

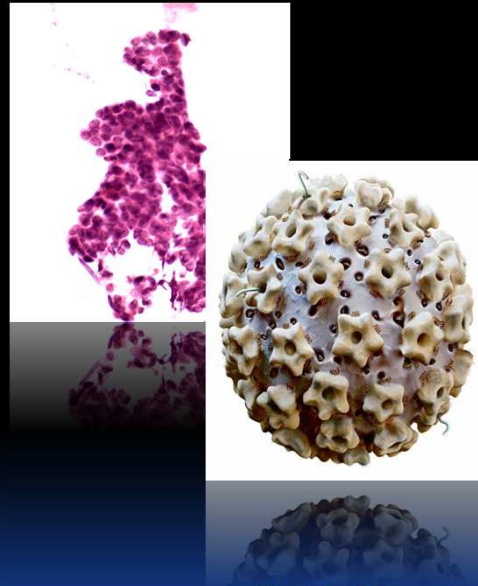


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HPV Testing of Head and Neck Squamous Cell Carcinoma

Disclosure of Relevant Financial Relationships

No financial or other conflicts to disclose.

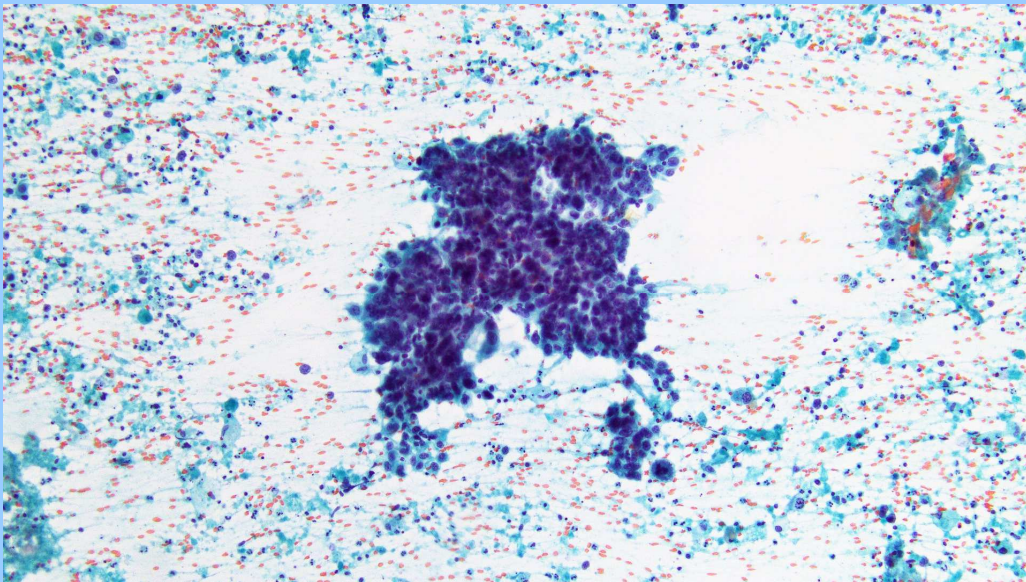
**Information presented includes work by
colleagues & collaborators at the MGH as well as
work from groups around the globe.**



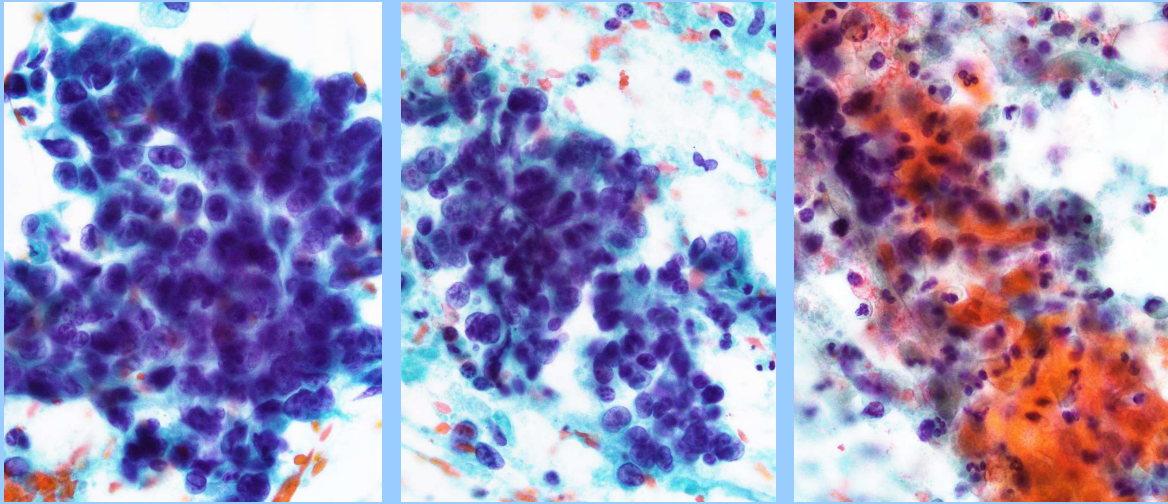
Overview

- **Pathologic features and clinical implications of HPV-associated head and neck cancer**
- **CAP testing guidelines for HPV in HN SCC**
- **Horizon: Cell free testing for HPV**

