Human Papillomavirus Testing in Head and Neck Carcinomas

Guideline From the College of American Pathologists

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• ***Context.***—**Human papillomavirus (HPV) is a major cause of oropharyngeal squamous cell carcinomas, and HPV (and/or surrogate marker p16) status has emerged as a prognostic marker that significantly impacts clinical management. There is no current consensus on when to test oropharyngeal squamous cell carcinomas for HPV/p16 or on which tests to choose.**

Objective.—**To develop evidence-based recommendations for the testing, application, interpretation, and reporting of HPV and surrogate marker tests in head and neck carcinomas.**

Design.—**The College of American Pathologists convened a panel of experts in head and neck and molecular pathology, as well as surgical, medical, and radiation oncology, to develop recommendations. A systematic review of the literature was conducted to address 6 key questions. Final recommendations were derived from strength of evidence, open comment period feedback, and expert panel consensus.**

Results.—**The major recommendations include (1) testing newly diagnosed oropharyngeal squamous cell carcinoma patients for high-risk HPV, either from the primary tumor or from cervical nodal metastases, using p16 immunohistochemistry with a 70% nuclear and cytoplasmic staining cutoff, and (2) not routinely testing nonsquamous oropharyngeal carcinomas or nonoropharyngeal carcinomas for HPV. Pathologists are to report tumors as HPV positive or p16 positive. Guidelines are provided for testing cytologic samples and handling of locoregional and distant recurrence specimens.**

Conclusions.—**Based on the systematic review and on expert panel consensus, high-risk HPV testing is recommended for all new oropharyngeal squamous cell carcinoma patients, but not routinely recommended for other head and neck carcinomas.**

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Transcriptionally active human papillomavirus (HPV) has been identified as an important cause of oropharyngeal carcinoma.[1–5] Human papillomavirus–positive oropharyngeal squamous cell carcinoma (OPSCC) has shown a significant increase in incidence during the past several decades, in contrast to conventional smoking- and alcohol-related head and neck squamous cell carcinoma (HNSCC), which has decreasing incidence.[1,5] The Centers for Disease Control and Prevention estimates that there are more than 16 000 cases of HPV-positive OPSCC per year in the United States.[6] These represent between 60% and 80% of all OPSCCs in the United States and Canada. Rates in many northern European countries also seem to be high, whereas rates in other parts of Europe are closer to 15% to 30%. Human papillomavirus–positive OPSCC rates are more variable in other continents but also appear to be substantially lower than for North America. For example, in India, rates may be less than 5%, and another large study found HPV-positive OPSCC rates of 16% across Europe, 36% in Central and South America, and 17% in Asia.[7–10] Patients with HPV-positive OPSCC tend to be younger, former- or nonsmokers, and male, with risk factors for exposure to high-risk HPV (HR-HPV).[2,11–13] The squamous

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Authors' disclosures of potential conflicts of interest and author contributions are found in the Appendix at the end of this article.

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cell carcinomas (SCCs) in these patients tend to have smaller primary tumors, but present with early nodal metastases.[1,3] As a group, patients with HPV-positive OPSCC have improved clinical outcomes compared with patients with conventional, HPV-negative HNSCC when managed by similar modalities.[2,11–13]

Testing for HR-HPV in HNSCC has become increasingly important during the past decade. Determining that an OPSCC is positive for HR-HPV (by strictly defined testing in the correct clinical and pathologic contexts) has significant implications for patient prognosis, and it is now integrated into the recently updated American Joint Committee on Cancer (AJCC) staging manual[14]; furthermore, HPV status determines patient eligibility for clinical trials investigating new treatment regimens and modalities.[15,16] In addition, determining that a metastatic SCC of unknown primary to a cervical lymph node is HPV positive strongly points to the oropharynx as the site of origin, with consequences for subsequent clinical management and treatment decisions.[15–19] For these reasons, several organizations, including the College of American Pathologists (CAP), the Royal College of Pathologists, and Cancer Care Ontario, have supported the establishment of evidence-based guidelines for HR-HPV testing in HNSCC.[17,20]

There are many important questions about HR-HPV testing that remain to be answered by evidence-based guidelines, including which anatomic sites and subtypes of HNSCC warrant HPV testing, when and how to test tissue specimens, and what should be done with fine-needle aspiration (FNA) samples.[21–26] In 2013, the CAP appointed an 11-person expert panel (EP) and a 9-person advisory panel to address these and other related questions to formulate a comprehensive set of recommendations.

METHODS

This evidence-based guideline was developed following the standards endorsed by the National Academy of Medicine, formerly the Institute of Medicine.[27] A detailed description of the methods and a systematic review (including the quality assessment and complete analysis of the evidence) used to create this guideline can be found in the supplemental digital content at www.archivesofpathology.org in the May 2018 table of contents.

Panel Composition

The CAP convened an EP consisting of members with expertise in head and neck and molecular pathology and surgical, medical, and radiation oncology to develop the guideline. In addition, a research methodologist consultant served on the EP for the systematic review of the evidence. An advisory panel consisting of 2 patient advocates, 4 pathologists, 1 medical oncologist/molecular epidemiologist, 1 radiation oncologist, and 1 methodologist assisted the EP. The following organizations provided official panel representation: the American Academy of Otolaryngology—Head and Neck Surgery Foundation, the American Society of Clinical Oncology (ASCO), and the American Society of Cytopathology.

In addition, the guideline was submitted to ASCO's Head and Neck Guideline Advisory Group and ASCO's Clinical Practice Guideline Committee for review of the final manuscript. No suggestions for revisions were proposed, and it was agreed that the guideline should be considered for endorsement by ASCO.

Conflict of Interest Policy

In accordance with the CAP conflict of interest policy (in effect April 2010), members of the EP disclosed all financial interests from 12 months prior to appointment throughout the development of this guideline. Individuals were instructed to disclose any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Complete disclosures of the EP members are listed in the Appendix. Disclosures of interest judged by the oversight group to be conflicts are as follows: R.R.S, research grants, National Institutes of Health (Bethesda, Maryland); W.H.W., consultancy, Merck & Co, (Kenilworth, New Jersey). The majority of EP members (9 of 11) were assessed as having no relevant conflicts of interest. The CAP provided funding for the administration of the project; no industry funds were used in the development of the guideline. All panel members volunteered their time and were not compensated for their involvement, except for the contracted methodologist. Please see the supplemental digital content for full details on the conflict of interest policy.

Objective

The scope of the panel was to develop evidence-based recommendations for the various methodologies and applications of HR-HPV testing in head and neck carcinomas. The key questions are listed as follows:

1. Should patients with newly diagnosed OPSCC, nonoropharyngeal SCC (non-OPSCC), oropharyngeal non-SCC, nonoropharyngeal non-SCC, and cervical nodal metastatic carcinomas of unknown and/or known primary be routinely tested for HR-HPV?
2. Do relevant clinical outcomes of specific tests or testing algorithms for HR-HPV differ based on items such as specimen size, type and length of tissue fixation, or the criteria/definition for a positive p16 immunohistochemistry (IHC) or in situ hybridization (ISH) test?
3. For patients with OPSCC, non-OPSCC, and cervical nodal metastatic SCC, what is the optimal method of reporting HPV test results to best inform patients and clinicians about the clinical significance of the results (including considerations about uncertainty)?
4. Should patients with recurrent/persistent OPSCC, non-OPSCC, and cervical nodal metastatic SCC be routinely tested for HR-HPV?
5. Should patients with locally and/or regionally recurrent OPSCC, non-OPSCC, and cervical nodal metastatic SCC be routinely tested for HR-HPV?
6. Should patients with distant disease be tested for HR-HPV?

Refer to the supplemental digital content for all of the subquestions under these main key questions.

Literature Search and Collection

A comprehensive search for literature was initially performed in MEDLINE using the OvidSP interface on March 3, 2014, encompassing the publication dates of January 1, 1995, to March 3, 2014. A supplemental literature search was completed in PubMed (March 26, 2014) encompassing the publication dates of January 1, 1995, to March 26, 2014. An additional literature search was performed using Scopus (March 29, 2014) to identify relevant articles published between January 1, 1995, and March 29, 2014, in journals not indexed in MEDLINE. The literature search of the electronic databases was conducted in 2 arms: the first combined medical subject headings and keywords to address the concepts *head and neck neoplasms*, *human papillomavirus (HPV)*, and *laboratory testing*, and the second combined medical subject headings and keywords for the concepts *head and neck neoplasms*, *human papillomavirus (HPV)*, and *outcomes*. The results of both arms of the search were combined and deduplicated. Limits were set for human studies published in English, and a publication filter was applied to exclude lower levels of evidence such as letters, commentaries, editorials, and case reports.

A search for gray (unindexed) literature included a review of guideline and systematic review repository sites (eg, Guidelines International Network, National Guideline Clearinghouse, Cochrane Library, Prospero, Centre for Reviews and Dissemination), and relevant medical organizations' Web sites to identify guidelines, protocols, and standards. A review of meeting abstracts

published in the years 2012–2014 from pathology and oncology organizations and EP recommendations completed the systematic literature review. The Ovid search was rerun on July 11, 2016, to identify articles published since March 1, 2014, that provided information that would alter the recommendations in any way.

Detailed information regarding the literature search strategy can be found in the supplemental digital content.

Inclusion and Exclusion Criteria

Practice guidelines, consensus documents, systematic reviews, meta-analyses, randomized controlled trials (RCTs), comparative studies, reviews, case-controlled studies, case series, and evaluation studies were eligible for inclusion.

Published studies were selected for full-text review if they met each of the following criteria:

1. Patients with tissue or cytology aspiration material taken from the workup of
   - Oropharyngeal primaries
   - Cervical nodal metastasis of unknown primary
   - Regional or distant metastasis from known or suspected oropharyngeal primary
   - Other head and neck sites (eg, sinonasal)
   - All carcinomas in the head and neck
2. Human studies
3. Patients of all ages and either sex
4. Studies published in English
5. The study compared, prospectively or retrospectively, laboratory testing methodologies or potential testing algorithms for HPV testing
6. The study addressed one of the key questions
7. The study included measurable data such as the negative predictive value or positive predictive value, if testing methodologies used to determine HPV status, alone and in combination; negative and positive concordance across the platforms; sensitivity and specificity of individual tests; and accuracy in determining HPV status.

Articles were excluded from the systematic review if they were noncomparative or qualitative studies, including editorials, commentaries, or letters; animal studies; full-text articles not available in English; studies that included patients with other tumor types not specified in the inclusion criteria; studies that did not include relevant measurable data; and studies that did not address at least one of the key questions.

Detailed information about the inclusion and exclusion criteria is available in the supplemental digital content.

Quality Assessment

An assessment of study quality was performed by a research methodologist for all fully published studies meeting inclusion criteria. Studies only available in abstract form did not undergo formal quality assessment. Formal quality assessment involved determining the risk of bias by assessing key indicators, based on study design and methodologic rigor. Refer to the supplemental digital content for the definitions of ratings for strength of evidence (Supplemental Table 1) and for the quality assessment results.

Assessing the Strength of Recommendations

Development of recommendations required that the panel review the identified evidence and make a series of key judgments. Grades for strength of recommendations were developed by the CAP Pathology and Laboratory Quality Center and are described in Table 1.

Guideline Revision

This guideline will be reviewed every 4 years, or earlier in the event of publication of substantive and high-quality evidence that could potentially alter the original guideline recommendations. If necessary, the entire panel will reconvene to discuss potential changes. When appropriate, the panel will recommend revision of the guideline to the CAP for review and approval.

Disclaimer

The CAP developed the Pathology and Laboratory Quality Center as a forum to create and maintain evidence-based practice guidelines and consensus statements. Practice guidelines and consensus statements reflect the best available evidence and expert consensus supported in practice. They are intended to assist physicians and patients in clinical decision making and to identify questions and settings for further research. With the rapid flow of scientific information, new evidence may emerge between the time a practice guideline or consensus statement is developed and when it is published or read. Guidelines and statements are not continually updated and may not reflect the most recent evidence. Guidelines and statements address only the topics specifically identified therein and are not applicable to other interventions, diseases, or stages of diseases. Furthermore, guidelines and statements cannot account for individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge, to determine the best course of treatment for the patient. Accordingly, adherence to any practice guideline or consensus statement is voluntary, with the ultimate determination regarding its application to be made by the physician in light of each patient's individual circumstances and preferences. The CAP makes no warranty, express or implied, regarding guidelines and statements and specifically excludes any warranties of merchantability and fitness for a particular use or purpose. The CAP assumes no responsibility for any injury or damage to persons or property arising out of or related to any use of this statement or for any errors or omissions.

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RESULTS

Of the 2803 unique studies identified in the systematic review, 503 met inclusion criteria and underwent data extraction. One hundred fifty-seven of these studies made up the evidentiary base and informed the guideline statements (recommendations). The vast majority were published, peer-reviewed articles, but 31 studies were published only in abstract form. All 157 underwent data extraction and qualitative analysis. Abstracts included in the 157 studies reported at least partial data. Those that did not report data for any of the outcomes of interest were excluded. Data from abstracts were used only in concert with peer-reviewed data, as they added support for recommendation statements. Abstract data alone were not used in the formulation of recommendations.

The EP met 16 times through Web-based meeting forums from November 22, 2013, through September 21, 2016. Additional work was completed via electronic mail. The EP met in person February 8 and 9, 2014, to formally initiate the project, and again April 9, 2016, to review the evidence to date and draft recommendations.

A public comment period was held from July 18 to August 8, 2016, on the CAP Web site. Fourteen draft recommendations, 2 demographic questions, and 3 questions about feasibility were posted for feedback.

Table 1. Grades for Strength of Recommendations[a]		
Designation	**Recommendation**	**Rationale**
Strong recommendation	Recommend for or against a particular practice (Can include "must" or "should")	Supported by convincing (high) or adequate (intermediate) quality of evidence and clear benefit that outweighs any harms
Recommendation	Recommend for or against a particular practice (Can include "should" or "may")	Some limitations in quality of evidence (adequate [intermediate] or inadequate [low]), balance of benefits and harms, values, or costs but panel concludes that there is sufficient evidence and/or benefit to inform a recommendation
Expert consensus opinion	Recommend for or against a particular practice (Can include "should" or "may")	Serious limitations in quality of evidence (inadequate [low] or insufficient), balance of benefits and harms, values, or costs, but panel consensus is that a statement is necessary
No recommendation	No recommendation for or against a practice	Insufficient evidence or agreement of the balance of benefits and harms, values, or costs to provide a recommendation

[a] Derived from Andrews et al.[262]

"Agree" and "disagree" responses were captured for every proposed recommendation. In addition, 269 written comments were captured. Seven draft recommendations achieved more than 90% agreement, 6 achieved between 80% and 90% agreement, and 1 received 57% agreement. Each EP member was assigned 1 or 2 draft recommendations for which to review the public comments and to present to the panel for group discussion. After consideration of the comments, 6 draft recommendations were maintained with the original language and 7 were revised. Resolution of all changes was obtained by unanimous consensus of the panel members using nominal group technique (rounds of subsequent teleconference webinars and email discussion). Final EP recommendations were approved by a formal vote. The panel considered laboratory efficiency and feasibility throughout the entire process, although neither cost nor cost-effectiveness analyses were performed. A description of the benefits and harms of implementing the guideline statements is included in the supplemental digital content.

An independent review panel, masked to the EP and vetted through the conflict of interest process, provided final approval on behalf of the CAP Council on Scientific Affairs. In addition, the guideline was submitted to ASCO's Head and Neck Guideline Advisory Group and ASCO's Clinical Practice Guideline Committee for review of the final manuscript. No suggestions for revisions were proposed and it was agreed that the guideline should be considered for endorsement by ASCO. The final recommendations are summarized in Table 2, and an algorithmic approach for the workup of patient specimens is provided in Figure 1.

GUIDELINE STATEMENTS

Statement 1.—*Strong Recommendation*.—Pathologists should perform HR-HPV testing on all patients with newly diagnosed OPSCC, including all histologic subtypes. This testing may be performed on the primary tumor or on a regional lymph node metastasis when the clinical findings are consistent with an oropharyngeal primary.

The strength of evidence is *convincing* to support this guideline statement.

The evidentiary base supporting this recommendation comprised 110 studies, of which 1 was a meta-analysis,[28] 8 were subgroup analyses from RCTs,[11,29–35] 77 were observational studies,[1,10,36–111] and 24 were studies only published in abstract form.[112–135] The meta-analysis received a quality score of 7 out of a possible 11 points, and all supporting RCT data had a risk of bias determined to be moderate. The supporting observational studies ranged from low to moderate risk of bias, with the exception of one study that was assessed to have a high risk of bias.[1] This study was a retrospective cohort with retrospective data collection and industry sponsorship. None of the other studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 2 in the supplemental digital content for the quality assessment results for studies included in the statement 1 evidentiary base.

Breaking from a tradition that has broadly grouped all carcinomas arising from the oral and oropharyngeal subsites as oral cancer, these guidelines maintain a sharp distinction between those carcinomas arising in the oropharynx and those arising in the oral cavity proper. Testing for the presence of HPV must be guided by a familiarity with head and neck anatomy, including those structures that define the oral cavity as separate from the oropharynx (Figure 2). The oral cavity proper comprises the lips, gingiva, retromolar trigone, hard palate, buccal mucosa, mobile tongue, and floor of the mouth, whereas the oropharynx comprises the palatine tonsils, soft palate, base of tongue (posterior to the circumvallate papillae), and lateral and posterior pharyngeal walls. Oropharyngeal tonsillar structures (ie, lingual and palatine tonsils), particular hot spots for HPV-related carcinogenesis, are present in the oropharynx, but not in the oral cavity.

Oropharyngeal SCCs with transcriptionally active HR-HPV represent a unique type of HNSCC. These HPV-positive OPSCCs have risk-factor, demographic, morphologic, molecular, and clinical profiles that stand apart from other HNSCCs.

Human papillomavirus status of a primary or metastatic OPSCC may have diagnostic, staging, and even therapeutic implications. Currently, however, the call for routine HPV testing reflects its standing as a powerful prognostic indicator for patients with OPSCC. The literature overwhelmingly supports the conclusion that HPV status is an important and independent predictor of overall and disease-specific survival for patients with OPSCC. The survival benefit of HPV-positive OPSCC is maintained across nearly all studies, despite significant heterogeneity in patient populations, sample size, methods of HPV detection, tumor stage, tumor treatment, comorbidity, and inclusion of various other prognostic factors in the analysis. In large prospective studies where patient populations with OPSCC are uniformly staged and treated, significant reduction in risk of progression and disease-related death is confirmed for HPV-positive tumors.[11,12,31,32,35]

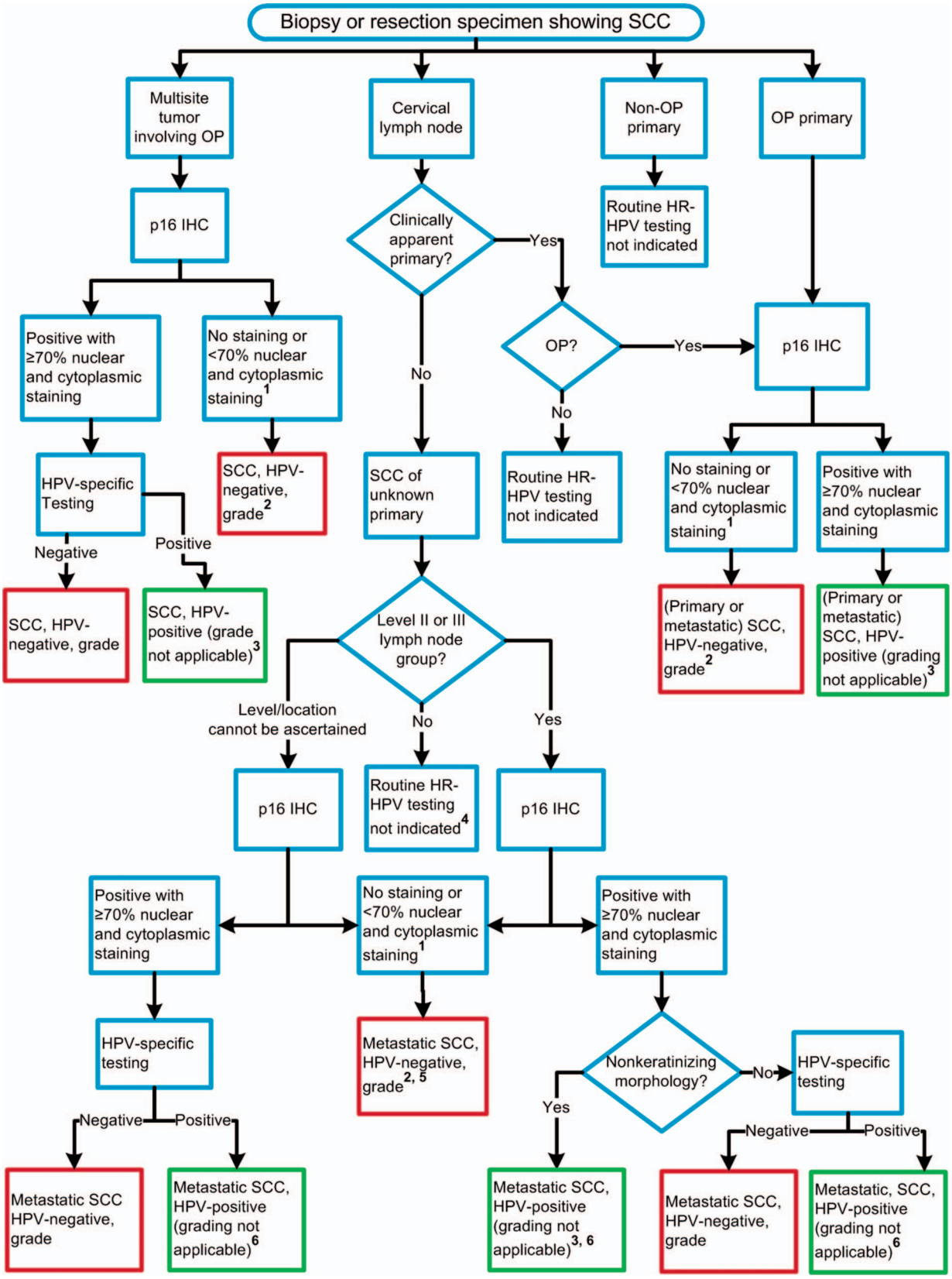

Figure 1. *High-risk human papillomavirus (HR-HPV) testing in head and neck squamous cell carcinomas (SCCs). Abbreviations: IHC, immunohistochemistry; OP, oropharyngeal. [1]Consider HR-HPV–specific testing for equivocal p16 results (50%–70% nuclear and cytoplasmic staining). [2]May also be reported as p16 negative with a comment specifying that the tumor is very likely HPV negative. [3]May also be reported as p16 positive with a comment specifying that the tumor is very likely HPV positive. [4]HR-HPV may be indicated in patients where the clinical suspicion for an HPV-positive SCC is high. [5]Consider Epstein-Barr encoding region (EBER) in situ hybridization for Epstein-Barr virus for the rare metastatic nonkeratinizing squamous cell carcinoma that is HR-HPV negative. [6]Include comment, "Likely oropharyngeal primary."*

Table 2. Summary of Guideline Statements	
Guideline Statement	**Strength of Recommendation**
1. Pathologists should perform HR-HPV testing on all patients with newly diagnosed OPSCC, including all histologic subtypes. This testing may be performed on the primary tumor or on a regional lymph node metastasis when the clinical findings are consistent with an oropharyngeal primary.	Strong recommendation
2. For oropharyngeal tissue specimens (ie, noncytology), pathologists should perform HR-HPV testing by surrogate marker p16 IHC. Additional HPV-specific testing may be done at the discretion of the pathologist and/or treating clinician, or in the context of a clinical trial.	Recommendation
3. Pathologists should *not* routinely perform HR-HPV testing on patients with nonsquamous carcinomas of the oropharynx.	Expert consensus opinion
4. Pathologists should *not* routinely perform HR-HPV testing on patients with nonoropharyngeal primary tumors of the head and neck.	Recommendation
5. Pathologists should routinely perform HR-HPV testing on patients with metastatic SCC of unknown primary in a cervical upper or mid jugular chain lymph node. An explanatory note on the significance of a positive HPV result is recommended.	Recommendation
6. For tissue specimens (ie, noncytology) from patients presenting with metastatic SCC of unknown primary in a cervical upper– or mid–jugular chain lymph node, pathologists should perform p16 IHC. *Note:* Additional HR-HPV testing on p16-positive cases should be performed for tumors located outside of level II or III (nonroutine testing) in the neck and/or for tumors with keratinizing morphology.	Expert consensus opinion
7. Pathologists should perform HR-HPV testing on head and neck FNA SCC samples from all patients with known OPSCC not previously tested for HR-HPV, with suspected OPSCC, or with metastatic SCC of unknown primary. *Note:* No recommendation is made for or against any specific testing methodology for HR-HPV testing in FNA samples. If the result of HR-HPV testing on the FNA sample is negative, testing should be performed on tissue if it becomes available. If pathologists use cytology samples for p16 IHC testing, they should validate the criteria (ie, cutoff) for a positive result.	Expert consensus opinion
8. Pathologists should report p16 IHC positivity as a surrogate for HR-HPV in tissue specimens (ie, noncytology) when there is at least 70% nuclear and cytoplasmic expression with at least moderate to strong intensity.	Expert consensus opinion
9. Pathologists should *not* routinely perform low-risk HPV testing on patients with head and neck carcinomas.	Expert consensus opinion
10. Pathologists should *not* repeat HPV testing on patients with locally recurrent, regionally recurrent, or persistent tumor if primary tumor HR-HPV status has already been established. If initial HR-HPV status was never assessed or results are unknown, testing is recommended. HPV testing may be performed on a case-by-case basis for diagnostic purposes if there is uncertainty regarding whether the tumor in question is a recurrence or a new primary SCC.	Expert consensus opinion
11. Pathologists should *not* routinely perform HR-HPV testing on patients with distant metastases if primary tumor HR-HPV status has been established. HPV testing may be performed on a case-by-case basis for diagnostic purposes if there is uncertainty regarding whether the tumor in question is a metastasis or a new primary SCC.	Expert consensus opinion
12. Pathologists should report primary OPSCCs that test positive for HR-HPV or its surrogate marker p16 as HPV positive and/or p16 positive.	Expert consensus opinion
13. Pathologists should *not* provide a tumor grade or differentiation status for HPV-positive/p16-positive OPSCCs.	Expert consensus opinion
14. Pathologists should *not* alter HR-HPV testing strategy based on patient smoking history.	Expert consensus opinion

Abbreviations: FNA, fine-needle aspiration; HPV, human papillomavirus; HR-HPV, high-risk HPV; IHC, immunohistochemistry; OPSCC, oropharyngeal SCC; SCC, squamous cell carcinoma.

