Advancing the Therapeutic Ratio in Head and Neck Cancer: Benchtop to Clinical Advances

Christine H. Chung, M.D.
Associate Professor of Oncology
Director, HNC Therapeutics Program
No Conflict of Interest
Learning Objectives

• To describe the differences in pathogenesis and clinical outcomes between HPV-related and -unrelated HNSCC

• To evaluate the process of translating the HPV-related diagnostic tests and cancer genetics data into prognostic and predictive evaluation in HNSCC management
Epidemiology of HNSCC

• The 6\textsuperscript{th} most common cancer worldwide
• Median age of Dx: 53-57
• Gender: Male Predominance (M:F=3:1)
• Approximately 2/3 of the cases are advanced stage III/IV at presentation
• Risk factors
  – Tobacco, Alcohol
  – Human Papillomavirus (HPV): oropharynx
  – Epstein-Barr Virus (EBV): nasopharynx
Common sites of squamous cell carcinoma:

- Oral cavity:
  - Oral tongue, floor of mouth, etc.

- Nasopharynx

- Oropharynx: base of tongue, soft palate, tonsil

- Hypopharynx: pyriform sinus, post-cricoid, posterior pharyngeal wall

- Sinonasal tumors
- Salivary gland tumors
- Lymphoma
- Mucosal melanoma
- Sarcoma
- Thyroid, etc.
HPV and oropharyngeal SCC
- Secondary lymphoid organs
- Tonsillar crypts: reticular crypt epithelium, extrafollicular area, mantle zones of lymphoid follicles and follicular germinal center
- The crypts increase the contact surface between the environment and lymphoid tissue
- Estimated surface area of crypts 295 cm$^2$ (entire OP surface area 45 cm$^2$)
Prevalence of Oral HPV Infection in the US, 2009-2010

- Cross-sectional study as a part of National Health and Nutrition Examination Survey
- 5579 men and women, aged 14-69 years
- 37 HPV type testing using DNA purified from oral exfoliated cells

Gillison, et al. JAMA, 2012
Prevalence by individual genotypes:
Overall: 6.9%, Low-risk HPV: 3.1%
High-risk HPV: 3.7%, HPV16: 1% (2.13 million)

Gillison, et al. JAMA, 2012
Overall Modeled Prevalence, any HPV

Gillison, et al. JAMA, 2012
Oral HPV Clearance

Multinational cohort of 4,074 healthy men

97% of infections cleared by 18 months

Kreimer et al. HPV 2010 IPV mtg
Incidence rates of oropharynx cancer during 1988 to 2004

- Approximately 16,000 cases per year
- At JHU, approximately 80% of newly diagnosed HNSCC are HPV-related

Diagnosis of HPV-Positive Oropharynx SCC

H&E

p16

In situ hybridization
Phase III Trial at Radiation Therapy Oncology Group (RTOG) 0129

**Tumor Site**
1. Larynx
2. Non-Larynx

**Nodal Stage**
1. N0
2. N1 or N2a-b
3. N2c or N3

**Zubrod Performance Status**
1. 0
2. 1

**Arm 1:**
Standard Fractionation (SFX)
70 Gy/35 Fx/7 weeks plus cisplatin 100 mg/m² on days 1, 22, 43

**Arm 2:**
Accelerated Fractionation by Concomitant Boost (AFX-C)
72 Gy/42 Fx/6 weeks plus cisplatin 100 mg/m² on days 1, 22

Ang, et al. NEJM 2010
Overall Survival based on Prognostic Stratification

- HPV+/non-smoker: 93% at 3-yr
- HPV+/smoker, HPV-/non-smoker: 71% at 3-yr
- HPV-/smoker: 46% at 3-yr

Ang, et al. NEJM 2010
Can you get HPV infection in non-oropharynx SCC?

