2016 [3]	Oropharynx, hypopharynx, and larynx	HPV+=75 vs. HPV-=107	<ul style="list-style-type: none"> <li>Overall survival at 3 years treated with radiotherapy alone               <ul style="list-style-type: none"> <li>72.3% vs. 33.5%; HR=0.40; 95% CI: 0.21 to 0.74</li> </ul> </li> <li>Progression-free survival at 3 years treated with radiotherapy alone               <ul style="list-style-type: none"> <li>64.7% vs. 15.6%; HR=0.30; 95% CI: 0.16 to 0.57</li> </ul> </li> <li>Overall survival at 3 years treated with radiotherapy plus cetuximab               <ul style="list-style-type: none"> <li>87.8% vs. 41.9%; HR=0.16; 95% CI: 0.07 to 0.36</li> </ul> </li> <li>Progression-free survival at 3 years treated with radiotherapy plus cetuximab               <ul style="list-style-type: none"> <li>82.1% vs. 29.1%; HR=0.18; 95% CI: 0.08 to 0.40</li> </ul> </li> </ul>
Nguyen-Tan et al, 2014 [4] (RTOG 0129)	Oral cavity, oropharynx, hypopharynx, larynx	HPV+=206 vs. HPV-=117	<ul style="list-style-type: none"> <li>Overall survival at 8 years               <ul style="list-style-type: none"> <li>70.9% vs. 30.2%; HR=0.34; 95% CI: 0.22 to 0.52; p&lt;0.001</li> </ul> </li> <li>Progression-free survival at 8 years               <ul style="list-style-type: none"> <li>64.0% vs. 23.3%; HR=0.43; 95% CI: 0.29 to 0.64; p&lt;0.001</li> </ul> </li> </ul>
Gilbert et al, 2015 [5]	Oral cavity, oropharynx, larynx, hypopharynx, paranasal sinus, unknown primary, nasopharynx	HPV+=9 vs. HPV-=35	<ul style="list-style-type: none"> <li>Median overall survival               <ul style="list-style-type: none"> <li>5.2 months vs. 5.9 months; p=0.39</li> </ul> </li> <li>Median progression-free survival               <ul style="list-style-type: none"> <li>1.6 months vs. 3.7 months; p=0.03</li> </ul> </li> </ul>

**Prevalence of HPV in HNSCC and subsites**

Study	Continent or country	No. of studies	Tumour site	No. of cases	No. of HPV+	HPV detection method	Prevalence
Mehanna et al, 2013 [6]	Europe, North America, other,	102	Oropharyngeal	5396	NR	PCR, ISH	47.7% (95% CI: 42.9 to 52.5)

Study	Continent or country	No. of studies	Tumour site	No. of cases	No. of HPV+	HPV detection method	Prevalence
	unknown, and mixed regions	236	Non-oropharyngeal	13972	NR		21.8% (95% CI: 18.9 to 25.1)
Haeggbloom et al, 2017 [7]	India, Switzerland, Italy, Spain, USA, UK, Sweden, Japan, Belgium, Canada, Netherlands, Australia, South Korea, New Zealand, China, Norway, France, Germany, Slovenia, Turkey	64	Tonsil and base of tongue	9719	NR	PCR, ISH, IHC	56% (95% CI: 55 to 57)
			Soft palate, uvula, walls of oropharynx, other	1991	NR		19% (95% CI: 17 to 20)
Gama et al, 2015 [8]	North America, Central and South America, Europe, other Asia and Pacific, Africa and Middle East, mixed regions, China	179	Larynx	7347	1830	PCR, IHC, DB, SB, ISH, FISH, NISH, HCII, CISH	26.9% (95% CI: 24.2 to 29.7)
Zhang et al, 2016 [9]	China	19	Larynx	964	379	PCR, ISH, IHC, WB, FISH	32% (95% CI: 22 to 44)
Shaikh et al, 2015 [10]	India, Pakistan, Bangladesh, Sri Lanka, Malaysia, Thailand, China, Hong Kong, Taiwan, South Korea, Japan, Australia	47	Oral cavity	3153	NR	PCR, SB, ISH, ICC, IHC	37.6% (95% CI: 35.9 to 39.2)
		26	Oropharynx	2768	NR		40.5% (95% CI: 38.7 to 42.4)
		19	Larynx	856	NR		23.6% (95% CI: 22.1 to 25.0)
Ndiaye et al, 2014 [11]	Asia, Central and South America, Europe, North America, Africa, Oceania	72	Oral cavity	5478	1360	PCR	24.2% (95% CI: 18.7 to 30.2)
		53	Oropharynx	3946	1828		45.8% (95% CI: 38.9 to 52.9)
		54	Larynx and hypopharynx	2739	649		22.1% (95% CI: 16.4 to 28.3)
Ragin et al, 2017 [12]	Europe, Asia, USA, Australia	6	Oropharynx	146	NR	PCR, ISH,	31.5% (95% CI: 17.7 to 47.1)