Testing for HR-HPV should be performed on all OPSCCs regardless of histologic type. The morphologic spectrum of HPV-positive OPSCC includes variants with papillary, adenosquamous, lymphoepithelioma-like, sarcomatoid, and basaloid features. Morphologic variation does not seem to influence clinical behavior, and it does not abrogate the need for HPV testing.[136–138]

In the open comment period, of the 168 respondents, 93.45% (n = 157) agreed with the recommendation and 5.36% (n = 9) disagreed. There were 22 written comments. Most of these comments were directed at the method of HPV testing and were, accordingly, taken into consideration in the final drafting of statement 2. Others reflected confusion about the anatomic definition of the oropharynx and its distinction from the oral cavity. To highlight this important distinction, a brief description of the anatomy of the oropharynx and oral cavity has been provided.

Statement 2.—*Recommendation*.—For oropharyngeal tissue specimens (ie, noncytology), pathologists should perform HR-HPV testing by surrogate marker p16 IHC. Additional HPV-specific testing may be done at the discretion of the pathologist and/or treating clinician, or in the context of a clinical trial.

The strength of evidence is *adequate*.

Sixty-seven studies (5 RCTs,[11,29,32,34,35] 48 observational studies,* and 14 studies published in abstract form†) make up the evidentiary base for statement 2. Of these studies, 31 reported on laboratory outcomes of interest (Table 3) and 51 on clinical outcomes (Table 4). The risk of bias assessment of the majority of the included studies ranged from low to

* *References 1, 36, 38, 42–44, 47–54, 56, 57, 59, 64, 65, 67, 69–78, 80, 81, 83, 84, 87, 89, 91, 92, 96–101, 104, 106, 108, 110, 111.*
† *References 112, 116, 118–120, 122, 125–127, 129–131, 134, 135.*

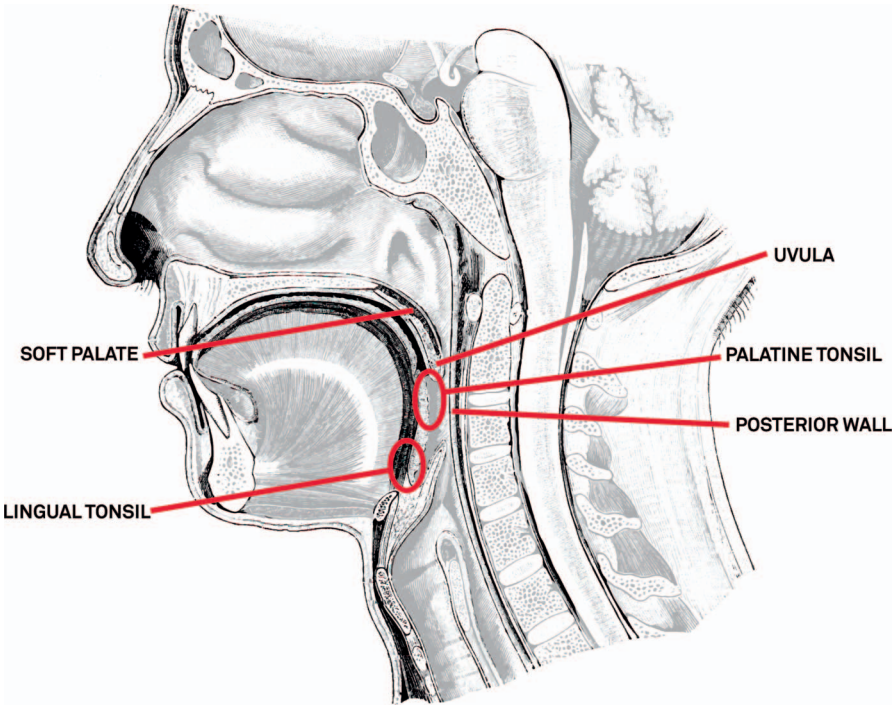

Figure 2. *Normal anatomy of the oropharynx, including specific features of the palatine tonsils and base of tongue.*

moderate, with only 2 studies assessed as high[1,68] because of industry funding. Although 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 but 2 studies.[1,38] None of the other studies were found to have methodologic flaws that would call into question the study findings. Refer to Supplemental Table 3 in the supplemental digital content for the quality assessment results for all studies included in the statement 2 evidentiary base.

Because the literature strongly supports that HR-HPV status is independently prognostic in OPSCC and that it should be routinely assessed, there is a need for consistency in clinical practice. Which test or combination of HR-HPV tests to perform is one of the more controversial issues in head and neck pathology. There are numerous HPV-specific tests, as well as the surrogate markers p16 IHC and hematoxylin-eosin morphology to consider. Although it is ideal to have a reference or standard criterion test, the current literature does not clearly support one. The test should be the one that best stratifies patient survival outcomes while also being practical and inexpensive. p16 IHC is the test the EP considers to best fit that role. p16 is markedly overexpressed in tumor cells with transcriptionally active HR-HPV because the viral E7 oncoprotein destabilizes pRb, functionally removing suppression of p16 expression and allowing tumor cells with high p16 levels to bypass pRb-dependent cell cycle arrest. The result is marked overexpression of p16, making it an excellent surrogate marker of viral infection in the correct context.[139,140] Based on abundant literature on p16 IHC as an independent predictor of improved patient prognosis in OPSCC,[‡] and on its widespread availability, ease and reproducibility of interpretation,[53] and excellent performance on small specimen samples such as small biopsies and tissue microarray punches,[11,89,142] the EP recommends that p16 testing be performed. Many consider the detection of HR-HPV E6 and E7 messenger RNA (mRNA) by ISH as the gold standard.[89,143,144] Although this is an excellent test, and perhaps even the ideal test from a purely scientific perspective, it isn't widely available for clinical use, is much more expensive than p16, and is more technically challenging to perform, and the data do not show statistically better performance than p16 IHC alone in OPSCC. Because p16 is only a surrogate marker for HR-HPV, and its overexpression is not always associated with the presence of HR-HPV,[53,75,81,89] as practice changes and HPV-specific tests such as RNA ISH become more widely available clinically, the latter may become the recommended test in the future.

For studies analyzing p16 IHC alone as a prognostic marker, the majority found it to be a marker of favorable outcome in multivariate analysis with univariate hazard ratios for death between 0.2 and 0.5[§] for overall, disease-free, and/or disease-specific survival compared with p16-negative patients. Several prospective and randomized controlled studies showed p16 IHC to be strongly prognostic alone as well.[11,12,32,145] In many of the studies, data extraction and summarization were complicated by p16 results, rather than being analyzed in isolation, being combined with results of HPV-specific test(s) for analysis and data reporting. Correlation rates between p16 IHC and HPV-specific tests were generally high, and best for HPV mRNA tests such as reverse transcriptase polymerase chain reaction and ISH.[‖]

In addition to the aforementioned studies, some additional studies not included in our systematic review support this recommendation. Sedghizadeh et al[141] performed a

[‡] *References 10–12, 29, 32, 70, 81, 141.*

[§] *References 30, 32, 34, 35, 42, 48, 69, 70, 73, 97, 99, 100, 110, 118, 119, 122, 126, 127, 129, 131, 134, 135.*

[‖] *References 44, 53, 81, 84, 89, 124, 125, 143.*

Table 3. Summary of Laboratory Data for Studies Analyzing p16 Immunohistochemistry and Human Papillomavirus (HPV)–Specific Tests

Source, y	No. of Patients or Specimens	Specimen Type	How HPV Positivity Was Defined
Gao et al,[44] 2013	150	. . .	p16 RNA-based PCR (eg, RT-PCR) RNA-based ISH
Ang et al,[11] 2010	721	. . .	p16 DNA ISH
Holzinger et al,[48] 2012	196 patients 199 specimens	Biopsy	p16 DNA PCR RNA-based PCR (eg, RT-PCR)
Isayeva et al,[52] 2014	102	Resection and biopsy	p16 RNA-based PCR (eg, RT-PCR)
Rietbergen et al,[76] 2013	86 to validate to testing algorithm, then 240 to conduct time trend analysis	Biopsy	p16 DNA PCR RNA-based PCR (eg, RT-PCR)
Xu et al, [120] 2013	93	. . .	p16 RNA-based PCR (eg, RT-PCR)
Schache et al,[81] 2013	79 cases, 78 of which were interpretable	Resection	HR-HPV RNAscope (Advanced Cell Diagnostics, Newark, California) (RNA ISH)
Shi et al,[84] 2009	111 patients 111 samples tested by qRT-PCR. 106 for ISH and p16	Biopsy	Comparison of qRT-PCR for E6 mRNA, DNA ISH and p16
Ukpo et al,[89] 2011	211	Biopsy or resection	Not clearly defined
Al-Swiahb et al,[36] 2010	220	Specimen type not reported	PCR alone
Chaturvedi et al,[1] 2011	271	. . .	p16 DNA PCR DNA ISH RNA-based PCR (eg, RT-PCR)
El-Mofty and Patil,[43] 2006	235 specimens	. . .	PCR alone
Holzinger et al,[47] 2013	188	. . .	PCR alone
Hong et al,[49] 2013	647	Resection and biopsy	p16 DNA PCR
Jordan et al,[53] 2012	235 patients 240 specimens	Biopsy	PCR alone
Licitra et al,[56] 2006	90	Resection	PCR alone
Lin et al,[57] 2013	60 patients 41 specimens	Resection and biopsy	ISH alone
Marklund et al,[59] 2012	69	Biopsy	HPV DNA PCR and then separately as p16 and HPV DNA PCR both (outcome data for latter group not provided)
Nasman et al,[64] 2013	439	Biopsy	PCR alone
Nasman et al,[65] 2013	290	Biopsy	PCR alone
Nichols et al,[67] 2010	68	Biopsy	ISH alone
Reimers et al,[74] 2007	106	Resection	p16 and HPV PCR were both done, and both were independently used for survival analysis

Table 3. Extended

Source, y	p16 Positivity Criteria	ISH Positivity Criteria	Control Method	Intervention
Gao et al,[44] 2013	>50% = p16$^+$	Punctate signals	ISH for E6/E7 RNA	PCR for HPV DNA
Ang et al,[11] 2010	>70% = p16$^+$	Punctate signals	ISH for HPV DNA	p16
Holzinger et al,[48] 2012	. . .	. . .	PCR for HPV DNA	p16
Isayeva et al,[52] 2014	>75% nuclear and cytoplasmic	. . .	RT-PCR	p16
Rietbergen et al,[76] 2013	>70% = p16$^+$	. . .	HPV DNA PCR, HPV genotype and RT-PCR for HPV 36 mRNA on frozen tissues	p16 IHC followed by GP5$^+$/6$^+$ PCR on p16$^+$ cases in FFPE tissues
Xu et al, [120] 2013	. . .	. . .	RT-PCR E6/E7 p16	p16
Schache et al,[81] 2013	. . .	For DNA ISH, any detectable chromogen in any of the malignant cells. For RNA ISH (RNAscope), a positive HPV test was defined as punctate staining that colocalized to the cytoplasm and/or nucleus of any of the malignant cells and, where staining was present in the control, was at least twice as strong as the dapB test	HPV RNA qRT-PCR	ISH for E6/E7 RNA
Shi et al,[84] 2009	Considered positive if strong signals were detected in both the tumor nuclei and cytoplasm	Punctate signals	qRT-PCR for E6 mRNA	ISH for HPV DNA
Ukpo et al,[89] 2011	Any + = p16$^+$	Blue nuclear dots (DNA ISH) and brown punctate dots in the nucleus or cytoplasm for RNA ISH	ISH for E6/E7 RNA	p16
Al-Swiahb et al,[36] 2010	>60%	. . .	PCR for HPV DNA	p16
Chaturvedi et al,[1] 2011	. . .	% of positive cells = 70, nuclear and cytoplasmic	PCR for HPV DNA	ISH for HPV DNA
El-Mofty and Patil,[43] 2006	Diffuse and strong staining	. . .	PCR for HPV DNA	p16
Holzinger et al,[47] 2013	. . .	. . .	PCR for HPV DNA	p16
Hong et al,[49] 2013	>70%	. . .	PCR for HPV DNA	p16
Jordan et al,[53] 2012	>70% = p16$^+$	Punctate and diffuse nuclear versus controls	PCR for HPV DNA	p16
Licitra et al,[56] 2006	. . .	. . .	PCR for HPV DNA	p16
Lin et al,[57] 2013	>50%	Punctate, nuclear signals	ISH for HPV DNA	p16
Marklund et al,[59] 2012	>75%	. . .	PCR for HPV DNA	p16
Nasman et al,[64] 2013	>70% = p16$^+$	. . .	PCR for HPV DNA	p16
Nasman et al,[65] 2013	>70%	. . .	PCR for HPV DNA	p16
Nichols et al,[67] 2010	>70%	Punctate signals	ISH for HPV DNA	p16
Reimers et al,[74] 2007	Strong nuclear and cytoplasmic staining in >60% of tumor cells	. . .	PCR for HPV DNA	p16

Table 3. Extended

Source, y	No. Test Positive Disease Positive	No. Test Positive Disease Negative	No. Test Negative Disease Positive	No. Test Negative Disease Negative	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Gao et al,[44] 2013	39	0	0	6	100 (92.3–100)	100 (50.0–100)	100 (92.3–100)	100 (50.0–100)
Ang et al,[11] 2010	192	22	14	95	93.2 (89.8–96.6)	81.2 (74.1–88.3)	89.7 (85.7–93.8)	87.2 (80.9–93.4)
Holzinger et al,[48] 2012	42	12	50	73	45.7 (35.5–55.8)	85.9 (78.5–93.3)	77.8 (66.7–88.9)	59.3 (50.7–68.0)
Isayeva et al,[52] 2014	47	11	14	21	77.0 (66.5–87.6)	65.6 (49.2–82.1)	81.0 (70.9–91.1)	60.0 (43.8–76.2)
Rietbergen et al,[76] 2013	23	1	1	61	95.8 (87.8–100)	98.4 (95.3–100)	95.8 (87.8–100)	98.4 (95.3–100)
Xu et al, [120] 2013	43	14	9	27	82.7 (72.4–93.0)	65.9 (51.3–80.4)	75.4 (64.3–86.6)	75.0 (60.9–89.1)
Schache et al,[81] 2013	32	3	1	42	97.0 (91.1–100)	93.3 (86.0–100)	91.4 (82.2–100)	97.7 (93.2–100)
Shi et al,[84] 2009	59	3	11	33	84.3 (75.8–92.8)	91.7 (82.6–100)	95.2 (89.8–100)	75.0 (62.2–87.8)
Ukpo et al,[89] 2011	148	3	4	37	97.4 (94.8–99.9)	92.5 (84.3–100)	98.0 (95.8–100)	90.2 (81.2–99.3)
Al-Swiahb et al,[36] 2010	31	5	2	182	93.9	97.3	86.1	98.9
Chaturvedi et al,[1] 2011	76	0	40	195	65.5	100.0	100.0	83.0
El-Mofty and Patil,[43] 2006	11	0	1	8	91.7	100.0	100.0	88.9
Holzinger et al,[47] 2013	31	23	8	114	79.5	83.2	57.4	93.4
Hong et al,[49] 2013	264	8	107	268	71.2	97.1	97.1	71.5
Jordan et al,[53] 2012	141	24	5	62	96.6	72.1	85.5	92.5
Licitra et al,[56] 2006	17	15	0	58	100.0	79.5	53.1	100.0
Lin et al,[57] 2013	23	4	0	14	100.0	77.8	85.2	100.0
Marklund et al,[59] 2012	8	9	4	48	66.7	84.2	47.1	92.3
Nasman et al,[64] 2013	246	15	57	121	81.2	89.0	94.3	68.0
Nasman et al,[65] 2013	203	8	22	57	90.2	87.7	96.2	72.2
Nichols et al,[67] 2010	53	3	0	12	100.0	80.0	94.6	100.0
Reimers et al,[74] 2007	25	4	2	65	92.6	94.2	86.2	97.0

Table 3. Continued

Source, y	No. of Patients or Specimens	Specimen Type	How HPV Positivity Was Defined
Rietbergen et al,[75] 2013 & Rietbergen et al,[77] 2014	906 total patients 841 had biopsy available for testing	Biopsy	p16 DNA PCR In addition to p16 and HPV positivity by PCR ($GP5^+/6^+$), this study focused on $p16^+$, HPV PCR^- cases and did additional HPV and non–HPV-related tests
Rischin et al,[32] 2010	861 in trial; 185 patients were studied	. . .	p16 alone
Thavaraj et al,[87] 2011	142	. . .	p16 DNA PCR DNA ISH Considered HPV related if p16 plus ISH or DNA PCR were positive
Weinberger et al,[91] 2006	107 patients 78 specimens	Biopsy	p16 DNA PCR
Weinberger et al,[92] 2009	77	. . .	p16 DNA PCR
Bledsoe et al,[96] 2013	121	Biopsy	p16 DNA ISH
Fujimaki et al,[98] 2013	66	Resection (n = 27) and biopsy (n = 39)	p16 DNA ISH
Song et al,[101] 2012	56	Resection	p16 DNA ISH
Maxwell et al,[130] 2011	77 patients	Specimen type NR	. . .

Abbreviations: dapB, dihydrodipicolinate reductase; FFPE, formalin-fixed, paraffin-embedded; HR, high risk; IHC, immunohistochemistry; ISH, in situ hybridization; NPV, negative predictive value; NR, not reported; PCR, polymerase chain reaction; PPV, positive predictive value; qRT-PCR, quantitative RT-PCR; RT-PCR, reverse transcription PCR.

systematic review and meta-analysis of studies examining p16 IHC expression and prognosis in OPSCC. Among the 18 studies finally included, they found that p16 status by IHC was prognostic for all survival metrics with hazard ratios between 0.3 and 0.4. Some newer studies compared HR-HPV RNA ISH with p16 IHC and showed high correlation between these tests (for high-incidence US study populations) in OPSCC patients.[146] In 3 large RNA ISH-based studies, correlation rates were 92%, 96%, and 100.0%, respectively.[89,143,144] Rooper et al[147] showed that almost all patients in their practice who were p16 positive but HPV deoxyribonucleic acid (DNA) ISH negative were actually positive for HR-HPV by mRNA using ISH, demonstrating that DNA ISH lacks sensitivity and that the correlation between p16 overexpression and presence of HPV mRNA is indeed high.

In the open comment period, of the 160 respondents, 89.44% (143) agreed with the recommendation, and 6.88% (11) disagreed. There were 32 written comments, the majority of which suggested that HPV-specific testing needs to be performed because p16 IHC is not truly a surrogate marker of HR-HPV. Additional comments pointed out that there are patients with p16-positive and HPV-negative tumors and vice versa. Although the latter 2 points are true, these reflect the concept that p16 IHC is being done specifically to ascertain if a patient's tumor is related to transcriptionally active HPV, rather than regarding it as a prognostic biomarker. All of the many HPV-specific tests that are available for use on tissue sections, such as DNA polymerase chain reaction, DNA ISH, RNA reverse transcriptase polymerase chain reaction, and RNA ISH, are independently prognostic.[11,12,29,70,141] There is no shortage of data. However, none of these tests are statistically significantly better than p16 IHC alone.[53,81,89,92,148] Some studies, particularly in lower–HPV-incidence regions (selected regions of Europe, for example), have demonstrated that p16-positive, HPV mRNA– and HPV DNA–negative patients have a poorer prognosis than those patients positive for both tests (although interestingly perhaps still better than for p16 and HPV mRNA/DNA double-negative patients).[75,77] Thus, p16 IHC alone may not be the right approach in these other geographic regions. To allow for discretion, the EP has provided the caveat that "HPV-specific testing may be done at the discretion of the

Table 3. Continued, Extended

Source, y	p16 Positivity Criteria	ISH Positivity Criteria	Control Method	Intervention
Rietbergen et al,[75] 2013 & Rietbergen et al,[77] 2014	>70%	. . .	PCR for HPV DNA	p16
Rischin et al,[32] 2010	Semiquantitative scoring of staining in the nucleus and cytoplasm. If 2 (moderate) or 3 (strong), the case was called positive. No percentage requirements were described	Punctate signals	p16	ISH for HPV DNA
Thavaraj et al,[87] 2011	>70%	Diffuse nuclear and cytoplasmic staining and punctate nuclear staining	HPV DNA PCR and DNA ISH	p16
Weinberger et al,[91] 2006	Strong and diffuse staining	. . .	PCR for HPV DNA	p16
Weinberger et al,[92] 2009	Dichotomous as strong and diffuse staining versus negative and they report no partial positive cases	. . .	PCR for HPV DNA	p16
Bledsoe et al,[96] 2013	. . .	. . .	ISH for HPV DNA	p16
Fujimaki et al,[98] 2013	>70%	Punctate signals	p16	ISH for HPV DNA
Song et al,[101] 2012	>70%	Diffuse nuclear and cytoplasmic staining and punctate nuclear staining	ISH for HPV DNA	p16
Maxwell et al,[130] 2011	. . .	. . .	ISH (not specified DNA versus RNA)	p16

pathologist and/or treating clinician, or in the context of a clinical trial.''

Doing both p16 IHC and HPV-specific testing will undoubtedly result in some patients with discrepant test results. There is not currently strong evidence for what to do in these situations, although it is probably good practice at this time not to label patients with discrepant p16- and HPV-specific test results as being in the prognostically favorable category of HPV positive. Particular caution should be exercised for laboratories currently using or considering DNA ISH, because it has been shown to lack sensitivity for HR-HPV and may lead to the above situation (of test discrepancy) more frequently than HPV-specific tests with higher sensitivity.[53,81,89,149]

Comments also dealt with method validation and proficiency testing for p16 IHC. Although the literature has little data comparing p16 IHC antibody clones and methods, most of the studies, despite differing methodologies and clones, show strong, independent, and statistically significant performance of p16 IHC alone as a prognostic marker.[11,32,81,141] Interestingly, most of the polled respondents from a CAP practice patterns survey and other surveys use the E6H4 antibody, and most of the studies in the literature also have used it, consistently showing it to have very good performance.[11,53,81,84,89] There are not sufficient data to recommend one antibody, platform, or set of test conditions over another. However, as p16 IHC becomes part of routine clinical practice in OPSCC, test validation and method comparison studies will be critical, and proficiency testing and benchmarking will likely become available. Laboratories should choose tests and model their technical performance after those large studies that validated p16 IHC testing, and must validate their p16 IHC performance in accordance with the IHC validation guideline previously published by the CAP.[150]

Refer to Tables 3 and 4 for the summary of data for laboratory and clinical outcomes for OPSCC tested with p16 IHC.

Statement 3.—*Expert Consensus Opinion*.—Pathologists should *not* routinely perform HR-HPV testing on patients with nonsquamous carcinomas of the oropharynx.

The strength of evidence is *insufficient*.

The vast majority of primary oropharyngeal carcinomas are SCCs derived from the epithelium lining the surface of the oropharynx and the tonsillar crypts, but a subset are carcinomas of minor salivary gland origin. Even less commonly, high-grade neuroendocrine (large and small

Table 3. Continued, Extended

Source, y	No. Test Positive Disease Positive	No. Test Positive Disease Negative	No. Test Negative Disease Positive	No. Test Negative Disease Negative	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Rietbergen et al,[75] 2013 & Rietbergen et al,[77] 2014	161	34	0	646 (were only tested by p16 and not by PCR)	100.0	95	82.6	100
Rischin et al,[32] 2010	44	3	58	67	43.1	95.7	93.6	53.6
Thavaraj et al,[87] 2011	88	2	2	50	97.8	96.2	97.8	96.2
Weinberger et al,[91] 2006	18	1	29	30	38.3	96.8	94.7	50.8
Weinberger et al,[92] 2009	18	0	29	30	38.3	100.0	100.0	50.8
Bledsoe et al,[96] 2013	93	4	0	24	100.0	85.7	95.9	100.0
Fujimaki et al,[98] 2013	30	0	8	28	78.9	100.0	100.0	77.8
Song et al,[101] 2012	15	13	3	15	83.3	53.6	53.6	83.3
Maxwell et al,[130] 2011	49	9	0	8	100.0	47.1	84.5	100.0

cell) carcinomas arise within the oropharynx, sometimes in association with an HPV-positive OPSCC.