**68 year-old man with 2.0 cm right neck (level II) mass.
An FNA is performed.**



FNA: Squamous cell carcinoma, focally keratinizing
Carcinoma of unknown primary (CUP)



HR-HPV analysis performed on FNA liquid-based SP specimen using BD-Onclarity PCR-based assay is **POSITIVE for HR-HPV 16**

A right tonsillar primary was identified. The patient elects to enter a clinical trial for patients with HPV-associated oropharyngeal carcinoma where he will receive reduced doses of radiation and chemotherapy.

Background to HPV-Associated Head and Neck Cancer

HPV “Epidemic” in HNSCC

HPV-Associated Head and Neck Cancer: A Virus-Related Cancer Epidemic

Trends in Head and Neck Cancer Incidence in Relation to Smoking Prevalence

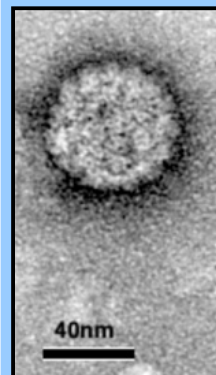
An Emerging Epidemic of Human Papillomavirus-Associated Cancers?

- Reflex testing for HR-HPV is indicated for certain HN cancers:
- Diagnosis
- Prognosis
- Guide Management
- Testing guidelines are needed to establish:
 - When should reflex testing be performed?
 - Which testing method(s) should be used?
 - How should HPV testing be applied to Cytology?

Human Papillomavirus

Small, non-enveloped, double-stranded DNA viruses that infect squamous (and other) epithelia.

- Approximately 200 HPV types
- Classified into two groups.
 - *Mucosal* (α)
 - *Cutaneous* (β, γ, μ, ν)
- Classification as low (HPV 6, 11) or high risk (HPV 16, 18, 33, 51, 53, others) based on risk of malignant progression.
- **HPV 16 followed by HPV 18 is the most common type associated with HN Cancer**



HPV expresses two oncoproteins: E6 and E7

- The process of malignant transformation arises from the continued function of the E6 and E7 viral oncoproteins.
- E6 and E7 target several critical cellular pathways, leading to deregulation of proliferation and evasion of apoptosis.
- HPV E7 inhibits the retinoblastoma tumor suppressor protein (pRb) and targets it for degradation allowing proliferation and resulting in high p16 expression levels.
- HPV E6 inactivates the p53 tumor suppressor, preventing cell death thru apoptosis.

Clinical presentation of HPV-associated HNSCC is different than smoking-related cancer

This pertains especially to the oropharynx

- More likely to be younger, male, married, and college educated
- >3:1-8:1 M:F
- Typically lack a significant history of tobacco or alcohol abuse.
- Sexual risk factors for oral or genital HPV exposure.
- Low T and high N stage tumors.

Survival in HPV-Associated OPSCC


- Retrospective analyses of clinical trials show a survival benefit in HPV(+) OPSCC.
- Meta-analysis shows **a 53% better overall and 74% better disease-specific survival for HPV(+) OPSCC**
- Subset of patients who have aggressive disease
- Smokers with HPV+ OPSCC have intermediate prognosis

HR-HPV has Major Implications for the Management of Head and Neck Cancer

Curr. Treat. Options in Oncol. (2022) 23:325–332
DOI 10.1007/s11864-022-00950-8

Head and Neck Cancer (PL Swiecicki, Section Editor)

HPV as a Carcinomic Driver in Head and Neck Cancer: a De-escalated Future?

James E. Bates, MD^{1,*} 
Conor E. Steuer, MD²

Clinical Trials for HPV-Associated OPSCC

"With improved prognosis in low-risk disease, clinical trials are implementing de-escalation strategies to maintain excellent survival outcomes while minimizing toxicity".

J. Rocco, MD 2022

Clinical Trials and Treatment:

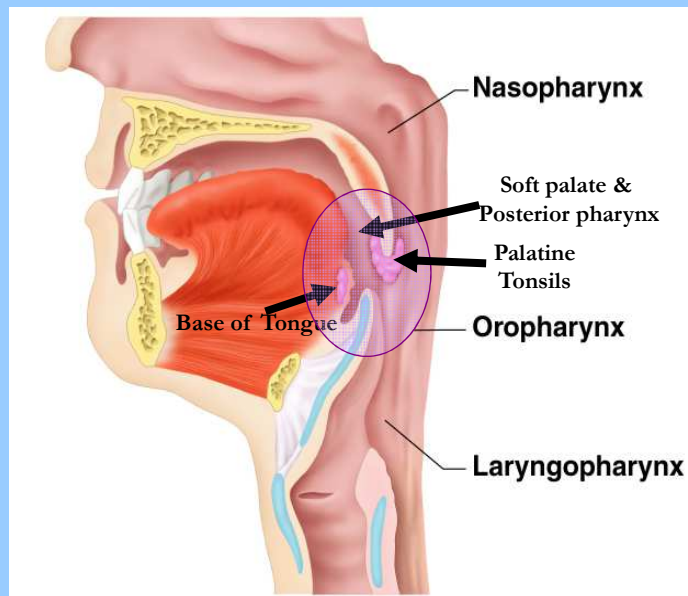
- De-Escalation
- Modified Doses
- Targeted Therapies
- Induction Approaches

Pathology of HPV-Associated Oropharyngeal Carcinomas

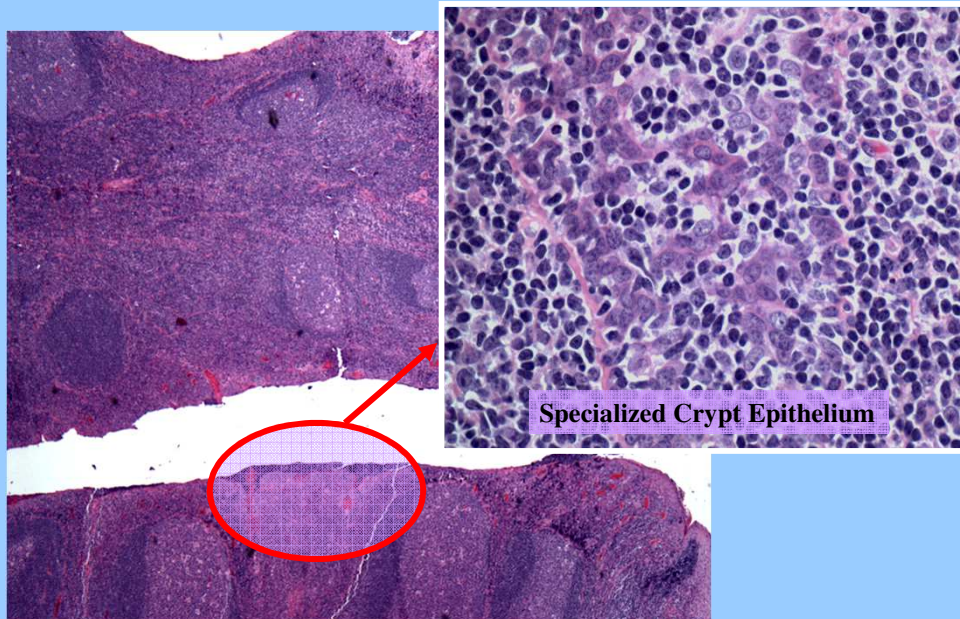
Role of HR-HPV in Head and Neck Cancer at Various Anatomic Subsites

- Association between HR-HPV and cancer at various HN sites:
 - Oropharynx: 80-90%
 - Sinonasal Cavity: 20-25%
 - Oral Cavity: 3-6%
 - Larynx: <5%
 - Other HN sites: e.g. Periocular, Nasopharynx