Study	Continent or country	No. of studies	Tumour site	No. of cases	No. of HPV+	HPV detection method	Prevalence
		6	Non- oropharynx	337	NR		14.5% (95% CI: 1.4 to 36.0)

#### HPV in neck nodal tissue of patients with metastatic SCC

Study	Patient population	Tissue samples	No. of cases	Primary tumour site	Prev of HPV+ in node mets	Testing method	Sensitivity, specificity, correlation, notes
Kobayashi et al, 2014 [13]	HNSCCUP with lymph node metastases	FFPE	33	Oropharynx=7, hypopharynx=4, larynx=2, maxilla=1, unknown=19	24%	IHC, ISH	Of the 8 (24%) patients with p16+ metastases, 5 (63%) had a primary lesion in the oropharynx. p16+ lymph node metastasis is significantly correlated with an occult primary lesion in the oropharynx (p<0.01).
Vent et al, 2013 [14]	CUP of the neck with lymph node metastases	FFPE	47	Oropharynx=11, bronchial=4, nasopharynx=1, larynx=1, oral cavity=1, parotid gland=1, esophagus=1, unknown=27	24.3% in SCC	IHC, PCR	In HPV-positive lymph node metastases, the primary tumour was more frequently detected (p=0.048) and more frequently found in the oropharynx (p=0.009).

#### Comparison of HPV detection methods

Study	No. of cases	Patient population	Tissue samples	Testing method (definition of positive result)	Sensitivity, specificity, concordance or correlation
Rosenthal et al, 2016 [3]	63	OPSCC	FFPE	1) p16 IHC (strong and diffuse nuclear and cytoplasmic staining $\geq 70\%$ of the tumour cells) 2) HPV ISH (specific staining of tumour cell nuclei for HPV)	<ul style="list-style-type: none"> <li>There was 78% concordance between p16+ and HPV+ tumours.</li> </ul>

Study	No. of cases	Patient population	Tissue samples	Testing method (definition of positive result)	Sensitivity, specificity, concordance or correlation
Riener et al, 2017 [15]	156	HNSCC	FFPE	1) IMP3 IHC (moderate to strong staining in $\geq 25\%$ of cells) 2) p16 IHC (strong nuclear and cytoplasmic staining in $> 50\%$ of cells) 3) Combination IMP3/p16 4) HPV DNA PCR	<ul style="list-style-type: none"> <li>• IMP3 <ul style="list-style-type: none"> <li>• Sensitivity=47%</li> <li>• Specificity=13%</li> </ul> </li> <li>• P16 <ul style="list-style-type: none"> <li>○ Sensitivity=63%</li> <li>○ Specificity=88%</li> <li>○ Significantly associated with HPV status (<math>p=0.017</math>)</li> </ul> </li> <li>• IMP3/p16 <ul style="list-style-type: none"> <li>○ Sensitivity=13%</li> <li>○ Specificity=77%</li> </ul> </li> </ul>
Tan et al, 2016 [16]	159	HNSCC	FFPE	1) p16 IHC ( $\geq 80\%$ of the tumour cells showed both cytoplasmic and nuclear p16INK4a staining) 2) HPV DNA GP5+/6+ PCR	<ul style="list-style-type: none"> <li>• There was 94.7% concordance between p16 IHC and HPV DNA GP5+/6+ PCR.</li> </ul>
Rietbergen et al, 2013 [17]	86	OPSCC	FFPE and fresh-frozen	1) p16 IHC + HPV DNA GP5+/6+ PCR (moderate to strong diffuse nuclear and cytoplasmic staining in $> 70\%$ of the carcinoma tissue) 2) HPV E6 mRNA RT-PCR	<ul style="list-style-type: none"> <li>• p16 IHC + HPV DNA GP5+/6+ PCR <ul style="list-style-type: none"> <li>○ Sensitivity=96%</li> <li>○ Specificity=98%</li> </ul> </li> </ul>
Meng et al, 2018 [18]	1470	OPSCC	FFPE	1) p16 IHC (strong and diffuse nuclear and cytoplasmic staining $\geq 80\%$ of the tumour cells) 2) HPV DNA PCR	<ul style="list-style-type: none"> <li>• p16 IHC <ul style="list-style-type: none"> <li>○ Sensitivity=100%</li> <li>○ Specificity=96%</li> </ul> </li> <li>• HPV status was significantly correlated with p16 overexpression.</li> </ul>
Ramshankar et al, 2014 [19]	167	OTSCC	FFPE	1) p16 IHC (intense nuclear and cytoplasmic staining in $> 50\%$ of tumour cells) 2) HPV DNA GP5+/6+ and SPF 10 consensus PCR 3) HPV16 E2/E6 qPCR	<ul style="list-style-type: none"> <li>• p16 IHC <ul style="list-style-type: none"> <li>○ Sensitivity=53%</li> <li>○ Specificity=50%</li> </ul> </li> <li>• There was 12.3% concordance between p16 IHC and HPV DNA GP5+/6+ and SPF 10 consensus PCR (<math>\kappa &lt; 0.2</math>).</li> </ul>
Young et al, 2015 [20]	307	LSCC	FFPE	1) p16 IHC (moderate or strong staining in $\geq 30\%$ of tumour cells) 2) HPV E6/E7 mRNA ISH (brown punctate cytoplasmic signals)	<ul style="list-style-type: none"> <li>• HPV E6/E7 mRNA ISH was significantly correlated with p16 IHC (<math>p &lt; 0.001</math>).</li> </ul>
Fonmarty et al, 2015 [21]	71	OPSCC	FFPE	1) p16 IHC (at pathologist's discretion) 2) HPV DNA PCR	<ul style="list-style-type: none"> <li>• There was 81.7% concordance between p16 IHC and HPV DNA PCR (<math>\kappa = 0.615</math>).</li> </ul>