Although HR-HPV may play an etiologic role in some oropharyngeal high-grade neuroendocrine carcinomas, the tumors tend to be clinically aggressive, regardless of HPV status.[151–153] In effect, HPV status does not appear to be a reliable marker for separating aggressive and nonaggressive tumors when it comes to high-grade neuroendocrine carcinomas of the oropharynx. For carcinomas of minor salivary gland origin, there is currently insufficient evidence to support an etiologic role of HPV in these tumors, or to validate the practice of HPV-testing them for prognostic purposes.[154–156] Almost all tested tumors have lacked transcriptionally active HR-HPV.[154,157,158] Accordingly, routine HPV testing is not recommended for nonsquamous carcinomas of the oropharynx, including minor salivary gland carcinomas and high-grade neuroendocrine carcinomas. On the other hand, the presence of glandular differentiation by itself should not be taken as an exclusion criterion for HPV testing. Oropharyngeal SCCs can sometimes exhibit glandular formations as a minor or predominant tumor component, and these adenosquamous carcinomas should undergo routine HPV testing as with other variant forms of OPSCC (see statement 1). Case reports of pure adenocarcinomas with transcriptionally active HR-HPV have been described in the oropharynx (similar to the uterine cervix), but they are so few that no recommendation for testing can be provided.[159,160]

Importantly, conventional squamous differentiation, such as surface dysplasia and keratinization, is not highly developed in most HPV-positive OPSCCs. Instead, HPV-positive OPSCCs are typically nonkeratinizing and show varying degrees of basaloid differentiation.[43,161] Human papillomavirus testing should not be suspended in an OPSCC because it lacks keratinization or exhibits basaloid features, as long as it is proven, with IHC if necessary, to be SCC and not a neuroendocrine or other nonsquamous poorly differentiated carcinoma.

In the open comment period, of the 158 respondents, 88.61% (n = 140) agreed with the recommendation, and 8.86% (n = 14) disagreed. There were 14 written comments. Most of these comments acknowledged the rarity of nonsquamous carcinomas in the oropharynx and encouraged continued HPV testing of these tumors in the research (not clinical) setting. Other comments expressed concerns that statement 3 would inappropriately promote nontesting of some OPSCCs because squamous differentiation is often not readily apparent in those that are HPV positive.

Table 4. Summary of Clinical Outcomes for Oropharyngeal Squamous Cell Carcinomas Tested With p16 Immunohistochemistry Alone or in Combination With Human Papillomavirus (HPV)–Specific Tests

Source, y	How HPV Positivity Was Defined	OS Median or % Survival HR (95% CI)	DFS Median or % Survival HR (95% CI)	PFS or RFS Median or % Survival HR (95% CI)	5-y Survival Median or % Survival HR (95% CI)
Ang et al,[11] 2010	p16 DNA ISH	HR, 0.38 (0.26–0.55); $P < .001$ $HR^{MVA} = 0.42$ Controlled for age, race, performance status, tumor stage, nodal stage, pack-years	. . .	HR, 0.40 (0.29–0.57); $P < .001$	. . .
Cerezo et al,[38] 2014	p16 alone	HR, 0.56 (0.22–1.4); $P = .22$ Controlled for age, tobacco, tumor site, stage, radiation therapy dose, chemotherapy	. . .	. . .	. . .
Chaturvedi et al,[1] 2011	p16 DNA PCR DNA ISH RNA-based PCR (eg, RT-PCR)	HPV^+: 131 mo; HPV^-: 20 mo; $P = .001$ HR, 0.31 (0.21–0.46); $P =$ NR Controlled for age; sex; race; registry; calendar period; stage at cancer diagnosis per SEER classification as localized, regional, or distant; and primary course of cancer-directed therapy	. . .	. . .	. . .
Cooper et al,[42] 2013	p16 alone	HR, 1.36 (1.04–1.77); $P = .03$ Controlled for basaloid features, male gender, age, treatment	. . .	. . .	. . .
Gao et al,[44] 2013	p16 RNA-based PCR RNA-based ISH	Univariate $P = .01$ MVA $P = .02$ Controlled for other genes	. . .	. . .	. . .
Gillison et al,[29] 2012	p16 alone	HR, 1.01 (1–1.01)	. . .	. . .	. . .
Holzinger et al,[48] 2012	p16 DNA PCR DNA ISH	HPV^+: 61 mo; HPV^-: 26 mo HR = 0.67 (0.44–1.03); $P = .07$ Controlled for age, gender, clinical stage, therapy status, and alcohol/tobacco consumption	. . .	HPV^+: 32 mo; HPV^-: 12 mo; $P =$ NR HR, 0.77 (0.53–1.12); $P = .2$ Controlled for age, gender, clinical stage, therapy status, and alcohol/tobacco consumption	. . .
Hong et al,[49] 2013	p16 DNA PCR	HR, 0.37 (0.25–0.54) Controlled for age 60 y or older, gender, T stage, N stage, site, smoking status, treatment	. . .	. . .	. . .
Hong et al,[50] 2013	p16 DNA PCR	HR, 0.37 (0.27–0.5); $P < .001$	HR = 0.39 (0.26–0.57); $P < .001$	. . .	. . .
Hong et al,[51] 2013	p16 DNA PCR	HR, 0.36 (0.26–0.5); $P < .001$	HR, 0.38 (0.25–0.59)	. . .	. . .
O'Sullivan et al,[70] 2013	p16 alone	HR, 0.33 (0.2–0.5); $P < .001$ Controlled for drinking, age, sex, T category, N category, treatment, smoking	. . .	. . .	. . .
Park et al,[71] 2013	p16 alone	HR, 2.17; $P = .13$ Controlled for age and T stage HPV^+: 78%; HPV^-: 63%; $P = .25$	. . .	HR, 1.75; $P = .20$	

Table 4. Continued

Source, y	How HPV Positivity Was Defined	OS Median or % Survival HR (95% CI)	DFS Median or % Survival HR (95% CI)	PFS or RFS Median or % Survival HR (95% CI)	5-y Survival Median or % Survival HR (95% CI)
Psychogios et al,[73] 2013	p16 alone	HPV^+: 80.8%; HPV^-: 79.5%; $P = .59$	. . .	. . .	. . .
Reimers et al,[74] 2007	p16 and HPV PCR both done, and both independently used for survival analysis	HR, 0.42 (0.10–1.76); $P = .24$ Controlled for HPV DNA, p16, EGFR, and tumor stage	HPV^+: 74%; HPV^-: 51%; $P = .08$ HR, 0.34 (0.06–1.85); $P = .21$ Controlled for HPV DNA, p16, EGFR, and tumor stage	. . .	HPV^+: 70%; HPV^-: 53%; $P = .23$
Rietbergen et al,[75] 2013	p16 DNA PCR Both p16 IHC and HPV PCR had to be positive to classify a tumor as positive	$HR^{UVA} = 0.34$ (0.25–0.48); $P < .001$ $HR^{MVA} = 0.35$ (0.25–0.5); $P < .001$ Controlled for age, gender, comorbidity (ACE-27 score), pack-years, unit years, tumor size, nodal stage	. . .	HR = 0.33 (0.24–0.46) $P < .001$ Controlled for age, gender, comorbidity (ACE-27 score), pack-years, unit years, tumor size, nodal stage) $p16^+$ and HPV PCR^+ group—5-y PFS: 70% $p16^+$ & HPV PCR^- group—5-y PFS: 42.6% $P < .001$	HPV^+: 73.5%; HPV^-: 40.9%; $P < .001$
Rischin et al,[32] 2010	p16 alone	At 2 y: HPV^+: 91%; HPV^-: 74%; $P = .01$ HR, 0.43 (0.2–0.93); $P = .03$ Controlled for hemoglobin, T category, N category, and ECOG performance status	. . .	. . .	. . .
Rodrigo et al,[78] 2014	p16 DNA PCR	$p16/PCR^+$ patients died of disease: 131/248 (52.8%) $p16/PCR^-$ patients died of disease: 3/248 (1.2%)	. . .	Local recurrence: $p16/PCR^+$: 4 patients; $p16/PCR^-$: 95 patients; $P = .72$	. . .
Scantlebury et al,[80] 2013	p16 RNA-based PCR (Either one or the other)	HR, 0.20 (0.06–0.69) $P = .01$ Controlled for race, smoking, HPV RNA ISH, treatment, D1 expression	HR, 0.25 (0.07–0.86) $P = .03$ Controlled for race, smoking, HPV RNA ISH, treatment, D1 expression	. . .	. . .
Schache et al,[81] 2013	HR-HPV RNAscope (Advanced Cell Diagnostics, Newark, California) (RNA ISH)	Based on RNA ISH: HR, 8.3 (1.9–35.9) $P = .01$ HPV^+ based on RNA ISH 0.91 (0.8–1) HPV^- based on RNA ISH 0.47 (0.33–0.68) $P < .001$	. . .	. . .	. . .

Table 4. Continued

Source, y	How HPV Positivity Was Defined	OS Median or % Survival HR (95% CI)	DFS Median or % Survival HR (95% CI)	PFS or RFS Median or % Survival HR (95% CI)	5-y Survival Median or % Survival HR (95% CI)
Semrau et al,[83] 2013	p16 DNA PCR	HPV DNA PCR and p16: $P = .41$ p16 only: $P = .13$ HPV-DNA PCR only: $P = .55$	. . .	HPV^+: 2-y PFS: HPV DNA PCR and p16: 69.2% 2-y PFS for p16 only: 70.1% HPV^-: 2-y PFS: HPV DNA PCR and p16: 46.2% 2-y PFS for p16 only: 37.1% 2-y PFS: HPV DNA PCR and p16: $P = .49$ 2-y PFS for p16 only: $P = .01$ HPV DNA PCR only: $P = .22$	. . .
Shi et al,[84] 2009	Comparison of qRT-PCR for E6 mRNA, DNA ISH, and p16	Based on qRT-PCR: HPV^+: 88%; HPV^-: 67%; $P = .001$ HR, 0.27 (0.1–0.7); $P = .007$ Based on p16: HPV^+: 88%; HPV^-: 68%; $P = .005$ HR, 0.42 (0.17–1.09); $P = .08$ Based on HPV16 DNA ISH: HPV^+: 86%; HPV^-: 74%; $P = .09$ HR, 0.65 (0.25–1.67); $P = .37$ Controlled for age, stage, and treatment	Based on qRT-PCR: HPV^+: 76%; HPV^-: 47%; $P < .001$ HR, 0.31 (0.15–0.63); $P = .001$ Based on p16: HPV^+: 77%; HPV^-: 46%; $P < .001$ HR, 0.32 (0.16–0.66); $P = .002$ Based on HPV16 DNA ISH: HPV^+: 78%; HPV^-: 47%; $P < .001$ HR, 0.35 (0.18–0.72); $P = .004$ Controlled for age, stage, and treatment	. . .	. . .
Weinberger et al,[91] 2006	p16 DNA PCR	HPV^+: 79% (PCR and p16 positive); HPV^-: 20% (PCR and p16 negative); $P = .01$ HR, 0.19 (HPV PCR positive and p16 positive group) (0.1–0.7); $P = .13$ Controlled for histologic grade, TNM stage, treatment type, primary versus recurrent tumor	HPV^+: 75% (PCR and p16 positive); HPV^-: 15% (PCR and p16 negative); $P = .01$ HR, 0.2 (HPV PCR^+ and $p16^+$ group) (0.1–0.6); $P = .01$ Controlled for histologic grade, TNM stage, treatment type (primary radiation versus surgery/radiation), primary versus recurrent disease	. . .	. . .
Bledsoe et al,[96] 2013	p16 DNA ISH	HPV^+: 93.9%; HPV^-: 73.2%; $P = .01$	HPV^+: 92.7% (86.9%–98.5); HPV^-: 63.5 (42.8%–84.1) $P = .001$	. . .	. . .
Cerezo et al,[97] 2014	p16 RNA-based PCR	HR, 0.55 HPV^+: 67.4%; HPV^-: 49.7; $P = .95$	HR, 0.65 (0.31–1.36) HPV^+: 54.6%; HPV^-: 46.6%; $P = .26$	. . .	. . .

Table 4. Continued

Source, y	How HPV Positivity Was Defined	OS Median or % Survival HR (95% CI)	DFS Median or % Survival HR (95% CI)	PFS or RFS Median or % Survival HR (95% CI)	5-y Survival Median or % Survival HR (95% CI)
Habbous et al,[99] 2014	p16 alone	HR, 0.36 (0.25–0.5); $P < .001$ Controlled for stage, smoking status, pack-years, alcohol consumption, age, marital status, treatment modality, and sex	. . .	. . .	. . .
Hess et al,[100] 2014	p16 alone	HPV$^+$: 86%; HPV$^-$: 71%; $P = .04$	. . .	. . .	. . .
Lassen et al,[34] 2013	p16 alone	HR, 0.30 (0.22–0.41) P = "independent significance" controlled for T classification, lymph node, EGFR expression, and treatment	HR, 0.29 for locoregional tumor control (0.19–0.44) controlled for T classification, lymph node, EGFR expression, and treatment HPV$^+$: 72%; HPV$^-$: 38%; $P < .001$	. . .	. . .
Song et al,[101] 2012	p16 DNA ISH	HPV ISH$^+$: 78.52 mo; HPV ISH$^-$: 63.83 mo; $P = .04$ HR, 5.34 for HPV$^-$ p16$^-$ (1.11–25.81); $P = .04$ Controlled for p16 status, HPV ISH status	HPV$^+$: 86.1 mo; HPV$^-$: 67.1 mo; $P = .12$ HR, 5.28 for p16$^-$ HPV$^-$ (1.09–25.56); $P = .04$ Controlled for p16 status, HPV ISH status	. . .	. . .
Fakhry et al,[35] 2014	p16 alone	HR, 0.57 (0.39–0.84); $P = .005$ Controlled for tumor stage, cigarette pack-years, progression type, salvage surgery	. . .	. . .	. . .
Ang et al,[116] 2012	p16 alone	HPV$^+$: not reached; HPV$^-$: 22.3 mo; $P < .001$ HR = 0.412; $P = .045$ Controlled for smoking, cyclin D1 expression, age, and stage	. . .	. . .	HPV$^+$: 100% for nonsmokers; HPV$^-$: 67% for nonsmokers
Knoedler et al,[119] 2011	p16 alone	HR, 0.44 (0.24–0.78)	. . .	HR, 0.44 (0.25–0.78) HPV$^+$: 79%; HPV$^-$: 52%; $P = .001$	. . .
Liu et al,[106] 2015	p16 RNA-based PCR	HPV$^+$/p16$^+$: 105.4 mo; HPV$^-$/p16$^-$: 14.1 mo HR, 4.65 (3.1–7.2); $P < .001$	. . .	. . .	. . .
Smith et al,[122] 2014	p16 alone	MVA: $P = .01$ Caucasian Americans; $P = .65$ African Americans Controlled for stage, gender, age, tobacco use, treatment	. . .	. . .	. . .
Rakusic et al,[126] 2012	p16 alone	HR = 0.33; $P = .01$ Controlled for T stage, age	. . .	. . .	HPV$^+$: 45%; HPV$^-$: 34%; $P = .07$
Rios Velazquez et al,[107] 2014	Both p16 IHC and PCR were used, but HPV positivity is not defined	HPV$^+$: 82%; HPV$^-$: 39%; $P < .001$	. . .	PFS: HPV$^+$: 83%; HPV$^-$: 35%; $P < .001$	HPV$^+$: 82%; HPV$^-$: 39%; $P < .001$
Brookes et al,[127] 2014	p16 alone	$P = .01$	. . .	RFS $P = .001$	. . .

Table 4. Continued

Source, y	How HPV Positivity Was Defined	OS Median or % Survival HR (95% CI)	DFS Median or % Survival HR (95% CI)	PFS or RFS Median or % Survival HR (95% CI)	5-y Survival Median or % Survival HR (95% CI)
Valduga et al,[129] 2012	p16 alone	HPV$^+$: 68.2%; HPV$^-$: 44.1%; $P = .01$	HPV$^+$: 76.2%; HPV$^-$: 58.4%; $P = .01$	. . .	. . .
Broglie et al,[131] 2012	p16 alone	$P = .001$	. . .	RFS: $P = .01$	. . .
Broglie et al,[134] 2011	p16 alone	$P < .001$	. . .	RFS: $P = .01$	. . .
Holzinger et al,[47] 2013	p16 RNA-based PCR	p16: HR, 0.73 (0.39–1.37); p16 high/HPV16 DNA$^+$: HR, 0.58 (0.28–1.20)	. . .	. . .	. . .
Lassen et al,[135] 2012	p16 alone	HR, 0.28 (0.18–0.48); HPV$^+$: 77%; HPV$^-$: 38%	HR = 0.17	HR = 0.22	. . .
Kuo et al,[54] 2008	p16 DNA PCR DNA ISH	. . .	. . .	. . .	HPV$^+$: 84%; HPV$^-$:59% $P = .01$ for p16 MVA $P = .04$ controlled for age, sex, histology, alcohol, betel nut, smoking, stage, and treatment
Oguejiofor et al,[69] 2013	p16 alone	. . .	6.07 (p16 negative versus positive) recurrence-free survival	. . .	. . .
Preuss et al,[72] 2008	p16 DNA PCR	NR	HR, 0.17 (0.02–1.34); $P = .06$, for PCR of HPV	. . .	HPV$^+$: 72%; HPV$^-$: 48%; $P = .13$
Trosman et al,[108] 2015	p16 DNA ISH	3-y projected OS: HPV$^+$: 89.9%; HPV$^-$: 62.0%; $P < .001$ Median OS: HPV$^+$: 25.6 mo; HPV$^-$: 11.1 mo; $P < .001$	. . .	3-y projected distant control rate: HPV$^+$: 88%; HPV$^-$: 74%; $P = .01$	. . .
Dunlap et al,[112] 2014	HPV DNA, p16	. . .	. . .	Distant recurrence: $P = .16$	. . .
Barasch et al,[110] 2016	p16 alone	. . .	HPV$^+$: 76%; HPV$^-$: 39%; $P < .001$	. . .	HPV$^+$: 80%; HPV$^-$: 39%; $P < .001$
Guihard et al,[125] 2011	RNA-based PCR	. . .	. . .	. . .	HR = 0.38 (0.2–0.72) HPV$^+$: 72%; HPV$^-$: 47%; $P = .003$ Controlled for T stage, nodal status, and age
Bussu et al,[104] 2014	p16 Digene HC2 DNA test (Gaithersburg, Maryland)	. . .	. . .	. . .	DSS: HPV DNA$^+$: 85%; HPV DNA$^-$: 33% HR, 0.19 (0.04–0.8); $P = .02$ p16 HR, 1.23 (0.4–3.7); $P = .71$ Controlled for age, sex, T stage, nodal involvement, primary subsite, and p16 IHC

Table 4. Continued

Source, y	How HPV Positivity Was Defined	OS Median or % Survival HR (95% CI)	DFS Median or % Survival HR (95% CI)	PFS or RFS Median or % Survival HR (95% CI)	5-y Survival Median or % Survival HR (95% CI)
Driessen et al,[111] 2016	p16 DNA PCR	HPV^+: 85%; HPV^-: 63%; $P = .18$	HPV^+: 85%; HPV^-: 51%; $P = .09$	. . .	
Isayeva et al,[52] 2014	p16 RNA-based PCR	. . .	HR, 0.31 (95% CI 0.09–1.06)	HR, 0.27 (disease progression); $P = .01$	. . .

Abbreviations: ACE-27, adult comorbidity evaluation-27; DFS, disease-free survival; DSS, disease-specific survival; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; HR, hazard ratio; IHC, immunohistochemistry; ISH, in situ hybridization; MVA, multivariate analysis; NR, not reported; OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival; qRT-PCR, quantitative RT-PCR; RFS, regression-free survival; RT-PCR, reverse transcriptase PCR; SEER, Surveillance, Epidemiology, and End Results Program; TNM, tumor-node-metastasis; UVA, univariate analysis.

Statement 4.—*Recommendation*.—Pathologists should *not* routinely perform HR-HPV testing on patients with nonoropharyngeal primary tumors of the head and neck.

The strength of evidence is *adequate*.

This recommendation is supported by 1 subgroup analysis of 3 RCTs and 28 observational studies that met the inclusion criteria for our systematic review.[66,158,162–187] Two of these studies were available only in abstract form and, as such, did not undergo formal quality assessment.[186] The evaluable 27 studies were deemed to have varying risk of bias: 5 were deemed low risk of bias,[162,164,169,176,183] 8 low to moderate,[¶] 10 moderate,[#] and 4 high.[66,158,175,179] The high risk of bias for the 4 studies was due to funding from industry and missing information on the other assessment criteria. Refer to Supplemental Table 4 for the quality assessment results for statement 4 studies.

There is confusion among many pathologists and treating physicians regarding the appropriateness of HPV testing in nonoropharyngeal head and neck carcinomas. Because of the considerable attention that HPV-positive OPSCC has received, it is understandable that pathologists and treating physicians may want to generalize that experience to carcinomas arising outside the oropharynx, but the EP did not find evidence to support this practice. Routine HPV testing for nonoropharyngeal head and neck carcinomas is not indicated because there is no proven prognostic or therapeutic difference based on its presence or absence (either by any of the various HPV-specific tests or by the surrogate marker p16). If HPV testing were to yield a positive result in a nonoropharyngeal carcinoma, it might mislead treating physicians and patients as to the origin and likely biologic behavior of the carcinoma. This does not mean that there is no potential biological and clinical significance for transcriptionally active HR-HPV in nonoropharyngeal head and neck carcinomas (particularly in specific anatomic subsites such as the nasopharynx or sinonasal tract); it simply means that the clinical significance and ramifications are not established at this time.

Although the EP does not recommend *routinely* testing nonoropharyngeal carcinomas for HPV, it does recognize that there are occasional situations where it may be indicated. For example, if the anatomic site of tumor origin is not provided, is ambiguous, and/or includes both an oropharyngeal and a nonoropharyngeal site (eg, for large tumors), then HPV testing may be appropriate. As another example, for a patient who had a prior HPV-positive OPSCC, HPV testing in a new non-OPSCC may be appropriate to understand the relationship between the 2 carcinomas (ie, recurrence versus new primary). In these settings, when HPV testing is performed, p16 IHC alone is insufficient because of its suboptimal positive predictive value in nonoropharyngeal sites. p16 IHC can be used to screen a tumor using the same criteria as in the oropharynx. If it is negative, then one can conclude that the tumor is not related to transcriptionally active HR-HPV. If it is positive, however, HPV-specific testing must be performed by one of the available platforms (see algorithm).

