“8th AJCC and WHO Updates for Head and Neck... and blowing up our planar world into 3D”
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- AJCC:
  - Oropharyngeal Cancer Staging
    - p16+ Oropharyngeal Carcinomas
  - Oral Cancer Staging
    - Depth of invasion
    - Extranodal extension
    - WPOI-5 as a recommended feature
- WHO Blue Book: Salivary
- Works in Progress:
  - Envisioning tumor pattern of invasion in three-dimensions

Chapter 10
Why p16 instead of HPV?

- Robust surrogate biomarker for transcriptionally active HR HPV infection ("Driver")
Chapter 10
Why p16 instead of HPV?

- Robust surrogate biomarker for transcriptionally active HR HPV infection ("Driver")
- Independent positive prognosticator with respect to p16/HPV discordance

p16\textsuperscript{INK4a} overexpression in HR-HPV infection

- Activated cyclin D kinase inactivates Rb, which promotes S phase progression.

** In HPV-mediated carcinogenesis HR-HPV E7 binds and inactivates Rb**

p16\textsuperscript{INK4a} overexpressed in HR HPV infection due to loss of feedback inhibition in the oncogenic stress that follows Rb inactivation

p16 as a Predictive Biomarker

- Lassen 2010, DAHANCA 5 trial, 331 patients, oropharynx, supraglottis SCC
- 10% "strong" nuclear & cytoplasmic staining, Santa Cruz 1:25 dilution, 25% of tumors were p16 +
- Nimorazole, hypoxic cell radiosensitizer, improved LR control for p16 negative patients, but no impact p16+ patients
- Disease control rate - significantly better for patients with p16+ tumors
Why HR-HPV / p16\(^{\text{INK4a}}\) Discordance?

- **“Driver infection”** HR-HPV genome (PCR, ISH) and p16\(^{\text{INK4a}}\) overexpression (IHC)
- **“Passenger infection”** HR-HPV genome and no p16\(^{\text{INK4a}}\) overexpression
- Analysis of HR-HPV transcripts challenges concept
  - Small subset oropharyngeal cancers harbor HR-HPV transcripts yet p16 is silenced (HPV/p16 discordant)
  - Outcomes for HPV/p16 discordant oropharyngeal cancer patients stratify according to p16 status

p16+ HPV-negative Oropharyngeal Cancers

102 patients (30 blacks, 72 whites), transcriptionally active HPV16 and HPV18 by reverse transcription and quantitative PCR

<table>
<thead>
<tr>
<th>Group</th>
<th>HPV16/18+ p16+</th>
<th>HPV16/18- p16+</th>
<th>HPV16/18+ p16-</th>
<th>HPV16/18- p16-</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.77 (1.70, 35.47)</td>
<td>19.00 (4.67, 77.28)</td>
<td>22.15 (4.99, 98.14)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.129 (0.028, 0.59)</td>
<td>2.45 (0.39, 15.17)</td>
<td>0.35 (0.53, 2.32)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.053 (0.013, 0.21)</td>
<td>0.41 (0.66, 2.54)</td>
<td>1.17 (0.19, 7.05)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.045 (0.01, 0.20)</td>
<td>0.35 (0.53, 2.32)</td>
<td>0.86 (0.14, 5.19)</td>
<td></td>
</tr>
</tbody>
</table>

Disease progression stratified by HPV16/18 and p16.

Kaplan Meier curve, disease-free survival over time (months after initial treatment) grouped by HPV16/18 and p16 status. X axis truncated at 60 months for demonstration purposes.

Human Pathol 45:310, 2014

p16: Quite usually all or nothing at all

The Histology of HPV+ OPSCC

The Histology of HPV+ OPSCC

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“Inside out”

MTM Labs (Roche) Dilution: 1:2

Anaplastic cells
The Histology of HPV+ Oropharyngeal SCC Lewis 2012

- 149 oropharyngeal SCC patients treated by primary surgery +/- adjuvant therapy
- Tumor anaplasia & multinucleated cells seen in nonkeratinizing SCC
- 12% recurrence, 6% distant mets

Potential Pitfall: “In-situ” Carcinoma

Reticulated tonsillar epithelium characterized by migrating intraepithelial lymphocytes, more subtle epithelial/lymphocyte boundary. Discontinuity of the supporting basement membrane enables lymphocyte trafficking. The basement membrane zone here lacks collagen IV deposition (E, F, black arrow).

