Thank you for inviting me to UCSF… After a long winter, finally the snow in Boston has melted!

Two different pathways to HN cancer: tobacco-associated vs. HPV-related
Background to HPV and Cancer

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, 51, 53, others) based on risk of malignant progression.
  - HPV16 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.
THE HPV-HNSCC EPIDEMIC
225% increase in HPV-positive SCC vs 50% decrease in HPV-negative SCC

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

- 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.

HPV-HNSCC is a sexually-transmitted disease

- Factors that increase oral or genital HPV exposure increase the risk of HNSCC
  - increasing age
  - increasing number of lifetime vaginal or oral sexual partners
  - ever having participated in casual sex
  - infrequent use of barriers during vaginal or oral sex
  - ever having had a sexually transmitted disease
  - Oral HPV infection and either
    - individuals who first performed oral sex at 18y or younger
    - or with increasing number of cigarettes smoked per day.
- Other risk factors for HPV-related HNSCC include
  - immuno-suppression
  - seropositivity for a HR-HPV
  - history of an HPV-associated malignancy
  - being the spouse of a woman with cervical cancer

Incidence of OPSCC in the USA:
Has surpassed cervical carcinoma

Chaturvedi, SEER data from 1984-2004
Role of HR-HPV in Head and Neck Cancer at Various Sites

- 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: Periorbital

Survival in HPV(+) OPSCC

- Retrospective analyses of clinical trials suggest that there is a survival benefit in HPV(+) OPSCC.
- Meta-analysis data reports a 53% better overall and 74% better disease-specific survival for HPV(+) OPSCC.
- There is still a subset of patients who have aggressive disease.

Survival in HPV(+) OPSCC

- Studies with Bcl-2 show association with poor prognosis

Pathology of HPV-Related Oropharyngeal Carcinomas

Nichols et al. 2010
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)
- There is no recognized precursor lesion in the oropharynx

OROPHARYNGEAL CARCINOMA AND HPV

The Specialized Crypt Epithelium: No precursor for HPV+ OPSCC

Westra, 2012
HPV-Positive Non-Keratinizing SCC of the Oropharynx – Subepithelial Cancer

Lobular growth but invasive

Other Histologic Patterns of HPV+ OPSCC:
- All patterns seem to share a good prognosis

Lymphoepithelial-Like
Basaloid

HPV-Related Non-Keratinizing SCC of the Oropharynx:
- Cohesive nests of mitotically active basoloid tumor cells

HPV-Related Non-Keratinizing SCC of the Oropharynx:
p16 IHC can be helpful for difficult/subtle patterns
HPV in Oropharyngeal SCC

- Should probably NOT be considered “poorly differentiated” – do not grade.
- Distinct from basaloid SCC
- Preferred term: “HPV-related non-keratinizing SCC”

What are some of the other forms of HPV-related oropharyngeal carcinoma?

Role of HR-HPV in Head and Neck Cancer:

Wide Variety of Carcinomas but non-keratinizing SCC is by far most common

- Association between HR-HPV and HN cancer:
  - Oropharynx:
    - Squamous cell carcinoma and variants
    - Non-keratinizing (predominant form)
    - Papillary SCC
    - Adenosquamous carcinoma
    - Adenocarcinoma
    - Small cell carcinoma
  - Sinonasal Cavity:
    - Schneiderian carcinoma (SCC) and variants
    - Sinonasal adenocarcinoma
    - Carcinoma with ACC-like features
    - SNUC
    - Small cell carcinoma
Papillary SCC:
Surface Papillary Growth

Papillary SCC:
CIS-Type Atypia and Mitoses

Papillary SCC:
Distinguish from Squamous Papilloma

HPV+ Adenosquamous Carcinoma:
Better prognosis than HPV- AdenoSCC
New Entity: Ciliated Non-Keratinizing Adenosquamous Carcinoma – HPV+

- Seethala et al:
- 10 cases
- Lower grade histology
- Better prognosis

Ciliated Non-Keratinizing Adenosquamous Carcinoma- P16+ IHC

HPV+ Ciliated Non-Keratinizing Adenosquamous Carcinoma

Sinonasal Carcinomas
Approx. 25% are HR-HPV +
Schneiderian Carcinoma

Clinical Features:
- A subset arise from inverted papilloma
- 34% are HR-HPV-related:
  - Non-keratinizing or partially keratinizing
- M>F
- 6th and 7th decades of life
- Complete surgical resection plus radiotherapy

Schneiderian Carcinoma of Sinonasal Cavity: Inverted Growth Pattern

Schneiderian Carcinoma of Sinonasal Cavity: CIS-Like Atypia

Sinonasal Carcinoma with AdCC-Like Features

- Morphologically similar to AdCC of salivary gland
- F>M, 3:1
- Mean age 55 years
  - Surface squamous dysplasia
  - Negative for MYB gene rearrangements
  - HPV types 33, 35, and indeterminate
HPV+ Sinonasal Carcinoma with ACC-Like Features