The systematic review uncovered 16 studies that investigated HPV testing in nonoropharyngeal head and neck carcinomas (Table 5). These studies were heterogeneous in anatomic subsites evaluated (eg, larynx, oral cavity, sinonasal tract, and other) and in the HPV testing methods used. The studies found that the prevalence of HPV-positive carcinomas, when considering all tests for HR-HPV, is generally low in these nonoropharyngeal sites, ranging from 5.9% to 58.3%.[**] When more specific, RNA-based methods for HPV detection were used, or p16 was combined with HPV-specific testing in order to establish the presence of transcriptionally active HR-HPV, the rates were 2.7% to 5.9%.[175,189] Importantly, when the interpretation criteria were reported and appropriate, the positive predictive value of p16 IHC in nonoropharyngeal carcinomas was low, ranging from 22% to 50%, because of the very low overall rates of transcriptionally active HR-HPV in these tumors.[166,173,175,183,189]

The systematic review identified 28 studies[††] that investigated the clinical outcomes of nonoropharyngeal HPV-positive carcinomas (Table 6). Once again, these studies were highly variable in anatomic subsites examined and HPV testing methods used and were also heterogeneous in their reported outcomes. Only 7 studies reported a statistically significant difference in overall, disease-free, and/or progression-free survival between HPV-positive and HPV-negative groups, including 5 that found that the patients with HPV-positive carcinomas had better survival[162,172,184,185] and 2 that actually found that the HPV-positive group had worse survival.[168,187,190] One additional study found that the HPV-positive group had significantly lower rates of recurrence.[169]

In addition to the aforementioned studies, systematic reviews, and meta-analyses, Li et al[192] and Syrjanen and

[¶] *References 163, 165–167, 170, 177, 180, 185.*

[#] *References 168, 171–174, 178, 181, 182, 184, 187.*

[**] *References 162, 164, 166, 168, 169, 173, 174, 176, 183, 185, 188.*

[††] *References 66, 162, 165, 167–173, 177–188, 190–195.*

Table 5. Summary of Laboratory Data for Human Papillomavirus (HPV) Testing in Nonoropharyngeal Carcinomas

Source, y	No. of Cases and Tumor Type	Method to Determine HPV Status	p16+ Criteria
Oral cavity			
Lingen et al,[176] 2013	409 Oral cavity	qRT-PCR for E6/E7 mRNA alone	H-score (combination of intensity and distribution) >60
Elango et al,[169] 2011	60 Oral tongue SCC	PCR alone	. . .
Duncan et al,[166] 2013	81 Oral cavity SCC	P16 DNA PCR	>50% = p16+
Kaminagakura et al,[173] 2012	114 Oral SCC	p16 DNA PCR	. . .
Ramshankar et al,[188] 2014	156 Oral tongue	p16 DNA PCR	>50% of cells = positive
Duray et al,[168] 2012	162 Oral cavity SCC	PCR alone	Any + = p16+
Chaudhary et al,[164] 2010	430 (222 specimens) Oral submucous fibrosis and oral SCC	PCR alone	. . .
Laryngeal and/or hypopharyngeal			
Laco et al,[174] 2008	88 Laryngeal lesions	ISH alone	Scaled, not clear what is positive
Wendt et al,[183] 2014	142 (109 specimens) Hypopharyngeal	PCR alone	>75% of cells = positive
Sinonasal			
Alos et al,[162] 2009	60 Sinonasal SCC	PCR alone	Only positive if "diffuse" in basal and parabasal layers excluding results in center
Larque et al,[185] 2014	70 Sinonasal SCC	PCR alone	Strong and diffuse cytoplasmic and nuclear in basal and suprabasal cells in all tumor nests
Bishop et al,[163] 2013	161 Sinonasal	p16 and HPV DNA ISH	≥70%
Other sites			
Lewis et al,[175] 2012	87 Oral cavity, laryngeal, hypopharyngeal SCC	RNA ISH alone	>70% = p16+
Skalova et al,[158] 2013	55 Salivary gland	PCR alone	They reported as 1%–25%, 26%–50%, more than 51%
Chung et al,[187] 2014	322 Non-OPSCC	p16 alone	>70% of cells = positive

Abbreviations: ISH, in situ hybridization; mRNA, messenger RNA; NPV, negative predictive value; OPSCC, oropharyngeal squamous cell carcinoma; PCR, polymerase chain reaction; PPV, positive predictive value; qRT-PCR, quantitative reverse transcription PCR; RTOG, Radiation Therapy Oncology Group; SCC, squamous cell carcinoma.

[a] Prevalence calculated by true positive + false negative/total.

Syrjanen[196] pooled data from numerous heterogeneous studies and found that the overall rates of HPV DNA in laryngeal and sinonasal carcinomas were 28% and 27%, respectively. Other studies using RNA-based HPV detection methods on oral cavity and laryngeal carcinomas reported HPV prevalences of 1.3% and 2.3%, respectively.[197,198]

In the open comment period, there were 154 respondents, of whom 79.7% (n = 123) agreed with the recommendation and 12.3% (n = 19) disagreed. There were 28 written comments, including some that expressed disagreement because they believed this statement included anogenital sites. The recommendation was revised to specify that it was applicable only to the head and neck. Some respondents disagreed with the recommendation because of their experience or reports of occasional HPV-positive tumors arising in other nonoropharyngeal head and neck sites (nasopharynx, oral cavity, hypopharynx, sinonasal tract, and conjunctiva/lacrimal sac were all mentioned). Finally, a few respondents felt the language should be changed to allow testing in cases for which the precise anatomic location was not given.

Table 5. Extended

Source, y	ISH+ Criteria	Control Method	Intervention	No. Test Positive, Disease Positive	No. Test Positive, Disease Negative
Oral cavity					
Lingen et al,[176] 2013	Punctate or diffuse nuclear signals	HPV qRT-PCR for E6/E7 mRNA	p16	19	27
Elango et al,[169] 2011	Punctate signals	PCR for HPV DNA	p16	10	8
Duncan et al,[166] 2013	. . .	PCR for HPV DNA	p16	7	7
Kaminagakura et al,[173] 2012	. . .	PCR for HPV DNA	p16	10	12
Ramshankar et al,[188] 2014	. . .	PCR for HPV DNA	p16	10	14
Duray et al,[168] 2012	. . .	PCR for HPV DNA	ISH for HPV DNA	13	0
Chaudhary et al,[164] 2010	. . .	PCR for HPV DNA	Digene Hybrid Capture II (Gaithersburg, Maryland)	61	9
Laryngeal and/or hypopharyngeal					
Laco et al,[174] 2008	Brown staining of nuclei	ISH for HPV DNA	p16	14	0
Wendt et al,[183] 2014	. . .	PCR for HPV DNA	p16	4	14
Sinonasal					
Alos et al,[162] 2009	. . .	PCR for HPV DNA	p16	12	0
Larque et al,[185] 2014	Punctate signals	PCR for HPV DNA	p16	14	0
Bishop et al,[163] 2013	Punctate signals localized to tumor nuclei	PCR for HPV DNA	p16	8	0
Other sites					
Lewis et al,[175] 2012	RNA ISH, granular cytoplasmic or punctate nuclear	ISH for E6/E7 RNA	p16	2	2
Skalova et al,[158] 2013	. . .	PCR for HPV DNA	p16	0	45
Chung et al,[187] 2014	Nuclear signals	ISH for HPV	p16	20	7

Statement 5.—*Recommendation*.—Pathologists should routinely perform HR-HPV testing on patients with metastatic SCC of unknown primary in a cervical upper– or mid–jugular chain lymph node. An explanatory note on the significance of a positive HPV result is recommended.

The strength of evidence is *adequate*.

This statement is supported by 4 observational studies that met the inclusion criteria for the systematic review.[199–202] Risk of bias assessments was deemed low in 2 studies[200,202] and low to moderate in 2.[199,201] None of these studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 5 in the supplemental digital content for the quality assessment results of included studies for statement 5.

Unknown primary is defined as any metastasis for which the primary site has not been clinically identified at the time the biopsy of the metastasis is performed. In this setting, HR-HPV testing may aid in determining the most likely primary site. Hence, HR-HPV status is important for patient management as it informs the clinical team where to search for the primary, or limits the likely area of primary if a definitive lesion is not identified. There are *inadequate* data at this time to support HR-HPV as a prognostic marker in this setting.

In patients who have an unknown primary SCC in the neck, it is common clinical practice to search for the primary site by performing a thorough endoscopic examination and directed biopsies of likely sources of disease, such as the base of tongue, tonsils, and nasopharynx, as well as

Table 5. Extended

Source, y	No. Test Negative, Disease Positive	No. Test Negative, Disease Negative	Prevalence, %[a]	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Oral cavity							
Lingen et al,[176] 2013	5	358	5.9	79.2 (62.9–95.4)	93.0 (90.4–95.5)	41.3 (27.1–55.5)	98.6 (97.4–99.8)
Elango et al,[169] 2011	19	18	52.7	34.5	69.2	55.6	48.6
Duncan et al,[166] 2013	0	67	8.6	100.0	90.5	50.0	100.0
Kaminagakura et al,[173] 2012	11	66	21.2	47.6	84.6	45.5	85.7
Ramshankar et al,[188] 2014	59	73	44.2	14.5	83.9	41.7	55.3
Duray et al,[168] 2012	7	20	50	65.0	100.0	100.0	74.1
Chaudhary et al,[164] 2010	11	141	32.4	84.7	94.0	87.1	92.8
Laryngeal and/or hypopharyngeal							
Laco et al,[174] 2008	0	10	58.3	100.0	100.0	100.0	100.0
Wendt et al,[183] 2014	3	88	6.4	57.1	86.3	22.2	96.7
Sinonasal							
Alos et al,[162] 2009	0	48	20	100.0	100.0	100.0	100.0
Larque et al,[185] 2014	0	56	20	100.0	100.0	100.0	100.0
Bishop et al,[163] 2013	0	0	100	100	0	100	0
Other sites							
Lewis et al,[175] 2012	0	69	2.7	100 (50.0–100)	97.2 (93.3–100)	50.0 (1.0–99.0)	100 (95.7–100)
Skalova et al,[158] 2013	5	5	9.1	0	10	0	50
Chung et al,[187] 2014	33	213	Data directly from study: p16 expression was positive in 14.1%, 24.2%, and 19.0% of non-OPSCC from RTOG 0129, 0234, and 0522, respectively. HPV ISH was positive in 6.5%, 14.6% and 6.9% of non-OPSCC from RTOG 0129, 0234, and 0522, respectively	37.7	96.8	74.1	86.6

tonsillectomy if a primary is not identified on biopsy. The majority of primary tumors can be identified with this approach. Factors that favor an oropharyngeal primary site include cervical (upper or mid jugular chain, synonymous with levels II and III) metastasis location and HR-HPV positivity.[203,204] Thus, HR-HPV status is important because positivity strongly favors oropharyngeal origin and can limit treatment fields, even if a definitive primary is not identified. In addition, more than 70% (to more than 90% in some studies) of SCCs that initially present as an unknown primary can ultimately be confirmed as oropharyngeal in origin after a thorough search.[203,205–208] Repeat HR-HPV testing of the primary tumor, once biopsied or resected, as a prognostic marker is not necessary because the metastasis would have already been tested in this setting.

Although the systematic review revealed limited data, it suggests better outcomes for patients with metastatic HPV-positive compared with HPV-negative SCC in metastases of unknown primary. Refer to Tables 7 and 8 for a summary of laboratory data and clinical outcomes for metastatic SCCs of unknown primary. However, most studies had small patient numbers and/or lacked statistical significance, and thus additional evidence is needed before HR-HPV status can be considered a reliable prognostic marker in SCCs of unknown primary.[199–202,209–211]

It is important that HR-HPV testing is routinely performed only in metastases of unknown primary located in the appropriate (cervical, specifically upper and mid jugular chain) lymph node groups. The upper and mid jugular chain includes level II and III lymph node groups. The pretest

probability of a positive HR-HPV result is high in this location because HPV-positive oropharyngeal SCCs almost always metastasize here, and the primary tumor is often clinically occult.[212,213] Up to one-third of HPV-positive OPSCCs present as an unknown primary (compared with 5%–10% of all head and neck cancers).[212,214] Thus, HR-HPV testing should be performed in all unknown primary cervical upper– or mid–jugular chain metastases.

In contrast, HR-HPV testing should not be routinely performed on metastases of unknown primary outside of the cervical upper– and mid–jugular chain region. Non–upper- and mid-jugular chain lymph node groups include level I (submandibular/submental), level IV (lower jugular), level V (posterior triangle), level VI (pretracheal/prelaryngeal/Delphian) and level VII (superior mediastinal), as well as parotid and supraclavicular nodes. The probability of an HPV-positive metastasis of unknown primary in the above locations is extremely low in the absence of concurrent upper/mid–jugular involvement, and therefore routine HR-HPV testing is not indicated. However, HR-HPV testing may be performed on a non–upper/mid-jugular chain metastasis when clinical suspicion for an HPV-positive SCC is high ("nonroutine" HR-HPV testing).

It is recognized that the specific lymph node group may not always be known to the pathologist examining the metastasis. Some may simply be labeled "right neck" or "left neck" by the ordering physician. Review of the medical record (clinical notes and/or radiology reports) or directly contacting the ordering physician will likely provide sufficient information to identify the involved lymph node group in most cases. There may be rare cases that cannot be localized even after attempts to obtain clinical information. In such cases, HR-HPV testing should be performed.

An explanatory note in the pathology report describing the clinical significance of a positive HR-HPV result is recommended, specifying that HPV-positive SCC metastases most likely originate from the oropharyngeal tonsils and/or base of tongue but rarely may be from other sites (ie, nasopharynx).

In the open comment period, there were 149 respondents; 85.23% (n = 127) agreed, and 11.41% (n = 17) disagreed. There were 22 written comments, including several comments questioning the method of HR-HPV testing, which is the subject of statement 6. Several comments were either in support of or against an explanatory note.

Statement 6.—*Expert Consensus Opinion*.—For tissue specimens (ie, noncytology) from patients presenting with metastatic SCC of unknown primary in a cervical upper– or mid–jugular chain lymph node, pathologists should perform p16 IHC.

Note: Additional HPV-specific testing on p16-positive cases should be performed for tumors located outside of level II or III (nonroutine testing) in the neck and/or for tumors with keratinizing morphology.

Strength of evidence is *insufficient*.

p16 IHC is a very sensitive surrogate marker for HR-HPV that also maintains high positive predictive value when the pretest probability of an HPV-positive SCC is high.[44,146,147] However, the positive predictive value decreases when the pretest probability is low because p16 overexpression can occur by mechanisms other than HR-HPV signaling. For example, as many as 20% to 30% of aggressive head and neck cutaneous SCCs overexpress p16 unrelated to HR-HPV.[215,216]

An algorithmic approach for HR-HPV testing, shown in Figure 1, efficiently triages SCC metastases of unknown primary and reduces unnecessary testing. Following the algorithm, p16 IHC alone is sufficient to determine HR-HPV tumor status when the metastasis is located in one of the upper– or mid–jugular chain (level II and III) lymph node groups and the tumor morphology is nonkeratinizing.[9,213,217] The pretest probability of an HR-HPV–positive SCC is very high in this setting (and therefore p16 IHC is an excellent test). High-risk HPV–specific testing is required to confirm a positive p16 IHC test result only when the tumor morphology is keratinizing and/or the metastasis is located outside of the upper or mid jugular chain (the latter would apply to the setting of nonroutine HPV testing). Confirmatory HR-HPV–specific testing should also be performed if the specific involved lymph node group cannot be determined, regardless of tumor morphology. If the p16 IHC is negative, no further HR-HPV testing is indicated, and the tumor is considered HPV negative because a p16-negative result essentially excludes a transcriptionally active HR-HPV–positive SCC.[44,146,147]

The ability to recognize keratinizing versus nonkeratinizing tumor morphology is important in order to apply the above HR-HPV testing algorithm. Nonkeratinizing SCC resembles transitional epithelium.[9,217] The tumor cells have oval to spindled nuclei, high nuclear to cytoplasmic ratios, and indistinct cell borders, often forming broad, pushing, ribbonlike nests. Limited keratinization may be present and does not mean that the tumor is keratinizing type (as long as the above described histologic features are present). Mitotic activity is typically brisk, with apoptotic debris and/or necrosis in the background. When in doubt as to whether a particular SCC is keratinizing or nonkeratinizing, as may be the case in small biopsy material, HR-HPV–specific testing is recommended. However, one should attempt to histologically classify most SCC metastases. In addition, one must consider that the morphology of the tumor may change in posttreatment specimens so that more keratinization may be present.

In the open comment period, there were 145 respondents; 84.14% (n = 122) agreed, and 11.72% (n = 17) disagreed. There were 21 written comments, with most weighing in on the method of HR-HPV testing: several comments favored p16 IHC testing alone in all settings, some were in support of HR-HPV–specific testing in all cases, and others felt the method should be at the discretion of the pathologist. Several pointed out that the location of the lymph node metastasis (needed to apply the HR-HPV testing algorithm) is not always known to the pathologist.

Statement 7.—*Expert Consensus Opinion*.—Pathologists should perform HR-HPV testing on head and neck FNA SCC samples from all patients with known oropharyngeal SCC not previously tested for HR-HPV, with suspected oropharyngeal SCC, or with metastatic SCC of unknown primary.

Note: No recommendation is made for or against any specific testing methodology for HR-HPV testing in FNA samples. If the result of HR-HPV testing on the FNA sample is negative, testing should be performed on tissue if it becomes available. If pathologists use cytology samples for p16 IHC testing, they should validate the criteria (ie, cutoff) for a positive result.

The strength of evidence is *adequate*.

This statement is supported by 16 studies[26,201,210,218–230] that met the inclusion criteria for our systematic review. Five

Table 6. Summary of Clinical Outcomes for Human Papillomavirus (HPV) Testing in Nonoropharyngeal Carcinomas

Source, y	Method to HPV Status	OS Median or % Survival HR (95% CI)	DFS, PFS, or RFS Median or % Survival HR (95% CI)	5-y Survival Median or % Survival HR (95% CI)	Other Clinical Outcome Reported
Zhao et al,[184] 2009	PCR alone	HR, 0.13 (0.02–0.98); $P = .048$ HPV, grade, stage, tobacco	. . .	. . .	. . .
Alos et al,[162] 2009	PCR alone	HR, 0.17 (0.04–0.81); $P = .07$ Controlling for age, sex, p53, previous papilloma, tumor grade, smoking, tumor location as sinus versus nasal, tumor stage at I–II versus III–IV	DFS: HPV$^+$: 36%; HPV$^-$: 14% PFS: HR, 0.21 (0.06–0.71); $P = .01$ Controlling for age, sex, p53, previous papilloma, tumor grade, smoking, tumor location as sinus versus nasal, tumor stage at I–II versus III–IV	. . .	. . .
Elango et al,[169] 2011	PCR alone	. . .	. . .	. . .	Recurrence: HPV$^+$: 7%; HPV$^-$: 32%; $P = .01$
Sugiyama et al,[182] 2007	PCR alone	. . .	. . .	OR, 0.30 (0.08–1.2); $P = .08$	DSS
Duray et al,[167] 2011	PCR alone	. . .	DFS: HPV$^+$: 67%; HPV$^-$: 77%; P = NS	. . .	. . .
Robinson et al,[180] 2013	p16 DNA PCR DNA ISH All 3 (p16, DNA PCR, DNA ISH) had to be positive to consider a case positive for HPV	The data were not reported purely based on HPV, but rather in combination with EBV Mean overall survival: EBV$^-$/HPV$^-$ = 47.6 mo (19.9–75.3) EBV$^+$/HPV$^-$: 67.9 mo (52.1–83.7) EBV$^-$/HPV$^+$: 53.6 mo (18.3–88.8) $P = .57$	. . .	. . .	. . .
Larque et al,[185] 2014	PCR alone	HPV$^+$: 156.8 mo (mean); HPV$^-$: 72 mo (mean); $P = .03$	DFS: HPV$^+$: mean 65.8 mo; HPV$^-$: mean 30.5 mo; $P = .01$	. . .	. . .
Jiang et al,[172] 2013	ISH alone	HPV overexpression: 66.2%; HPV normal: 86.6%; $P = .06$	HPV overexpression: 60.0%; HPV normal: 83.9%; $P = .03$	. . .	. . .
Ernoux-Neufcoeur et al,[170] 2011	PCR alone	. . .	5-y DFS: HPV$^+$: 58%; HPV$^-$: 88%; P = NS RFS: HPV$^+$: 32% recurred; HPV$^-$: 8% recurred	. . .	5-y DFS in p16; p16$^+$: 100%; p16$^-$: 58%; P = NS
Nemes et al,[178] 2006	PCR alone	. . .	. . .	. . .	2-y survival: HPV$^+$: 45.1%; HPV$^-$: 52.2%; $P = .73$
Stephen et al,[181] 2012	DNA PCR	HPV$^+$: 79.7 mo; HPV$^-$: 75 mo; $P = .35$	. . .	. . .	. . .
Morshed et al,[177] 2008	PCR alone	OS: 0.66 (HPV$^+$ versus HPV$^-$)	. . .	. . .	DSS: 0.49 (HPV$^+$ versus HPV$^-$)
Huang et al,[171] 2012	PCR alone	$P = .44$	$P = .43$	. . .	. . .
Kirby et al,[186] 2014	p16 DNA ISH RNA-based ISH	$P = .17$	. . .	P = NS	. . .
Kaminagakura et al,[173] 2012	p16 DNA PCR	HPV$^+$: 66.8%; HPV$^-$: 38.4%; $P = .12$	DFS: HPV$^+$: 52.7%; HPV$^-$: 40.4%; $P = .36$	. . .	. . .
Wendt et al,[183] 2014	PCR alone	OS: .16 (any HPV DNA positive) (0.03–0.70); $P = .15$ Controlling for age, stage, sex, p16	DFS for patients with hypopharyngeal cancer stratified HPV16 status: $P = .06$ DFS for patients with hypopharyngeal cancer stratified p16 status: $P = .86$	. . .	. . .
Xu et al,[193] 2014	p16 RNA-based PCR	P = NS	DFS: P = NS	. . .	DSS: P = NS

Table 6. Continued

Source, y	Method to HPV Status	OS Median or % Survival HR (95% CI)	DFS, PFS, or RFS Median or % Survival HR (95% CI)	5-y Survival Median or % Survival HR (95% CI)	Other Clinical Outcome Reported
Duray et al,[168] 2012	PCR alone	. . .	5-y DFS HPV$^+$: 40%; HPV$^-$: 76%; $P = .01$ HR, 2.81; $P = .01$ Controlling for TNM staging and node status	. . .	. . .
Lee et al,[191] 2012	PCR alone	$P = .11$	DFS: HPV$^+$: 55%; HPV$^-$: 61%; $P = .21$	HPV$^+$: 37%; HPV$^-$: 50%; $P = .11$	DSS: HPV$^+$: 58%; HPV$^-$: 68%; $P = .21$
Chuang et al,[190] 2012	PCR alone	Only a graph provided: HPV$^+$ did worse; $P = .13$	Only a graph provided for RFS: HPV$^+$ did worse; $P = .03$	HR, 1.77 (0.79–03.95) HPV$^+$: 46.3%; HPV$^-$: 72.6%; $P = .17$ Controlling for sex, smoking, drinking, betel quid use	. . .
Leidy et al,[194] 2012	p16 alone	At 10 y: HPV$^+$: 31%; HPV$^-$: 34%; $P = .28$	. . .	. . .	. . .
Reuschenbach et al,[179] 2013	Not specified	HPV EIA (PCR): $P = .88$ HPV-EIA (PCR and diffuse p16 IHC): $P = .80$ No staining versus focal p16 staining: $P = .09$ No staining versus diffuse p16 staining: $P = .25$	DFS: HPV EIA (PCR): $P = .86$ HPV-EIA (PCR and diffuse p16 IHC): $P = .35$	. . .	. . .
Li et al,[192] 2013	. . .	. . .	. . .	. . .	Risk of laryngeal SCC; OR, 5.39; (3.25–8.94)
Nichols et al,[66] 2013	PCR alone	HR, 0.19 (0.06–0.60); $P = .004$ Controlling for age, sex, site, TNM stage, smoking, alcohol, p16, HPV 16 only	DFS: HR, 0.24 (0.1–0.56); $P = .001$ Controlling for age, sex, site, TNM stage, smoking, alcohol, p16, HPV 16 only	. . .	. . .
Chernock et al,[165] 2013	HPV DNA ISH HPV PCR	$P = .06$	DFS = NS	. . .	. . .
Chung et al,[187] 2014	p16 alone	For p16: HR, 0.56 (0.35–0.89); $P = .01$ Controlling for age, sex, TNM stage For ISH: HR, 0.64 (0.34–1.21), $P = .17$	For p16 PFS: HR, 0.63 (0.42–0.95); $P = .03$ Controlled for age, sex, TNM stage For ISH: HR, 0.77 (0.44–1.33); $P = .35$	. . .	. . .
Ramshankar et al,[188] 2014	p16 DNA PCR	For p16: HR, 2.4 (1.3–4.4); $P = .01$ Controlling for age, sex, stage For HPV16: HR, 0.6 (0.38–0.10); $P = .049$ Controlling for age, sex, stage	DFS for p16: HR, 2.6 (1.4–4.6); $P = .01$ Controlling for age, sex, stage	. . .	. . .
Stenmark et al,[195] 2014	p16 DNA PCR	HR, 1.83 (0.71–4.72) Controlling for age, tobacco exposure, WHO grade, and viral status Median OS: HPV$^+$ = 71%; HPV$^-$ = 47.2%; $P = .39$ Controlling for age, tobacco exposure, WHO grade, and viral status	PFS: HR, 1.86 (0.77–4.47); $P = .36$ Controlling for age, tobacco exposure, WHO grade, and viral status	. . .	Locoregional control: HR, 3.0 (0.78–11.5); $P = .24$ Controlling for age, tobacco exposure, WHO grade, and viral status

Abbreviations: DFS, disease-free survival; DSS, disease-specific survival; EBV, Epstein-Barr virus; EIA, enzyme immunoassay; HR, hazard ratio; ISH, in situ hybridization; NS, not significant (no exact *P* value reported); OR, odds ratio; OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival; RFS, recurrence-free survival; SCC, squamous cell carcinoma; TNM, tumor-node-metastasis; WHO, World Health Organization.

Table 7. Summary of Laboratory Data for Metastatic Squamous Cell Carcinomas of Unknown Primary						
Source, y	No. of Patients or Specimens	Specimen Type	How HPV Was Defined	p16+ Criteria	ISH Criteria	PCR Assay
Compton et al,[199] 2011	25	Resection and biopsy	p16 DNA ISH	. . .	Nuclear signals	. . .
Tribius et al,[200] 2012	63	Specimen type not reported	p16 DNA PCR	<1% is negative, isolated cells <5% is sporadic, focal is small clusters <25%, and diffuse is >25% nuclear and cytoplasmic staining	. . .	Qualitative PCR assay
Vent et al,[201] 2013	47	Specimen type not reported	p16 versus HPV alone	>60%	. . .	Qualitative PCR assay
Sivars et al,[202] 2014	50	Resection and biopsy	PCR alone	>70%	. . .	Quantitative PCR assay—MFI 100

Abbreviations: HPV, human papillomavirus; ISH, in situ hybridization; MFI, median fluorescence intensity; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.

of these studies[210,224–227] were reported only in abstract form and did not undergo quality assurance. Of the 11 studies assessed, all were retrospective cohort designs, with the exception of 1 cross-sectional study.[222] All included studies had prospective data collection, 2 reported blinding of outcome assessors,[222,230] and only 1 reported funding from industry.[230] Ultimately, 4 studies[219,222,229,230] were deemed to have a low to moderate risk of bias, 6 moderate,[‡‡] and 1 high,[228] although this study did not have methodologic flaws that would raise concerns about its findings. See Supplemental Table 6 in the supplemental digital content for the quality assessment results for all studies included in the statement 7 evidentiary base.

