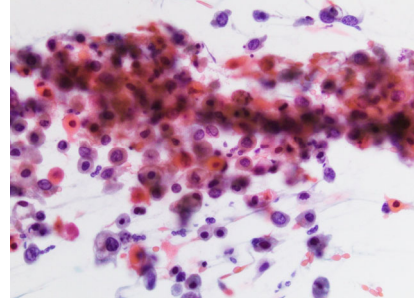


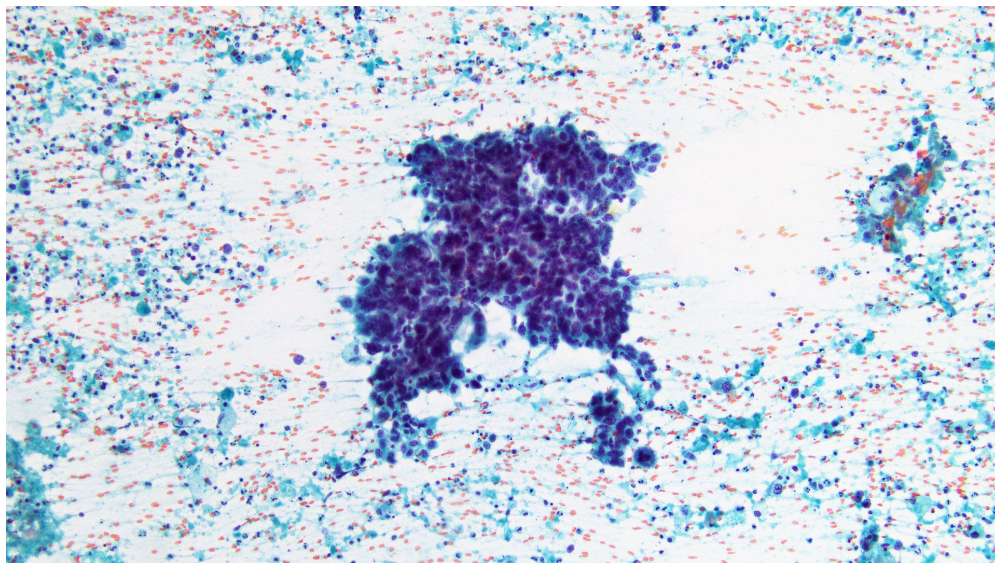


William C. Faquin, MD, PhD
Professor of Pathology
Harvard Medical School
Massachusetts General Hospital
Director of Head and Neck Pathology
Massachusetts Eye and Ear

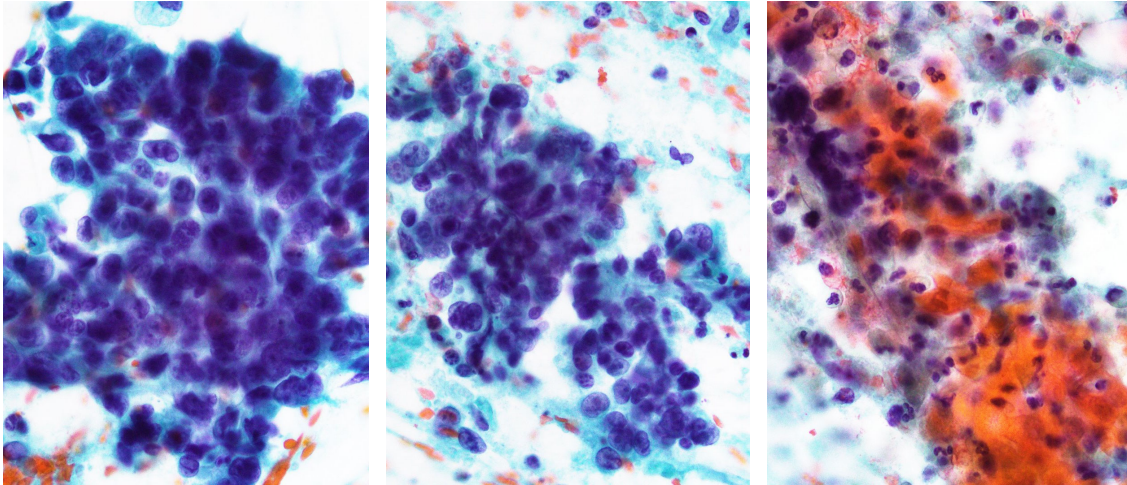


HPV Testing in Head and Neck Squamous Cell Carcinomas

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)

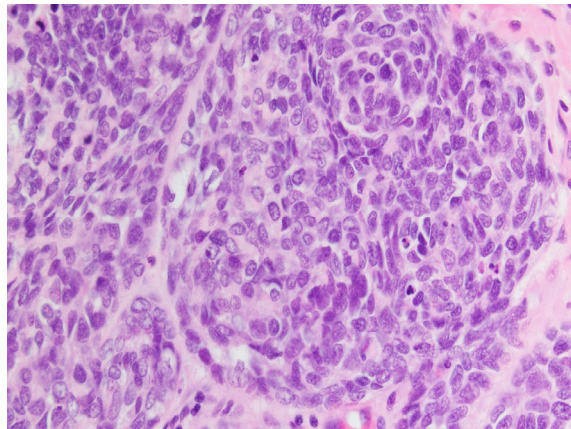


CYTOLOGY DIAGNOSIS:
MALIGNANT
Metastatic squamous cell carcinoma.