Sinonasal Carcinoma with ACC-Like Features: Pleomorphic high grade histology

Sinonasal Carcinoma with ACC-Like Features: Focal Solid High-Grade Areas

HPV-Positive Peri-Orbital Tumors
- Conjunctival SCC
- Lacrimal sac NK SCC
- Possible role in sebaceous carcinomas of meibomian gland
**HPV-Related SCC of the Conjunctiva:**
35% are positive for HR-HPV

**HPV-Related SCC In Situ of the Conjunctiva:**
Most SCC occur at transformation zone between limbus and cornea

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**Why Should We Test for HR-HPV in HNSCC?**
- Improved prognosis among many patients
- Identify primary site of metastatic SCC
- Distinguish metastatic SCC from branchial cleft cyst or other benign HN cysts
- Distinguish HPV- from EBV-related carcinomas
- Patient eligibility for clinical trials/de-escalation therapy
Head and Neck Squamous Cell Carcinoma

When should testing for HR-HPV be performed, and which tests should be used?

Guidelines for the Detection of HR-HPV in Head and Neck SCC

- **Cancer Care Ontario Recommendations (2013)**
  - The tumors of all adults 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 IHC p16 should be used as an initial screening method

HR-HPV Detection in HNSCC

- Many unanswered questions
- **Reflex Testing:**
  - No general consensus on which method(s) for diagnosing HPV-positive cancer should be used.
  - Techniques differ in sensitivity, specificity, cost, and other technical considerations.

College of American Pathologists (CAP): EBG’s for HPV Testing in HNSCC

- **Multidisciplinary Committee**
  - Which test or combination of tests should be performed?
  - Is IHC for p16 sufficient?... *Probably NOT!... And definitely NOT outside the oropharynx*
  - Which specimens should be routinely tested?
  - How should these tests be applied to FNA?
- **CAP EB Guidelines are expected in late 2015**
Methods for determining HR-HPV status in HNSCC

- **IHC for p16**
  - Reduced specificity, esp outside OP
- **PCR for HPV DNA**
  - High sensitivity but low specificity
- **ISH for HPV DNA**
  - High specificity; reduced sensitivity at low viral load
- **RT-PCR E6/E7 mRNA**
  - Needs fresh frozen tissue
- **ISH for HPV RNA**
  - Reduced sensitivity at low viral load
- **IHC for E6/E7**
  - Low sensitivity/poor performance
- **Cytology Test Platforms**
  - Validation studies needed; automated

**OROPHARYNGEAL CARCINOMA AND HPV:**
Common algorithm is p16 IHC followed by confirmation with ISH or PCR using HPV 16 HR Cocktail

- **P16 Immunohistochemistry for HR-HPV**

  ![Antibody manufacturer comparison chart](chart.png)

- **HPV in Oropharyngeal SCC:**
  - Sensitivity approaches 100%
  - Specificity is low: 79-82%
  - Lewis et al: p16+ HPV- associated with good prognosis
    - Other studies have not supported this
    - Considered equivocal prognostically
  - Low viral load and rare HR-HPV types may explain some cases
The **GOLD STANDARD** is the demonstration of transcriptionally active HR-HPV

**VIEWRNA ASSAY FOR E6/E7 HPV mRNA**

- Amplifies target-specific signal
- Very high specificity
- High sensitivity
- Single-molecule RNA detection

**ISH for HPV E6/E7 mRNA**

Potential to apply ISH for E6/E7 HPV mRNA to cytologic preparations.

**Is there a role for HR-HPV testing in FNA specimens?**
HPV-Related Oropharyngeal SCC:
The only tissue biopsy may be an FNA!

HR-HPV in FNAs of HNSCC

- Recommended testing strategies not defined for FNA
- Cell blocks can be used
- Caveats for p16 and cell blocks:
  - P16 alone should NOT be used for FNA of mets
  - Criteria for percentage of stained cells less defined
  - Branchial cleft cysts can be p16 positive

HR-HPV in FNAs of HNSCC

- Liquid-phase testing:
  - More efficient than cell block (FFPE)
  - Objective result with clear-cut scoring
  - Can be automated
Severals have already been validated:
  » Hybrid Capture II
  » CervistaTM HPV HR
  » CervistaTM HPV 16/18
  » Roche cobas® HPV test
  » APTIMA® HPV Assay

HPV Testing in FNA Specimens
FUTURE DIRECTIONS:
A Subset of HPV-Related HNSCC are Still Aggressive

- Identify Biomarkers to detect patients with HPV+ HNSCC who are likely to fail therapy.
  - **Bel-2**
  - **P53 mutational status**
  - **EGFR**
  - **BH3-profiling**
  - **NGS and intra-tumor heterogeneity**

**SUMMARY**

- HPV-related HNSCC represents a distinct disease from traditional smoking-related HNSCC.
- Patients are typically male, younger, non-drinkers and non-smokers with risk factors associated with sexual exposure to the HPV virus.
- The OP and sinonasal cavity are most common HN sites: Non-keratinizing SCC
- HPV 16 is the most common subtype, esp. in the OP
- P16 IHC and ISH or PCR for HPV 16 are used in combination for detection
- The gold standard is demonstration of transcriptionally active HR-HPV – ISH for mRNA

Thank You!