The Immunohistochemical Markers and Ancillary Tests your Lab Needs Now: Learn from the Experts
March 22, 2015

CAP Companion Meeting at USCAP 2015

From Top to Bottom: Biomarkers and HPV-Associated Tumors of the Anogenital Region and Head & Neck

Teresa M. Darragh, MD
UCSF

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<td>Introduction — Robert M. Najarian</td>
<td>8:30AM</td>
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<tr>
<td>Immunostains for Prognostication and Identification of Gastrointestinal Tumors — Aatur D. Singhi</td>
<td>8:40AM</td>
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<td><strong>From Top to Bottom: Biomarkers and Human Papillomavirus Associated Tumors of the Anogenital Region and Head &amp; Neck — Teresa M. Darragh</strong></td>
<td><strong>9:05AM</strong></td>
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<td>Critical Issues in Breast Cancer ER and HER2 IHC Testing — Allen M. Gown</td>
<td>9:30AM</td>
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<td>Break</td>
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<td>Best practices recommendations in the application of immunohistochemistry in urologic pathology: the International Society of Urological Pathology (IUSP) consensus — Ming Zhou</td>
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<td>Practical Immunohistochemistry in Dermatopathology — Mark R. Wick</td>
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<td>The Workup of the Metastatic Tumor of Unknown Primary Site — Andrew M. Bellizzi</td>
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<td>Closing Comments and Final Q&amp;A — Moderator &amp; Faculty</td>
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**Agenda**
Faculty Disclosures: Teresa M. Darragh, MD

- Hologic: Research supplies for anal cytology
- OncoHealth: Advisory Board (*Ended August 2014*)
- Roche: Advisory Board (*October 2013*)
  - Honorarium paid to UCSF
- Ventana-Roche: Speaker’s Bureau (*August 2014*)
  - Honorarium paid to UCSF
- TheVax: Advisory Board (*August 2014*)
  - Honorarium paid to UCSF
Objectives

• Review biomarker use in HPV-associated squamous cell cancers of the head & neck
  o p16 immunostains
  o HPV testing
    – HPV in situ hybridization
    – High-risk HPV testing and typing on FNA

• Review the CAP-ASCCP LAST Project’s recommendations for biomarker use for HPV-associated squamous lesions of the lower anogenital tract
  o HPV pathogenesis, in brief
  o Use of p16 immunohistochemistry
HPV–associated cancers
United States, 2009

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Head & Neck
HPV-associated Squamous Cell Carcinoma

• Younger patients
  o Men > Women
  o Nonsmokers, nondrinkers

• Localized to oropharynx
  o ~80% HPV-associated cancers
    – >90% due to HPV16
  o Tonsils
  o Base of tongue

• Better prognosis than non-HPV SCC
  o Even though higher stage at presentation
  o Candidates for de-escalation therapy
Human papillomavirus is a favourable prognostic factor in tonsillar cancer and its oncogenic role is supported by the expression of E6 and E7. Mol Oncol. 2007;1:350–5

Incidence of HPV-positive and HPV-negative tonsillar squamous cell carcinoma

In US, incidence expected to surpass incidence of cervical cancer by 2020

Lindquist D, Romanitan M, Hammarstedt L, Nasman A, Dahlstrand H, Lindholm J. Human papillomavirus is a favourable prognostic factor in tonsillar cancer and its oncogenic role is supported by the expression of E6 and E7. Mol Oncol. 2007;1:350–5
HPV and H&N SCC

- In oropharyngeal SCC
  - p16 and HPV: Excellent correlation
  - p16 has been used as a surrogate marker for HPV.

- “The expression of p16, or lack thereof, has been found to be superior to all other clinical or IHC parameters for predicting prognosis for patients with OP-SCC.”

