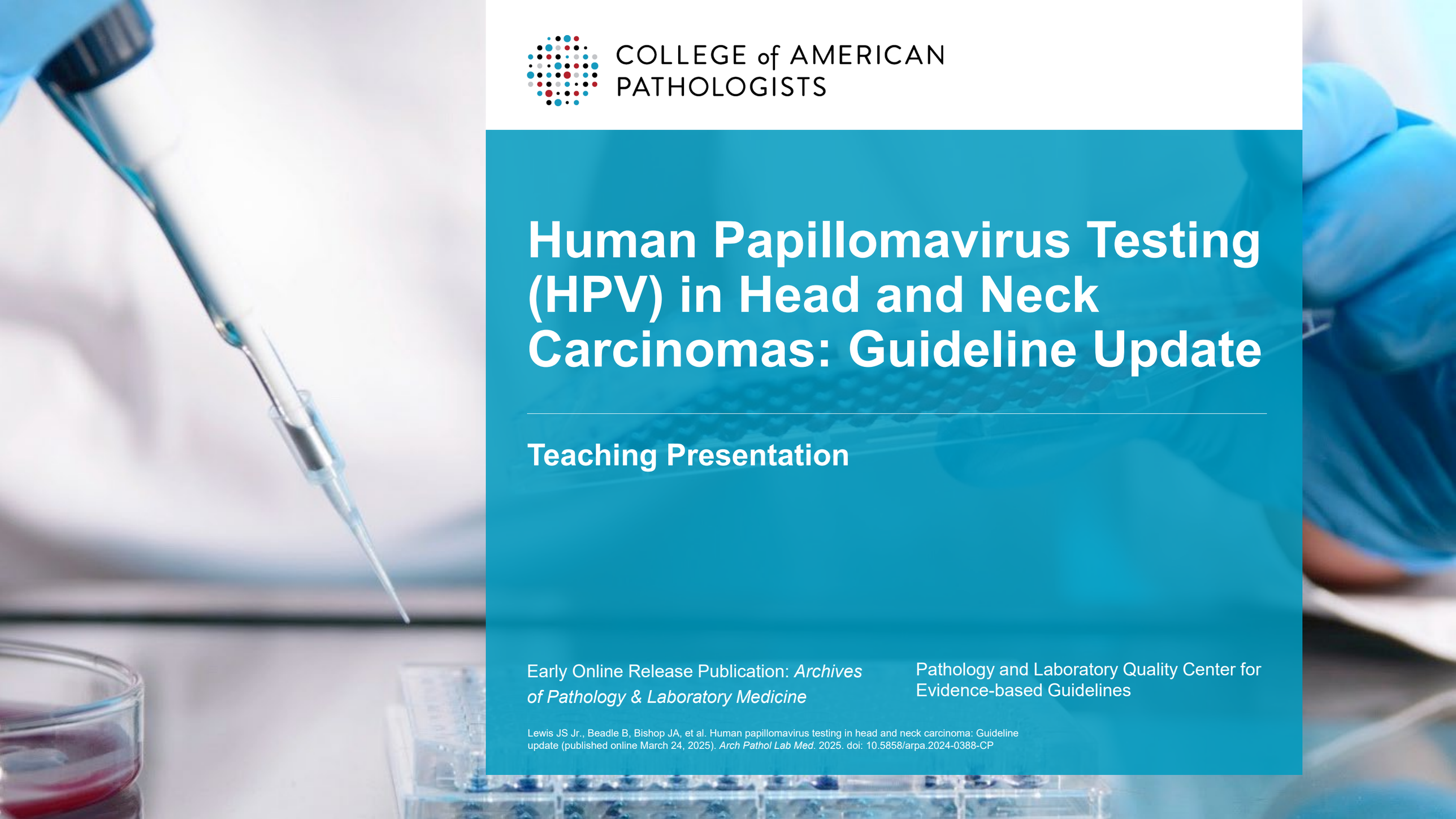


COLLEGE of AMERICAN PATHOLOGISTS

Human Papillomavirus Testing (HPV) in Head and Neck Carcinomas: Guideline Update

Teaching Presentation

Early Online Release Publication: *Archives of Pathology & Laboratory Medicine*

Pathology and Laboratory Quality Center for Evidence-based Guidelines

Lewis JS Jr., Beadle B, Bishop JA, et al. Human papillomavirus testing in head and neck carcinoma: Guideline update (published online March 24, 2025). *Arch Pathol Lab Med*. 2025. doi: 10.5858/arpa.2024-0388-CP