- Oral Cavity: oral tongue, floor of mouth, etc.
- Nasopharynx: base of tongue, soft palate, tonsil
- Oropharynx: tonsil
- Hypopharynx: pyriform sinus, post-cricoid, posterior pharyngeal wall

Sinonasal tumors
- Salivary gland tumors
- Lymphoma
- Mucosal melanoma
- Sarcoma
- Thyroid, etc.
Can you use p16 staining as a surrogate marker of HPV infection in non-Oropharynx SCC?
P16 expression in HNSCC

• p16 protein is encoded by CDKN2A (Cyclin-dependent kinase inhibitor 2A) located on chromosome 9p21

• p16 inhibits the kinase activities of CDK4 and CDK6 and induces cell cycle arrest; therefore, functions as a tumor suppressor

Chung, et al. ASCO 2013
HPV-Negative

- p16 loss (~80%)
  - Chromosomal/gene deletion (29%)
  - Hypermethylation (23-58%)
  - Gene mutation (9-12%)

HPV-Positive

- p16 gain
  - CDK4
  - CDK6
  - Cyclin D

- Chromosomal/gene deletion (29%)
- Hypermethylation (23-58%)
- Gene mutation (9-12%)

Chung, et al. ASCO 2013
p16 as a prognostic biomarker

- p16 is a validated surrogate marker of HPV infection in oropharyngeal SCC (OPSCC): \( \text{Kappa} = 0.80, \ 95\% \ CI \ 0.73-0.87^* \)
- p16 is a biomarker of favorable outcome in OPSCC
- Its role in non-OPSCC (oral cavity, hypopharynx and larynx) is unclear

*Ang, et al. NEJM 2010
Chung, et al. ASCO 2013
Methods

• Obtained non-OPSCC tumor samples from:
  – RTOG 0129: Phase III concurrent cisplatin with either standard FX XRT or accelerated FX by concomitant boost XRT in LA HNSCC

  – RTOG 0234: Phase II XRT + cetuximab with either cisplatin or docetaxel following surgical resection of LA HNSCC with high risk of recurrence

  – RTOG 0522: Phase III concurrent cisplatin + XRT +/- cetuximab in LA HNSCC

Chung, et al. ASCO 2013
Methods

- p16 expression determined by IHC (positive if >70% of the tumor cells demonstrated diffuse staining)

- High-risk HPV status determined by *in situ* hybridization using a cocktail probe including HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66

Chung, et al. ASCO 2013
<table>
<thead>
<tr>
<th>Study ID (n=Total # non-OPSCC)</th>
<th>RTOG 0129 (n=288)</th>
<th>RTOG 0234 (n=129)</th>
<th>RTOG 0522 (n=266)</th>
<th>Total (n=683)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>p16 data available</strong></td>
<td>85 (30%)</td>
<td>95 (74%)</td>
<td>142 (53%)</td>
<td>322 (47%)</td>
</tr>
<tr>
<td><strong>p16-positive</strong></td>
<td>12 (14%)</td>
<td>23 (24%)</td>
<td>27 (19%)</td>
<td>62 (19%)</td>
</tr>
<tr>
<td><strong>p16-negative</strong></td>
<td>73 (86%)</td>
<td>72 (76%)</td>
<td>115 (81%)</td>
<td>260 (81%)</td>
</tr>
<tr>
<td><strong>HPV data available</strong></td>
<td>93 (32%)</td>
<td>103 (80%)</td>
<td>101 (38%)</td>
<td>297 (43%)</td>
</tr>
<tr>
<td><strong>HPV-positive</strong></td>
<td>6 (6%)</td>
<td>15 (15%)</td>
<td>7 (7%)</td>
<td>28 (9%)</td>
</tr>
<tr>
<td><strong>HPV-negative</strong></td>
<td>87 (94%)</td>
<td>88 (85%)</td>
<td>94 (93%)</td>
<td>269 (91%)</td>
</tr>
</tbody>
</table>
### p16 and HPV Status in non-OPSCC