OROPHARYNGEAL CARCINOMA AND HPV

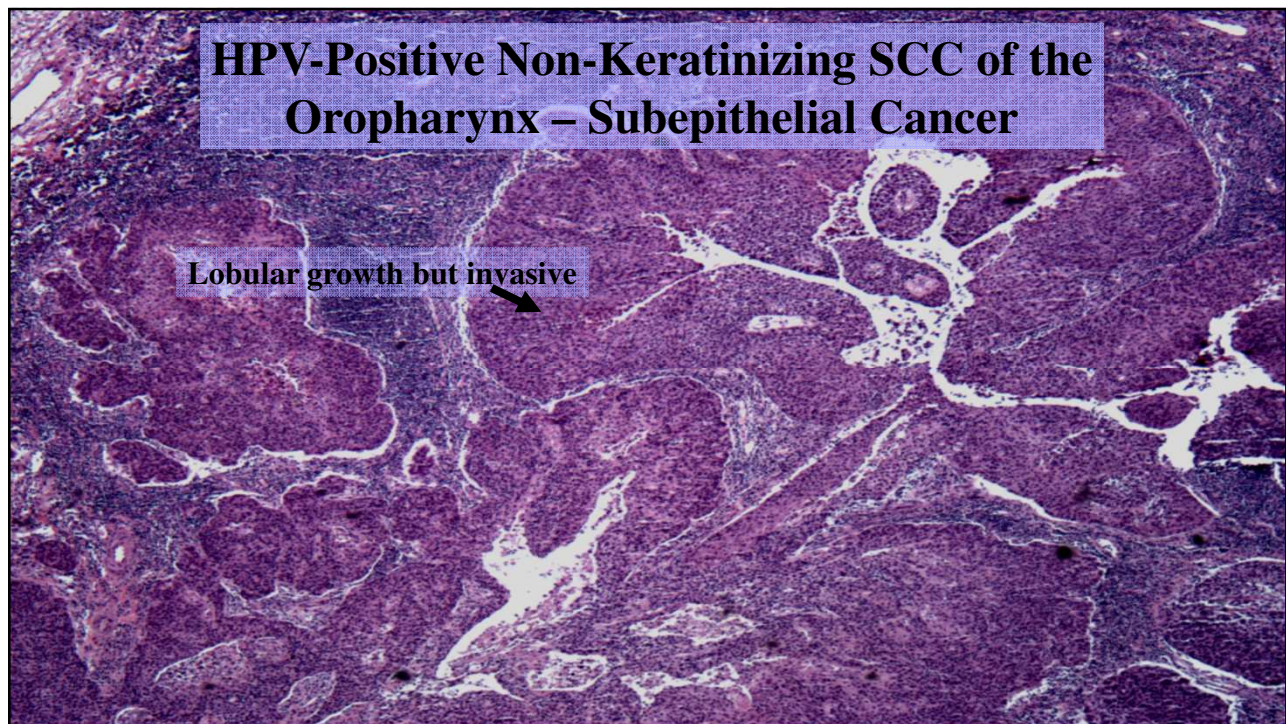


OROPHARYNGEAL CARCINOMA



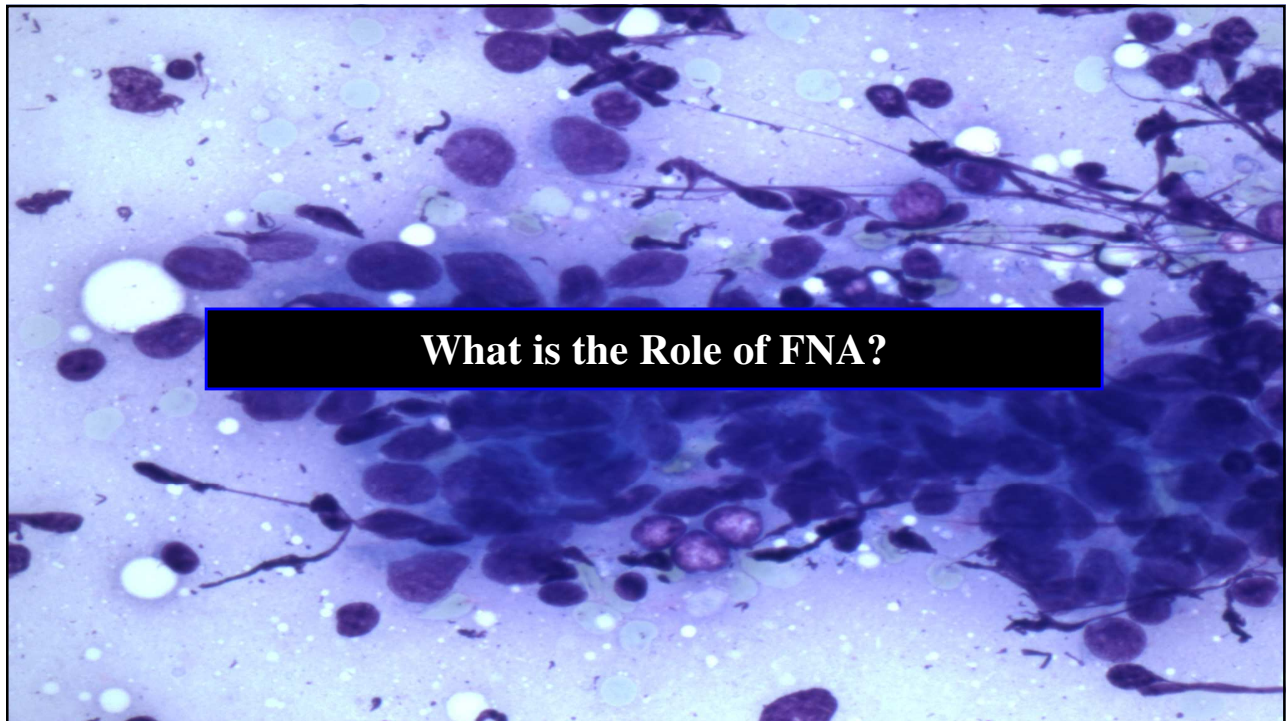
HPV in Oropharyngeal SCC

- Non-keratinizing or partially keratinizing
- Basaloid appearance
- 90-95% are due to HPV type 16
- Small subset due to HPV 18 and other HR-HPV types (31, 33, 53 etc)
 - Must include adequate “cocktail” in any HPV-specific test



HPV in Oropharyngeal SCC

- Should not be considered “poorly differentiated” –
– **Do NOT grade!**
- Distinct from HPV-independent basaloid SCC
- Preferred terms:
 - **HPV-Associated SCC (adopted by 2022 WHO)**
 - **HPV-Positive SCC**
 - **P16-Positive SCC**



What is the Role of FNA?