Outline

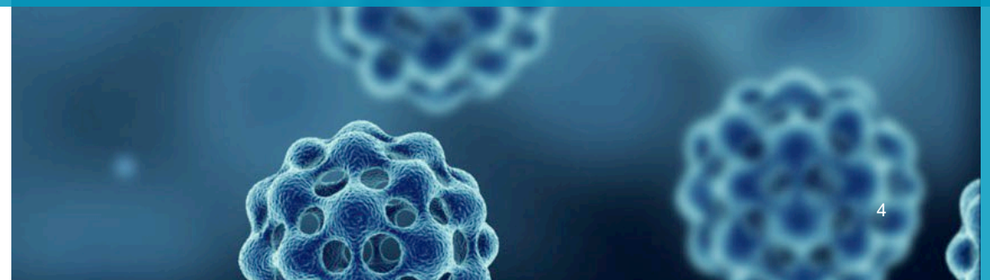
- Introduction
- Objective
- Key Questions and Results
- Guideline Recommendations and Good Practice Statements
- Guideline Development Process
- Conclusion

Introduction

- In 2018, the Pathology and Laboratory Quality Center for Evidence-based Guidelines (The Center) published a clinical practice guideline[1] to develop recommendations for the testing, application, interpretation, and reporting of high-risk human papillomavirus (HR-HPV) and surrogate marker tests in head and neck carcinomas.
- Since the 2018 publication substantial evidence has been published on human papillomavirus (HPV) in non-oropharyngeal anatomic sites, HPV global rates, p16 immunohistochemistry (IHC) and HPV testing performance in cytology specimens, and performance of p16 IHC as a surrogate marker.

Objective

- To assess research published since the release of the original 2018 guideline and to update evidence-based recommendations for HPV testing in head and neck carcinomas.



Key Questions and Results

Key Questions (KQs)

In patients with OPSCC, non-oropharyngeal SCC, metastatic SCC of unknown primary in the neck, non-squamous head and neck carcinoma are clinical outcomes improved in HPV-associated carcinoma compared to HPV-independent carcinoma?

In patients with newly diagnosed OPSCC including multi-site overlapping tumors, non-OPSCC, non-squamous head and neck carcinoma, and cervical nodal metastatic carcinomas of unknown and/or known primary do relevant outcomes differ based on the type(s) of HR-HPV testing performed?

Does performance of specific tests or testing algorithms for HR-HPV differ based on specimen characteristics such as its size, age, type of fixation, time-to-fixation and length of tissue fixation, the criteria/definition for a “positive” p16 IHC or HPV in situ hybridization (ISH) test result?

In patients with OPSCC, can HR-HPV status be used to determine if a cancer is a recurrence versus new primary?

Results

Seven Strong Recommendations

Four Conditional Recommendations

Five Good Practice Statements

Guideline Recommendations and Good Practice Statements

Guideline Statement 1 & 2

- **Strong Recommendation 1 -** Pathologists should perform high-risk human papillomavirus (HR-HPV) testing on all patients with newly diagnosed oropharyngeal squamous cell carcinoma (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, non-cytology), including regional lymph nodes with metastatic squamous cell carcinoma (SCC) and clinical findings consistent with an oropharyngeal primary, pathologists should perform HR-HPV testing by surrogate marker p16 IHC. In certain scenarios HPV-specific testing should be performed: a) in geographic regions with a low prevalence of HR-HPV-associated OPSCC b) when p16 immunostaining is equivocal (50-70% staining or when staining is extensive but weak) c) when there is a discrepancy between p16 staining and morphology, d) for large, multisite tumors overlapping the oropharynx, e) when specimens are from a non-tonsillar, non-base of tongue oropharyngeal site, and f) when required by clinical trials. Additional HPV-specific testing may be done at the discretion of the pathologist and/or treating clinician.

Rationale/Discussion

- Literature supports that HR-HPV status is a strong and independent predictor of overall and disease-specific survival for patients with OPSCC.
- Morphologic variation does not seem to influence clinical behavior, and so testing is indicated for all SCC subtypes.
- For geographic regions with a low prevalence of HR-HPV-associated OPSCC, also referred to as the HPV attributable fraction (HPV AF), HPV-specific testing should be performed in patients with positive p16 IHC.
  - p16 is not a suitable standalone surrogate in geographic regions where the HPV AF under 50% and certainly where it is 20% or lower. For these regions, routine confirmatory HPV-specific testing is recommended.