Correlation (phi coefficient): 0.46

<table>
<thead>
<tr>
<th></th>
<th>p16-Neg</th>
<th>p16-Pos</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>HPV-Neg</td>
<td>213 (78.0%)</td>
<td>33 (12.1%)</td>
<td>246 (90.1%)</td>
</tr>
<tr>
<td>HPV-Pos</td>
<td>7 (2.6%)</td>
<td>20 (7.3%)</td>
<td>27 (9.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>220 (80.6%)</td>
<td>53 (19.4%)</td>
<td>273 (100%)</td>
</tr>
</tbody>
</table>

Chung, et al. ASCO 2013
### p16 and HPV Status Correlation by primary sites

**Oral cavity: Phi 0.35**

<table>
<thead>
<tr>
<th></th>
<th>p16-Neg</th>
<th>p16-Pos</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>HPV-Neg</td>
<td>53 (67%)</td>
<td>13 (16%)</td>
<td>66 (84%)</td>
</tr>
<tr>
<td>HPV-Pos</td>
<td>5 (6%)</td>
<td>8 (10%)</td>
<td>13 (16%)</td>
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<tr>
<td>Total</td>
<td>58 (73%)</td>
<td>21 (27%)</td>
<td>79 (100%)</td>
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</table>

**Hypopharynx: Phi 0.54**

<table>
<thead>
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<th>p16-Neg</th>
<th>p16-Pos</th>
<th>Total</th>
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<tbody>
<tr>
<td>HPV-Neg</td>
<td>45 (83%)</td>
<td>6 (11%)</td>
<td>51 (94%)</td>
</tr>
<tr>
<td>HPV-Pos</td>
<td>0 (0%)</td>
<td>3 (6%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Total</td>
<td>45 (83%)</td>
<td>9 (17%)</td>
<td>54 (100%)</td>
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</table>

**Larynx: Phi 0.52**

<table>
<thead>
<tr>
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<th>p16-Neg</th>
<th>p16-Pos</th>
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<tbody>
<tr>
<td>HPV-Neg</td>
<td>115 (82%)</td>
<td>14 (10%)</td>
<td>129 (92%)</td>
</tr>
<tr>
<td>HPV-Pos</td>
<td>2 (1%)</td>
<td>9 (6%)</td>
<td>11 (8%)</td>
</tr>
<tr>
<td>Total</td>
<td>117 (84%)</td>
<td>23 (16%)</td>
<td>140 (100%)</td>
</tr>
</tbody>
</table>

Chung, et al. ASCO 2013
Progression-Free Survival

HR (95% CI) [p16-positive/p16-negative]
0.65 (0.44-0.98)
2-sided log-rank p=0.04

Patients at Risk
p16-positive 62 45 38 34 23 10
p16-negative 260 167 131 106 74 23

Chung, et al. ASCO 2013
Overall Survival

HR (95% CI) [p16-positive/p16-negative]
0.57 (0.36-0.90)
2-sided log-rank p=0.01

Patients at Risk
p16-positive  62  57  44  41  28  13
p16-negative  260 212 170 140 91  33

Chung, et al. ASCO 2013
Progression-Free Survival

Patients at Risk

<table>
<thead>
<tr>
<th>Condition</th>
<th>Years after Randomization</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
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<tr>
<td>p16-pos oropharynx (op)</td>
<td>493</td>
</tr>
<tr>
<td>p16-pos non-oropharynx (nonop)</td>
<td>62</td>
</tr>
<tr>
<td>p16-neg oropharynx (op)</td>
<td>198</td>
</tr>
<tr>
<td>p16-neg non-oropharynx (nonop)</td>
<td>260</td>
</tr>
</tbody>
</table>

Chung, et al. ASCO 2013
Overall Survival

Patients at Risk

<table>
<thead>
<tr>
<th>Condition</th>
<th>Years after Randomization</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>p16-pos op</td>
<td>493</td>
</tr>
<tr>
<td>p16-pos nonop</td>
<td>62</td>
</tr>
<tr>
<td>p16-neg op</td>
<td>198</td>
</tr>
<tr>
<td>p16-neg nonop</td>
<td>260</td>
</tr>
</tbody>
</table>

Chung, et al. ASCO 2013
Clinical Implications of HPV/p16 Status in HNSCC

• Understanding HPV-induced carcinogenesis may provide less toxic treatment for oropharynx SCC
  – Long-term toxicities of intense treatments in young patients are problematic
  – Chronic pain, shoulder dysfunction, xerostomia, dysphagia, odynophagia, etc.