Study	No. of cases	Patient population	Tissue samples	Testing method (definition of positive result)	Sensitivity, specificity, concordance or correlation
Meshman et al, 2017 [22]	31	LSCC and HPSCC	NR	1) p16 IHC (nuclear and cytoplasmic staining >70% of the cells) 2) HPV ISH (nuclear-specific staining)	<ul style="list-style-type: none"> <li>• p16 IHC               <ul style="list-style-type: none"> <li>○ Sensitivity=100%</li> <li>○ Specificity=52.9%</li> </ul> </li> </ul>
Liu et al, 2015 [23]	185	OPSCC	FFPE	1) p16 IHC (nuclear and cytoplasmic staining $\geq$ 50% of tumour cells) 2) HPV16 E6/E7 DNA PCR 3) HPV DNA PCR	<ul style="list-style-type: none"> <li>• p16 IHC               <ul style="list-style-type: none"> <li>○ Sensitivity=92%</li> <li>○ Specificity=92%</li> </ul> </li> <li>• There was 92% concordance between p16 IHC and HPV DNA PCR.</li> <li>• There was 89% concordance between p16 IHC and HPV16 E6/E7 DNA PCR.</li> </ul>
Salazar et al, 2014 [24]	163	HNSCC	FFPE	1) p16 IHC (>50% of tumour cells presented with a strong nuclear stain) 2) HPV16 E6/E7 mRNA PCR 3) HPV16 MY09/11/HMB01 DNA PCR	<ul style="list-style-type: none"> <li>• There was moderate agreement between p16 IHC and HPV16 E6/E7 mRNA PCR (kappa=0.64).</li> <li>• There was moderate agreement between p16 IHC and HPV16 MY09/11/HMB01 DNA PCR (kappa=0.63).</li> </ul>
Schache et al, 2013 [25]	78	OPSCC	FFPE and fresh-frozen	1) HR-HPV RNAScope (strong staining in the majority of cells in the section) 2) p16 IHC (strong and diffuse nuclear and cytoplasmic staining in $\geq$ 70% of the tumour and an <i>H</i> score of >60) 3) HR-HPV DNA ISH (any detectable chromogen in any of the malignant cells) 4) DNA qPCR 5) Combined p16 IHC/HR-HPV DNA ISH 6) Combined p16 IHC/DNA qPCR	<ul style="list-style-type: none"> <li>• HR-HPV RNAScope               <ul style="list-style-type: none"> <li>○ Sensitivity=97%</li> <li>○ Specificity=93%</li> </ul> </li> <li>• p16 IHC               <ul style="list-style-type: none"> <li>○ Sensitivity=97%</li> <li>○ Specificity=82%</li> </ul> </li> <li>• HR-HPV DNA ISH               <ul style="list-style-type: none"> <li>○ Sensitivity=94%</li> <li>○ Specificity=91%</li> </ul> </li> <li>• DNA qPCR               <ul style="list-style-type: none"> <li>○ Sensitivity=91%</li> <li>○ Specificity=87%</li> </ul> </li> <li>• Combined p16 IHC/HR-HPV DNA ISH               <ul style="list-style-type: none"> <li>○ Sensitivity=94%</li> <li>○ Specificity=91%</li> </ul> </li> <li>• Combined p16 IHC/DNA qPCR               <ul style="list-style-type: none"> <li>○ Sensitivity=91%</li> <li>○ Specificity=93%</li> </ul> </li> </ul>
Walline et al, 2013 [26]	338	HNSCC	FFPE	1) p16 IHC (moderate to high intensity nuclear and cytoplasmic staining in $\geq$ 51% of tumour cells) 2) HPV ISH	<ul style="list-style-type: none"> <li>• p16 IHC               <ul style="list-style-type: none"> <li>○ Sensitivity=94.2%</li> <li>○ Specificity=85.5%</li> </ul> </li> <li>• HPV ISH               <ul style="list-style-type: none"> <li>○ Sensitivity=82.9%</li> </ul> </li> </ul>