Nodal Metastases in HPV-positive OP SCC

Ang et al. *NEJM* 2010; 363: 24.
Jordan et al. *Am J Surg Pathol* 2012; 36: 945.
Lewis Jr. et al. *Am J Surg Pathol* 2010; 1044:38.

Nodal metastases to Level II or III are present at presentation in approx 80-85% of all HPV-associated OPSCC.

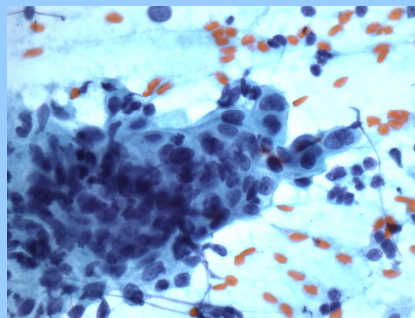
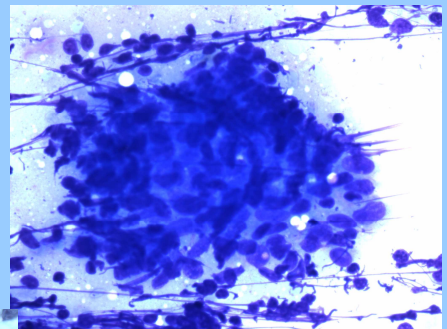
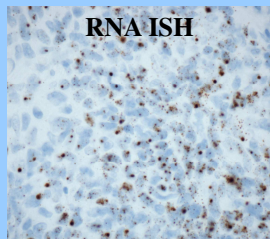
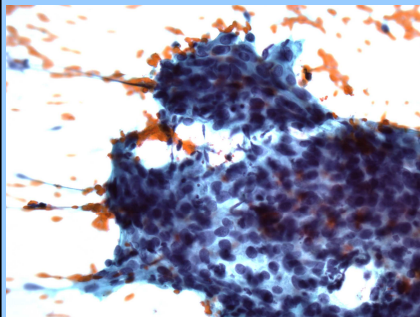
HPV-Positive Oropharyngeal SCC:

Often first detected and diagnosed by FNA !



FNA is a key method used in the initial detection of these metastatic cancers.

Cytologic Features of HPV-Associated HN SCC





FNA Pitfall: Many HPV+ SCC Metastases are Cystic and Can Mimic Benign Squamous Cysts

Given the role of FNA in the initial detection of these cancers, appropriate HPV testing modalities for cytology are needed....

Role of HR-HPV in HN Cancer

... The oropharynx is the head and neck site where HPV-positivity has the **strongest link** to improved patient outcome.

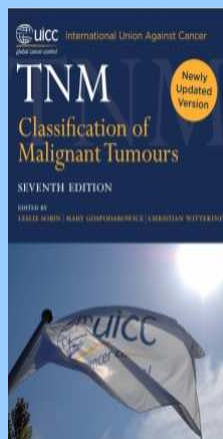
Should we do reflex testing for HR-HPV in HN SCC??? **YES!!!**

Why Should We Test for HR-HPV in HNSCC?

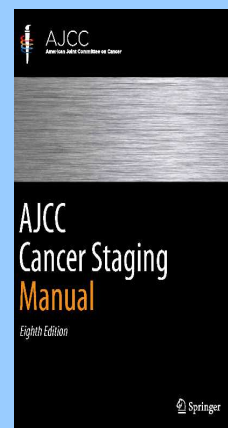
- Improved prognosis among many patients
- Identify primary site of metastatic SCC (CUP)
- Distinguish metastatic SCC from benign head and neck squamous cysts
- Distinguish HPV- from EBV-related carcinomas
- **Determine patient eligibility for clinical trials/de-escalation therapy**

8th Edition: AJCC Staging Update for HPV-Associated OP Cancer

- 1) Patient Prognosis and Etiology Counseling
- 2) UICC/AJCC Staging



**Specific, Separate
Staging System
for HPV-
Associated
OPSCC**





The CAP EBG HPV Testing Committee was Formed to Establish a Uniform Approach

Human Papillomavirus Testing in Head and Neck Carcinomas

Guideline From the College of American Pathologists

James S. Lewis Jr, MD; Beth Beadle, MD, PhD; Justin A. Bishop, MD; Rebecca D. Churnock, MD; Carol Colasacco, MLIS, SCT(ASCP); Christina Lacchetti, MHSC; Joel Todd Moncur, MD, PhD; James W. Rocco, MD, PhD; Mary R. Schwartz, MD; Raja R. Seethala, MD; Nicole E. Thomas, MPH, CT(ASCP)^{CM}; William H. Westra, MD; William C. Faquin, MD, PhD