- No FDA-approved test to detect oral HPV
  - No routine testing recommend
  - ISH (DNA, mRNA) & PCR
  - Liquid-phase HPV tests → Per cervical cytology

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HPV-associated SCC of the Oropharynx

Non-keratinized SCC

Lobular pattern with high-grade histology & central necrosis

“basaloid”

Hybrid Capture 2 human papilloma virus testing for head and neck cytology specimens.
Deborah J. Chute, MD, Ghada T. Aramouni, CT (ASCP), Jennifer A. Brainard, MD, Aaron P. Hoschar, MD, Anita Kroeger, SM (ASCP), Belinda Yen-Lieberman, PhD. Journal of the American Society of Cytopathology Volume 3, Issue 4, Pages 173-182 (July 2014)
Keratinizing OP-SCC

- +p16 in 10% to 15% of oropharyngeal KSCC
- Correlates with the presence of high-risk HPV
- Associated with favorable outcomes

Keratinizing-Type Squamous Cell Carcinoma of the Oropharynx: p16 Overexpression Is Associated With Positive High-Risk HPV Status and Improved Survival. Cai, Chunyu; MD, PhD; Chernock, Rebecca; Pittman, Meredith; El-Mofty, Samir; DMD, PhD; Thorstad, Wade; Lewis, James American Journal of Surgical Pathology. 38(6):809-815, June 2014.
Metastatic SCC: Primary Site?

FNA of cervical lymph node → cell block

Liquid-phase sample → HPV testing and typing

p16 & HPV testing
Head and Neck Cancer

- **Oropharyngeal** tumor with non-keratinizing SCC
  - p16+ helps confirm HPV-associated

- **Oropharyngeal** tumors with overlapping morphology
  - Keratinized and/or well-differentiated
  - p16+ helps confirm HPV-associated (less common)

- **Unknown primary non-keratinizing SCC** with cervical node metastasis
  - p16+ helps localize primary site of tumor origin to oropharynx
  - +/- Confirmatory HPV testing

- **H&N sites other than oropharynx** with non-keratinizing SCC
  - p16+ \(\rightarrow\) Screen for HPV-association
  - Confirmatory HPV testing

Prognostic significance of HPV outside of the OP remains unclear.

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  o HPV pathogenesis, in brief
  o Use of p16 immunohistochemistry
The LAST Project

Lower Anogenital Squamous Terminology standardization project for histopathologic diagnoses of HPV-associated lesions of the lower anogenital tract
The CAP-ASCCP LAST Project

1. A unified histopathological nomenclature with a **single set of diagnostic terms** is recommended for all HPV-associated preinvasive squamous lesions of the lower anogenital tract (LAT).

2. A **2-tiered nomenclature** is recommended for non-invasive HPV-associated squamous proliferations of the LAT which may be further qualified with the appropriate –IN terminology.

3. The recommended terminology for HPV-associated squamous lesions of the LAT is:
   - *Low-grade squamous intraepithelial lesion (LSIL)* and
   - *High-grade squamous intraepithelial lesion (HSIL)*
LSIL: Virion production & transient lesions

LSIL (CIN1)  LSIL

Productive infection
HSIL: HPV E6/E7 expression & risk of cancer

HSIL (CIN3)

Transforming infection

HSIL
2-tiered system: LSIL & HSIL

Schematic Representation of SIL

<table>
<thead>
<tr>
<th>Normal</th>
<th>Low-grade squamous intraepithelial lesion (LSIL)</th>
<th>High-grade squamous intraepithelial lesion (HSIL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Condyloma, Very mild to mild dysplasia</td>
<td>Moderate dysplasia, Severe dysplasia, In Situ carcinoma</td>
</tr>
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</table>

Reflects HPV biology and clinical management

Infection & Precancer
# Diagnostic Variation

![Schematic Representation of SIL](image)

## Interobserver variability & Diagnostic (un)certainty
Morphologic interpretation = Art

Can the science of medicine make the art of medicine more reliable?

Can we use our knowledge of HPV biology to make histopathologic diagnoses more objective?
Effect of HPV on the cell cycle

- Combined effect of high-risk HPV E6 and E7
  - Via interactions with pRb and p53
  - Maintain damaged cells in a hyper-proliferative state
  - Immortalize cells with un-repaired DNA damage
  - Overexpression of p16
What is p16?

It is a tumor suppressor protein that is a biomarker for transforming HPV infection and can be used as a surrogate marker of HPV-associated precancer.

**p16 and Normal cell cycle progression**

- Release of E2F from pRB results in cell cycle progression, mitotic replication, and low level expression of p16.