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

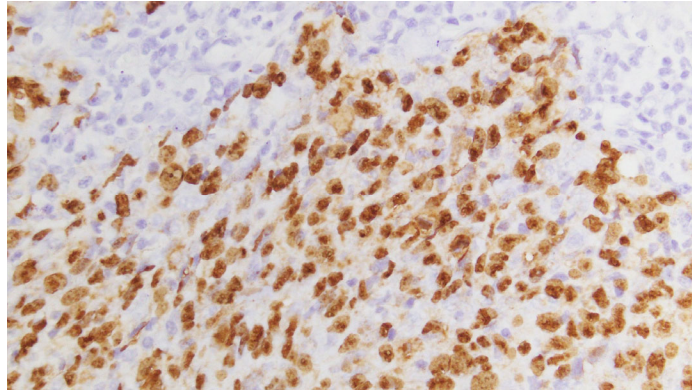
This defines the carcinoma as arising from the oropharynx, and it has implications for prognosis and management

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.



ISH for EBER in Metastatic NPC

NOTE:
When HPV testing is
NEGATIVE, consider
testing for **EBV** to
diagnose
**nasopharyngeal
carcinoma**.



Overview of 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?

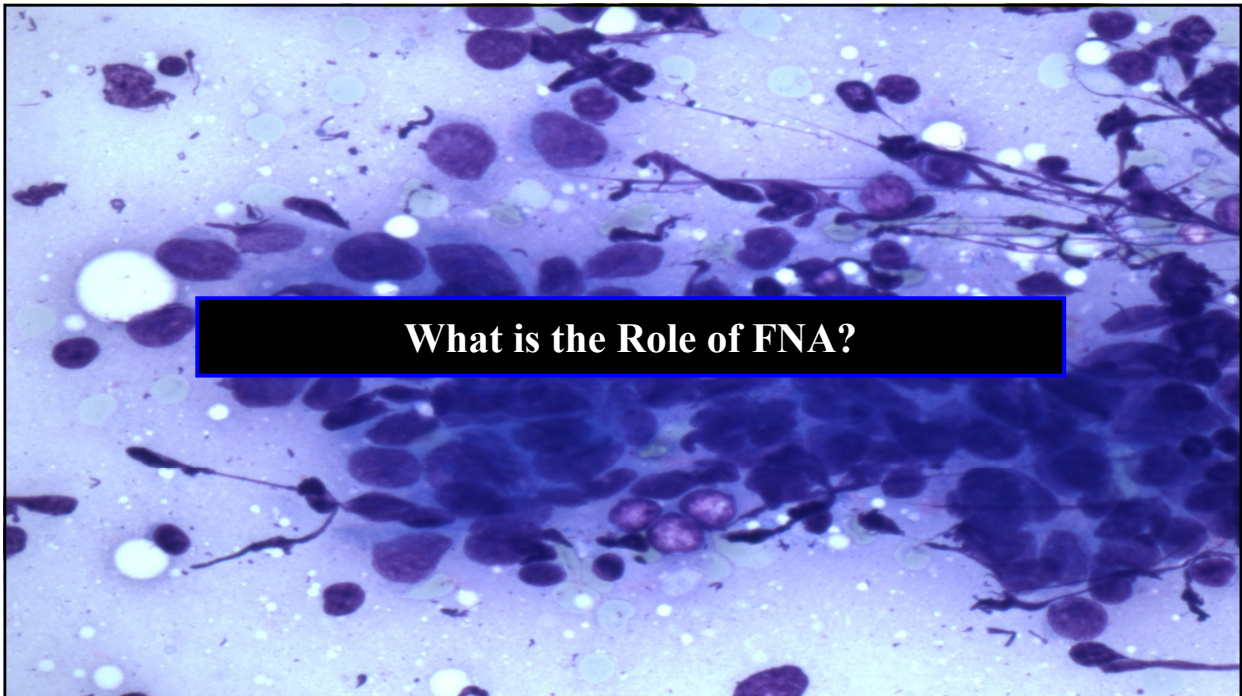
Clinical presentation of HPV-associated HNSCC is different than smoking-related cancer

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



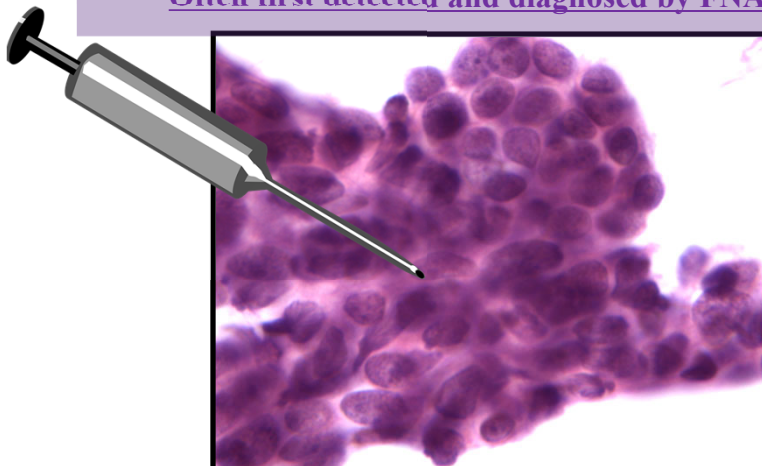
Nodal Metastases in HPV-positive OP SCC

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.