CAP EBG HPV Testing Committee



14 CAP Recommendations 2018 (Updates Coming in late 2023)

1. Pathologists should perform 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.
2. For oropharyngeal tissue specimens (i.e., non-cytology), 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.
3. Pathologists should not routinely perform HR-HPV testing on patients with non-squamous carcinomas of the oropharynx.
4. Pathologists should not routinely perform HR-HPV testing on patients with non-oropharyngeal primary tumors of the head and neck.
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.
6. For tissue specimens (i.e., non-cytology) from patients presenting with metastatic SCC of unknown primary in a cervical upper or mid jugular chain lymph node, pathologists should perform p16 IHC.
7. Pathologists should perform HR-HPV testing on head and neck fine needle aspiration (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.
8. Pathologists should report p16 IHC positivity as a surrogate for HR-HPV in tissue specimens (i.e., non-cytology) when there is at least 70% nuclear and cytoplasmic expression with at least moderate to strong intensity.
9. Pathologists should not routinely perform low-risk HPV testing on patients with head and neck carcinomas.
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.
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.
12. Pathologists should report primary OPSCCs that test positive for HR-HPV or its surrogate marker p16 as "HPV-positive" and/or "p16-positive."
13. Pathologists should not provide a tumor grade or differentiation status for HPV-positive/p16-positive OPSCC.
14. Pathologists should not alter HR-HPV testing strategy based on patient smoking history.

Current CAP Recommendations for HPV Testing in Head and Neck Cancer

General Overview:

- The tumors of all patients presenting with **oropharyngeal SCC** should be tested for HR-HPV
- Neck nodal tissue from all patients with **metastatic SCC of unknown primary** should be tested for HR-HPV
- **Staining with p16** can be used as the sole initial screening method but confirmatory testing may be necessary in selected cases
- HR-HPV **Testing of FNA specimens** is recommended

CAP Guideline Endorsed by ASCO

Human Papillomavirus Testing in Head and Neck Carcinomas: ASCO Clinical Practice Guideline Endorsement of the College of American Pathologists Guideline

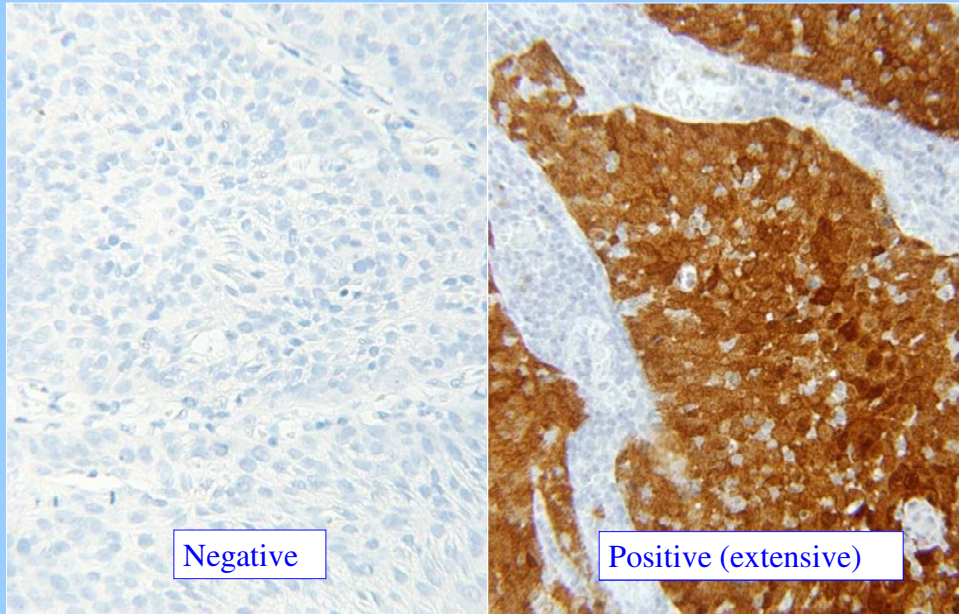
Carole Fakhry, Christina Lacchetti, Lisa M. Rooper, Richard C. Jordan, Danny Rischin, Erich M. Sturgis, Diana Bell, Mark W. Linen, Seema Harichand-Herd, John Thibo, Jose Zevallos, and Bayardo Perez-Ordonez

J Clin Oncol 2018;36:3152-3161.

HPV in Oropharyngeal SCC: p16 Immunohistochemistry

- **Sensitivity approaches 100%... But there are problems!**
- **Specificity is high in OP (>90%) but low outside OP (79-82%)**
- **In the OP in USA, p16 correlates well with HPV+**
- **In mets to level II/III and NK morphology, p16 has a “high rate” of correlation with HPV+ but not perfect**
- **Application of p16 to FNA is problematic**

p16 Immunohistochemistry- 70% Threshold



*The **GOLD STANDARD** is the demonstration of transcriptionally active HR-HPV*

**ISH for HPV E6/E7 mRNA has become standard practice
for cases where HPV-specific testing is needed**

Can be applied to cytologic preparations.