Rationale for New Stage Grouping

- 704 patients with surgically managed p16+ oropharyngeal cancer, +/- adjuvant therapy, USA and UK
- Performance of 7th AJCC staging criteria compared with proposed, new pathologic staging system by conjunctive consolidation method:
  - Stage I: pT1/T2, ≤ 4 + LN (pN0/pN1)
  - Stage II: pT1/T2, > 4 + LN (pN2) or pT3/T4, ≤ 4 + LN (pN0/pN1)
  - Stage III: pT3/T4 and > 4 + LN (pN2)

Conjunctive consolidation method: combines and consolidates significant data
Chapter 10: HPV-Mediated (p16+)
Oropharyngeal Cancer

AJCC
Oropharyngeal Cancer Staging

**T Category**
- **T0**: No primary identified
- **T1**: Tumor ≤ 2 cm
- **T2**: Tumor > 2 cm ≤ 4 cm
- **T3**: Tumor > 4 cm extending to lingual surface of epiglottis
- **T4**: Moderately advanced local disease (invades larynx, extrinsic muscle of tongue, pterygoid, palatine, mandible, or beyond)

**N Category**
- **NX**: Regional lymph nodes cannot be assessed
- **pN0**: No regional lymph node metastasis
- **pN1**: Metastasis in < 4 lymph nodes
- **pN2**: Metastasis in > 4 lymph nodes

When **T** is... And **N** is... And **M** is... Then stage group is...
- **T0 / T1 / T2** N0 / N1 M0 I
- **T0 / T1 / T2** N2 M0 II
- **T3 / T4** N0 / N1 M0 II
- **T3 / T4** N2 M0 III
- Any T Any N M1 IV

**Depth of Invasion**
- AJCC:
  - Oropharyngeal Cancer Staging
    - p16+ Oropharyngeal Carcinomas
  - Oral Cancer Staging
    - Depth of Invasion

**Oral cavity / Lip: Summary pathology changes**
- Depth of invasion to increase the T category
- No longer using extrinsic tongue muscle invasion in T4 as this is a feature of DOI
- Separate N staging for HPV-related and HPV-unrelated cancers
- ENE is introduced as a descriptor in all HPV-unrelated cancers
- Pathologically ENE is classified as either ENE<sub>mi</sub> (< 2 mm) or ENE<sub>ma</sub> (> 2 mm)

**Depth of invasion has been used synonymously with tumor thickness**
- Horizon = mucosal surface
- Plumb line to lowest point

- Depth of invasion has been used synonymously with tumor thickness
  - Horizon = mucosal surface
  - Plumb line to lowest point

- Depth of invasion has been used synonymously with tumor thickness.
Depth of invasion (continuous variable) significantly predictive of DSS when adjusted for confounders.

Cut points of 5mm for T1, and 10mm for T2, T3 demonstrated best fit of data (AIC).

- **T1**: ≤ 2 cm, DOI ≤ 5 mm
- **T2**: ≤ 2 cm, DOI > 5 mm and ≤ 10 mm or > 2 cm but ≤ 4 cm and DOI ≤ 10 mm
- **T3**: Tumor > 4 cm or any DOI > 10 mm
- **T4a**: Moderately advanced local disease, invading through cortical bone, or involving the inferior alveolar nerve, floor of mouth, or skin of face. Tumor invades adjacent structures only (e.g., through cortical bone of the mandible or maxilla, or involves the maxillary sinus or skin of the face) Note: Superficial erosion of bone/tooth socket (alone) by a gingival primary is not sufficient to classify a tumor as T4.
- **T4b**: Very advanced local disease, invading masticator space, pterygoid plates, or skull base and/or encases the internal carotid artery.

**AJCC, AJCC Cancer Staging System**
- **Oral Cancer Staging**: p16+ Oropharyngeal Carcinomas
- **Depth of Invasion**
- **Extranodal extension (ENE)**: extension of metastatic carcinoma though lymph node capsule into surrounding connective tissue, regardless of stromal reaction.
- **Histopathologic designations for ENE as follows**:
  - ENE<sub>n</sub> (none)
  - ENE<sub>m</sub> (microscopic ENE ≤ 2 mm)
  - ENE<sub>g</sub> (ENE > 2 mm or gross ENE).