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

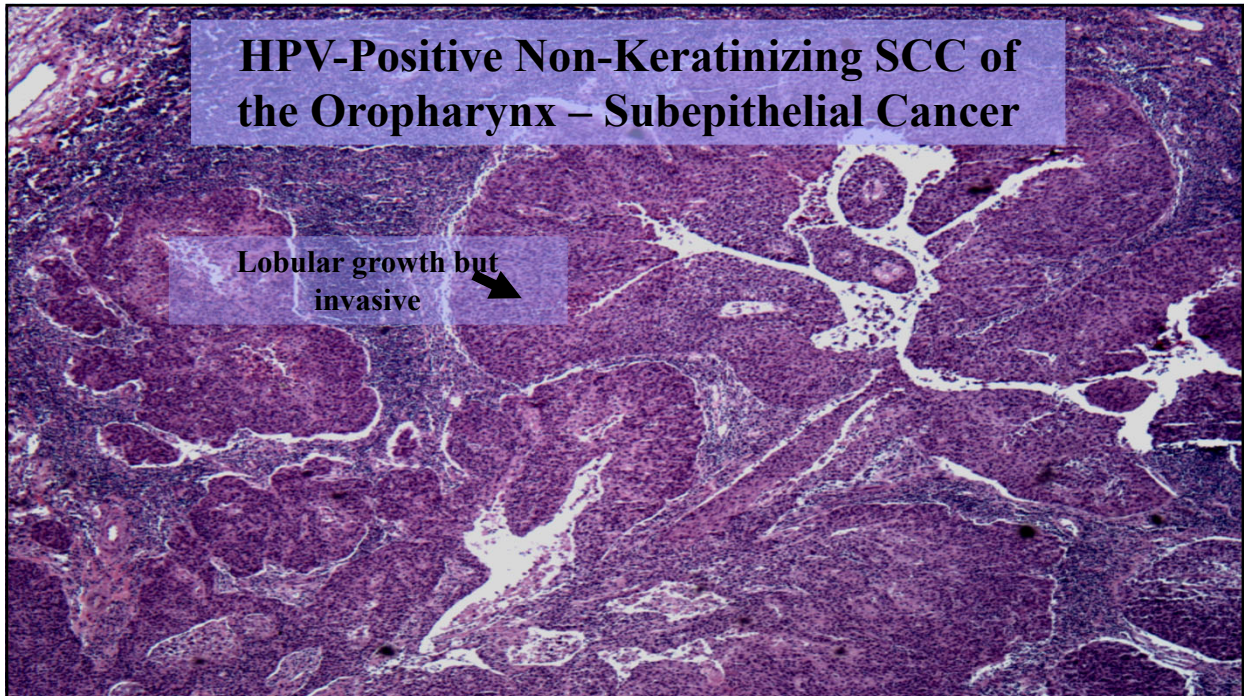
**Pathology of HPV-Associated
Oropharyngeal Carcinomas**

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**

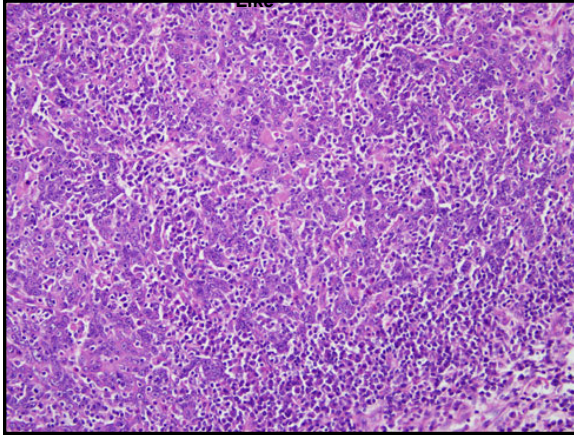
HPV-Positive Non-Keratinizing SCC of the Oropharynx – Subepithelial Cancer

Lobular growth but
invasive

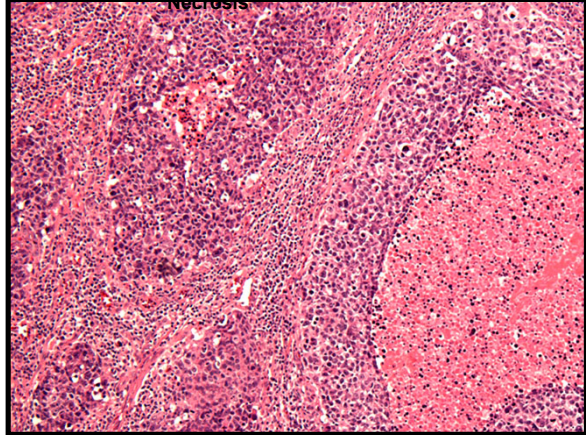


Other Histologic Patterns of HPV+ OPSCC:
Most patterns seem to share good prognosis