Among patients with HPV-positive OPSCC, approximately 85% develop lymph node metastases, and approximately 50% of these patients are first diagnosed based upon an enlarged cervical lymph node.[11,53,55,231–233] Fine-needle aspiration is frequently used to sample and diagnose metastatic head and neck carcinomas in cervical lymph nodes. Because of the marked tendency for HPV-positive HNSCC to metastasize to cervical lymph nodes, FNA plays a very important diagnostic role in the initial detection of these cancers.[234–237] In some cases, cytologic material may be the only tumor specimen available for diagnostic workup, and in a subset of cases, a primary site of origin will not be identified even after exhaustive clinical and radiologic evaluation. The aspirated material obtained by FNA can be tested by any of a variety of methods for HR-HPV and used to classify the metastatic HNSCC as HPV positive or HPV negative, thus indicating an oropharynx origin.

The systematic review identified a limited number of studies that used samples obtained by FNA for analysis of HR-HPV status of metastatic HNSCC. The range of cytologic studies includes HPV determination using formalin-fixed, paraffin-embedded FNA material in a cell block; liquid-based specimens (Surepath, Becton, Dickinson and Company, Franklin Lakes, New Jersey, and ThinPrep, Hologic, Marlborough, Massachusetts); and scrapes from air-dried or alcohol-fixed smears. Testing methodologies evaluated included p16 IHC,[222,229,230,238] DNA ISH,[239] cobas HPV test (Roche Molecular Systems, Inc, Pleasanton, California),[26,228] Cervista HPV HR and Cervista HPV16/18 assays (Hologic),[220,240,241] and Hybrid-Capture 2 assay (Qiagen, Germantown, Maryland).[219,224,242] The literature supports the use of FNA as a valid method for obtaining material for HR-HPV testing.[218,221,223] Sensitivities and specificities of HPV assays for detecting HR-HPV in FNA samples are reported to be greater than 90%; however, there are limited data about the accuracy of any one particular HPV testing method.[§§]

A particular difficulty in the assessment of HR-HPV in FNA samples pertains to the interpretation of p16 immunoreactivity in cell block specimens. Although use of the standard criterion of more than 70% positive cells is accepted when applied to tissue biopsy, 3 recent studies suggest that thresholds as low as 10% to 15% for the percentage of positive cells may be valid for cell blocks.[23,229,230] Whichever method is selected to assess the HR-HPV status of an FNA sample, individual laboratory validation is required. Because of the limited data available pertaining to HR-HPV testing in FNA specimens at the current time, HR-HPV testing is recommended on any subsequent tissue specimens that may become available if HR-HPV testing was negative in the FNA specimen.

In the open comment period there were 142 respondents; 84.51% (n = 120) agreed, and 13.38% (n = 19) disagreed. There were 16 written comments, including a number that suggested that either p16 IHC or liquid-based testing methodologies be used. Others commented that confirmatory testing should be performed for FNA samples testing positive by p16 IHC, particularly in cases where the patient is known to have a nonoropharyngeal HNSCC.

Refer to Tables 9 and 10 for the summaries of laboratory data and clinical outcomes for studies where FNAs were used.

Statement 8.—*Expert Consensus Opinion*.—Pathologists should report p16 IHC positivity as a surrogate for HR-HPV in tissue specimens (ie, noncytology) when there is at least 70% nuclear and cytoplasmic expression with at least moderate to strong intensity.

The strength of evidence is *insufficient*.

p16 is a tumor suppressor protein that inhibits CDK4 and CDK6–dependent/cyclin D–mediated phosphorylation of RB required for cell proliferation.[243] Overexpression of p16

[‡‡] *References 26, 201, 218, 220, 221, 223.*

[§§] *References 26, 219, 220, 222, 224, 228, 229.*

Table 7. Extended

Source, y	Control Method	Intervention	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Compton et al,[199] 2011	ISH for HPV DNA	p16	100 (57.1–100)	77.8 (58.6–97.0)	63.6 (35.2–92.1)	100 (78.6–100)
Tribius et al,[200] 2012	PCR for HPV DNA	p16	72.7 (57.5–87.9)	60.0 (42.5–77.5)	66.7 (51.3–82.1)	66.7 (48.9–84.4)
Vent et al,[201] 2013	ISH for HPV DNA	p16	100 (66.7–100)	96.4 (89.6–100)	90.0 (71.4–100)	100 (88.9–100)
Sivars et al,[202] 2014	PCR for HPV DNA	p16	100.0	90.6	85.7	100.0

Table 8. Clinical Outcomes of Human Papillomavirus Testing in Metastatic Squamous Cell Carcinomas of Unknown Primary

Source, y	Method to Determine HPV Status	OS Median or % Survival HR (95% CI)	DFS Median or % Survival HR (95%CI)	PFS or RFS Median or % Survival HR (95%CI)	3-y or 5-y Survival Median or % Survival HR (95% CI)	Other Clinical Outcome Reported
Compton et al,[199] 2011	p16 DNA ISH	. . .	HPV^+: 66.7%; HPV^-: 48.5%; $P = .54$	. . .	5-y: HPV^+: 66.7%; HPV^-: 48.5%; $P = .35$	. . .
Tribius et al,[200] 2012	p16 DNA PCR	2-y OS: HPV^+: 75.7%; HPV^-: 75.3%; $P = .53$	. . .	PFS: HPV^+: 79.5%; HPV^-: 67.8%; $P = .30$	. . .	. . .
Vent et al,[201] 2013	p16 versus HPV alone	5-y: $p16^+$: 83%; $p16^-$: 32%; P = NS	$p16^+$: 83%; $P16^-$: 32.4%; $P = .18$	. . .	. . .	. . .
Sivars et al,[202] 2014	PCR alone	. . .	HPV^+: 85.0%; HPV^-: 63.3%; $P = .05$	. . .	5-y: HPV^+: 80%; HPV^-: 36.7%; $P = .01$ HR = 0.29 (0.09–0.91); $P = .03$ Controlled for p53 expression, gender, age, and smoking habits 5-y survival HPV DNA and $p16^+$: 77%; HPV DNA and $p16^-$: 40.6%; $P = .02$	. . .
Straetmans et al,[209] 2014	p16 DNA PCR HPV 16 FISH	. . .	. . .	RFS: HPV^+: 100%; HPV^-: 77.8%; P = NS	5-y: HPV^+: 75%; HPV^-: 66.7%; P = NS	Distant metastasis HPV^+: 0%; HPV^-: 5.6%
Fowler et al,[210] 2012 [abstract]	p16 DNA ISH	$P = .001$	$P = .07$	. . .	3-y: HPV^+: 83%; HPV^-: 40% 5-y: HPV^+: 71%; HPV^-: 40%	1-y survival: HPV^+: 97%; HPV^-: 64%
Straetmans et al,[211] 2011 [abstract]	p16 DNA PCR DNA ISH	. . .	. . .	. . .	5-y: $p16^+$:69%; $p16^-$: 33%; $P = .05$ HPV^+: 65%; HPV^-: 37%; $P = .09$	42% of primaries found during follow-up. Significant correlation between HPV^+ and later detection of oropharyngeal primary ($P = .04$)

Abbreviations: DFS, disease-free survival; FISH, fluorescence in situ hybridization; HPV, human papillomavirus; HR, hazard ratio; ISH, in situ hybridization; NS, not significant (no exact *P* value reported); OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival; RFS, regression-free survival.

Table 9. Summary of Laboratory Data for Studies Using Fine-Needle Aspirations

Source, y	No. of Patients or Specimens	Specimen Type	Cancer Type	Method to Determine HPV Status
Vent et al,[201] 2013	47 patients	. . .	CUP	p16 versus HPV alone
Begum et al,[218] 2007	77 specimens	FNA	. . .	. . .
Bishop et al,[219] 2012	24 patients	Cytologic preparations (FNAs and brushes) were obtained from surgical resections	OPSCC, non-OPSCC, cervical nodal metastatic carcinoma of known primary, CUP	p16 DNA PCR DNA ISH
Guo et al,[220] 2014	64 patients	FNA	OPSCC, non-OPSCC, cervical nodal metastatic carcinoma of known primary, CUP	p16 DNA PCR DNA ISH
Lau et al,[263] 2011	67 patients	FNA	Cervical nodal metastatic carcinoma of known primary, CUP	Cervista on FNA fluid for HR-HPV DNA
Jakscha et al,[222] 2013	OPSCC	Resection, biopsy, FNA	. . .	p16 alone
Jannapureddy et al,[223] 2010	40 patients	FNA	OPSCC, non-OPSCC	p16 DNA ISH
Smith et al,[224] 2014	25 patients	Resection, FNA	OPSCC	p16 DNA ISH
Kerr et al,[26] 2014	33 patients	Resection, biopsy, FNA	OPSCC, non-OPSCC	Compared Roche cobas (Roche, Pleasanton, California), ISH, and p16
Davis et al,[225] 2014	74 patients	Surgical specimens, FNA	OPSCC, cervical nodal metastatic carcinoma of known primary	p16 DNA ISH HPV L1 IHC
Fatima et al,[226] 2012 [abstract]	. . .	FNA	Cervical nodal metastatic carcinoma of known primary	p16 DNA ISH
Baldassarri et al,[228] 2015	37 patients	FNA with correlating biopsy or resection specimens	OPSCC, non-OPSCC, cervical nodal metastatic carcinoma of known primary, CUP	Roche cobas on cytology fluid
Holmes et al,[229] 2015	85 patients	Metastatic specimens sampled by FNA and primary tumors by resection or biopsy	CUP	p16 DNA ISH
Jalaly et al,[230] 2015	48 patients	Resection, biopsy, FNA	OPSCC, cervical nodal metastatic carcinoma of known primary	p16 RNA-based ISH

Abbreviations: CUP, cancer of unknown primary; FNA, fine-needle aspiration; HPV, human papillomavirus; HR-HPV, high-risk human papillomavirus; IHC, immunohistochemistry; ISH, in situ hybridization; L1, level 1; NA, not applicable; non-OPSCC, nonoropharyngeal squamous cell carcinoma; NPV, negative predictive value; OPSCC, oropharyngeal squamous cell carcinoma; PCR, polymerase chain reaction; PPV, positive predictive value.

is an established surrogate marker for transcriptionally active HR-HPV infection.[139,140,243,244]

The criteria for p16 IHC have been established in multiple retrospective and prospective studies that validated the association of p16 immunopositivity with a more favorable prognosis in OPSCCs compared with p16-negative carcinomas.[11,35,81,245] It is important to note that staining must be both nuclear and cytoplasmic to be considered positive. Definitions for what percentage of positive cells is necessary have varied substantially; however, some of the largest and prospective studies, such as Ang et al,[11] have supported a stringent cutoff of 70% to 75%. However, in high-incidence countries such as the United States, lesser staining cutoffs may function similarly. With these criteria, the sensitivity of p16 IHC for transcriptionally active HR-HPV approaches 100%. The specificity of p16 IHC in the oropharynx is lower (~85%–95%) for transcriptionally active HR-HPV, in part because of p16 expression unrelated to HPV.[53,81,246] The interrater agreement among pathologists for p16 IHC interpretation is excellent ($\kappa = 0.95$–0.98).[53]

Rare tissue specimens may exhibit an equivocal pattern of p16 staining that fails to meet the recommended threshold for positivity. For instance, cases can exhibit more than 50% and less than 70% moderate to strong nuclear and cytoplasmic staining or diffuse low-intensity nuclear and cytoplasmic staining. Limited evidence suggests a subset of these cases may have transcriptionally active HR-HPV.[53,247]

Table 9. Extended

Source, y	p16+ Criteria	ISH Criteria	PCR assay	Control Method
Vent et al,[201] 2013	>60%	. . .	Qualitative	ISH for HPV DNA
Begum et al,[218] 2007	Any + = p16+	Punctate signals	. . .	ISH for HPV DNA
Bishop et al,[219] 2012	>70% = p16+	Punctate signals	Quantitative—>1 genome copy per 10 cells	ISH for HPV DNA
Guo et al,[220] 2014	. . .	. . .	Qualitative	PCR for HPV DNA
Lau et al,[263] 2011	100% = p16+	. . .	. . .	Cervista
Jakscha et al,[222] 2013	. . .	. . .	. . .	p16 expression in primary tumor
Jannapureddy et al,[223] 2010	Nuclear and cytoplasmic staining	Punctate signals	. . .	ISH for HPV DNA
Smith et al,[224] 2014	>70%	Punctate signals	. . .	p16
Kerr et al,[26] 2014	. . .	. . .	Qualitative	ISH for HPV DNA
Davis et al,[225] 2014	Nuclear and cytoplasmic staining	Punctate signals	. . .	ISH for HPV DNA
Fatima et al,[226] 2012 [abstract]	>70%	Punctate signals	. . .	Status on surgical specimens
Baldassarri et al,[228] 2015	. . .	. . .	. . .	Roche cobas
Holmes et al,[229] 2015	>70%	Punctate signals	NA	ISH for HPV
Jalaly et al,[230] 2015	>15% for cell block but >70% for tissue	Punctate signals	. . .	ISH for E6/E7 RNA

In these situations, an HPV-specific test can be performed at the discretion of the pathologist.

The specificity of p16 IHC outside of the oropharynx is not as well characterized but is lower, in part because of entities that can mimic p16/HPV–positive metastatic OPSCC. For instance, p16 IHC is positive (using the above high expression cutoff) in as many as 20% to 30% of cutaneous head and neck SCCs, which are unrelated to HPV and have no association with clinical outcomes.[216] Approximately 40% of lymphoepithelial cysts are p16 immunopositive within the epithelial lining, although the staining is typically patchy and involves less than 50% of the epithelium.[248]

In the open comment period there were 140 respondents; 90.00% (n = 126) agreed and 4.29% (n = 6) disagreed with statement 8. There were 22 written comments, including comments that the data supporting the 70% threshold should be referenced. Several comments suggested there should be a description of equivocal patterns of p16 staining that do not meet the threshold for positivity. There was a comment that p16, as a surrogate marker for HPV, can be positive in non–HPV-related tumors. Lastly, there were comments that p16 IHC should be standardized to include the antibody clone used.

Statement 9.—*Expert Consensus Opinion*.—Pathologists should *not* routinely perform low-risk HPV testing on patients with head and neck carcinomas.

The strength of evidence is *insufficient*.

There is persistent confusion about whether low-risk HPV types (6 and 11) should be tested in HNSCCs, as indicated by published studies from reference laboratories that indicate that this testing is frequently requested.[249] Low-risk types of HPV are biologically distinct from high-risk types, largely because of different binding and signaling properties of their respective E6 and E7 proteins.[250–253]

Table 9. Extended

Source, y	Intervention	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Vent et al,[201] 2013	p16	100 (66.7–100)	96.4 (89.6–100)	90.0 (71.4–100)	100 (88.9–100)
Begum et al,[218] 2007	p16	90.0 (71.4–100)	55.6 (23.1–88.0)	69.2 (44.1–94.3)	83.3 (53.5–100)
Bishop et al,[219] 2012	Hybrid Capture II (Digene, Gaithersburg, Maryland)	100 (76.9–100)	90.9 (73.9–100)	92.9 (79.4–100)	100 (70–100)
Guo et al,[220] 2014	Cervista (Hologic, Marlborough, Massachusetts)	97.4 (92.3–100)	90.9 (73.9–100)	97.4 (92.3–100)	100 (73.9–100)
Lau et al,[263] 2011	p16	72.4 (56.1–88.7)	83.3 (53.5–100)	95.5 (86.8–100)	38.5 (12.0–64.9)
Jakscha et al,[222] 2013	FNA—p16 expression in lymph node metastasis	92.3 (77.8–100)	95.1 (88.5–100)	85.7 (67.4–100)	97.5 (92.7–100)
Jannapureddy et al,[223] 2010	p16	100 (66.7–100)	77.4 (62.7–92.1)	56.3 (31.9–80.6)	100 (87.5–100)
Smith et al,[224] 2014	Hybrid Capture II	100 (62.5–100) cytologic tumor cells required	100 (62.5–100)	100 (62.5–100)	100 (62.5–100)
		66.7 (40–93.3) without requiring cytologic cells	100 (62.5–100)	100 (62.5–100)	66.7 (40–93.3)
Kerr et al,[26] 2014	Roche cobas	100 (75–100)	86.4 (72–100)	80 (59.8–100)	100 (84.2–100)
Davis et al,[225] 2014	p16	75.0 (63.2–86.8)	63.6 (43.5–83.7)	83.0 (72.2–93.7)	51.9 (33.0–70.7)
	HPV L1	76.6 (64.5–88.7)	31.6 (10.7–52.5)	73.5 (61.1–85.8)	35.3 (12.6–58.0)
Fatima et al,[226] 2012 [abstract]	p16 and HPV ISH	64	100	100	34
		43	100	100	25
Baldassarri et al,[228] 2015	p16	100 (72.7–100)	100 (66.7–100)	100 (72.7–100)	100 (66.7–100)
Holmes et al,[229] 2015	p16	100 (94.5–100)	92 (81.4–100)	96.5 (91.7–100)	100 (87–100)
Jalaly et al,[230] 2015	p16	. . .	. . .	. . .	. . .

Although low-risk HPV types are an established etiologic agent in benign squamous papillomas and warts of various sites, they do not play a significant role in the development of HPV-positive OPSCC.[253]

Because there is little (if any) benefit of identifying low-risk HPV types in the head and neck, the EP determined that there is no role for routine low-risk HPV in this context. Although the systematic review did not specifically address low-risk HPV types, the expert consensus opinion is that low-risk testing should not be routinely performed.

In the open comment period, there were 140 respondents; 95.71% (n = 134) agreed, and 4.29% (n = 6) disagreed. There were 7 written comments, 2 of which focused on a belief that low-risk HPV can lead to dysplasia. The published literature does not support this association. One comment suggested that low-risk HPV testing should be done in patients with HIV; however, there is no evidence that low-risk HPV results in carcinoma in HIV-positive patients.

Statement 10.—*Expert Consensus Opinion*.—Pathologists should not repeat HPV testing on patients with locally recurrent, regionally recurrent, or persistent tumor if primary tumor HR-HPV status has already been established. If initial HR-HPV status was never assessed or results are unknown, testing is recommended. Testing for HPV may be performed on a case-by-case basis for diagnostic purposes if there is uncertainty regarding whether the tumor in question is a recurrence or a new primary SCC.

The strength of evidence is *insufficient*.

High-risk HPV status established on the primary tumor is the basis for its prognostic value. Recurrences are readily testable and have been demonstrated to show the same HR-HPV status.[254] As such, there is no documented value of repeating testing for HR-HPV on locoregionally recurrent or persistent HNSCC. However, because of this consistency of phenotype, HR-HPV testing on a recurrence when the status of the primary OPSCC is unknown would accurately

Table 10. Summary of Clinical Outcomes for Studies Using Fine-Needle Aspiration

Source, y	Method to Define HPV Status	OS, Median or % HR (95% CI)	DFS, Median or % HR (95% CI)	3-y Survival, Median or % HR (95% CI)	5-y Survival, Median or % HR (95% CI)	Other Clinical Outcomes
Vent et al,[201] 2013	p16 versus HPV alone	$p16^+$: 83%; $p16^-$: 32%; $P = NS$	$p16^+$: 83%; $p16^-$: 32.4%; $P = .18$ HPV^+: 67%; HPV^-: 48%; $P = .94$	. . .	. . .	. . .
Fowler et al,[210] 2012	p16 DNA ISH	$P = .001$	5-y: HPV/$p16^+$: 75%; HPV/$p16^-$: 56%; $P = .07$	HPV^+: 83%; HPV^-: 40%	HPV^+: 71%; HPV^-:40%	1-y survival: HPV^+: 97%; HPV^-: 64%
Davis et al,[225] 2014	p16 DNA ISH HPV L1 IHC	$P = .10$	. . .	. . .	. . .	. . .
Inohara et al,[227] 2012	PCR alone	. . .	. . .	HPV^+: 100%; HPV^-: 89%; $P = .67$	. . .	Complete response of advanced nodal metastases (N2c) to concomitant chemoradiotherapy HPV^+: 100%; HPV^-: 40%

Abbreviations: DFS, disease-free survival; HPV, human papillomavirus; HR, hazard ratio; IHC, immunohistochemistry; ISH, in situ hybridization; L1, level 1; NS, not significant (no exact *P* value reported); OS, overall survival; PCR, polymerase chain reaction.

reflect the HPV status and is thus recommended. Particularly with delayed recurrences, a logical clinical question that may arise is the possibility of a new primary tumor. Such scenarios require correlation with clinical and morphologic features, and HPV status may be informative in separating a recurrence from a new primary SCC.

Our systematic review yielded limited data addressing the level of concordance between the HPV status of the primary tumor and the corresponding recurrence. In one study,[254] 16 locoregional recurrences and 21 metastases were tested along with their untreated primaries. Thirty-six of 37 cases (97%) demonstrated a concordant HPV status. For the 1 discordant case, a second primary was not entirely excluded. However, the study also suggests that technical variability in testing may affect findings in a recurrent site.

In the open comment period there were 94 respondents; 92.47% (n = 86) agreed, and 7.53% (n = 7) disagreed. There were 8 comments, most notably the suggestion to indicate that HPV testing may help separate true recurrence from a separate primary tumor. Concern about technical differences among laboratories affecting repeat testing was raised, as well as a concern about treatment effect.

Statement 11.—*Expert Consensus Opinion*.—Pathologists should *not* routinely perform HR-HPV testing on patients with distant metastases if primary tumor HR-HPV status has been established. Testing for HPV may be performed on a case-by-case basis for diagnostic purposes if there is uncertainty regarding whether the tumor in question is a metastasis or a new primary SCC.

The strength of evidence is *insufficient*.

High-risk HPV status established on the primary tumor is the basis for its prognostic value. Distant metastases are readily testable and limited data show that they retain the same HR-HPV status, including p16 overexpression.[137,254,255] As such, there is no documented value of repeating testing on a metastatic tumor. However, because of this consistency of phenotype, HR-HPV testing on a metastasis when the status of the primary is unknown would accurately reflect the HPV status of the primary HNSCC and is thus recommended. In some cases, however, there is the possibility that the metastasis represents a new primary SCC. Such scenarios require correlation with clinical and morphologic features, and HPV status may be informative in separating a distant metastasis from a new primary SCC.

Our systematic review yielded limited data addressing the level of concordance between the HPV status of primary tumor and corresponding distant metastasis. Collectively, concordance is noted in about 44 of 45 (97.7%) tested paired primary tumors and metastases in the literature.[137,254,255] In one study,[137] 20 of 20 tested metastases demonstrated concordant HPV status as compared with their primaries. In another study,[254] 16 locoregional recurrences and 21 distant metastases were tested along with their untreated primaries. Thirty-six of 37 cases (97%) demonstrated concordant HPV status. For the 1 discordant case, a second primary was not entirely excluded. One small series of 4 patients outside of our systematic review confirmed concordance in HPV status between 4 primary and metastatic SCCs.[255]

However, as with recurrences, technical variability in testing may affect findings in a metastatic site,[254] and the findings are insufficient to recommend a specific testing algorithm at recurrent sites. Particularly with possible lung metastases, an approach that includes HPV-specific testing for p16-positive tumors should be considered, because of p16 expression in a subset of lung SCCs not associated with HR-HPV.

In the open comment period there were 90 respondents; 95.56% (n = 86) agreed, and 4.44% (n = 4) disagreed. There were 7 comments, most notably echoing the statement that HPV testing may help separate true recurrence from a separate primary tumor. Concern about p16 IHC testing as a stand-alone test in this context was raised, as well as concern about the effect of tumor heterogeneity.

Statement 12.—*Expert Consensus Opinion*.—Pathologists should report primary OPSCCs that test positive for HR-HPV or its surrogate marker p16 as HPV positive and/or p16 positive.

The strength of evidence is *insufficient*.

Oropharyngeal SCC with transcriptionally active HR-HPV is a distinct subtype of head and neck cancer. Because HPV defines this subtype, the HR-HPV status (by HPV-specific and/or surrogate marker p16 testing) should be included in the pathologic diagnosis. In the literature, a number of different terms have been used to describe the HPV status of

these tumors, including *HPV positive*, *HPV related*, *HPV driven*, *HPV mediated*, and *p16 positive*.[12,55,148,256] The above expert consensus opinion is consistent with the terminology used in contemporary classifications of OPSCCs.