Study	No. of cases	Patient population	Tissue samples	Testing method (definition of positive result)	Sensitivity, specificity, concordance or correlation
				3) HPV E6 DNA PCR-MassArray 4) HPV L1 PGMY DNA PCR (consensus PCR)	<ul style="list-style-type: none"> <li>○ Specificity=81.0%</li> <li>• HPV E6 DNA PCR-MassArray               <ul style="list-style-type: none"> <li>○ Sensitivity=99.5%</li> <li>○ Specificity=100%</li> </ul> </li> </ul>
Lingen et al, 2013 [27]	409	OCSCC	FFPE	1) p16 IHC (an <i>H</i> score of $\geq 60$ ) 2) HR-HPV E6/7 mRNA qRT-PCR	<ul style="list-style-type: none"> <li>• p16               <ul style="list-style-type: none"> <li>○ Sensitivity=79.2%</li> <li>○ Specificity=93.0%</li> </ul> </li> </ul>
Hooper et al, 2015 [28]	87	OSCC and OPSCC	FFPE and fresh-frozen	1) HR-HPV Hybrid Capture 2 (a RLU/CO value $>1$ ) 2) HR-HPV Cervista (a fluorescent signal) 3) HPV E6/7 DNA PCR 4) p16 IHC ( $\geq 10\%$ of tumours with strong diffuse staining) 5) Agreement between at least 2 of Capture 2, Cervista, or PCR (gold standard)	<ul style="list-style-type: none"> <li>• HR-HPV Hybrid Capture 2               <ul style="list-style-type: none"> <li>○ Sensitivity=100%</li> <li>○ Specificity=100%</li> </ul> </li> <li>• HR-HPV Cervista               <ul style="list-style-type: none"> <li>○ Sensitivity=100%</li> <li>○ Specificity=100%</li> </ul> </li> <li>• HPV E6/7 DNA PCR               <ul style="list-style-type: none"> <li>○ Sensitivity=94%</li> <li>○ Specificity=100%</li> </ul> </li> <li>• p16 IHC               <ul style="list-style-type: none"> <li>○ Sensitivity=92%</li> <li>○ Specificity=90%</li> </ul> </li> </ul>
Duncan et al, 2013 [29]	81	OSCC	NDPE	1) p16 IHC (medium-intensity cytoplasmic staining with or without nuclear staining in 10% to 50% of tumor cells or strong diffuse nuclear and cytoplasmic staining in $>50\%$ of tumour cells) 2) HPV DNA PCR	<ul style="list-style-type: none"> <li>• p16 IHC               <ul style="list-style-type: none"> <li>○ Sensitivity=50%</li> <li>○ Specificity=100%</li> </ul> </li> <li>• There was strong correlation between p16 IHC and HPV DNA PCR (<math>r=0.77</math>).</li> </ul>
Drumheller et al, 2019 [30]	27	HNSCC	FFPE	1) p16 IHC (strong and diffuse staining present in $>70\%$ of tumour cells, involving the nuclei and cytoplasm) 2) RNA ISH (presence of brown punctate dots in the nucleus and/or cytoplasm of malignant cells)	<ul style="list-style-type: none"> <li>• There was 88.9% concordance between p16 IHC and RNA ISH.</li> </ul>
Prigge et al, 2017 [31]	24 studies	OPSCC	FFPE	1) p16 IHC (varied among included studies) 2) HPV DNA PCR 3) HPV DNA ISH 4) Combined p16 IHC/HPV DNA PCR 5) HPV E6/7 mRNA PCR (gold standard)	<ul style="list-style-type: none"> <li>• p16 IHC               <ul style="list-style-type: none"> <li>○ Pooled sensitivity=94%</li> <li>○ Pooled specificity=83%</li> </ul> </li> <li>• HPV DNA PCR               <ul style="list-style-type: none"> <li>○ Pooled sensitivity=98%</li> <li>○ Pooled specificity=84%</li> </ul> </li> <li>• HPV DNA ISH</li> </ul>