Lymphoepithelial-
Like

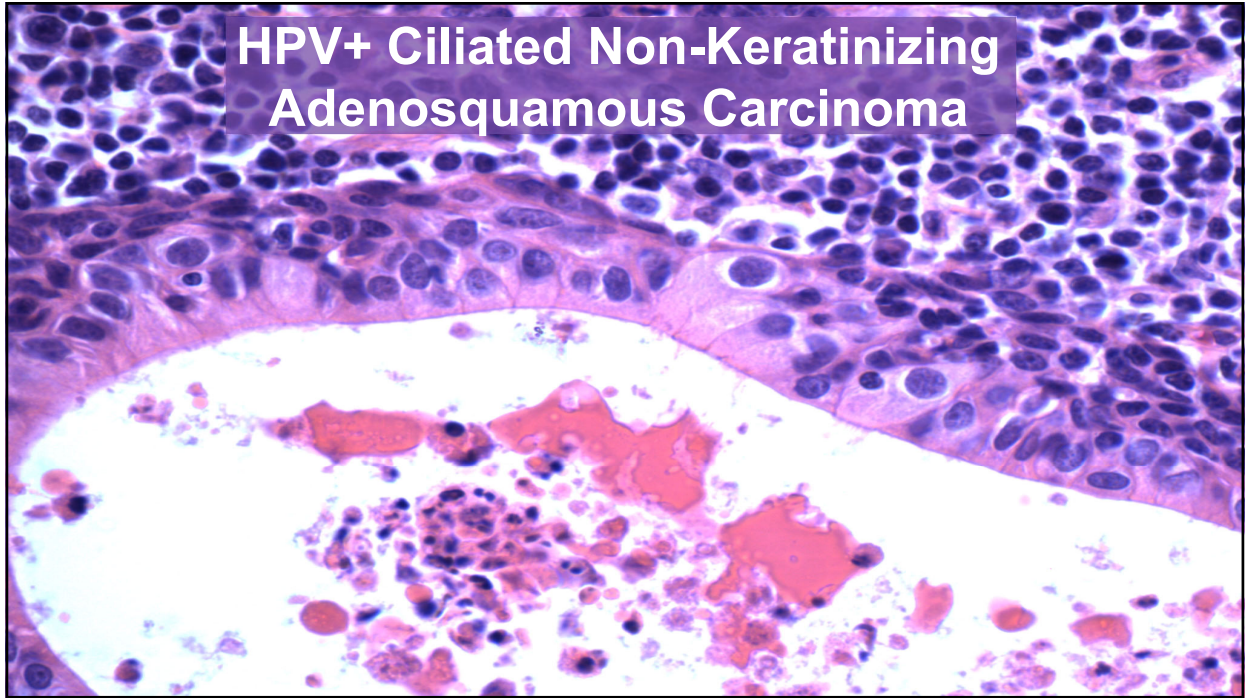


Basaloid with Comedo
Necrosis

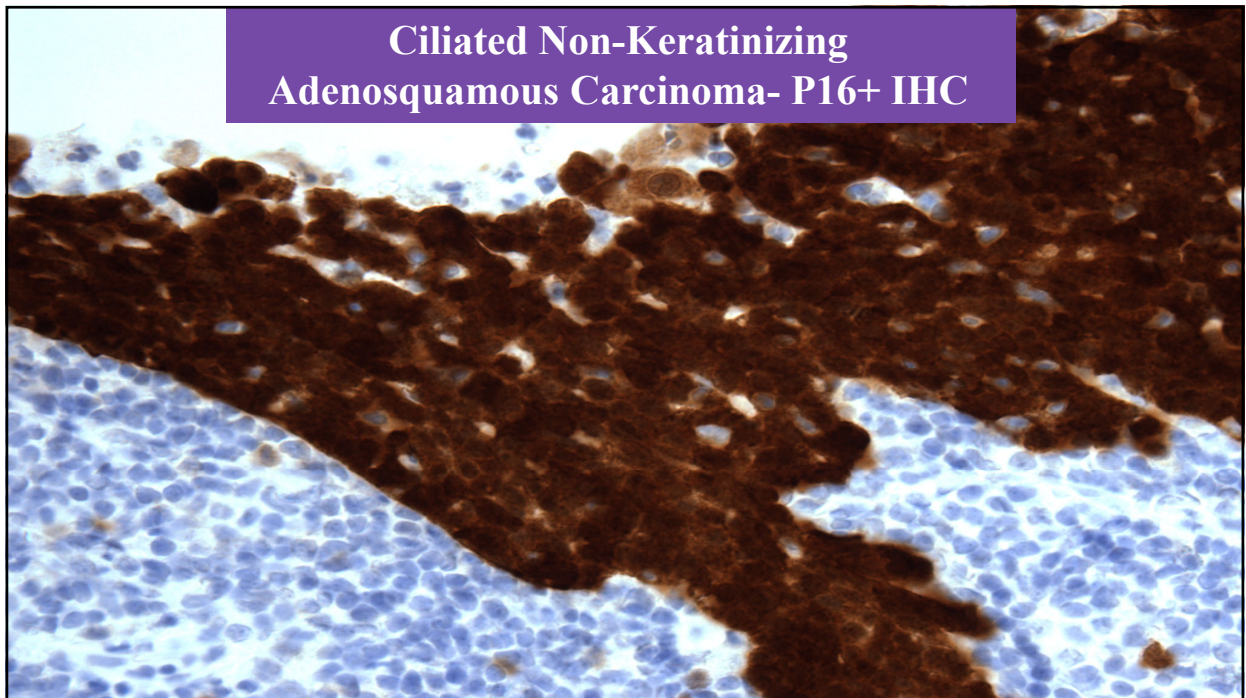