The term *HPV positive* refers to OPSCCs with detectable virus by HPV-specific methods in a tumor that is already established to be p16 positive, referring thus to transcriptionally active HPV. It can also be used to describe tumors that are just positive for p16 by IHC. Although p16 IHC is a surrogate marker for HR-HPV, a positive result in the appropriate clinical and pathologic context is sufficient to classify a tumor as HPV positive for risk stratification.[55] If the term *p16 positive* is used in clinical reporting on its own, a comment should be added that describes the strong relationship between p16 immunopositivity and HPV in the respective setting. For tumors that are positive for p16 by IHC with or without accompanying HPV-specific testing, both HPV positive and p16 positive can be used for reporting (ie, HPV-positive or p16-positive OPSCC).

The World Health Organization (WHO) also recommends the term *SCC, HPV positive* for patients who are either p16 positive (when it is the only test performed) or p16 plus HPV-specific test positive. The new Union for International Cancer Control[257] and AJCC staging systems[14] prefer a hybrid term, *HPV-mediated (p16$^+$) oropharyngeal cancer*. The panel considers these synonymous.

The above terminology should be used at the time of diagnosis, if possible. Delays in the reporting of HPV status should be avoided, as the HPV status of OPSCCs defines the tumor subtype, predicts prognosis, and may affect therapeutic decisions. Given the widespread availability and rapid turnaround time of p16 IHC, the HPV status of OPSCC should be rapidly available in the majority of cases.[243]

In the open comment period there were 136 respondents; 92.65% (n = 126) agreed, and 5.15% (n = 7) disagreed. There were 22 written comments. There were several comments that HPV positive and p16 positive are not equivalent, in part because of a small percentage of p16-positive tumors that are unrelated to HPV. Others commented that reporting terminology needs to explicitly describe the HPV status, given that some clinicians may not understand the relationship between p16 IHC and HPV status. There was a suggestion to avoid the term HPV positive because of the stigma of sexually transmitted diseases. Lastly, there was a suggestion to use the terminology approved by the *WHO Classification of Head and Neck Tumours*,[258] which this guideline agrees with, in accordance with the new 4th edition.

Statement 13.—*Expert Consensus Opinion*.—Pathologists should *not* provide a tumor grade or differentiation status for HPV-positive/p16-positive OPSCC.

The strength of evidence is *insufficient*.

Tumor grade is a measurement of differentiation, that is, how closely a tumor resembles the normal tissue from which it presumably arises. Tumor grade generally correlates with biologic behavior, so that well-differentiated tumors typically behave less aggressively than poorly differentiated tumors. For SCCs, highly keratinizing tumors with keratin pearl formation and small nuclei are considered well differentiated. Most HPV-positive OPSCCs have a characteristic morphologic appearance. As they are usually nonkeratinizing, with high nuclear to cytoplasmic ratio sand hyperchromatic nuclei, and are arranged in lobules and sheets, they have often been classified as poorly differentiated or high-grade carcinomas. However, these classifiers were developed in head and neck SCC in general and not specifically for HPV-positive OPSCC. In these tumors, this morphology does not predict poor outcomes, but rather is paradoxically associated with a better prognosis in a majority of cases because it predicts HPV positivity.[2,55,90]

Human papillomavirus–positive/p16-positive OPSCCs arise from the tonsillar crypts rather than the surface epithelium. The tonsillar crypts are lined by a specialized reticulated epithelium with an associated lymphocytic infiltrate. Deep in the normal crypts, this epithelium has basaloid cytologic features, absent keratinization, high nuclear to cytoplasmic ratio, and permeating lymphocytes. Westra[161] has proposed that HPV-positive oropharyngeal SCCs might best be considered as well-differentiated carcinomas given their resemblance to the nonneoplastic reticulated crypt lining epithelium.

In the open comment period there were 136 respondents; 57.35% (n = 78) agreed, and 36.03% (n = 49) disagreed, making this the recommendation with the lowest agreement rate. There were 26 written comments. Eight comments were acknowledgment of the respondents' lack of expertise to answer the question. Thirteen respondents stated that grading should be performed. Some of these 13 felt that tumor grade and differentiation might assist in staging and future therapy. Some saw "no harm" in providing tumor grade even if it did not correlate with clinical behavior. Some of the respondents confused tumor grade and staging. Several respondents pointed out that the CAP protocol for the examination of specimens from patients with carcinomas of the pharynx[259] and the 7th edition of the AJCC staging manual[260] require histologic grading on all head and neck cancers. The concerns regarding AJCC 7th edition will be less relevant as the 8th edition of the AJCC staging manual[14] comes into clinical use by early 2018, and familiarity with the 4th edition of the *WHO Classification of Head and Neck Tumours*[258] grows. The latter states that grading is not applicable for HPV-positive OPSCC. In addition, some pointed out the practical complication that one might not know what the p16 status was before signing out a case, and that one would have to provide tumor grade in an addendum if the tumor turned out to be p16 negative.

Statement 14.—*Expert Consensus Opinion*.—Pathologists should *not* alter HR-HPV testing strategy based on patient smoking history.

The strength of evidence is *insufficient*.

Patients with HPV-positive OPSCC often have improved disease-free and overall survival when compared with those with HPV-negative OPSCC. The strong historical association of OPSCC with tobacco and alcohol use has led to the examination of these and other potential prognostic factors to further risk stratify HPV-positive OPSCC to allow the identification of patients who might benefit from treatment deintensification and subsequent reduction in short- and long-term side effects without compromising overall survival. The initial observation that tobacco use significantly decreased the improved survival seen in HPV-positive OPSCC resulted from a retrospective analysis of Radiation Therapy Oncology Group 0129 data using recursive partition analysis. Although HPV status was the main predictor of survival, tobacco use as measured by pack-years increased the risk of death by 1% per year, independent of OPSCC HPV status.[11] The authors concluded that tumor HPV status and tobacco use (>10 pack-years) were robust and independent predictors of survival after chemoradiation therapy. A subsequent retrospective analysis based on the

subset of OPSCC patients from Radiation Therapy Oncology Groups 9003 and 0129 confirmed these findings.[29] Careful and exhaustive quantitation of tobacco exposure in this analysis demonstrated that the risk of death increased linearly with tobacco smoking as measured by pack-years in HPV-related OPSCC. The increased rate of locoregional failure seen in this cohort suggests a likely direct effect of tobacco use on treatment effectiveness, rather than from competing causes of mortality commonly seen in smokers. Since these initial studies, a number of additional analyses based on recursive partitioning analysis have validated tobacco use as an important variable in treatment response in HPV-positive OPSCC.[70,261] There is also no published evidence that smoking changes the results of any of the HPV-specific tests or p16 IHC. Consequently, the EP does not recommend altering the HR-HPV testing strategy based on smoking history. Rather, tobacco use, as measured by pack-years, is one of several variables, along with HR-HPV status, that the treating physician will use when counseling patients regarding likely treatment outcomes and potentially when selecting therapy in the context of a clinical trial.

In the open comment period there were 134 respondents; 97.76% (n = 131) agreed, and 1.49% (n = 2) disagreed. Of all the recommendations, this one had the highest agreement rate. There were only 8 written comments. Two respondents commented that the smoking history was irrelevant, one respondent expressed concern regarding pathology access to the smoking history, and another expressed concern about the lack of standards in reporting a smoking history. One respondent agreed that HPV and smoking may coexist, and another stressed smoking does not rule out HPV infection. One respondent suggested performing HPV testing in non-OPSCC in the absence of a smoking history and limited keratinization.

CONCLUSIONS

The emergence of HPV-positive OPSCC, which is biologically and clinically a unique type of HNSCC, has made it critical that such patients be identified by routine testing for HR-HPV in clinical practice. Knowledge of the HPV tumor status is important for patient prognosis and for the establishment of specific treatments better matched to such tumors. This EP, through a rigorous systematic review, has provided 14 formal recommendations or expert consensus opinions on the nature of HPV testing in various head and neck specimens, scenarios, and settings, with the goal of standardizing what is performed across diverse pathology practice settings. These recommendations will be expected to evolve with future research, literature updates, and reviews in the coming years.

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References

1. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol*. 2011; 29(32):4294–4301.

2. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst*. 2000;92(9):709–720.

3. Adelstein DJ, Ridge JA, Gillison ML, et al. Head and neck squamous cell cancer and the human papillomavirus: summary of a National Cancer Institute State of the Science Meeting, November 9–10, 2008, Washington, D.C. *Head Neck*. 2009;31(11):1393–1422.

4. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med*. 2007;356(19):1944–1956.

5. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol*. 2010;11(8):781–789.

6. Viens LJ, Henley SJ, Watson M, et al. Human papillomavirus-associated cancers—United States, 2008–2012. *MMWR Morb Mortal Wkly Rep*. 2016; 65(26):661–666.

7. Bhosale PG, Pandey M, Desai RS, et al. Low prevalence of transcriptionally active human papilloma virus in Indian patients with HNSCC and leukoplakia. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2016;122(5):609–618.e7.

8. Castellsague X, Alemany L, Quer M, et al. HPV involvement in head and neck cancers: comprehensive assessment of biomarkers in 3680 patients. *J Natl Cancer Inst*. 2016;108(6):djv403. doi:10.1093/jnci/djv403.

9. Gondim DD, Haynes W, Wang X, Chernock RD, El-Mofty SK, Lewis JS Jr. Histologic typing in oropharyngeal squamous cell carcinoma: a 4-year prospective practice study with p16 and high-risk HPV mRNA testing correlation *Am J Surg Pathol*. 2016;40(8):1117–1124.

10. O'Sullivan B, Huang SH, Perez-Ordonez B, et al. Outcomes of HPV-related oropharyngeal cancer patients treated by radiotherapy alone using altered fractionation. *Radiother Oncol*. 2012;103(1):49–56.

11. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. 2010;363(1):24–35.

12. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus–positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst*. 2008;100(4):261–269.

13. Ragin CCR, Taioli E. Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: review and meta-analysis. *Int J Cancer*. 2007;121(8):1813–1820.

14. Amin MB, Edge SB, Greene F, et al. *AJCC Cancer Staging Manual*. 8th ed. New York, NY: Springer; 2017.

15. Bonilla-Velez J, Mroz EA, Hammon RJ, Rocco JW. Impact of human papillomavirus on oropharyngeal cancer biology and response to therapy: implications for treatment. *Otolaryngol Clin North Am*. 2013;46(4):521–543.

16. Mirghani H, Amen F, Blanchard P, et al. Treatment de-escalation in HPV-positive oropharyngeal carcinoma: ongoing trials, critical issues and perspectives. *Int J Cancer*. 2015;136(7):1494–1503.

17. Westra W. Detection of human papillomavirus (HPV) in clinical samples: evolving methods and strategies for the accurate determination of HPV status of head and neck carcinomas. *Oral Oncol*. 2014;50(9):771–779.

18. Begum S, Gillison ML, Ansari-Lari MA, Shah K, Westra WH. Detection of human papillomavirus in cervical lymph nodes: a highly effective strategy for localizing site of tumor origin. *Clin Cancer Res*. 2003;9(17):6469–6475.

19. Bishop JA, Ogawa T, Chang X, et al. HPV analysis in distinguishing second primary tumors from lung metastases in patients with head and neck squamous cell carcinoma. *Am J Surg Pathol*. 2012;36(1):142–148.

20. Min KW, Houck JR, Jr; Cancer Committee, College of American Pathologists. Protocol for the examination of specimens removed from patients with carcinomas of the upper aerodigestive tract: carcinomas of the oral cavity including lip and tongue, nasal and paranasal sinuses, pharynx, larynx, salivary glands, hypopharynx, oropharynx, and nasopharynx. *Arch Pathol Lab Med*. 1998; 122(3):222–230.

21. Hou Y, Chaudhary S, Shen R, Li Z. Fine-needle aspiration of cervical lymph nodes yields adequate materials for accurate HPV testing in metastatic head and neck squamous cell carcinomas. *Diagn Cytopathol*. 2016;44(10):792–798.

22. Channir HI, Gronhoj Larsen C, Ahlborn LB, et al. Validation study of HPV DNA detection from stained FNA smears by polymerase chain reaction: improving the diagnostic workup of patients with a tumor on the neck. *Cancer*. 2016;124(11):820–827.

23. Xu B, Ghossein R, Lane J, Lin O, Katabi N. The utility of p16 immunostaining in fine needle aspiration in p16-positive head and neck squamous cell carcinoma. *Hum Pathol*. 2016;54:193–200.

24. Sivars L, Landin D, Haeggblom L, et al. Human papillomavirus DNA detection in fine-needle aspirates as indicator of human papillomavirus–positive oropharyngeal squamous cell carcinoma: a prospective study. *Head Neck*. 2016; 39(3):419–426.

25. Takes RP, Kaanders JH, van Herpen CM, Merkx MA, Slootweg PJ, Melchers WJ. Human papillomavirus detection in fine needle aspiration cytology of lymph node metastasis of head and neck squamous cell cancer. *J Clin Virol*. 2016;85:22–26.

26. Kerr DA, Pitman MB, Sweeney B, Arpin RN III, Wilbur DC, Faquin WC. Performance of the Roche cobas 4800 high-risk human papillomavirus test in cytologic preparations of squamous cell carcinoma of the head and neck. *Cancer Cytopathol*. 2014;122(3):167–174.

27. Institute of Medicine (IOM). *Clinical Practice Guidelines We Can Trust*. Washington, DC: National Academies Press; 2011.

28. O'Rorke MA, Ellison MV, Murray LJ, Moran M, James J, Anderson LA. Human papillomavirus related head and neck cancer survival: a systematic review and meta-analysis. *Oral Oncol*. 2012;48(12):1191–1201.

29. Gillison ML, Zhang Q, Jordan R, et al. Tobacco smoking and increased risk of death and progression for patients with p16-positive and p16-negative oropharyngeal cancer. *J Clin Oncol*. 2012;30(17):2102–2111.

30. Kumar B, Cordell KG, Lee JS, et al. EGFR, p16, HPV titer, Bcl-xL and p53, sex, and smoking as indicators of response to therapy and survival in oropharyngeal cancer. *J Clin Oncol*. 2008;26(19):3128–3137.

31. Posner MR, Lorch JH, Goloubeva O, et al. Survival and human papillomavirus in oropharynx cancer in TAX 324: a subset analysis from an international phase III trial. *Ann Oncol*. 2011;22(5):1071–1077.

32. Rischin D, Young RJ, Fisher R, et al. Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. *J Clin Oncol*. 2010;28(27):4142–4148.

33. Wu Y, Posner MR, Schumaker LM, et al. Novel biomarker panel predicts prognosis in human papillomavirus-negative oropharyngeal cancer: an analysis of the TAX 324 trial. *Cancer*. 2012;118(7):1811–1817.

34. Lassen P, Overgaard J, Eriksen JG. Expression of EGFR and HPV-associated p16 in oropharyngeal carcinoma: correlation and influence on prognosis after radiotherapy in the randomized DAHANCA 5 and 7 trials. *Radiother Oncol*. 2013;108(3):489–494.

35. Fakhry C, Zhang Q, Nguyen-Tan PF, et al. Human papillomavirus and overall survival after progression of oropharyngeal squamous cell carcinoma. *J Clin Oncol*. 2014;32(30):3365–3373.

36. Al-Swiahb JN, Huang C-C, Fang F-M, et al. Prognostic impact of p16, p53, epidermal growth factor receptor, and human papillomavirus in oropharyngeal cancer in a betel nut-chewing area. *Arch Otolaryngol Head Neck Surg*. 2010;136(5):502–508.

37. Attner P, Du J, Nasman A, et al. Human papillomavirus and survival in patients with base of tongue cancer [published correction appears in *Int J Cancer*. 2012;131(9):E1182]. *Int J Cancer*. 2011;128(12):2892–2897.

38. Cerezo L, de la Torre A, Hervas A, et al. Oropharyngeal cancer related to human papilloma virus: incidence and prognosis in Madrid, Spain. *Clin Transl Oncol*. 2014;16(3):301–306.

39. Cheng NM, Chang JTC, Huang CG, et al. Prognostic value of pretreatment 8F-FDG PET/CT and human papillomavirus type 16 testing in locally advanced oropharyngeal squamous cell carcinoma. *Eur J Nucl Med Mol Imaging*. 2012; 39(11):1673–1684.

40. Chien CY, Su CY, Fang FM, et al. Lower prevalence but favorable survival for human papillomavirus-related squamous cell carcinoma of tonsil in Taiwan. *Oral Oncol*. 2008;44(2):174–179.

41. Cohen MA, Weinstein GS, O'Malley BW Jr, Feldman M, Quon H. Transoral robotic surgery and human papillomavirus status: oncologic results. *Head Neck*. 2011;33(4):573–580.

42. Cooper T, Biron V, Adam B, Klimowicz AC, Puttagunta L, Seikaly H. Prognostic utility of basaloid differentiation in oropharyngeal cancer. *J Otolaryngol Head Neck Surg*. 2013;42:57. doi:10.1186/1916-0216-42-57.

43. El-Mofty SK, Patil S. Human papillomavirus (HPV)-related oropharyngeal nonkeratinizing squamous cell carcinoma: characterization of a distinct phenotype. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101(3): 339–345.

44. Gao G, Chernock RD, Gay HA, et al. A novel RT-PCR method for quantification of human papillomavirus transcripts in archived tissues and its application in oropharyngeal cancer prognosis. *Int J Cancer*. 2013;132(4):882–890.

45. Granata R, Miceli R, Orlandi E, et al. Tumor stage, human papillomavirus and smoking status affect the survival of patients with oropharyngeal cancer: an Italian validation study. *Ann Oncol*. 2012;23(7):1832–1837.

46. Hannisdal K, Schjolberg A, De Angelis PM, Boysen M, Clausen OPF. Human papillomavirus (HPV)-positive tonsillar carcinomas are frequent and have a favourable prognosis in males in Norway. *Acta Otolaryngol*. 2010;130(2):293–299.

47. Holzinger D, Flechtenmacher C, Henfling N, et al. Identification of oropharyngeal squamous cell carcinomas with active HPV16 involvement by immunohistochemical analysis of the retinoblastoma protein pathway. *Int J Cancer*. 2013;133(6):1389–1399.

48. Holzinger D, Schmitt M, Dyckhoff G, Benner A, Pawlita M, Bosch FX. Viral RNA patterns and high viral load reliably define oropharynx carcinomas with active HPV16 involvement. *Cancer Res*. 2012;72(19):4993–5003.

49. Hong A, Jones D, Chatfield M, et al. HPV status of oropharyngeal cancer by combination HPV DNA/p16 testing: biological relevance of discordant results. *Ann Surg Oncol*. 2013;20(suppl 3):S450–S458.

50. Hong AM, Martin A, Armstrong BK, et al. Human papillomavirus modifies the prognostic significance of T stage and possibly N stage in tonsillar cancer. *Ann Oncol*. 2013;24(1):215–219.

51. Hong AM, Martin A, Chatfield M, et al. Human papillomavirus, smoking status and outcomes in tonsillar squamous cell carcinoma. *Int J Cancer*. 2013; 132(12):2748–2754.

52. Isayeva T, Xu J, Dai Q, et al. African Americans with oropharyngeal carcinoma have significantly poorer outcomes despite similar rates of human papillomavirus-mediated carcinogenesis. *Hum Pathol*. 2014;45(2):310–319.

53. Jordan RC, Lingen MW, Perez-Ordonez B, et al. Validation of methods for oropharyngeal cancer HPV status determination in US cooperative group trials. *Am J Surg Pathol*. 2012;36(7):945–954.

54. Kuo KT, Hsiao CH, Lin CH, Kuo LT, Huang SH, Lin MC. The biomarkers of human papillomavirus infection in tonsillar squamous cell carcinoma—molecular basis and predicting favorable outcome. *Mod Pathol*. 2008;21(4): 376–386.

55. Lewis JS Jr, Thorstad WL, Chernock RD, et al. p16 positive oropharyngeal squamous cell carcinoma: an entity with a favorable prognosis regardless of tumor HPV status. *Am J Surg Pathol*. 2010;34(8):1088–1096.

56. Licitra L, Perrone F, Bossi P, et al. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. *J Clin Oncol*. 2006;24(36):5630–5636.

57. Lin RJ, Lubpairee T, Liu KY, Anderson DW, Durham S, Poh CF. Cyclin D1 overexpression is associated with poor prognosis in oropharyngeal cancer. *J Otolaryngol Head Neck Surg*. 2013;42:23. doi:10.1186/1916-0216-42-23.

58. Lindel K, Beer KT, Laissue J, Greiner RH, Aebersold DM. Human papillomavirus positive squamous cell carcinoma of the oropharynx: a radiosensitive subgroup of head and neck carcinoma. *Cancer*. 2001;92(4):805–813.

59. Marklund L, Nasman A, Ramqvist T, Dalianis T, Munck-Wikland E, Hammarstedt L. Prevalence of human papillomavirus and survival in oropharyngeal cancer other than tonsil or base of tongue cancer. *Cancer Med*. 2012; 1(1):82–88.

60. Maxwell JH, Kumar B, Feng FY, et al. Tobacco use in human papillomavirus–positive advanced oropharynx cancer patients related to increased risk of distant metastases and tumor recurrence. *Clin Cancer Res*. 2010;16(4):1226–1235.

61. Mills AM, Beck AH, Pourmand N, Le QT, Kong CS. Evaluation of ProExC as a prognostic marker in oropharyngeal squamous cell carcinomas. *Am J Surg Pathol*. 2012;36(8):1158–1164.

62. Mizumachi T, Kano S, Sakashita T, et al. Improved survival of Japanese patients with human papillomavirus-positive oropharyngeal squamous cell carcinoma. *Int J Clin Oncol*. 2013;18(5):824–828.

63. Mooren JJ, Kremer B, Claessen SMH, et al. Chromosome stability in tonsillar squamous cell carcinoma is associated with HPV16 integration and indicates a favorable prognosis. *Int J Cancer*. 2013;132(8):1781–1789.

64. Nasman A, Andersson E, Marklund L, et al. HLA class I and II expression in oropharyngeal squamous cell carcinoma in relation to tumor HPV status and clinical outcome. *PLoS One*. 2013;8(10):e77025. doi:10.1371/journal.pone.0077025.

65. Nasman A, Nordfors C, Grun N, et al. Absent/weak CD44 intensity and positive human papillomavirus (HPV) status in oropharyngeal squamous cell carcinoma indicates a very high survival. *Cancer Med*. 2013;2(4):507–518.

66. Nichols AC, Dhaliwal SS, Palma DA, et al. Does HPV type affect outcome in oropharyngeal cancer? *J Otolaryngol Head Neck Surg*. 2013;42:9. doi:10.1186/1916-0216-42-9.

67. Nichols AC, Finkelstein DM, Faquin WC, et al. Bcl2 and human papilloma virus 16 as predictors of outcome following concurrent chemoradiation for advanced oropharyngeal cancer. *Clin Cancer Res*. 2010;16(7):2138–2146.

68. Nichols AC, Palma DA, Dhaliwal SS, et al. The epidemic of human papillomavirus and oropharyngeal cancer in a Canadian population. *Curr Oncol*. 2013;20(4):212–219.

69. Oguejiofor KK, Hall JS, Mani N, et al. The prognostic significance of the biomarker p16 in oropharyngeal squamous cell carcinoma. *Clin Oncol (R Coll Radiol)*. 2013;25(11):630–638.

70. O'Sullivan B, Huang SH, Siu LL, et al. Deintensification candidate subgroups in human papillomavirus–related oropharyngeal cancer according to minimal risk of distant metastasis. *J Clin Oncol*. 2013;31(5):543–550.

71. Park K, Cho KJ, Lee M, Yoon DH, Kim S-B. Importance of FOXP3 in prognosis and its relationship with p16 in tonsillar squamous cell carcinoma. *Anticancer Res*. 2013;33(12):5667–5673.

72. Preuss SF, Weinell A, Molitor M, et al. Nuclear survivin expression is associated with HPV-independent carcinogenesis and is an indicator of poor prognosis in oropharyngeal cancer. *Br J Cancer*. 2008;98(3):627–632.

73. Psychogios G, Mantsopoulos K, Agaimy A, et al. Prognostic factors in limited (T1-2, N0-1) oropharyngeal carcinoma treated with surgery ± adjuvant therapy. *Head Neck*. 2013;35(12):1752–1758.

74. Reimers N, Kasper HU, Weissenborn SJ, et al. Combined analysis of HPV-DNA, p16 and EGFR expression to predict prognosis in oropharyngeal cancer. *Int J Cancer*. 2007;120(8):1731–1738.

75. Rietbergen MM, Brakenhoff RH, Bloemena E, et al. Human papillomavirus detection and comorbidity: critical issues in selection of patients with oropharyngeal cancer for treatment de-escalation trials. *Ann Oncol*. 2013;24(11): 2740–2745.

76. Rietbergen MM, Leemans CR, Bloemena E, et al. Increasing prevalence rates of HPV attributable oropharyngeal squamous cell carcinomas in the Netherlands as assessed by a validated test algorithm. *Int J Cancer*. 2013;132(7): 1565–1571.

77. Rietbergen MM, Snijders PJ, Beekzada D, et al. Molecular characterization of p16-immunopositive but HPV DNA-negative oropharyngeal carcinomas. *Int J Cancer*. 2014;134(10):2366–2372.

78. Rodrigo JP, Heideman DAM, Garcia-Pedrero JM, et al. Time trends in the prevalence of HPV in oropharyngeal squamous cell carcinomas in northern Spain (1990–2009). *Int J Cancer*. 2014;134(2):487–492.