**Cancer. 2014**
- Extent of Pathologic Extracapsular Extension and Outcomes in Patients With Nonopharyngeal Head and Neck Cancer Treated With Initial Surgical Resection

**Head & Neck. 2015**
- Influence of extracapsular nodal spread extent on prognosis of oral squamous cell carcinoma
350 patients with oral cavity and laryngeal cancer
- Extranodal extension: Grade 3 > 1mm, Grade 4 no residual LN
- MVA Grade 4 independently associated with DSS, (p = 0.01, HR 2.44)

245 patients with oral cavity cancer
- ENE continuous variable
- 44% (n = 109) +ENE
- ROC analysis: 1.7 mm extension best performance cut-point on MVA
Extranodal extension

<table>
<thead>
<tr>
<th>N Category</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph node metastasis</td>
</tr>
<tr>
<td>N1</td>
<td>One ipsilateral lymph node ≤ 3 cm no ENE</td>
</tr>
<tr>
<td>N2</td>
<td>One ipsilateral lymph node ≤ 3 cm and ENE+ or multiple ipsilateral, bilateral, or contralateral lymph nodes ≤ 6 cm no ENE</td>
</tr>
<tr>
<td>N2a</td>
<td>One ipsilateral or contralateral node ≤ 3 cm and ENE+ or one ipsilateral node &gt; 3 cm but ≤ 6 cm no ENE</td>
</tr>
<tr>
<td>N2b</td>
<td>Multiple ipsilateral nodes ≤ 6 cm, no ENE</td>
</tr>
<tr>
<td>N2c</td>
<td>Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension and ENE-</td>
</tr>
<tr>
<td>N3</td>
<td>Metastasis in lymph node more than 6 cm in greatest dimension and ENE+ or multiple ipsilateral, bilateral, or contralateral lymph nodes any with ENE+</td>
</tr>
<tr>
<td>N3a</td>
<td>One LN &gt; 6 cm, no ENE</td>
</tr>
<tr>
<td>N3b</td>
<td>Metastasis in a single ipsilateral node more than 6 cm in greatest dimension and ENE+, or multiple ipsilateral, bilateral, or contralateral lymph nodes any with ENE+</td>
</tr>
</tbody>
</table>

AJCC:
- Oropharyngeal Cancer Staging
  - p16+ Oropharyngeal Carcinomas
- Oral Cancer Staging
  - Depth of Invasion
  - Extranodal extension
  - WPOI-5 as a recommended feature

WPOI:
- Worst pattern of invasion (WPOI)
  - Nonaggressive (WPOI 1-3)
  - Aggressive (WPOI 4, 5)
    - WPOI 5 = Dispersed carcinoma (>1 mm)
- Lymphocytic immune response
- Perineural invasion

Aggressive Pattern of Invasion

- WPOI 4: Tumor satellites, convincingly separately from main tumor, and small (≤ 15 cells)
- WPOI 5: > 1 mm scatter between satellites

The risk model predicts different combinations of histological variables associated with high-risk tumor phenotypes.
Print out millimeter rulers on acetate paper to lay over glass slides.

Phase II Validation: Low-Stage Cohort

<table>
<thead>
<tr>
<th>Disease-specific survival</th>
<th>DF</th>
<th>Standard error</th>
<th>P</th>
<th>Hazard ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPOI 5</td>
<td>1</td>
<td>0.0001</td>
<td>6.34</td>
<td></td>
<td>2.50, 16.09</td>
</tr>
<tr>
<td>Risk Category</td>
<td>1</td>
<td>0.0005</td>
<td>9.16</td>
<td></td>
<td>2.65, 31.66</td>
</tr>
</tbody>
</table>

Both the High-Risk and WPOI 5 are significantly predictive of disease-free survival and disease-specific survival, adjusted for stage, age, margins, medical center, and treatment, in regression analysis, considering competing risks.

Can WPOI-5 predict occult cervical disease?

- No standardized approach for addressing cervical lymph nodes in T1/T2 cN0 oral squamous cancer patients
- Observation or elective neck dissection (END)
- Depth of invasion > 5 mm associated with a 20% rate of occult cervical metastases and is acceptable criterion for END
- Majority of patients treated with END are overtreated
Can WPOI-5 predict occult cervical disease?

152 patients, T1/T2, (> 5mm DOI), n=138 undergoing neck dissection

Table 1: Sensitivity of sentinel node biopsy (SNB) of 86%, NPV 95%, false negative rate 14%

<table>
<thead>
<tr>
<th>WPOI 5</th>
<th>Positive predictive value 68%, negative predictive value 78%</th>
</tr>
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<tbody>
<tr>
<td>N0</td>
<td>51</td>
</tr>
<tr>
<td>N1</td>
<td>13</td>
</tr>
<tr>
<td>N2</td>
<td>8</td>
</tr>
<tr>
<td>N3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
</tr>
</tbody>
</table>

WPOI 5: Positive predictive value 68%, negative predictive value 78%

- If END performed only for WPOI 5: 3% occult lymph nodes missed
- The Sentinel European Node Trial, Tc-99m lymphoscintigraphy: sensitivity of sentinel node biopsy (SNB) of 86%, NPV 95%, false negative rate 14%
- SNB more cost effective than END for DOI > 5mm
- Tc-99m lymphoscintigraphy may detect aberrant drainage patterns or contralateral metastases
- However, SNB operator dependent, not widely accepted in US
- WPOI5 might impact SNB decision paradigms?