**What are other forms of HPV-positive
head and neck carcinoma?**

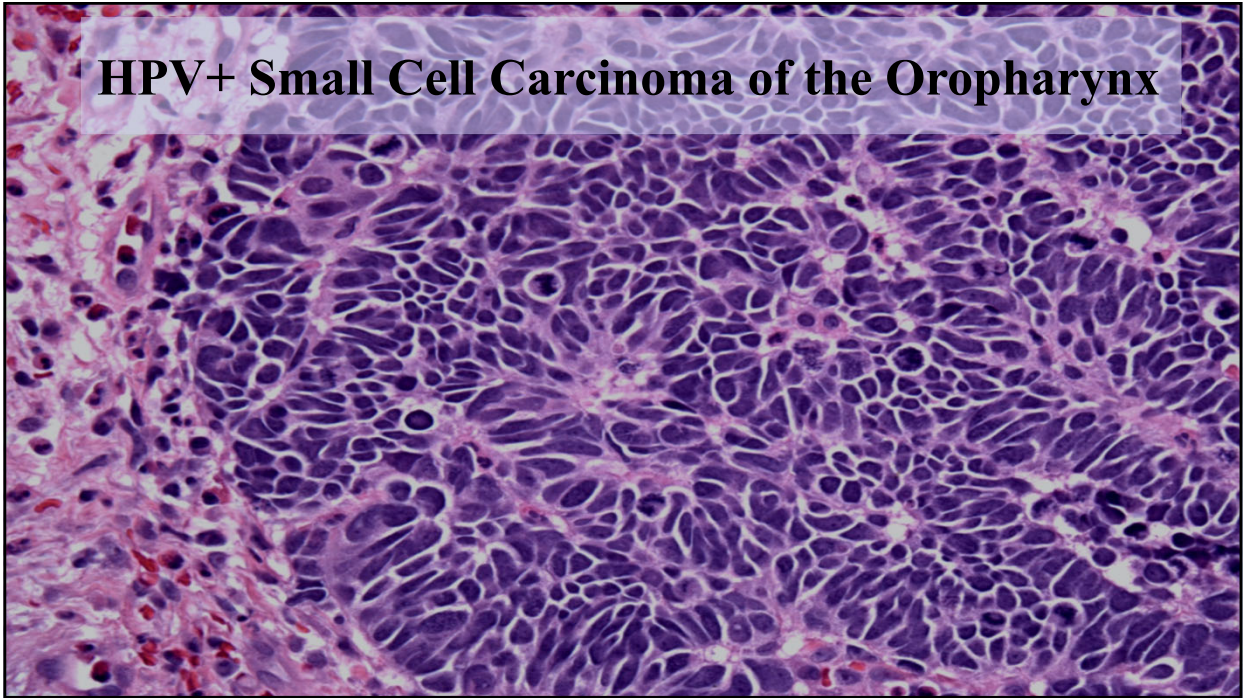
**HPV+ Ciliated Non-Keratinizing
Adenosquamous Carcinoma**