Study	No. of cases	Patient population	Tissue samples	Testing method (definition of positive result)	Sensitivity, specificity, concordance or correlation
					<ul style="list-style-type: none"> <li>○ Pooled sensitivity=85%</li> <li>○ Pooled specificity=88%</li> <li>• Combined p16 IHC/HPV DNA PCR</li> <li>○ Pooled sensitivity=93%</li> <li>○ Pooled specificity=96%</li> </ul>

**Abbreviations:**

CI, confidence interval; CISH, chromogene in situ hybridization; CUP, cancer of unknown primary DB, dot blot hybridization; DNA, deoxyribonucleic acid; FFPE, formalin-fixed, paraffin-embedded; FISH, filter in situ hybridization; HCII, hybrid capture II; HNSCC, head and neck squamous cell carcinoma; HNSCCUP: head and neck squamous cell carcinoma of an unknown primary site; HPSCC, hypopharyngeal squamous cell carcinoma; HPV, human papillomavirus; HR, hazard ratio; HR-HPV: high risk human papillomavirus; ICC, immunocytochemistry; IHC, immunohistochemistry; IMP3: insulin-like growth factor II mRNA binding protein 3; ISH, in situ hybridization; LSCC, laryngeal squamous cell carcinoma; mRNA, messenger ribonucleic acid; NDPE, nondecalcified paraffin-embedded; NISH, non-isotopic in situ hybridization; NR, not reported; OSCC, oral cavity squamous cell carcinoma; OPSCC, oropharyngeal squamous cell carcinoma; OSCC, oral squamous cell carcinoma; OTSCC, oral tongue squamous cell carcinoma; PCR, polymerase chain reaction; qPCR: quantitative polymerase chain reaction; qRT, quantitative reverse transcription; RLU/CO, relative light unit/cutoff; RT-PCR: reverse transcription polymerase chain reaction; SB, southern blot hybridization; SCC: squamous cell carcinoma; WB, western blot

**Ongoing Trials**

Interventions	Official Title	Status	Protocol ID	Estimated primary completion date	Last updated
Reduced radiotherapy + Pacitaxel/Cisplatin  vs.  Standard radiotherapy + 5- Fluorouracil/Cisplatin	Randomised Phase-III-trial of Simultaneous Radiochemotherapy (RCT) of Locally Advanced Head and Neck Cancer in the Stages III and IV A-B: Comparing Dose Reduced Radiotherapy (63,6 Gy) With Paclitaxel/Cisplatin to Standard Radiotherapy (70,2 Gy) With 5-Fluorouracil/Cisplatin	Unknown	NCT01126216	February 2015	August 11, 2017
Resection + adjuvant radio(chemo)therapy  vs.	Comparative Effectiveness Trial of Transoral Head and Neck Surgery Followed by Adjuvant Radio(Chemo)Therapy Versus Primary	Recruiting	NCT03691441	June 5, 2023	July 9, 2019

Primary radio(chemo)therapy + salvage neck dissection	Radiochemotherapy for Oropharyngeal Cancer				
cetuximab + radiation therapy vs. durvalumab + radiation therapy	Randomized Phase II/III Trial of Radiotherapy With Concurrent MEDI4736 (Durvalumab) vs. Radiotherapy With Concurrent Cetuximab in Patients With Locoregionally Advanced Head and Neck Cancer With a Contraindication to Cisplatin	Recruiting	NCT03258554	December 31, 2025	September 12, 2019