79. Rotnaglova E, Tachezy R, Salakova M, et al. HPV involvement in tonsillar cancer: prognostic significance and clinically relevant markers. *Int J Cancer*. 2011;129(1):101–110.

80. Scantlebury JB, Luo J, Thorstad WL, El-Mofty SK, Lewis JS Jr. Cyclin D1—a prognostic marker in oropharyngeal squamous cell carcinoma that is tightly associated with high-risk human papillomavirus status. *Hum Pathol*. 2013;44(8): 1672–1680.

81. Schache AG, Liloglou T, Risk JM, et al. Validation of a novel diagnostic standard in HPV-positive oropharyngeal squamous cell carcinoma. *Br J Cancer*. 2013;108(6):1332–1339.

82. Sedaghat AR, Zhang Z, Begum S, et al. Prognostic significance of human papillomavirus in oropharyngeal squamous cell carcinomas. *Laryngoscope*. 2009;119(8):1542–1549.

83. Semrau R, Duerbaum H, Temming S, et al. Prognostic impact of human papillomavirus status, survivin, and epidermal growth factor receptor expression on survival in patients treated with radiochemotherapy for very advanced nonresectable oropharyngeal cancer. *Head Neck*. 2013;35(9):1339–1344.

84. Shi W, Kato H, Perez-Ordonez B, et al. Comparative prognostic value of HPV16 E6 mRNA compared with in situ hybridization for human oropharyngeal squamous carcinoma. *J Clin Oncol*. 2009;27(36):6213–6221.

85. Straetmans JM, Olthof N, Mooren JJ, de Jong J, Speel EJM, Kremer B. Human papillomavirus reduces the prognostic value of nodal involvement in tonsillar squamous cell carcinomas. *Laryngoscope*. 2009;119(10):1951–1957.

86. Tahtali A, Hey C, Geissler C, et al. HPV status and overall survival of patients with oropharyngeal squamous cell carcinoma—a retrospective study of a German head and neck cancer center. *Anticancer Res*. 2013;33(8):3481–3485.

87. Thavaraj S, Stokes A, Guerra E, et al. Evaluation of human papillomavirus testing for squamous cell carcinoma of the tonsil in clinical practice. *J Clin Pathol*. 2011;64(4):308–312.

88. Tural D, Elicin O, Batur S, et al. Human papillomavirus is independent prognostic factor on outcome of oropharyngeal squamous cell carcinoma. *Tumour Biol*. 2013;34(6):3363–3369.

89. Ukpo OC, Flanagan JJ, Ma XJ, Luo Y, Thorstad WL, Lewis JS Jr. High-risk human papillomavirus E6/E7 mRNA detection by a novel in situ hybridization assay strongly correlates with p16 expression and patient outcomes in oropharyngeal squamous cell carcinoma. *Am J Surg Pathol*. 2011;35(9):1343–1350.

90. Ukpo OC, Pritchett CV, Lewis JE, Weaver AL, Smith DI, Moore EJ. Human papillomavirus–associated oropharyngeal squamous cell carcinomas: primary tumor burden and survival in surgical patients. *Ann Otol Rhinol Laryngol*. 2009; 118(5):368–373.

91. Weinberger PM, Yu Z, Haffty BG, et al. Molecular classification identifies a subset of human papillomavirus–associated oropharyngeal cancers with favorable prognosis. *J Clin Oncol*. 2006;24(5):736–747.

92. Weinberger PM, Yu Z, Kountourakis P, et al. Defining molecular phenotypes of human papillomavirus–associated oropharyngeal squamous cell carcinoma: validation of three-class hypothesis. *Otolaryngol Head Neck Surg*. 2009;141(3):382–389.

93. Worden FP, Kumar B, Lee JS, et al. Chemoselection as a strategy for organ preservation in advanced oropharynx cancer: response and survival positively associated with HPV16 copy number. *J Clin Oncol*. 2008;26(19):3138–3146.

94. Worsham MJ, Stephen JK, Chen KM, et al. Improved survival with HPV among African Americans with oropharyngeal cancer. *Clin Cancer Res*. 2013; 19(9):2486–2492.

95. Ali SMA, Awan MS, Ghaffar S, et al. Human papillomavirus infection in oral squamous cell carcinomas: correlation with histologic variables and survival outcome in a high risk population. *Oral Surg*. 2008;1(2):111–113.

96. Bledsoe TJ, Noble AR, Hunter GK, et al. Oropharyngeal squamous cell carcinoma with known human papillomavirus status treated with definitive chemoradiotherapy: patterns of failure and toxicity outcomes. *Radiat Oncol*. 2013;8:174. doi:10.1186/1748-717X-8-174.

97. Cerezo L, Lopez C, de la Torre A, et al. Incidence of human papillomavirus-related oropharyngeal cancer and outcomes after chemoradiation in a population of heavy smokers. *Head Neck*. 2014;36(6):782–786.

98. Fujimaki M, Fukumura Y, Mitani K, et al. Histological subtypes and characteristic structures of HPV-associated oropharyngeal carcinoma; study with Japanese cases. *Diagn Pathol*. 2013;8(1):211. doi:10.1186/1746-1596-8-211.

99. Habbous S, Harland LT, La Delfa A, et al. Comorbidity and prognosis in head and neck cancers: differences by subsite, stage, and human papillomavirus status. *Head Neck*. 2014;36(6):802–810.

100. Hess CB, Rash DL, Daly ME, et al. Competing causes of death and medical comorbidities among patients with human papillomavirus–positive vs human papillomavirus–negative oropharyngeal carcinoma and impact on adherence to radiotherapy. *JAMA Otolaryngol Head Neck Surg*. 2014;140(4): 312–316.

101. Song JS, Kim MS, Park JW, Lee YS, Kang CS. Expression of human papillomavirus-related proteins and its clinical implication in tonsillar squamous cell carcinoma. *Korean J Pathol*. 2012;46(2):177–186.

102. Spector ME, Gallagher KK, Bellile E, et al. Patterns of nodal metastasis and prognosis in human papillomavirus–positive oropharyngeal squamous cell carcinoma. *Head Neck*. 2013;36(9):1233–1240.

103. Vainshtein JM, Spector ME, McHugh JB, et al. Refining risk stratification for locoregional failure after chemoradiotherapy in human papillomavirus–associated oropharyngeal cancer. *Oral Oncol*. 2014;50(5):513–519.

104. Bussu F, Sali M, Gallus R, et al. Human papillomavirus (HPV) infection in squamous cell carcinomas arising from the oropharynx: detection of HPV DNA and p16 immunohistochemistry as diagnostic and prognostic indicators—a pilot study. *Int J Radiat Oncol Biol Phys*. 2014;89(5):1115–1120.

105. Cai C, Chernock RD, Pittman ME, El-Mofty SK, Thorstad WL, Lewis JS Jr. Keratinizing-type squamous cell carcinoma of the oropharynx: p16 overexpression is associated with positive high-risk HPV status and improved survival. *Am J Surg Pathol*. 2014;38(6):809–815.

106. Liu SZ, Zandberg DP, Schumaker LM, Papadimitriou JC, Cullen KJ. Correlation of p16 expression and HPV type with survival in oropharyngeal squamous cell cancer. *Oral Oncol*. 2015;51(9):862–869.

107. Rios Velazquez E, Hoebers F, Aerts HJ, et al. Externally validated HPV-based prognostic nomogram for oropharyngeal carcinoma patients yields more accurate predictions than TNM staging. *Radiother Oncol*. 2014;113(3):324–330.

108. Trosman SJ, Koyfam SA, Ward MC, et al. Effect of human papillomavirus on patterns of distant metastatic failure in oropharyngeal squamous cell carcinoma treated with chemoradiotherapy. *JAMA Otolaryngol Head Neck Surg*. 2015;141(5):457–462.

109. Hatekeyama H, Mizumachi T, Sakashita T, Kano S, Homma A, Fukuda S. Epithelial-mesenchymal transition in human papillomavirus-positive and -negative oropharyngeal squamous cell carcinoma. *Oncol Rep*. 2014;32(6): 2673–2679.

110. Barasch S, Mohindra P, Hennrick K, Hartig GK, Harari PM, Yang DT. Assessing p16 status of oropharyngeal squamous cell carcinoma by combined assessment of the number of cells stained and the confluence of p16 staining: a validation by clinical outcomes. *Am J Surg Pathol*. 2016;40(9):1261–1269.

111. Driessen CM, Janssens GO, van der Graaf WT, et al. Toxicity and efficacy of accelerated radiotherapy with concurrent weekly cisplatin for locally advanced head and neck carcinoma. *Head Neck*. 2016;38(suppl 1):E559–E565.

112. Dunlap NE, Narayan R, Shaughnessy J, et al. HPV/p16 status and patterns of failure for squamous cell carcinoma of the oropharynx after definitive chemoradiation: establishing a relationship to elective nodal irradiation: definitive management of head-and-neck squamous cell carcinoma [ASTRO abstract 216]. *Int J Radiat Oncol Biol Phys*. 2014;88(suppl 2):483–484.

113. Zandberg DP, Liu SZ, Goloubeva O, Schumaker LM, Cullen KJ. HPV-positive oropharyngeal cancer increased for both black and white patients over time, 1992–2007: epidemiology and prevention [ASTRO abstract 107]. *Int J Radiat Oncol Biol Phys*. 2014;88(suppl 2):494–495.

114. Sweeney BJ, Ring L, Rego M, Smith HL, Faquin W, Wilbur DC. Automated extraction of FFPE (formalin fixed paraffin embedded) tissue for head and neck HR-HPV (high risk human papillomavirus) testing on the Roche cobas® 4800 System [ASC abstract 120]. *J Am Soc Cytopathol*. 2013;2(1):S52–S53.

115. Bahl A, Dar L, Mohanti B, et al. Prevalence, trends, and survival impact of human papillomavirus on oropharyngeal cancer in Indian population [ASCO abstract 5540]. *J Clin Oncol*. 2012;30(suppl 15):5540.

116. Ang M-K, Ang SH, Krishna SS, et al. Association of smoking status with p16 and cyclin D1 (CCND1) expression with clinical characteristics and overall survival (OS) in oropharyngeal squamous cell carcinoma (OSC) [ASCO abstract 5551]. *J Clin Oncol*. 2012;30(suppl 15):5551.

117. Lorch JH, Hanna G, Dai W, et al. HPV and survival in patients with oropharyngeal squamous cell cancer of the head and neck (OPC) treated with induction chemotherapy followed by chemoradiotherapy (ST) versus chemoradiotherapy alone (CRT): the Dana-Farber experience [ASCO abstract 5582]. *J Clin Oncol*. 2012;30(suppl 15):5582.

118. Austin M, Schmidt R, Parvathaneni U, et al. Expression of p16, ERCC1, and EGFR amplification as predictors of responsiveness of locally advanced squamous cell carcinomas of head and neck (SCCHN) to cisplatin, radiotherapy, and erlotinib: a phase II randomized trial [ASCO abstract 5515]. *J Clin Oncol*. 2012;30(suppl 15):5515.

119. Knoedler M, Zakarneh A, Zimmermann U, Woelke K, Kaschke O, Keilholz U. Effects of human papillomavirus (HPV) and other potential risk factors on survival in patients with oropharyngeal cancer [ASCO abstract 5577]. *J Clin Oncol*. 2011;29(suppl 15):5577.

120. Xu J, Isayeva T, Brandwein-Gensler M. p16 overexpression: a better prognostic discriminator than transcriptionally active human papillomavirus 16 [CAP abstract 4]. *Arch Pathol Lab Med*. 2013;137(10):1481.

121. Shaw R, Schache A, Jones T, Risk J, Robinson M, Liloglou L. Smoking may not influence prognosis in HPV-16 mediated oropharyngeal squamous cell carcinoma [AAO-AHNS abstract 1948]. Presented at the 8th International Conference on Head & Neck Cancer; July 21–25, 2012; Toronto, ON, Canada.

122. Smith G, Muller S, Moore C, et al. Racial disparity in p16 positive oropharyngeal squamous cell carcinoma [USCAP abstract 1354]. *Mod Pathol*. 2014;27(suppl 2):328A.

123. Xu J, Dai Q, Isayeva T, Hebert-Magee S, Brandwein-Gensler M. African Americans with oropharyngeal carcinoma: decreased transcriptionally active high-risk human papillomavirus contributes to poorer survival [USCAP abstract 1340]. *Mod Pathol*. 2012;92(suppl 1):318A.

124. Hasegawa M, Meda H, Agena S, Suzuki M. HPV E6/E7 mRNA expression in oropharyngeal carcinoma [AAO-HNS abstract]. *Otolaryngol Head Neck Surg*. 2011;145(suppl 2):P168.

125. Guihard S, Jung AC, Abecassis J, et al. Prognostic value of HPV E6/E7 mRNA expression in a retrospective series of 144 French patients [ASTRO abstract 2589]. *Int J Radiat Oncol Biol Phys*. 2011;81(2):S491–S492.

126. Rakusic Z, Seiwerth S, Jakovčević A, Prgomet D, Juretić A. Impact of human papillomavirus on clinicopathological characteristics of oropharyngeal carcinomas [ASTRO abstract 2677]. *Int J Radiat Oncol Biol Phys*. 2012;84(3): S473–S474.

127. Brookes L, Allibone R, Christian JA. The impact of human papillomavirus on oropharyngeal cancer in Nottingham, UK [ESTRO abstract EP-1142]. *Radiother Oncol*. 2014;111(suppl 1):S34–S35.

128. Lorch J, Thotakura V, Posner M, et al. HPV and survival in patients with oropharyngeal squamous cell cancer of the head and neck (OPC) treated with induction chemotherapy followed by chemoradiotherapy (ST) versus chemoradiotherapy alone (CRT): a retrospective analysis [ASTRO abstract 145].

Multidisciplinary Head and Neck Cancer Symposium; January 26–28, 2012; Phoenix, AZ. http://www.abstractsonline.com/Plan/ViewAbstract.aspx?sKey=b1c590bb-c362-40ea-bf96-b8f21b8936d8&cKey=2c0d661a-453a-4dbd-8d42-32fa94f6c890&mKey=82897973-8a41-4f26-bb8e-7e358fc53b94. Accessed June 6, 2017.

129. Valduga F, Caldara A, Vanoni V, et al. Clinical outcome according to p16 status and treatment modalities in oropharyngeal cancer (OC) patients (PTS) [ASTRO abstract 220]. Multidisciplinary Head and Neck Cancer Symposium; January 26–28, 2012; Phoenix, AZ. http://www.abstractsonline.com/Plan/ViewAbstract.aspx?sKey=b1c590bb-c362-40ea-bf96-b8f21b8936d8&cKey=da2c4be7-f301-46c7-9ff2-93d0285d561e&mKey=%7b82897973-8A41-4F26-BB8E-7E358FC53B94%7d. Accessed June 6, 2017.

130. Maxwell J, Ferris R. Effect of HPV and smoking on survival and recurrence in oropharyngeal cancer patients [AHNS abstract S035]. Presented at the annual meeting of the American Head and Neck Society; April 27–28, 2011; Chicago, IL.

131. Broglie M, Soltermann A, Pawlita M, Huber G, Studer G, Stoeckli S. Impact of p16, p53, smoking, alcohol and staging on survival in oropharyngeal squamous cell carcinoma [AHNS abstract S220]. Presented at the 8th International Conference on Head & Neck Cancer; July 21–25, 2012; Toronto, ON, Canada.

132. Upile N, Lancaster J, Kinshuck A, et al. Is human papillomavirus (HPV) status really important? Transoral laser surgery in the management of oropharyngeal squamous cell carcinoma (OPSCC) [AHNS abstract P467]. Presented at the 8th International Conference on Head & Neck Cancer; July 21–25, 2012; Toronto, ON, Canada.

133. Saraiya M, Thompson T, Lynch C, et al. HPV genotype-specific survival of oropharyngeal cancers—United States, 1994–2005 [IPS abstract]. Presented at the 28th International Papillomavirus Conference; November 30–December 6, 2012; San Juan, PR.

134. Broglie M, Soltermann A, Pawlita M, Probst R, Stoeckli S. Risk stratification based on p16-expression and other factors in OPSCC [IPS abstract P-16.23]. Presented at the 27th Annual International Papillomavirus Conference & Clinical Workshops; September 17–22, 2011; Berlin, Germany.

135. Lassen P, Primdahl H, Johansen J, et al. HPV, smoking and RT-outcome in advanced OPC treated without chemotherapy—analysis of DAHANCA patients [ESTRO abstract OC-0149]. *Radiother Oncol*. 2012;103(suppl 1):S58.

136. Chernock RD, Lewis JS Jr, Zhang Q, El-Mofty SK. Human papillomavirus–positive basaloid squamous cell carcinomas of the upper aerodigestive tract: a distinct clinicopathologic and molecular subtype of basaloid squamous cell carcinoma. *Hum Pathol*. 2010;41(7):1016–1023.

137. Mehrad M, Carpenter DH, Chernock RD, et al. Papillary squamous cell carcinoma of the head and neck: clinicopathologic and molecular features with special reference to human papillomavirus. *Am J Surg Pathol*. 2013;37(9):1349–1356.

138. Begum S, Westra WH. Basaloid squamous cell carcinoma of the head and neck is a mixed variant that can be further resolved by HPV status. *Am J Surg Pathol*. 2008;32(7):1044–1050.

139. Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. *Nat Rev Cancer*. 2010;10(8):550–560.

140. Munger K, Baldwin A, Edwards KM, et al. Mechanisms of human papillomavirus–induced oncogenesis. *J Virol*. 2004;78(21):11451–11460.

141. Sedghizadeh PP, Billington WD, Paxton D, et al. Is p16-positive oropharyngeal squamous cell carcinoma associated with favorable prognosis?: a systematic review and meta-analysis. *Oral Oncol*. 2016;54:15–27.

142. Ma C, Lewis J Jr. Small biopsy specimens reliably indicate p16 expression status of oropharyngeal squamous cell carcinoma. *Head Neck Pathol*. 2012;6(2):208–215.

143. Bishop JA, Ma X-J, Wang H, et al. Detection of transcriptionally active high-risk HPV in patients with head and neck squamous cell carcinoma as visualized by a novel E6/E7 mRNA in situ hybridization method. *Am J Surg Pathol*. 2012;36(12):1874–1882.

144. Kerr DA, Arora KS, Mahadevan KK, et al. Performance of a branch chain RNA in situ hybridization assay for the detection of high-risk human papillomavirus in head and neck squamous cell carcinoma. *Am J Surg Pathol*. 2016;39(12):1643–1652.

145. Rosenthal DI, Harari PM, Giralt J, et al. Association of human papillomavirus and p16 status with outcomes in the IMCL-9815 phase III registration trial for patients with locoregionally advanced oropharyngeal squamous cell carcinoma of the head and neck treated with radiotherapy with or without cetuximab. *J Clin Oncol*. 2016;34(12):1300–1308.

146. Mirghani H, Casiraghi O, Amen F, et al. Diagnosis of HPV-driven head and neck cancer with a single test in routine clinical practice. *Mod Pathol*. 2015;28(12):1518–1527.

147. Rooper LM, Gandhi M, Bishop JA, Westra WH. RNA in-situ hybridization is a practical and effective method for determining HPV status of oropharyngeal squamous cell carcinoma including discordant cases that are p16 positive by immunohistochemistry but HPV negative by DNA in-situ hybridization. *Oral Oncol*. 2016;5511–5516.

148. Schache AG, Liloglou T, Risk JM, et al. Evaluation of human papilloma virus diagnostic testing in oropharyngeal squamous cell carcinoma: sensitivity, specificity, and prognostic discrimination. *Clin Cancer Res*. 2011;17(19):6262–6271.

149. Schlecht NF, Brandwein-Gensler M, Nuovo GJ, et al. A comparison of clinically utilized human papillomavirus detection methods in head and neck cancer. *Mod Pathol*. 2011;24(10):1295–1305.

150. Fitzgibbons PL, Bradley LA, Fatheree LA, et al. Principles of analytic validation of immunohistochemical assays: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med*. 2014;138(11):1432–1443.

151. Bishop JA, Westra WH. Human papillomavirus-related small cell carcinoma of the oropharynx. *Am J Surg Pathol*. 2011;35(11):1679–1684.

152. Kraft S, Faquin WC, Krane JF. HPV-associated neuroendocrine carcinoma of the oropharynx: a rare new entity with potentially aggressive clinical behavior. *Am J Surg Pathol*. 2012;36(3):321–330.

153. Bates T, McQueen A, Iqbal MS, Kelly C, Robinson M. Small cell neuroendocrine carcinoma of the oropharynx harbouring oncogenic HPV-infection. *Head Neck Pathol*. 2014;8(1):127–131.

154. Isayeva T, Said-Al-Naief N, Ren Z, Li R, Gnepp D, Brandwein-Gensler M. Salivary mucoepidermoid carcinoma: demonstration of transcriptionally active human papillomavirus 16/18. *Head Neck Pathol*. 2013;7(2):135–148.

155. Boland JM, McPhail ED, Garcia JJ, Lewis JE, Schembri-Wismayer DJ. Detection of human papilloma virus and p16 expression in high-grade adenoid cystic carcinoma of the head and neck. *Mod Pathol*. 2012;25(4):529–536.

156. Bishop JA, Yonescu R, Batista D, Yemelyanova A, Ha PK, Westra WH. Mucoepidermoid carcinoma does not harbor transcriptionally active high risk human papillomavirus even in the absence of the MAML2 translocation. *Head Neck Pathol*. 2014;8(3):298–302.

157. Brunner M, Koperek O, Wrba F, et al. HPV infection and p16 expression in carcinomas of the minor salivary glands. *Eur Arch Otorhinolaryngol*. 2012;269(10):2265–2269.

158. Skalova A, Kaspirkova J, Andrle P, Hosticka L, Vanecek T. Human papillomaviruses are not involved in the etiopathogenesis of salivary gland tumors. *Cesk Patol*. 2013;49(2):72–75.

159. Perez-Ordonez B, Irish JC, Yu ES, Gillison ML. Human papillomavirus-16 associated adenocarcinoma NOS of base of tongue. *Head Neck Pathol*. 2012;7(3):268–273.

160. Chang AM, Nikiforova MN, Johnson JT, et al. Human papillomavirus-associated adenocarcinoma of the base of tongue: potentially actionable genetic changes. *Head Neck Pathol*. 2013;8(2):151–156.

161. Westra WH. The pathology of HPV-related head and neck cancer: implications for the diagnostic pathologist. *Semin Diagn Pathol*. 2015;32(1):42–53.

162. Alos L, Moyano S, Nadal A, et al. Human papillomaviruses are identified in a subgroup of sinonasal squamous cell carcinomas with favorable outcome. *Cancer*. 2009;115(12):2701–2709.

163. Bishop JA, Guo TW, Smith DF, et al. Human papillomavirus-related carcinomas of the sinonasal tract. *Am J Surg Pathol*. 2013;37(2):185–192.

164. Chaudhary AK, Pandya S, Mehrotra R, Bharti AC, Singh M, Singh M. Comparative study between the Hybrid Capture II test and PCR based assay for the detection of human papillomavirus DNA in oral submucous fibrosis and oral squamous cell carcinoma. *Virol J*. 2010;7:253. doi:10.1186/1743-422X-7-253.

165. Chernock RD, Wang X, Gao G, et al. Detection and significance of human papillomavirus, CDKN2A(p16) and CDKN1A(p21) expression in squamous cell carcinoma of the larynx. *Mod Pathol*. 2013;26(2):223–231.

166. Duncan LD, Winkler M, Carlson ER, Heidel RE, Kang E, Webb D. p16 immunohistochemistry can be used to detect human papillomavirus in oral cavity squamous cell carcinoma. *J Oral Maxillofac Surg*. 2013;71(8):1367–1375.

167. Duray A, Descamps G, Arafa M, et al. High incidence of high-risk HPV in benign and malignant lesions of the larynx. *Int J Oncol* 2011;39(1):51–59.

168. Duray A, Descamps G, Decaestecker C, et al. Human papillomavirus DNA strongly correlates with a poorer prognosis in oral cavity carcinoma. *Laryngoscope*. 2012;122(7):1558–1565.

169. Elango KJ, Suresh A, Erode EM, et al. Role of human papilloma virus in oral tongue squamous cell carcinoma. *Asian Pac J Cancer Prev*. 2011;12(4):889–896.

170. Ernoux-Neufcoeur P, Arafa M, Decaestecker C, et al. Combined analysis of HPV DNA, p16, p21 and p53 to predict prognosis in patients with stage IV hypopharyngeal carcinoma. *J Cancer Res Clin Oncol*. 2011;137(1):173–181.

171. Huang SF, Li HF, Liao CT, et al. Association of HPV infections with second primary tumors in early-staged oral cavity cancer. *Oral Dis*. 2012;18(8):809–815.

172. Jiang H, Lin P-F. Human papillomavirus infection a favorable prognostic factor in laryngeal squamous cell carcinoma is associated with the expression of proliferating cell nuclear antigen. *Pak J Med Sci*. 2013;29(5):1173–1177.

173. Kaminagakura E, Villa LL, Andreoli MA, et al. High-risk human papillomavirus in oral squamous cell carcinoma of young patients. *Int J Cancer*. 2012;130(8):1726–1732.