Guideline Statement 3

- **Strong Recommendation 3 - For tissue specimens, when p16 IHC is indicated, pathologists should report it as positive (and as a surrogate for HR-HPV) when there is at least 70% nuclear and cytoplasmic expression with at least moderate to strong intensity.**

Rationale/Discussion

- **HR-HPV specific testing is recommended for equivocal cases where staining for p16 is 50-70% moderate to strong nuclear and cytoplasmic staining or diffuse weak nuclear and cytoplasmic staining.**
- **Data is insufficient to recommend a particular antibody, testing platform or set of test conditions. It is important that each laboratory validate immunohistochemical assays, including specific clones, used in their laboratory, in accordance with CAP "Principles of Analytic Validation of Immunohistochemical Assays: Guideline Update".[2]**

Guideline Statements 4 & 5

- **Conditional Recommendation 4 - Pathologists should routinely perform HR-HPV testing on sinonasal SCC.**
- **Conditional Recommendation 5 - When testing a sinonasal SCC specimen for HR-HPV, pathologists should test directly for transcriptionally-active HR-HPV (RNA in situ hybridization [ISH]); positivity for the surrogate marker p16 IHC may be used to screen tumors for confirmatory HPV-specific testing.**

Rationale/Discussion

- Surveillance, Epidemiology, and End Results (SEER) data indicate an annual increase in prevalence of HR-HPV-associated SNSCC of approximately 2% over the period of 1995 to 2018.[3]
- A National Cancer Database case control design utilizing propensity score matched pairs demonstrates significantly better outcomes in HR-HPV-associated SNSCC patients as compared to HR-HPV-independent.[4]
- If one is screening with p16 IHC, then, if positive, one needs to use one of the recommended HPV-specific tests. Literature supports that RNA-ISH is a sufficient testing strategy.

Guideline Statements 6 & 7

- **Strong Recommendation 6 -** Pathologists should routinely perform HR-HPV testing on patients with metastatic SCC of unknown primary in a cervical lymph node.
- **Conditional Recommendation 7 -** For tissue specimens (ie, non-cytology) from patients presenting with metastatic SCC of unknown primary in a cervical lymph node, pathologists should perform HPV-specific testing or surrogate marker p16 IHC, followed by HPV-specific testing for p16 positive tumors. An explanatory note on the significance of a positive HPV result is recommended.

Rationale/Discussion

- Up to one-third of HR-HPV-associated OPSCCs present as metastases from an unknown primary, compared with 5%–10% of head and neck cancers overall.[5,6]
- Some SCCs from non-oropharyngeal sites (eg, from cutaneous or lung primaries) often overexpress p16 due to mechanisms unrelated to HR-HPV. They can present as cervical lymph node metastases of unknown primary and without confirmation by HR-HPV specific testing a p16-only testing strategy could lead to misclassification and mismanagement.

Guideline Statements 8

- **Strong Recommendation 8 - Pathologists should not routinely perform HR-HPV testing on patients with primary oral cavity, laryngeal, nasopharyngeal, or hypopharyngeal SCCs of the head and neck for prognostic purposes.**

  Note: **HR-HPV testing in nasopharyngeal SCCs can be used at the discretion of the pathologist and/or treating clinician.**

Rationale/Discussion

- Even though 2-5% of oral cavity and larynx and up to 25% of nasopharyngeal carcinomas are HPV-associated, the literature continues to lack consistent evidence of a prognostic benefit.[7,8]
- The data is particularly consistent in oral cavity and laryngeal SCC
- The data on nasopharynx is limited by small, heterogeneous studies with frequent lack of comparison of EBV-associated and EBV and HPV negative tumors with HPV-associated ones; while there is a suggestion of a prognostic benefit relative to double negative patients, there is not sufficient data
  - This was left as an optional testing at the discretion of the pathologist and/or treating clinician
- Conjunctival SCC was not covered as it is under ocular pathology

Guideline Statement 9

- **Good Practice Statement 9 - Pathologists should not routinely perform HR-HPV testing on patients with non-squamous carcinomas of the head and neck for prognosis.**

  Note: **HR-HPV testing is used in certain diagnostic settings (eg, HPV-related multiphenotypic sinonasal carcinoma) and/or for establishing primary site.**