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**Appendix 1. Members of the Expert Panel**

<b>Name</b>	<b>Affiliation</b>	<b>Conflict of Interest Declaration</b>
Kevin Higgins	Head & Neck Surgeon Sunnybrook Hospital, Toronto	None declared
Ken Schneider	Radiation Oncologist Windsor	None declared
John Waldron	Radiation Oncologist Princess Margaret Cancer Centre, Toronto	None declared
Bret Wehrli	Pathologist London	None declared
Eric Winkvist	Medical Oncologist London	None declared
John Yoo	Head & Neck Surgeon London	On the board of directors of Cotting Pharmaceuticals. This is a drug development company in clinical stage. The primary agent is a novel molecule that targets P53 mutations. The company is publicly traded. I have received stock options only and no salary. I came off the board in September, 2019 and am no longer an insider.

## Appendix 2. Search Strategy

### Medline

1. exp "head and neck neoplasms"/
2. exp \*carcinoma, squamous cell/
3. HNSCC.ab,mp,tw.
4. (oropharyngeal adj2 (cancer? or carcinoma? or neoplasm? or tumor? or malignan\$)).mp,tw.
5. (laryngeal adj2 (cancer? or carcinoma? or neoplasm? or tumor? or malignan\$)).mp,tw.
6. (hypopharyngeal adj2 (cancer? or carcinoma? or neoplasm? or tumor? or malignan\$)).mp,tw.
  
7. exp Oropharynx/
8. exp larynx/
9. exp hypopharynx/
10. exp oral cavity/
11. 1 and 2
12. or/4-6
13. (or/7-10) and 11
14. 3 or 12 or 13
15. (P16 adj2 protein).mp,tw.
16. immunohistochemistry.mp,tw.
17. PCR.mp,tw.
18. polymerase chain reaction.mp,tw.
19. (polymerase adj2 chain adj2 reaction).mp,tw.
20. \*In Situ Hybridization/
21. (in adj2 situ adj2 hybridization).mp,tw.
22. \$ISH.mp,tw.
23. or/15-22
24. HPV.mp,tw.
25. human papillomavirus.mp,tw.
26. papillomavirus.mp,tw.
27. or/24-26
28. "sensitivity and specificity"/
29. 14 and 23 and 27 and 28
30. 14 and 27
31. or/29-30
32. meta-analysis.pt,sh,tw.
33. (meta-analy\$ or meta analy\$ or metaanaly\$).tw.
34. 32 or 33
35. 31 and 34
36. guideline\$.pt,sh,tw.
37. 31 and 36
38. exp randomized controlled trials/
39. random\$.pt,sh,tw.
40. 38 or 39
41. 31 and 40
42. 35 or 37 or 41
43. exp clinical trial/
44. exp longitudinal studies/
45. retrospective studies.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism

supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

46. exp cohort studies/

47. 43 or 44 or 45 or 46

48. 31 and 47

49. 42 or 48

50. (case report\$ or editorial\$ or comment\$ or letter\$ or news).pt.

51. 49 not 50

52. limit 51 to (english language and humans)

53. (201304: or 201305: or 201306: or 201307: or 201308: or 201309: or 201310: or 201311: or 201312: or 2014: or 2015: or 2016: or 2017: or 2018: or 2019:).dc. or (201304: or 201305: or 201306: or 201307: or 201308: or 201309: or 201310: or 201311: or 201312: or 2014: or 2015: or 2016: or 2017: or 2018: or 2019:).ed.

54. 52 and 53

55. remove duplicates from 54

#### **(Additional literature search strategy on CUPs)**

1. exp "head and neck neoplasms"/

2. exp \*carcinoma, squamous cell/

3. neoplasm metastasis.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

4. or/1-3

5. (unknown adj2 primary).mp,tw.

6. (occult adj2 tumor).mp,tw.

7. (unknown adj2 origin?).mp,tw.

8. or/5-7

9. or/5-7

10. human papillomavirus.mp,tw.

11. papillomavirus infection/ge

12. papillomavirus infection/pa

13. papillomavirus infection/vi

14. p16.mp,tw.

15. or/9-14

16. lymph nodes/pa

17. lymph nodes/vi

18. or/16-17

19. 4 and 8 and 15

20. 8 and 15

21. 15 and 18

22. 4 and 8 and 15 and 18

23. 19 or 20 or 21 or 22

24. (P16 adj2 protein).mp,tw.

25. immunohistochemistry.mp,tw.