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

Darcy A. Kerr, MD,† Kshitij S. Arora, MBBS,‡ Krishnan K. Mahadevan, MBBS,‡
Jason L. Hornick, MD, PhD,†§ Jeffrey F. Krane, MD, PhD,†§ Miguel N. Rivera, MD,*†
David T. Ting, MD,†|| Vikram Deshpande, MD,*† and William C. Faquin, MD, PhD*†*

The Problem:

P16 versus HPV-Specific Testing for Biopsies and Resections

- Especially in low prevalence areas, p16 lacks specificity even in OP
- In cases of CUP, p16 alone has a risk of being a false positive
- **Information about BOTH p16 and HR-HPV has implications for prognosis**

Prognostic implications of p16 and HPV discordance in oropharyngeal cancer (HNCIG-EPIC-OPC): a multicentre, multinational, individual patient data analysis

Hisham Mehanna*, Miren Taberna*, Christian von Buchwald, Sara Tous, Jill Brooks, Marisa Mena, Francisca Morey, Christian Grønhoj, Jacob Høygaard Rasmussen, Martin Garset-Zamani, Laia Bruni, Nikolaos Batis, Ruud H Brakenhoff, C René Leemans, Robert J Baatenburg de Jong, Jens Peter Klusmann, Nora Wuerdemann, Steffen Wagner, Tina Dalianis, Linda Marklund, Haitham Mirghani, Andrew Schache, Jaqueline A James, Shao Hui Huang, Brian O'Sullivan, Paul Nankivell, Martina A Broglie, Markus Hoffmann, Elgar Susanne Quabius, Laia Alemany, on behalf of the HNCIG-EPIC group

Lancet Oncol. 2023

Implications of all the available evidence

Along with routine p16 immunohistochemistry, HPV testing is strongly recommended where HPV status determines eligibility for clinical trials, where it affects patient counselling, and where treatment de-escalation or intensification are being considered, especially in areas with low HPV-attributable fractions.

P16/HPV STATUS	5-year overall survival
P16+/HPV+	81.1%
P16+/HPV-	54.7%
P16-/HPV+	53.2%
P16-/HPV-	42.4%

Potential Changes to HPV Testing in Head and Neck Cancer in 2023

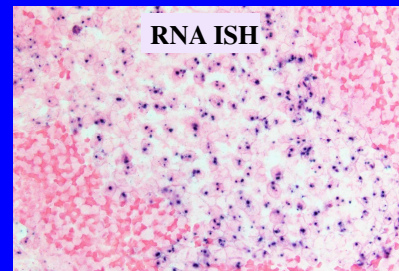
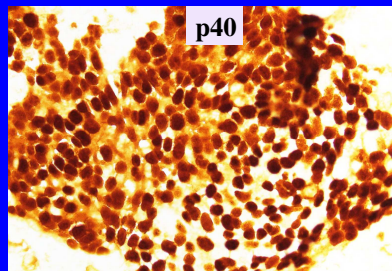
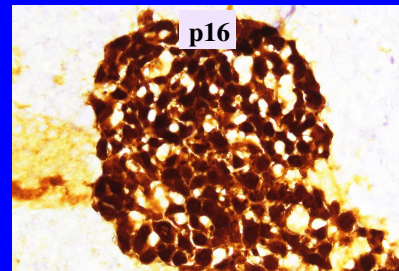
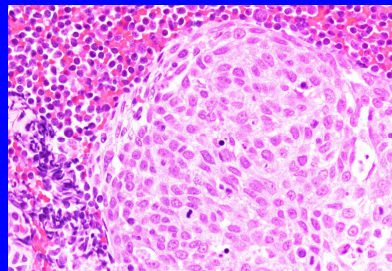
Highlights of Some Potential Changes to CAP Guideline:

- **More HPV-specific testing for OPSCC:**
 - Clinical trials, Low overall HPV attributable fraction regions, Equivocal p16, Discrepant p16 and morphology, Multisite tumors, Non-tonsillar/non-base of tongue oropharyngeal sites.
 - Cervical LN CUP
 - FNA specimens
- **Reflex testing for sinonasal SCC**
 - Should include HPV-specific testing

How should HR-HPV testing be done in FNA specimens?

CELL BLOCK

**Testing for
HR-HPV
in FNAs of
HNSCC**



P16 IHC and RNA ISH in FNAs of HNSCC

Heterogeneity of p16 Immunohistochemistry and Increased Sensitivity of RNA In Situ Hybridization in Cytology Specimens of HPV-Related Head and Neck Squamous Cell Carcinoma

Kristine S. Wong, MD¹; Jeffrey F. Krane, MD, PhD ²; and Vickie Y. Jo, MD ¹

- P16 sensitivity of 93% for any positive result (as low as 5%!)
- 38% sensitivity using CAP criteria (>70%) for p16
- RNA ISH was >97% sensitive

HR-HPV testing in FNAs of HNSCC CUP: Which test to use?