**Ciliated Non-Keratinizing
Adenosquamous Carcinoma- P16+ IHC**



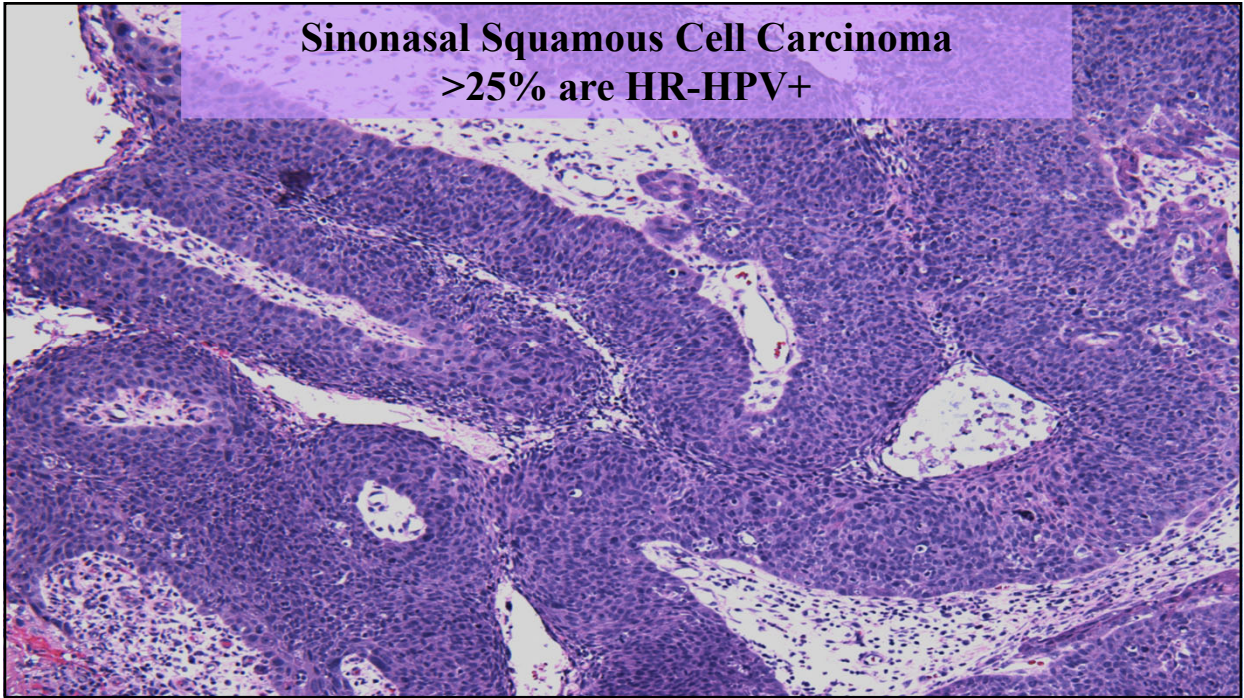
HPV+ Small Cell Carcinoma of the Oropharynx



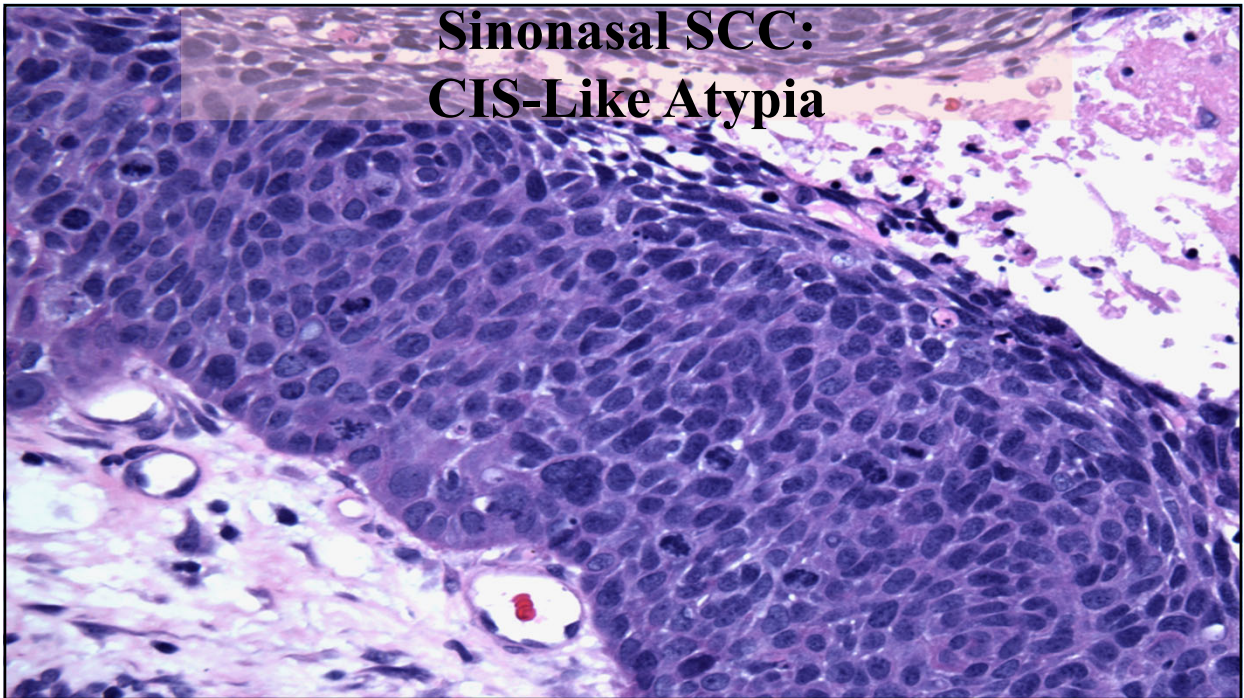
HR-HPV in Head and Neck Neuroendocrine Carcinomas

- Neuroendocrine carcinomas of HN are rare
- HPV+ cases represent 5-10% of NEC
 - Small cell carcinoma
 - Large cell carcinoma
 - Mixed patterns: small cell/SCC; small cell/lg cells
- Primarily HPV type 16 – **probably NOT the driver!**
- **Aggressive behavior despite being HPV+**
 - **Uniformly lethal**