26. PCR.mp,tw.

27. polymerase chain reaction.mp,tw.

28. (polymerase adj2 chain adj2 reaction).mp,tw.

29. \*In Situ Hybridization/

30. (in adj2 situ adj2 hybridization).mp,tw.

31. \$ISH.mp,tw.
32. or/24-31
33. HPV.mp,tw.
34. human papillomavirus.mp,tw.
35. papillomavirus.mp,tw.
36. or/33-35
37. "sensitivity and specificity"/
38. 23 and 32 and 36 and 37
39. 23 and 36
40. or/38-39
41. meta-analysis.pt,sh,tw.
42. (meta-analy\$ or meta analy\$ or metaanaly\$).tw.
43. 41 or 42
44. 40 and 43
45. guideline\$.pt,sh,tw.
46. 40 and 45
47. exp randomized controlled trials/
48. random\$.pt,sh,tw.
49. 47 or 48
50. 40 and 49
51. 44 or 46 or 50
52. exp clinical trial/
53. exp longitudinal studies/
54. retrospective studies.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
55. exp cohort studies/
56. or/52-55
57. 40 and 56
58. 51 or 57
59. (case report\$ or editorial\$ or comment\$ or letter\$ or news).pt.
60. 58 not 59
61. limit 60 to (english language and humans)
62. (201304: or 201305: or 201306: or 201307: or 201308: or 201309: or 201310: or 201311: or 201312: or 2014: or 2015: or 2016: or 2017: or 2018: or 2019:).dc. or (201304: or 201305: or 201306: or 201307: or 201308: or 201309: or 201310: or 201311: or 201312: or 2014: or 2015: or 2016: or 2017: or 2018: or 2019:).ed.
63. 61 and 62

#### Embase

1. exp "head and neck cancer"/
2. exp "squamous cell carcinoma"/
3. HNSCC.ab,mp,tw.
4. (oropharyngeal adj2 (cancer: or carcinoma: or neoplasm: or tumo?: or malignan:)).mp,tw.
5. (laryngeal adj2 (cancer: or carcinoma: or neoplasm: or tumo?: or malignan:)).mp,tw.
6. (hypopharyngeal adj2 (cancer: or carcinoma: or neoplasm: or tumo?: or malignan:)).mp,tw.
7. exp oropharynx/
8. exp larynx/

9. exp hypopharynx/
10. exp oral cavity/
11. 1 and 2
12. or/4-6
13. (or/7-10) and 11
14. 3 or 12 or 13
15. (p16 adj2 protein).mp,tw.
16. immunohistochemistry.mp,tw.
17. polymerase chain reaction.mp,tw.
18. PCR.mp,tw.
19. \*in situ hybridization/
20. (in adj2 situ adj2 hybridization).mp,tw.
21. :ISH.tw.
22. or/15-21
23. HPV.mp,tw.
24. human papillomavirus.mp,tw.
25. papillomavirus.mp,tw.
26. or/23-25
27. "sensitivity and specificity"/
28. 14 and 22 and 26 and 27
29. 14 and 26
30. or/28-29
31. meta-analysis.ti,tw.
32. (meta-analy: or meta analy: or metaanaly:).ti,tw.
33. 31 or 32
34. 30 and 33
35. guideline:.ti,tw.
36. 30 and 35
37. exp Randomized Controlled Trial/
38. random:.ti,tw.
39. 37 or 38
40. 30 and 39
41. 34 or 36 or 40
42. exp controlled Study/
43. logitudinal studies/
44. retrospective studies.ti,tw.
45. exp Cohort Studies/
46. 42 or 43 or 44 or 45
47. 30 and 46
48. 41 or 47
49. (case report: or editorial: or comment: or letter: or news).ti,tw.
50. 48 not 49
51. limit 50 to (english language and humans)
52. (201304: or 201305: or 201306: or 201307: or 201308: or 201309: or 201310: or 201311: or 201312: or 2014: or 2015: or 2016: or 2017: or 2018: or 2019:).dd.
53. 51 and 52
54. remove duplicates from 53