Rationale/Discussion

- There is insufficient evidence to support routine HR-HPV testing in any type of non-squamous carcinoma for prognostic or predictive purposes.
- Non-routine (case specific) HR-HPV testing of non-squamous head and neck carcinomas is important in certain scenarios:
  - HR-HPV tumor status may be helpful in neuroendocrine carcinomas and adenocarcinomas when the primary tumor site is unclear
  - HR-HPV specific testing is also important in poorly-differentiated malignant neoplasms for which the tumor type is uncertain and includes HR-HPV-associated carcinoma
  - HR-HPV testing is also critical to establish the correct diagnosis of HPV-related multiphenotypic sinonasal carcinoma, a tumor type for which HR HPV positivity is definitional

Guideline Statements 10 & 11

- **Conditional Recommendation 10 - Pathologists should perform HR-HPV testing on head and neck fine needle aspirations (FNA) of nodal SCC samples from all patients with: (a) clinical findings of an oropharyngeal or sinonasal primary or (b) metastatic SCC of unknown primary.**
- **Strong Recommendation 11 - For FNA specimens, pathologists should perform HPV-specific testing.**

  Note: **In selected circumstances p16 IHC can be performed instead of HPV-specific testing. If the result of HR-HPV testing on the FNA sample is negative, testing should be performed on tissue if it becomes available.**

Rationale/Discussion

- Recommendation applies to patients for whom no prior SCC sampling with HR-HPV has been performed.
- p16 IHC does not function in the same way on cell block material as it does in formalin-fixed, paraffin embedded non-FNA tissue biopsies and resections.
  - Using the same cutoff of >70% for p16 IHC as is recommended for surgical pathology tissue specimens, the accuracy of p16 IHC for predicting HR-HPV was only 38% compared to the use of RNA-ISH which had an accuracy of 97%.[9]
- HPV-specific testing methodologies have consistently proven to be accurate and more effective than p16 IHC for FNA and cell block material.
- In selected circumstances such as when HPV-specific testing methodologies are not available, p16 IHC can be performed; however, given the risk of a false negative result, repeat HR-HPV testing should be performed on tissue if it becomes available whenever p16 IHC is negative.

Guideline Statement 12

- **Strong Recommendation 12 - For HPV specific testing, pathologists should utilize tests that exhibit optimal performance characteristics, such as RNA-ISH or DNA PCR; and have adequate coverage of non-HPV16 high-risk types.**

  DNA-ISH is not recommended.

Rationale/Discussion

- Not all HPV-specific tests perform similarly.
  - The literature continues to show that HR-HPV DNA ISH lacks analytic sensitivity relative to HR-HPV RNA ISH.[10]
- DNA ISH is not recommended to be used in any scenario.
- DNA PCR is adequate only in conjunction with p16 immunohistochemistry, therefore in p16 positive tumors, pathologists can use DNA PCR as the HPV-specific confirmatory test (never as a standalone test).
- Only 85% to 90% of HPV-associated head and neck SCC is caused by HPV type 16. The remainder is a mix of several different types including HPV type 18, 31, and 33.[11]
- HR-HPV ISH or PCR should include at least types 16, 18, 26, 33, 35, and 58.

Guideline Statement 13

- **Good Practice Statement 13 - Pathologists should not routinely perform low-risk HPV testing on patients with head and neck carcinomas.**

Rationale/Discussion

- **There is no evidence to support low-risk HPV-testing for either prognostic purposes or to guide therapy.**

Guideline Statement 14

- **Good Practice Statement 14 - 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.**

Rationale/Discussion

- For cancers that are clinically persistent after treatment or clearly recurrent in nature, there is no documented value for repeating testing on these specimens. Some exceptions include:
  - If the initial HR-HPV status was never assessed or the results are unknown, testing is recommended.
  - There are cases in which new tumors cannot clearly be delineated as recurrences versus new primary tumors. This can be due to clinical location, behavior, or significant time interval between first treatment and second lesion. HR-HPV may be used to help distinguish recurrence from a new primary SCC.