The protective effect of p16INK4a in oral cavity carcinomas: p16INK4a dampens tumor invasion—integrated analysis of expression and kinomics pathways

Sutamay Basu1, Jie Xu1, Cameron Rueger1, Qian Du1, Tiffany Cooper1, William Carroll1, Dan Buettner2, Martha Vose3, Bruce Wert4, Bryan Rosenblatt1, William Griffin3, Joshua Anderson1, Christopher Kellner3, Hadi S. Yang1,2,3

1Department of Pathology, Surgery, Medicine, Radiology Oncology, University of Alabama at Birmingham, Birmingham, AL. 2NCI-sponsored Tissue Bank Program, Fox Chase Cancer Center, Philadelphia, PA. 3RK3, The Arizona State University at Tempe, Tempe, AZ. 4The Arizona State University at Tempe, Tempe, AZ. 5Department of Oral Medicine, Indiana University School of Dental Medicine, Indianapolis, IN. 6Baylor Medical Center, Continuum Health Partners, New York, NY, USA

Histological observations on pattern of invasion and HPV-mediated carcinomas: Can E6/E7 impact tumor invasion?

Modern Pathology 2015: HPV 16 and HPV 18 detected in 22.6% and 11%, respectively

Table 2: The relationship between HPV detection and outcome in oral cavity carcinoma

<table>
<thead>
<tr>
<th>Disease progression</th>
<th>HPV 16 detection</th>
<th>HPV 18 detection</th>
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<tbody>
<tr>
<td>0</td>
<td>22.6%</td>
<td>11%</td>
</tr>
<tr>
<td>1</td>
<td>20.4%</td>
<td>9.8%</td>
</tr>
<tr>
<td>2</td>
<td>18.7%</td>
<td>8.5%</td>
</tr>
<tr>
<td>3</td>
<td>17.9%</td>
<td>7.4%</td>
</tr>
<tr>
<td>4</td>
<td>17.1%</td>
<td>6.3%</td>
</tr>
<tr>
<td>5</td>
<td>16.4%</td>
<td>5.2%</td>
</tr>
<tr>
<td>6</td>
<td>15.7%</td>
<td>4.1%</td>
</tr>
</tbody>
</table>

High-risk, low-risk and unknown HPV status

Oral Cavity Carcinoma, p16INK4a, HPV16/18, Outcome

Table 3: The relationship between HPV detection and outcome in oral cavity carcinoma

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<td>8.5%</td>
</tr>
<tr>
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</tr>
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<td>5.2%</td>
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<tr>
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<td>15.7%</td>
<td>4.1%</td>
</tr>
</tbody>
</table>

High-risk, low-risk and unknown HPV status
Cell line invasiveness varies with WPOI

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>UAB1</th>
<th>UAB2</th>
<th>UAB3</th>
<th>UAB4</th>
<th>UAB5</th>
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</thead>
<tbody>
<tr>
<td>Site of origin</td>
<td>Oropharynx</td>
<td>Oral</td>
<td>Oral</td>
<td>Oral</td>
<td>Oral</td>
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<tr>
<td>Pattern of Invasion</td>
<td>WPOI 3</td>
<td>WPOI 3</td>
<td>WPOI 5</td>
<td>WPOI 5</td>
<td>WPOI 4</td>
</tr>
<tr>
<td>HPV status</td>
<td>HPV16</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

Control (blue bar) 24h TGFβ stimulation (red bar)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAB-1 vs UAB-4</td>
<td>0.0014</td>
</tr>
<tr>
<td>UAB-4 vs UAB-6</td>
<td>0.0206</td>
</tr>
</tbody>
</table>

Can Viral E6E7 Oncoproteins Impact Invasion?

Tumor proliferation assay (Promega)

HPV16 E6E7 oncoproteins decrease tumor invasion in some SCC cell lines.

Differences in p16<sup>INK4a</sup> sensitive invasion pathways

MMP1 and COL1A1 were decreased more than 40-fold in UAB-4.