- p16 protein facilitates the re-binding of pRB to E2F, leading to cell cycle arrest.
Transforming HPV Infection: Oncogenesis

- Since pRb is deactivated by HPV’s E7 → p16 is overexpressed

- In cells with transforming HPV infections, HPV viral oncoprotein E7 impairs the function of pRB, disrupting its ability to bind to E2F
- This leads to deregulated cell proliferation, genetic instability and p16 over-expression detectible by immunohistochemistry staining

• p16 screams STOP
Art of Interpretation + Current Science Hypotheses

• Diagnostic variation can be improved by:
  • Limiting the number of tiers
  • The use of biologic markers, such as:
    • p16
    • Ki-67
    • ProEx C

• *Add objectivity to the art...*
Use of p16

• Diffuse strong (block positive) staining with p16 showed similar accuracy for high grade disease when compared to an adjudicated histology standard.

• p16 immunohistochemistry improves the accuracy of a single pathologist’s interpretation of high grade vs. low grade disease relative to an adjudicated pathology panel of experts.

• Objective measure of high grade disease.
The published literature indicates improved interobserver agreement of the diagnosis of CIN2+ with the conjunctive use of H&E morphology with p16^{INK4a} immunohistochemistry compared with H&E morphology alone.
p16 positive stain = “Block positive”

- Strong and diffuse staining
- Nucleus or nucleus plus cytoplasmic staining
- Of the basal cell layer with upward extension involving at least 1/3 of the epithelium

Anal HSIL
When do we use p16?

LAST Recommendations

1. HSIL vs. Mimic
2. Query -IN2
3. Difference in opinion
4. NOT for obvious –IN1 or –IN3
   4a. “a priori”: When no histologic HSIL is found on biopsy in “high-risk” situations – prior Pap with HSIL, ASC-H, HPV16+ ASC-US, AGC (NOS)
LAST: Biomarkers Recommendations

1. p16 IHC is *recommended* when the H&E morphologic **differential diagnosis** is between **precancer** (–IN2 or –IN3) and a **mimic** of precancer (e.g., processes known to be not related to neoplastic risk such as immature squamous metaplasia, atrophy, reparative epithelial changes, tangential cutting).

   ➢ Strong and diffuse block-positive p16 results support a categorization of precancerous disease.
DDx: HSIL vs. Mimic of HSIL

1. HSIL
2. Mimic of HSIL
DDx: HSIL vs. Mimic

p16 positive = HSIL

Anal biopsy
DDx: HSIL vs Reactive

1. HSIL
2. Reactive
DDx: HSIL vs Reactive

p16 negative = Reactive

Cervical Biopsy
LAST: Biomarkers Recommendations

2. If the pathologist is entertaining an H&E morphologic interpretation of –IN 2 (under the old terminology, which is a biologically equivocal lesion falling between the morphologic changes of HPV infection [low-grade lesion] and precancer), p16 IHC is recommended to help clarify the situation.

- Strong and diffuse block positive p16 results support a categorization of precancer. Negative or non-block-positive staining strongly favors an interpretation of low-grade disease or a non-HPV associated pathology.
Query CIN 2

1. LSIL
2. HSIL
Query CIN 2

p16 negative = LSIL
Query AIN 2

1. LSIL
2. HSIL

43%
57%
Query AIN 2

HSIL (AIN2)

p16 +
Recommendation 4: Don’t use!

If BIOPSY is morphologically **unequivocal**: Negative
- IN 1
- IN 3

**NO**

p16 stain

LSIL

**X**

HSIL
HPV Biology: Infection vs. Precancer
Biomarkers – Add Objectivity: Reduce diagnostic variation

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<td>Condyloma</td>
<td>CIN/AIIN grade 1</td>
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<td>Normal</td>
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Biology & Management
### Biomarkers: p16

**Surrogate for transforming infection**

#### Schematic Representation of SIL

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<td></td>
<td>CIN/AIN grade 1</td>
<td>CIN/AIN grade 2</td>
<td>CIN/AIN grade 3</td>
</tr>
<tr>
<td></td>
<td>Very mild to mild dysplasia</td>
<td>Moderate dysplasia</td>
<td>Severe dysplasia</td>
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- **Productive infection**
- **Transforming infection**
Updates: WHO Blue Book

- Adopted the LAST Project’s terminology for the cervix, vulva and vagina
- 4th edition
- Published April 2014
The LAST Project

Lower Anogenital Squamous Terminology Standardization Project

[Logos for CAP and ASCCP]
The LAST Project:

The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: Background and Consensus Recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology.


• Int J Gynecol Pathol. 2013 Jan;32(1):76-115
HPV Vaccination!!!

9-valent vaccine
FDA-approved December 2014