Guideline Statement 15

- **Good Practice Statement 15 - Pathologists should not routinely perform HR-HPV testing on patients with distant metastases if primary tumor HR-HPV status has been established. HR-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. A positive p16 IHC result, in this setting, should be confirmed with an HPV-specific test.**

Rationale/Discussion

- Given that the HR-HPV status of metastatic oropharyngeal SCC is not expected to change (supported by limited data) in regional or distant metastases, routine repeat testing of distant metastases for HR-HPV in metastatic HPV-associated oropharyngeal SCC is not indicated when the HR-HPV status has already been established.
- In the lung, in particular, where primary SCC can sometimes be difficult to tell from metastatic SCC, HR-HPV testing may be helpful. If used for this purpose (typically in solitary lung lesions in patients with a history of oropharyngeal SCC), p16 can be a screening test. If positive, then HPV-specific testing must be utilized as up to 10 to 20% of lung and cutaneous primary SCCs can be positive for p16.

Guideline Statement 16

- **Good Practice Statement 16 - Pathologists should not provide a tumor grade or differentiation status for HPV-associated OPSCCs.**

Rationale/Discussion

- **Providing a grade or differentiation status for HPV-associated OPSCC patients is not recommended, however the expert panel supports providing a histologic subtype, as per the World Health Organization (WHO) classification.[12]**

Guideline Development Process

Expert Panel (EP)

- James S. Lewis Jr. MD*
- William Faquin MD, PhD*
- Beth Beadle MD, PhD
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- Rebecca Chernock MD
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- Mary Schwartz, MD
- Raja Seethala, MD

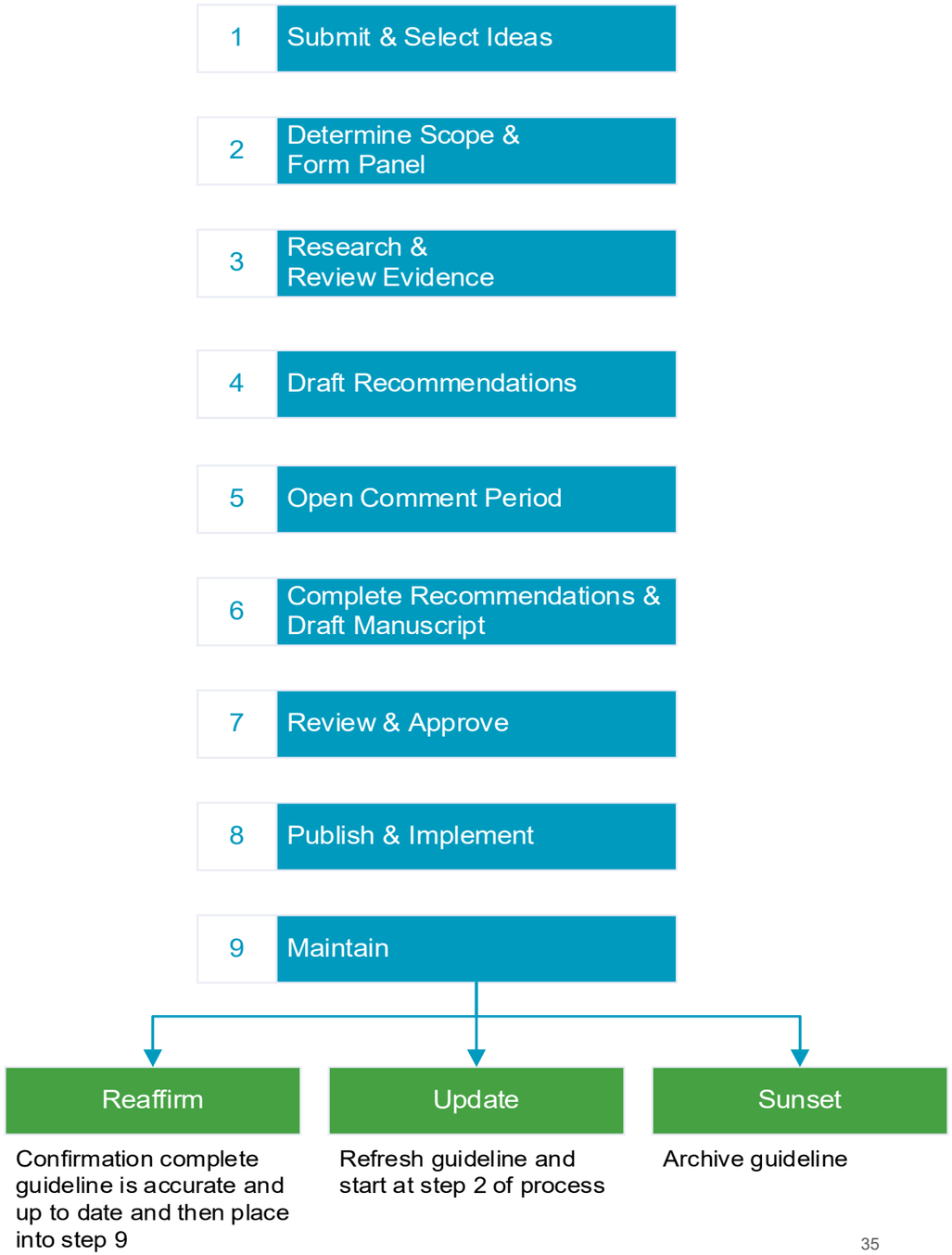
Advisory Panel (AP)