174. Laco J, Slaninka I, Jirasek M, Celakovsky P, Vosmikova H, Ryska A. High-risk human papillomavirus infection and p16INK4a protein expression in laryngeal lesions. *Pathol Res Pract*. 2008;204(8):545–552.

175. Lewis JS Jr, Ukpo OC, Ma XJ, et al. Transcriptionally-active high-risk human papillomavirus is rare in oral cavity and laryngeal/hypopharyngeal squamous cell carcinomas—a tissue microarray study utilizing E6/E7 mRNA in situ hybridization. *Histopathology*. 2012;60(6):982–991.

176. Lingen MW, Xiao W, Schmitt A, et al. Low etiologic fraction for high-risk human papillomavirus in oral cavity squamous cell carcinomas. *Oral Oncol*. 2013;49(1):1–8.

177. Morshed K, Polz-Dacewicz M, Szymanski M, Polz D. Short-fragment PCR assay for highly sensitive broad-spectrum detection of human papillomaviruses in laryngeal squamous cell carcinoma and normal mucosa: clinico-pathological evaluation. *Eur Arch Otorhinolaryngol*. 2008;265(suppl 1):S89–S96.

178. Nemes JA, Deli L, Nemes Z, Marton IJ. Expression of p16(INK4A), p53, and Rb proteins are independent from the presence of human papillomavirus genes in oral squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;102(3):344–352.

179. Reuschenbach M, Kansy K, Garbe K, et al. Lack of evidence of human papillomavirus-induced squamous cell carcinomas of the oral cavity in southern Germany. *Oral Oncol*. 2013;49(9):937–942.

180. Robinson M, Suh YE, Paleri V, et al. Oncogenic human papillomavirus-associated nasopharyngeal carcinoma: an observational study of correlation with ethnicity, histological subtype and outcome in a UK population. *Infect Agents Cancer*. 2013;8(1):30. doi:10.1186/1750-9378-8-30.

181. Stephen JK, Chen KM, Shah V, et al. Human papillomavirus outcomes in an access-to-care laryngeal cancer cohort. *Otolaryngol Head Neck Surg*. 2012; 146(5):730–738.

182. Sugiyama M, Bhawal UK, Kawamura M, et al. Human papillomavirus-16 in oral squamous cell carcinoma: clinical correlates and 5-year survival. *Br J Oral Maxillofac Surg*. 2007;45(2):116–122.

183. Wendt M, Romanitan M, Nasman A, et al. Presence of human papillomaviruses and p16 expression in hypopharyngeal cancer. *Head Neck*. 2014;36(1):107–112.

184. Zhao D, Xu QG, Chen XM, Fan MW. Human papillomavirus as an independent predictor in oral squamous cell cancer. *Int J Oral Sci*. 2009;1(3): 119–125.

185. Larque AB, Hakim S, Ordi J, et al. High-risk human papillomavirus is transcriptionally active in a subset of sinonasal squamous cell carcinomas. *Mod Pathol*. 2014;27(3):343–351.

186. Kirby S, Marcinow A, Teknos T, Iwenofu O. Does p16 status matter in the larynx? A study of overall survival in p16 positive squamous cell carcinomas of the larynx regardless of HPV status [USCAP abstract 1326]. *Mod Pathol*. 2014; 27(suppl 2):321A.

187. Chung CH, Zhang Q, Kong CS, et al. p16 protein expression and human papillomavirus status as prognostic biomarkers of nonoropharyngeal head and neck squamous cell carcinoma. *J Clin Oncol*. 2014;32(35):3930–3938.

188. Ramshankar V, Soundara VT, Shyamsundar V, Ramani P, Krishnamurthy A. Risk stratification of early stage oral tongue cancers based on HPV status and p16 immunoexpression. *Asian Pac J Cancer Prev*. 2014;15(19):8351–8359.

189. Furrer VE, Benitez MB, Furnes M, Lanfranchi HE, Modesti NM. Biopsy vs. superficial scraping: detection of human papillomavirus 6, 11, 16, and 18 in potentially malignant and malignant oral lesions. *J Oral Pathol Med*. 2006;35(6): 338–344.

190. Chuang CY, Sung WW, Wang L, et al. Differential impact of IL-10 expression on survival and relapse between HPV16-positive and -negative oral squamous cell carcinomas. *PLoS One*. 2012;7(10):e47541. doi:10.1371/journal.pone.0047541.

191. Lee LA, Huang CG, Liao CT, et al. Human papillomavirus-16 infection in advanced oral cavity cancer patients is related to an increased risk of distant metastases and poor survival. *PLoS One*. 2012;7(7):e40767. doi:10.1371/journal.pone.0040767.

192. Li X, Gao L, Li H, et al. Human papillomavirus infection and laryngeal cancer risk: a systematic review and meta-analysis. *J Infect Dis*. 2013;207(3):479–488.

193. Xu J, Isayeva T, BrandweinGensler M. P16Ink4A: no correlation with transcriptionally active HPV16/18 or outcomes in oral cavity carcinoma [USCAP abstract 1367]. *Mod Pathol*. 2014;27(suppl 2):332A.

194. Leidy J, Chen K, Stockl T, et al. p16 Expression and prognostic value in laryngeal squamous cell carcinomas—a large cohort study from Chinese patients [USCAP abstract 1311]. *Mod Pathol*. 2012;25(suppl 2):311A.

195. Stenmark MH, McHugh JB, Schipper M, et al. Nonendemic HPV-positive nasopharyngeal carcinoma: association with poor prognosis. *Int J Radiat Oncol Biol Phys*. 2014;88(3):580–588.

196. Syrjanen K, Syrjanen S. Detection of human papillomavirus in sinonasal carcinoma: systematic review and meta-analysis. *Hum Pathol*. 2013;44(6):983–991.

197. Poling JS, Ma XJ, Bui S, et al. Human papillomavirus (HPV) status of non-tobacco related squamous cell carcinomas of the lateral tongue. *Oral Oncol*. 2014;50(4):306–310.

198. Young RJ, Urban D, Angel C, et al. Frequency and prognostic significance of p16(INK4A) protein overexpression and transcriptionally active human papillomavirus infection in laryngeal squamous cell carcinoma. *Br J Cancer*. 2015;112(6):1098–1104.

199. Compton AM, Moore-Medlin T, Herman-Ferdinandez L, et al. Human papillomavirus in metastatic lymph nodes from unknown primary head and neck squamous cell carcinoma. *Otolaryngol Head Neck Surg*. 2011;145(1):51–57.

200. Tribius S, Hoffmann AS, Bastrop S, et al. HPV status in patients with head and neck of carcinoma of unknown primary site: HPV, tobacco smoking, and outcome. *Oral Oncol*. 2012;48(11):1178–1184.

201. Vent J, Haidle B, Wedemeyer I, et al. p16 expression in carcinoma of unknown primary: diagnostic indicator and prognostic marker. *Head Neck*. 2013;35(11):1521–1526.

202. Sivars L, Nasman A, Tertipis N, et al. Human papillomavirus and p53 expression in cancer of unknown primary in the head and neck region in relation to clinical outcome. *Cancer Med*. 2014;3(2):376–384.

203. Cianchetti M, Mancuso AA, Amdur RJ, et al. Diagnostic evaluation of squamous cell carcinoma metastatic to cervical lymph nodes from an unknown head and neck primary site. *Laryngoscope*. 2009;119(12):2348–2354.

204. Zengel P, Assmann G, Mollenhauer M, et al. Cancer of unknown primary originating from oropharyngeal carcinomas are strongly correlated to HPV positivity. *Virchows Arch*. 2012;461(3):283–290.

205. Byrd JK, Smith KJ, de Almeida JR, et al. Transoral robotic surgery and the unknown primary: a cost-effectiveness analysis. *Otolaryngol Head Neck Surg*. 2014;150(6):976–982.

206. Durmus K, Rangarajan SV, Old MO, Agrawal A, Teknos TN, Ozer E. Transoral robotic approach to carcinoma of unknown primary. *Head Neck*. 2014; 36(6):848–852.

207. Karni RJ, Rich JT, Sinha P, Haughey BH. Transoral laser microsurgery: a new approach for unknown primaries of the head and neck. *Laryngoscope*. 2011; 121(6):1194–1201.

208. Patel SA, Magnuson JS, Holsinger FC, et al. Robotic surgery for primary head and neck squamous cell carcinoma of unknown site. *JAMA Otolaryngol Head Neck Surg*. 2013;139(11):1203–1211.

209. Straetmans J, Vent J, Lacko M, et al. Management of neck metastases of unknown primary origin united in two European centers. *Eur Arch Otorhinolaryngol*. 2014;272(1):1–11.

210. Fowler N, Manzoor N, Rajasekaran K, et al. Prevalence of human papilloma virus & p16 and predictors of survival in patients with cervical unknown primary squamous cell carcinoma [AAO-AHNS abstract S168]. 8th International Conference on Head and Neck Cancer; July 21–25, 2012; Toronto, ON, Canada. http://ahns.jnabstracts.com/2012/Detail?ID=0168. Accessed June 6, 2017.

211. Straetmans J, Vent J, Henfling M, et al. Value of HPV in neck metastasis of unknown primary tumours [IPS abstract O-16.08]. Presented at the 27th International Papillomavirus Conference and Clinical Workshop; September 17–22, 2011; Berlin, Germany.

212. Weiss D, Koopmann M, Rudack C. Prevalence and impact on clinicopathological characteristics of human papillomavirus-16 DNA in cervical lymph node metastases of head and neck squamous cell carcinoma. *Head Neck*. 2011;33(6):856–862.

213. Sanguineti G, Pai S, Agbahiwe H, et al. HPV-related oropharyngeal carcinoma with overt level II and/or III metastases at presentation: the risk of subclinical disease in ipsilateral levels IB, IV and V. *Acta Oncol*. 2014;53(5):662–668.

214. Pavlidis N, Briasoulis E, Hainsworth J, Greco FA. Diagnostic and therapeutic management of cancer of an unknown primary. *Eur J Cancer*. 2003; 39(14):1990–2005.

215. Beadle BM, William WN Jr, McLemore MS, Sturgis EM, Williams MD. p16 expression in cutaneous squamous carcinomas with neck metastases: a potential pitfall in identifying unknown primaries of the head and neck. *Head Neck*. 2013;35(11):1527–1533.

216. McDowell LJ, Young RJ, Johnston ML, et al. p16-positive lymph node metastases from cutaneous head and neck squamous cell carcinoma: no association with high-risk human papillomavirus or prognosis and implications for the workup of the unknown primary. *Cancer*. 2016;122(8):1201–1208.

217. Chernock RD, El-Mofty SK, Thorstad WL, Parvin CA, Lewis JS Jr. HPV-related nonkeratinizing squamous cell carcinoma of the oropharynx: utility of microscopic features in predicting patient outcome. *Head Neck Pathol*. 2009; 3(3):186–194.

218. Begum S, Gillison ML, Nicol TL, Westra WH. Detection of human papillomavirus-16 in fine-needle aspirates to determine tumor origin in patients with metastatic squamous cell carcinoma of the head and neck. *Clin Cancer Res*. 2007;13(4):1186–1191.

219. Bishop JA, Maleki Z, Valsamakis A, et al. Application of the hybrid capture 2 assay to squamous cell carcinomas of the head and neck: a convenient liquid-phase approach for the reliable determination of human papillomavirus status. *Cancer Cytopathol*. 2012;120(1):18–25.

220. Guo M, Khanna A, Dhillon J, et al. Cervista HPV assays for fine-needle aspiration specimens are a valid option for human papillomavirus testing in patients with oropharyngeal carcinoma. *Cancer Cytopathol*. 2014;122(2):96–103.

221. Lastra RR, Pramick MR, Nakashima MO, et al. Adequacy of fine-needle aspiration specimens for human papillomavirus infection molecular testing in head and neck squamous cell carcinoma. *Cytojournal*. 2013;10:21. doi:10.1186/1750-9378-8-30.

222. Jakscha J, Zlobec I, Storck C, et al. The clinical impact of p16 status in fine-needle aspirates of cervical lymph node metastasis of head and neck squamous cell carcinomas. *Eur Arch Otorhinolaryngol*. 2013;270(2):661–667.

223. Jannapureddy S, Cohen C, Lau S, Beitler JJ, Siddiqui MT. Assessing for primary oropharyngeal or nasopharyngeal squamous cell carcinoma from fine needle aspiration of cervical lymph node metastases. *Diagn Cytopathol*. 2010; 38(11):795–800.

224. Smith DF, Maleki Z, Coughlan D, et al. Human papillomavirus status of head and neck cancer as determined in cytologic specimens using the hybrid-capture 2 assay. *Oral Oncol*. 2014;50(6):600–604.

225. Davis D, Braxton D, Fatima N, Momin S, Cynthia C. HPV L1 in oropharyngeal squamous cell carcinomas: comparison and correlation with p16, HPV ISH, and outcome [USCAP abstract 1309]. *Mod Pathol*. 2014;27(suppl 2): 317A.

226. Fatima N, Cohen C, Siddiqui M. Comparing HPV ISH and p16 in assessing metastatic oropharyngeal carcinoma [USCAP abstract 361]. *Mod Pathol*. 2012;25(suppl 2):89A.

227. Inohara H, Yasui T, Maruyama H. Human papilloma virus in neck metastasis from head and neck carcinoma and unknown primary carcinoma

[AHNS abstract P346]. Presented at the 8th International Conference on Head & Neck Cancer; July 21–25, 2012; Toronto, ON, Canada.

228. Baldassarri R, Aronberg R, Levi AW, Yarbrough WG, Kowalski D, Chhieng D. Detection and genotype of high-risk human papillomavirus in fine-needle aspirates of patients with metastatic squamous cell carcinoma is helpful in determining tumor origin. *Am J Clin Pathol*. 2015;143(5):694–700.

229. Holmes BJ, Maleki Z, Westra WH. The fidelity of p16 staining as a surrogate marker of human papillomavirus status in fine-needle aspirates and core biopsies of neck node metastases: implications for HPV testing protocols. *Acta Cytol*. 2015;59(1):97–103.

230. Jalaly JB, Lewis JS Jr, Collins BT, et al. Correlation of p16 immunohistochemistry in FNA biopsies with corresponding tissue specimens in HPV-related squamous cell carcinomas of the oropharynx. *Cancer Cytopathol*. 2015;123(12):723–731.

231. Goldenberg D, Begum S, Westra WH, et al. Cystic lymph node metastasis in patients with head and neck cancer: an HPV-associated phenomenon. *Head Neck*. 2008;30(7):898–903.

232. McIlwain WR, Sood AJ, Nguyen SA, Day TA. Initial symptoms in patients with HPV-positive and HPV-negative oropharyngeal cancer. *JAMA Otolaryngol Head Neck Surg*. 2014;140(5):441–447.

233. O'Sullivan B, Huang SH, Su J, et al. Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. *Lancet Oncol*. 2016;17(4):440–451.

234. Holmes BJ, Westra WH. The expanding role of cytopathology in the diagnosis of HPV-related squamous cell carcinoma of the head and neck. *Diagn Cytopathol*. 2014;42(1):85–93.

235. Krane JF. Role of cytology in the diagnosis and management of HPV-associated head and neck carcinoma. *Acta Cytol*. 2013;57(2):117–126.

236. Pusztaszeri MP, Faquin WC. Cytologic evaluation of cervical lymph node metastases from cancers of unknown primary origin. *Semin Diagn Pathol*. 2015;32(1):32–41.

237. Allison DB, Miller JA, Coquia SF, Maleki Z. Ultrasonography-guided fine-needle aspiration with concurrent small core biopsy of neck masses and lymph nodes yields adequate material for HPV testing in head and neck squamous cell carcinomas. *J Am Soc Cytopathol*. 2016;5(1):22–30.

238. Grimes R, Garcia-Buitrago MT, Jorda M, Ganjei-Azar P, Ferrell A, Gomez-Fernandez C. P16INKa immunocytochemistry in fine-needle aspiration cytology smears of metastatic head and neck squamous cell carcinoma. *Acta Cytol*. 2013;57(1):33–37.

239. Zhang MQ, El-Mofty SK, Davila RM. Detection of human papillomavirus-related squamous cell carcinoma cytologically and by in situ hybridization in fine-needle aspiration biopsies of cervical metastasis: a tool for identifying the site of an occult head and neck primary. *Cancer*. 2008;114(2):118–123.

240. Anderson CE, McLaren KM, Rae F, Sanderson RJ, Cuschieri KS. Human papilloma virus in squamous carcinoma of the head and neck: a study of cases in south east Scotland. *J Clin Pathol*. 2007;60(4):439–441.

241. Han M, Bernadt CT, Murray B, et al. Aptima HR-HPV testing from Diff-Quik-stained fine-needle aspiration smears of oropharyngeal squamous cell carcinoma. *J Am Soc Cytopathol*. 2016;5(4):221–226.

242. Chute DJ, Aramouni GT, Brainard JA, Hoschar AP, Kroeger A, Yen-Lieberman B. Hybrid Capture 2 human papilloma virus testing for head and neck cytology specimens. *J Am Soc Cytopathol*. 2014;3(4):173–182.

243. Bishop JA, Lewis JS Jr, Rocco JW, Faquin WC. HPV-related squamous cell carcinoma of the head and neck: an update on testing in routine pathology practice. *Semin Diagn Pathol*. 2015;32(5):344–351.

244. Venuti A, Paolini F. HPV detection methods in head and neck cancer. *Head Neck Pathol*. 2012;6(suppl 1):S63–S74.

245. Miah AB, Schick U, Bhide SA, et al. A phase II trial of induction chemotherapy and chemo-IMRT for head and neck squamous cell cancers at risk of bilateral nodal spread: the application of a bilateral superficial lobe parotid-sparing IMRT technique and treatment outcomes. *Br J Cancer*. 2015;112(1):32–38.

246. Gronhoj Larsen C, Gyldenlove M, Jensen DH, et al. Correlation between human papillomavirus and p16 overexpression in oropharyngeal tumours: a systematic review. *Br J Cancer*. 2014;110(6):1587–1594.

247. Lewis JS Jr, Chernock RD, Ma XJ, et al. Partial p16 staining in oropharyngeal squamous cell carcinoma: extent and pattern correlate with human papillomavirus RNA status. *Mod Pathol*. 2012;25(9):1212–1220.

248. Cao D, Begum S, Ali SZ, Westra WH. Expression of p16 in benign and malignant cystic squamous lesions of the neck. *Hum Pathol*. 2010;41(4):535–539.

249. Witt BL, Albertson DJ, Coppin MG, Horrocks CF, Post M, Gulbahce HE. Use of in situ hybridization for HPV in head and neck tumors: experience from a national reference laboratory. *Head Neck Pathol*. 2015;9(1):60–64.

250. Munger K, Phelps WC, Bubb V, Howley PM, Schlegel R. The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. *J Virol*. 1989;63(10):4417–4421.

251. Hawley-Nelson P, Vousden KH, Hubbert NL, Lowy DR, Schiller JT. HPV16 E6 and E7 proteins cooperate to immortalize human foreskin keratinocytes. *EMBO J*. 1989;8(12):3905–3910.

252. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer*. 2002;2(5):342–350.

253. Rautava J, Syrjanen S. Biology of human papillomavirus infections in head and neck carcinogenesis. *Head Neck Pathol*. 2012;6(suppl 1):S3–S15.

254. Vainshtein J, McHugh JB, Spector ME, et al. Human papillomavirus–related oropharyngeal cancer: HPV and p16 status in the recurrent versus parent tumor. *Head Neck*. 2015;37(1):8–11.

255. Muller S, Khuri FR, Kono SA, Beitler JJ, Shin DM, Saba NF. HPV positive squamous cell carcinoma of the oropharynx. Are we observing an unusual pattern of metastases? *Head Neck Pathol*. 2012;6(3):336–344.

256. El-Naggar AK, Westra WH. p16 expression as a surrogate marker for HPV-related oropharyngeal carcinoma: a guide for interpretative relevance and consistency. *Head Neck*. 2012;34(4):459–461.

257. Brierley JD, Gospodarowicz MK, Wittekind E. *TNM Classification of Malignant Tumours*. 8th ed. Chichester, UK: John Wiley & Sons; 2017.

258. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ, eds. *WHO Classification of Head and Neck Tumours*. 4th ed. Geneva, Switzerland: IARC/WHO Press; 2017.

259. Seethala RR, Weinreb I, Carlson DL, et al; for Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with carcinomas of the pharynx. http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution%20Folders/WebContent/pdf/pharynx-13protocol-3300.pdf. Published October 2013. Accessed June 6, 2017.

260. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. *AJCC Cancer Staging Manual*. 7th ed. New York, NY: Springer; 2011.

261. Huang SH, Xu W, Waldron J, et al. Refining American Joint Committee on Cancer/Union for International Cancer Control TNM stage and prognostic groups for human papillomavirus–related oropharyngeal carcinomas. *J Clin Oncol*. 2015;33(8):836–845.

262. Andrews J, Guyatt G, Oxman AD, et al. GRADE guidelines, 14: going from evidence to recommendations: the significance and presentation of recommendations. *J Clin Epidemiol*. 2013;66(7):719–725.

263. Lau HY, Brar S, Klimowicz AC, et al. Prognostic significance of p16 in locally advanced squamous cell carcinoma of the head and neck treated with concurrent cisplatin and radiotherapy. *Head Neck*. 2011;33(2):251–256.

APPENDIX. Disclosed Interests and Activities From April 2013 to December 2016

Name	Interest/Activity Type	Entity
Justin A. Bishop, MD	Grants/contracted research/collaborative agreements	NCI SPORE
	Royalties	Springer
Rebecca D. Chernock, MD	Grants/contracted research/collaborative agreements	Affymetrix
		Barnes Jewish Hospital–Cancer Frontier Fund
	Lecture fees/honoraria	USCAP
	Remuneration from relevant commercial entities	Elsevier
	Other	International Collaboration on Cancer Reporting
William C. Faquin, MD, PhD	Boards, advisory boards	*Acta Cytologica*
		Advances in Anatomic Pathology
		Archives of Pathology & Laboratory Medicine
		Cancer Cytopathology
		Diagnostic Cytopathology
		Head and Neck Pathology
		Journal of the American Society of Cytopathology
	Consultancies	Navigant Consulting
		Guidepoint Global Consulting

APPENDIX. Continued

Name	Interest/Activity Type	Entity
	Expert witness	Schochor, Federico and Staton, PA
		Roxanne Ward, PC
		Martin, Magnuson, McCarthy & Kenney
		Judith A. Berman, PLLC
		Katherine E. Poindexter
	Grants/contracted research/collaborative agreements	Adenoid Cystic Carcinoma Research Foundation
		NCI
	Lecture fees/honoraria	ASC
		ASCP
		CAP
		Harvard Medical School
		New England Thyroid Club
		Pacific Northwest Society of Pathology
		USCAP
	Intellectual properties/patents	USPTO
	Royalties	Springer-Verlag
James S. Lewis Jr, MD	Boards, advisory boards	*The American Journal of Surgical Pathology*
		Head and Neck Pathology
		Annals of Otology, Rhinology, and Laryngology
	Lecture fees/honoraria	ASCP
		USCAP
		AAOMP
		The Ohio State University Medical Center
	Grants/contracted research/collaborative agreements	NCCN
		Barnes Jewish Hospital–Cancer Frontier Fund
		Affymetrix
		NIH
	Expert witness	Fox Galvin
		Stamos & Trucco, LLP
		Hare, Wynn, Newell, and Newton
	Other	International Collaboration on Cancer Reporting
Joel Todd Moncur, MD, PhD	Board, advisory boards	Murtha Cancer Center
James W. Rocco, MD, PhD	Board, advisory boards	Johns Hopkins Head and Neck SPORE
		MD Anderson Head and Neck SPORE
		Oral Oncology
	Grants/contracted research/collaborative agreements	NIDCR
	Intellectual properties/patents	USPTO
	Royalties	UpToDate
Mary R. Schwartz, MD	Board, advisory boards	ASC Foundation
		Archives of Pathology & Laboratory Medicine
		Cancer Cytopathology
		Center for Medicine After the Holocaust
		Diagnostic Cytopathology
		Journal of American Society of Cytopathology
	Grants/contracted research/collaborative agreements	Houston Methodist Department of Pathology and Genomic Medicine microgrants
		United States Department of Defense
Raja R. Seethala, MD	Lecture fees/honoraria	ASCP
	Grants/contracted research/collaborative agreements	NIH
		NIDCR
	Royalties	Demos Medical Publishing
William H. Westra, MD	Consultancies	Merck
	Board, advisory boards	AstraZeneca
	Expert witness	Robert E. Schack PA
	Lecture fees/honoraria	AAOMP
		ASC
		Massachusetts General Hospital
		Memorial Sloan Kettering Cancer Center
		Yale University School of Medicine

Abbreviations: AAOMP, American Academy of Oral and Maxillofacial Pathology; ASC, American Society of Cytopathology; ASCP, American Society of Clinical Pathology; CAP, College of American Pathologists; NCCN, National Comprehensive Cancer Network; NCI, National Cancer Institute; NIDCR, National Institute of Dental and Craniofacial Research; NIH, National Institutes of Health; SPORE, Specialized Programs of Research Excellence; USCAP, United States and Canadian Academy of Pathology; USPTO, United States Patent and Trademark Office.