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- WHO Blue Book: Salivary
• Concepts behind some of the changes:
  – No “analogue” as definitional = “It is what it is”
• MASC: added as a definite entity, but under the nomenclature of “Secretory carcinoma”
  – Cribriform Adenocarcinoma of Tongue (CAT) still provisional entity
• Grade not definitional, to allow for grade variance
• Polymorphous low-grade adenocarcinoma = Polymorphous adenocarcinoma

• Duct carcinoma:
  – Low-grade versus high-grade
  – Invasive versus intraductal
  – Low-grade salivary duct carcinoma: no longer subcategory of cystadenocarcinoma
    • Cystadenocarcinoma goes away
• Sialoblastoma = Sialoblastic Carcinoma
• Sclerosing polycystic adenosis added as definite entity

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• WHO Blue Book: Salivary
• Works in Progress:
  – Envisioning tumor pattern of invasion in three-dimensions

• Traditional pathology quantifies disease via study of glass slides
  • Traditional pathologists experientially identify and quantify complex processes
  • Pathologists interpret planar images correlated with other data to arrive at three-dimensional mental imagery of complex processes.

Work in Progress: Envisioning Tumor Pattern of Invasion in three Dimensions
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Envisioning Tumor Pattern of Invasion in three Dimensions

• Traditional pathology quantifies disease via study of glass slides
• Pathologists experientially identify and quantify complex processes by interpreting planar images correlated with other data to arrive at three dimensional mental imagery of complex processes.
• We believe that concrete 3D modeling of tissue morphology has the potential to enhance our understanding of diseases.

WPOI represents specific architecture at the cancer/non-cancer interface. Classification is based solely on 2D planar analysis of complex 3D structures.

Possibly, important diagnostic information is revealed in a fully 3D model.

Nonaggressive Pattern of Invasion

In the context of this case, likely that deeper sections will demonstrate connection between these satellites, therefore not convincingly separated.

Nonaggressive Pattern of Invasion

Aim: Validate contextual planar assumptions on tumor invasion by 3D modeling
Work in Progress: Envisioning Tumor Pattern of Invasion in 3 Dimensions

- Developing machine classifiers to identify squamous carcinoma in digital images.
- Digital classifiers = “Tumor Masks” developed by “Texture analysis”
- Texture in a glass slide???
- Texture = Rich source of visual information = Complex visual patterns, sub-patterns, brightness, color, slope, size, etc.
- 156 texture-based features extracted from “ground truth” to capture local color, greyscale, and texture characteristics

Examples of classifiers:
A. Median greyscale: intensity compared with neighborhood values
B. Haralick features: information measure of value frequency in the neighborhood (microtexture) and hierarchical spatial arrangement (macrotexture)
C. Gabor features: information measure on directional changes, includes spatial localization


Envisioning Tumor Pattern of Invasion in 3 Dimensions

- Bayes classifier trained to recognize classes, pixel by pixel @ low resolution
- Minimum redundancy and maximum relevance method
  – Fewest number of filters that achieve classification
- Produce classification map: intensities = likelihood of target class
- Thresholded binary map
Envisioning Tumor Pattern of Invasion in 3 Dimensions

- A two-pass classifier for tumor masks: 1st - tissue vs. background. 2nd - tumor vs. non-tumor
- To ensure a smooth 3D model, interpolation performed
- Tumor surface visualized as 3D topographical map, or surface mesh. The features extracted via the 3D Object Counter plugin for ImageJ, and then visualized with Fiji or Meshlab

Machine Learning: Tumor classification using low-resolution data

"Ground truth" = The control!

- The "ground truth" models do not require machine learning classifiers; they become the control for comparison with computer generated models
- Generated on a tiff stack processed with Photoshop magic wand tool

"Ground truth"
Machine learning: Tumor classification based on low-resolution data

Region of interest

Tumor mask from corresponding ROI
Carcinoma plus lymphocytes

Machine Learning: Tumor classification using low-resolution data

Transformed

Likelihood mask
Threshold Binary

Machine Learning: Tumor classification using low-resolution data

Transformed

Likelihood mask
Threshold Binary

Build stack

Build stack
Machine Learning: Tumor classification using low-resolution data

Tumor classifier sensitive but “over recognizes”

Work in Progress: Envisioning Tumor Pattern of Invasion in three Dimensions

- Refine model by extracting regions of interest at the invasive interface – and examine other segmentation models on corresponding high-resolution data
- Is WPOI-5 the best classifier? Large scale interrogation of pattern of invasion – which cut-point has best performance?
- Rationale for clinical trial: Continued observational accrual low-stage WPOI-5 oral cancers
- Multidimensional manifold: Imagine fruits hanging from a tree
15 patients, T2 or T2 oral cavity SCC
8 WPOI-5 versus 7 WPOI-4
Four developed disease progression
Thank you