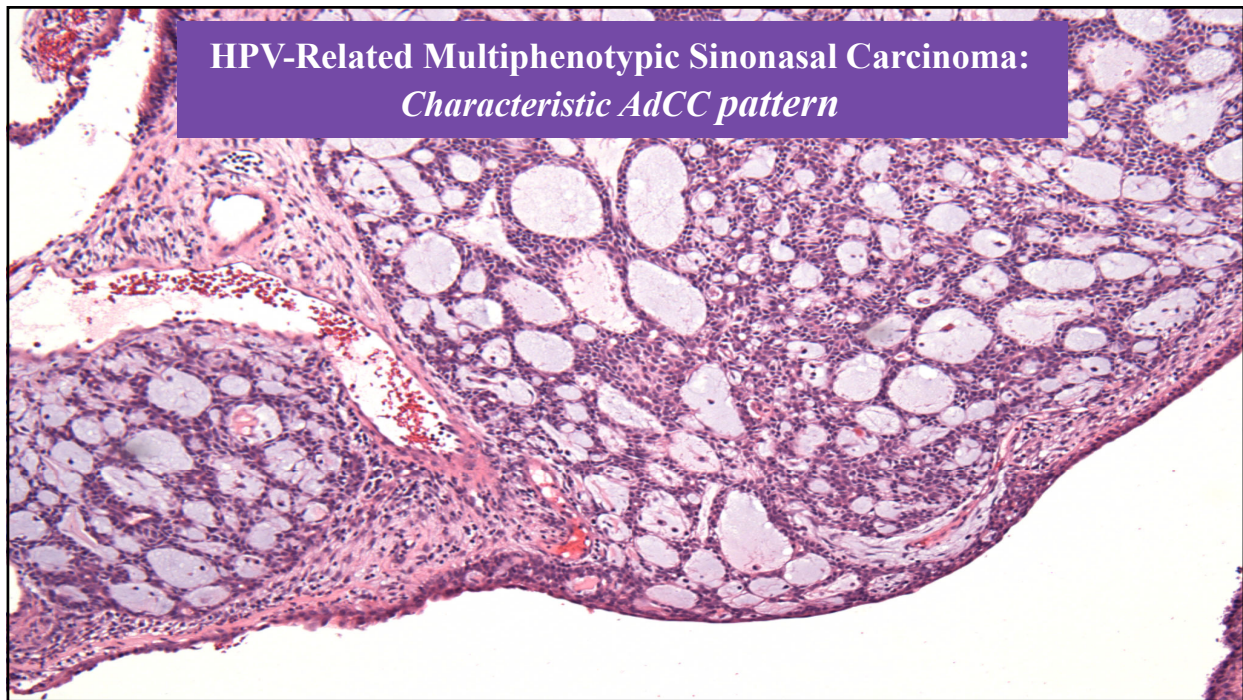
Sinonasal Squamous Cell Carcinoma
>25% are HR-HPV+



Sinonasal SCC:
CIS-Like Atypia



Abstract: Human papillomavirus (HPV)-related carcinoma of the head and neck are characterized by a predilection for the oropharynx, a nonkeratinizing squamous morphology, and infection with the HPV 18 type, but comprehensive HPV testing using the head and neck tissue has not been performed. The percentages of HPV-related carcinoma may be more wide ranging than initially appreciated. In particular, a subset of sinonasal carcinoma are HPV positive, and these include a variant that is histologically similar to adenoid cystic carcinoma (ACC). Cases were identified by retrospective and prospective analysis of head



HPV in Oral Cavity SCC

Ukpo et al. *Histopathol* 2012; 60:982.
Lopes et al. *Oral Oncol* 2011; 47: 698.
Bishop et al. *Am J Surg Pathol* 2012;
36:1874.

- HR-HPV DNA in 5-50% (average 33%)
 - DNA PCR and ISH testing
 - No survival benefit for patients with HPV DNA
- **Transcriptionally-active HPV uncommon (3-5%)**
- **No clear association with morphology or patient outcome**

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.**

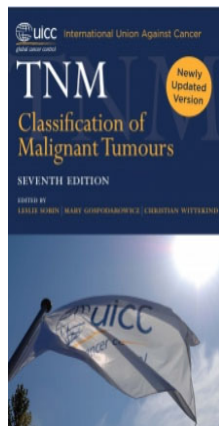
Should we do reflex testing for HR-HPV in HN SCC??? **YES!!!**

Why Should We Test for HR-HPV in HNSCC?

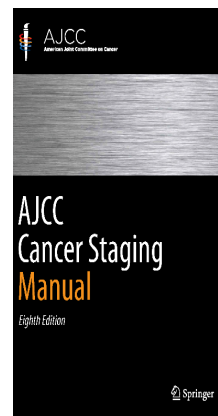
- Improved prognosis among many patients
- Identify primary site of metastatic SCC (CUP)
- Distinguish metastatic SCC from benign head and neck squamous cysts
- Distinguish HPV- from EBV-related carcinomas
- **Determine patient eligibility for clinical trials/de-escalation therapy**