**(Additional literature search strategy on CUPs)**

1. exp "head and neck neoplasms"/

2. exp \*carcinoma, squamous cell/
3. neoplasm metastasis.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]
4. or/1-3
5. (unknown adj2 primary).mp,tw.
6. (occult adj2 tumor?).mp,tw.
7. (unknown adj2 origin?).mp,tw.
8. or/5-7
9. HPV.mp,tw.
10. human papillomavirus.mp,tw.
11. papillomavirus infection/pa
12. papillomavirus infection/vi
13. papillomavirus infection/
14. p16.mp,tw.
15. or/9-14
16. lymph nodes/pa
17. lymph nodes/vi
18. or/16-17
19. 4 and 8 and 15
20. 8 and 15
21. 15 and 18
22. 4 and 8 and 15 and 18
23. 19 or 20 or 21 or 22
24. (p16 adj2 protein).mp,tw.
25. immunohistochemistry.mp,tw.
26. polymerase chain reaction.mp,tw.
27. PCR.mp,tw.
28. \*in situ hybridization/
29. (in adj2 situ adj2 hybridization).mp,tw.
30. :ISH.tw.
31. or/24-30
32. HPV.mp,tw.
33. human papillomavirus.mp,tw.
34. papillomavirus.mp,tw.
35. or/32-34
36. "sensitivity and specificity"/
37. 23 and 31 and 35 and 36
38. 23 and 35
39. or/37-38
40. meta-analysis.ti,tw.
41. (meta-analy: or meta analy: or metaanaly:).ti,tw.
42. 40 or 41
43. 39 and 42
44. guideline:.ti,tw.
45. 39 and 44
46. exp Randomized Controlled Trial/
47. random:.ti,tw.
48. 46 or 47
49. 39 and 48

50. 43 or 45 or 49
51. exp controlled Study/
52. logitudinal studies/
53. retrospective studies.ti,tw.
54. exp Cohort Studies/
55. or/51-54
56. 39 and 55
57. 50 or 56
58. (case report: or editorial: or comment: or letter: or news).ti,tw.
59. 57 not 58
60. limit 59 to (english language and humans)
61. (201304: or 201305: or 201306: or 201307: or 201308: or 201309: or 201310: or 201311: or 201312: or 2014: or 2015: or 2016: or 2017: or 2018: or 2019:).dd.
62. 60 and 61

#### **Cochrane Database of Systematic Reviews**

1. HNSCC.ab,mp,tw.
2. (oropharyngeal adj2 (cancer? or carcinoma? or neoplasm? or tumor? or malignan\$)).mp,tw.
3. (laryngeal adj2 (cancer? or carcinoma? or neoplasm? or tumor? or malignan\$)).mp,tw.
4. (hypopharyngeal adj2 (cancer? or carcinoma? or neoplasm? or tumor? or malignan\$)).mp,tw.
5. or/1-4
6. (P16 adj2 protein).mp,tw.
7. immunohistochemistry.mp,tw.
8. PCR.mp,tw.
9. polymerase chain reaction.mp,tw.
10. (polymerase adj2 chain adj2 reaction).mp,tw.
11. (in adj2 situ adj2 hybridization).mp,tw.
12. \$ISH.mp,tw.
13. or/6-12
14. HPV.mp,tw.
15. human papillomavirus.mp,tw.
16. papillomavirus.mp,tw.
17. or/14-16
18. 5 and 13 and 17
19. 5 and 17
20. or/18-19

Searched <https://meetinglibrary.asco.org/> (ASCO) and <https://elibrary.estro.org/> (ESTRO) with keywords: “randomized” AND “phase III” AND “head and neck” AND “HPV”.



## DEFINITIONS OF REVIEW OUTCOMES

1. **ARCHIVE** - ARCHIVE means that a Clinical Expert and/or Expert Panel has reviewed new evidence pertaining to the guideline topic and determined that the guideline is out of date or has become less relevant. The document, however, may still be useful for education or other information purposes. The document is designated archived on the CCO website and each page is watermarked with the words “ARCHIVED.”
2. **ENDORSE** - ENDORSE means that a Clinical Expert and/or Expert Panel has reviewed new evidence pertaining to the guideline topic and determined that the guideline is still useful as guidance for clinical decision making. A document may be endorsed because the Expert Panel feels the current recommendations and evidence are sufficient, or it may be endorsed after a literature search uncovers no evidence that would alter the recommendations in any important way.
3. **UPDATE** - UPDATE means the Clinical Expert and/or Expert Panel recognizes that the new evidence pertaining to the guideline topic makes changes to the existing recommendations in the guideline necessary but these changes are more involved and significant than can be accomplished through the Document Assessment and Review process. The Expert Panel advises that an update of the document be initiated. Until that time, the document will still be available as its existing recommendations are still of some use in clinical decision making, unless the recommendations are considered harmful.