- Jessica Geiger MD
- Kumarasen Cooper MD, PhD
- Carole Fakhry MD, MPH
- Vickie Jo MD
- Christina Kong MD
- Amy Lynn MD
- Cherie Paquette MD
- Michael Prystowsky MD, PhD
- Stephen Smith MD
- Bert Noojin, JD

*Guideline Co-Chair

Guideline Development Process

- The Center follows the standards endorsed by the National Academy of Medicine for developing Clinical Practice Guidelines.
- Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach was utilized in updating the guideline.
- A detailed description of the guideline development process can be found online [Evidence-based Guidelines Development Methodology Manual]

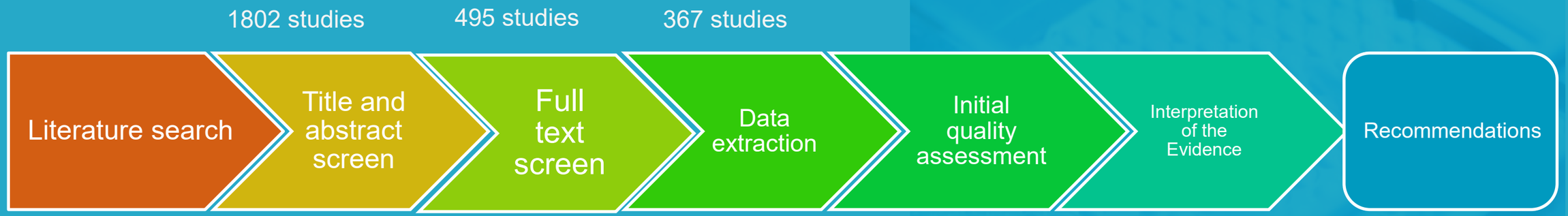


Literature Search

- Search was conducted in Ovid MEDLINE, Embase, Cochrane Library.
- Initial literature search ran on: July 6, 2021
  - 1445 studies from January 1, 2016 to July 6, 2021
- Literature refresh ran on: August 2, 2023
  - 560 studies from July 6, 2021 to August 2, 2023

Systematic Review of the Literature

- **Each level of systematic review (title-abstract screening, full-text review, and data extraction) was performed in duplicate by two members of the expert panel.**



Quality Assessment

- Systematic Reviews (SRs) and Meta-analyses questions were assessed as per the Assessing the Methodological Quality of SRs (AMSTAR) tool[13]
- Non-randomized studies were assessed using the Risk of Bias in Non-Randomized Studies – of Intervention (ROBINS-I) tool.[14]
- Diagnostic studies were assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool.[15]

Open Comment Period

- Open Comment Period held from August 14 to September 1, 2023
  - ~63 respondents for each draft statement
  - 153 written comments

Review and Approval

- The AP reviewed and provided feedback on the draft recommendations and manuscript.
- The EP approved the final recommendations and good practice statements with a formal vote.
- The independent review panel (IRP) representing the Council on Scientific Affairs reviewed and approved the guideline for the CAP.
  - IRP members were masked to the expert panel and vetted through the conflicts of interest (COI) process

Conclusion

Conclusion

- **The guideline update provides direction on HPV testing in various head and neck carcinomas, to improve and standardize, where possible, HPV testing across diverse pathology practice settings and different countries.**

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- **The CAP developed the Pathology and Laboratory Quality Center for Evidence-based Guidelines as a forum to create and maintain laboratory practice guidelines (LPGs). Guidelines 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 an LPG is developed and when it is published or read. LPGs are not continually updated and may not reflect the most recent evidence. LPGs address only the topics specifically identified therein and are not applicable to other interventions, diseases, or stages of diseases. Furthermore, guidelines 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 LPG 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. CAP makes no warranty, express or implied, regarding LPGs and specifically excludes any warranties of merchantability and fitness for a particular use or purpose. 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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