- P16 will no longer be recommended as a stand-alone test or a screening test for FNA specimens
- HPV-specific testing is preferred!!!
 - RNA ISH for HR-HPV works well
 - Liquid-based HR-HPV testing works well

HR-HPV in FNAs of HNSCC

- **HPV-Specific Liquid-phase testing:**

- Advantages over cell block (FFPE)
- Objective result with clear-cut scoring
- Can be automated
- Covers a broad range of HR-HPV

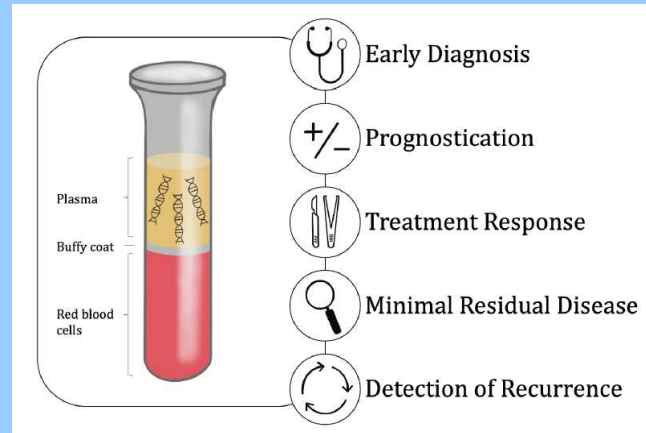
Several have already been validated:

- » Hybrid Capture II
- » Cervista™ HPV HR
- » Cervista™ HPV 16/18
- » Roche cobas® HPV test
- » APTIMA® HPV Assay
- » **BD Onclarity**

**On the Horizon: Cell Free DNA Testing
for HPV-Associated SCC**

Emerging Test: Cell Free DNA and HPV+ HN Cancer

- Circulating tumor HPV DNA detectable in blood plasma
- Blood-based molecular diagnostics – qPCR and droplet digital (dd)PCR
- **High sensitivity and specificity** compared to prior attempts
- Low cost: Approx \$45 per test



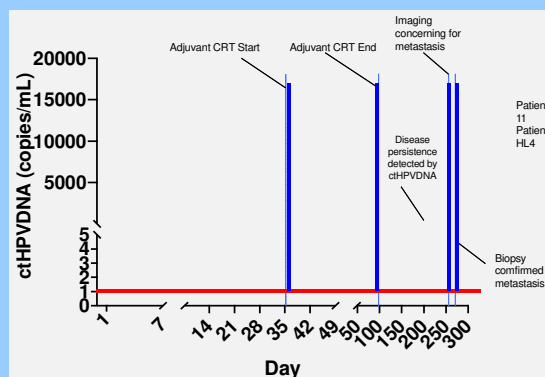
Cell Free DNA and HPV+ HN Cancer

- 140 patients with HN SCC
- **Sensitivity: 98.4%**
- **Specificity: 98.6%**
- Median diagnostic interval of 26 days less
- Reduced cost

Cell-Free HPV DNA Provides an Accurate and Rapid Diagnosis of HPV-Associated Head and Neck Cancer

Giulia Siravegna^{1,2}, Connor J. O'Boyle³, Shohreh Varmeh^{3,4}, Natalia Queenan³, Alexa Michel², Jarrod Stein³, Julia Thierauf⁵, Peter M. Sadow^{1,3,5}, William C. Faquin^{1,3,5}, Simon K. Perry⁵, Adam Z. Bard⁵, Wei Wang⁶, Daniel G. Deschler^{1,3}, Kevin S. Emerick^{1,3}, Mark A. Varvares^{1,3}, Jong C. Park^{1,7}, John R. Clark^{1,7}, Annie W. Chan^{1,8}, Vanessa Carlota Andreu Arasa⁹, Osamu Sakai⁹, Jochen Lennerz^{1,2,5}, Ryan B. Corcoran^{1,2,7}, Lori J. Wirth^{1,7}, Derrick T. Lin^{1,3}, A. John Iafrate^{1,2,5}, Jeremy D. Richmon^{1,3}, and Daniel L. Faden^{1,3,10}

2022



SUMMARY

- HPV-associated OPSCC represents a distinct disease from traditional smoking-related HNSCC.
- Many head and neck cancers have HPV+ forms, but **HPV+ OPSCC has the strongest link** to better patient outcome
- **Reflex testing** for HR-HPV should be performed for histologic and cytologic specimens
- Many testing options/scenarios – in many cases, both p16 and HPV-specific testing are best
- **Updated CAP testing guidelines in late 2023.**

**Thank
You!**