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

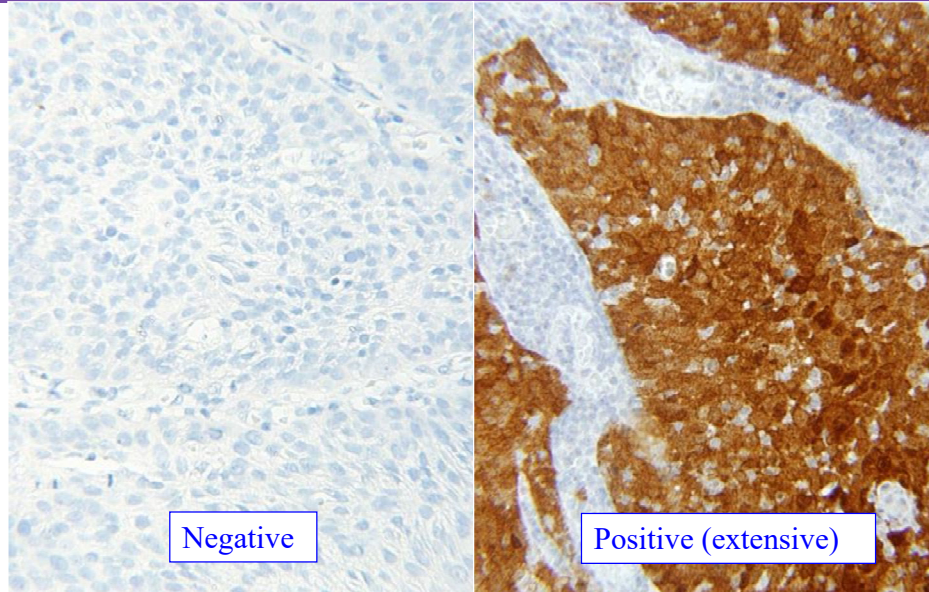
Guideline From the College of American Pathologists

James S. Lewis Jr, MD; Beth Beadle, MD, PhD; Justin A. Bishop, MD; Rebecca D. Chernock, 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

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



Prior 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

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**

Changes to HPV Testing in Head and Neck Cancer in 2025

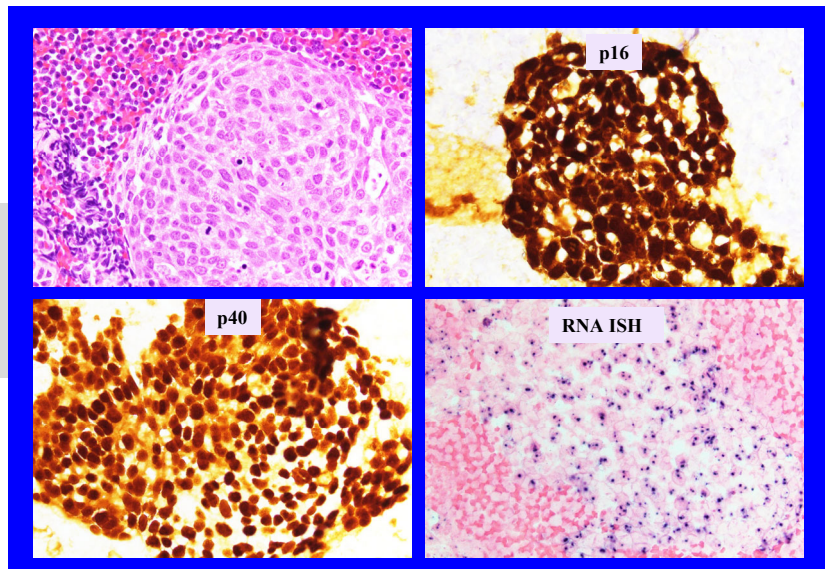
Highlights of Changes to CAP Guideline:

- **More HPV-specific testing for OPSCC:**
 - Low overall HPV attributable fraction regions, Equivocal p16, Discrepant p16 and morphology, Multisite tumors, Non-tonsillar/non-base of tongue oropharyngeal sites, when required by clinical trials
 - 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, PhD²; and Vickie Y. Jo, MD¹

- **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**

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*†

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

- **P16 is no longer 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**
 - **PCR testing**

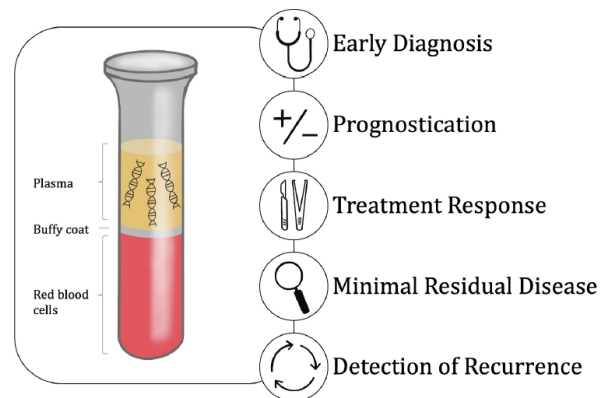
HR-HPV in FNAs of HNSCC

- **HPV-Specific Liquid-phase testing:**
 - **Advantages over cell block (FFPE)**
 - **Objective result with clear-cut scoring**
 - **Can be automated**
- 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**
- **Can detect HPV months before biopsy**



SUMMARY

- HR-HPV plays a major etiologic role in head and neck cancers of the oropharynx
- Most cases are first diagnosed by FNA!!!!**
- Updated CAP Guidelines for HPV testing, recommend **more HPV-specific tests:**
 - Lymph node mets of unknown primary
 - Sites outside the oropharynx
 - FNA specimens of LN mets**

Thank You