• p16 IHC is NOT a reliable surrogate for HPV infection in non-OPSCC and treatments should not be de-intensified

• HPV/p16 assays are limited as prognostic markers: Not all HPV+/p16+ HNSCC do well and not all HPV-/p16- HNSCC do poorly. How about HPV-/p16+ patients?
Molecular classification of HNSCC (n=60)

Basal  Mesenchymal  Atypical: HPV +  Classical

These 4 molecular subtypes are validated by two independent datasets:
Univ. North Carolina (n=138)
The Cancer Genome Atlas (n=279)

Group 3: Atypical HPV +

Five HNC Expression Subtypes (n=132, HPV+ 44%)

MS = Mesenchymal  
CL = Classical

<table>
<thead>
<tr>
<th>HPV+MS</th>
<th>MS</th>
<th>Basal</th>
<th>HPV+CL</th>
<th>Classical</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8A</td>
<td>CD8B</td>
<td>HLA-DRA</td>
<td>VIM</td>
<td>MMP9</td>
</tr>
</tbody>
</table>

Kech, et al  
ASCO 2013
Molecular classification for Prognosis in HNSCC

3-yr PFS

- HPV+CL ~90%
- HPV+MS, MS ~70%
- BA, CL ~50%

CL: Classical
MS: Mesenchymal
BA: Basal

Kech, et al ASCO 2013
Laboratory biomarkers must provide additional information beyond routinely obtained clinical data.
Molecular classification for Prognosis in HNSCC

3-yr PFS

- HPV+CL ~90%
- HPV+MS, MS ~70%
- BA, CL ~50%

3-yr OS

- HPV+/non-smoker: 93%
- HPV+/smoker, HPV-/non-smoker: 71%
- HPV-/smoker: 46%

Survival Time in Days

p = 0.044

Kech, et al ASCO 2013

Ang, et al. NEJM 2010
Now we can ask what the biological differences are between HPV+ smokers vs. non-smokers.
Both BA and CL subtypes are in the high risk group, but they may need two different therapeutic approaches based on their underlying biology.
Clinical Application

• Newly diagnosed patients
  – Clinical trials based on biologically defined subtypes
    • Choice of treatment modalities - surgical vs. non-surgical
    • Choice of chemotherapy and targeted agents
    • Novel induction or maintenance regimens

• Immediate application may be in recurrent/metastatic patients
Five HNC Expression Subtypes (n=132, HPV+ 44%)

MS = Mesenchymal
CL = Classical

Kech, et al
ASCO 2013
Oncogenes (accelerators) vs. Tumor Suppressor Genes (breaks)

B Vogelstein et al. Science 2013;339:1546-1558
<table>
<thead>
<tr>
<th>PIK3CA Variants</th>
<th>HPV Negative # (%) N=244</th>
<th>HPV Positive # (%) N=35</th>
</tr>
</thead>
<tbody>
<tr>
<td>C604R</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>C901F</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>C971R</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>E110del</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>E365V</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>E453K</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>E542K</td>
<td>9 (3.7%)</td>
<td>5 (14.3%)</td>
</tr>
<tr>
<td>E545K</td>
<td>11 (5.5%)</td>
<td>7 (20.0%)</td>
</tr>
<tr>
<td>E726K</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>E970K</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>G1007R</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>G363A</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>G451R</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>H1047L</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>H1047R</td>
<td>6 (2.5%)</td>
<td>0</td>
</tr>
<tr>
<td>K111E</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>K111N</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>Q75E,Q75E</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>R335G</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>R88Q,M1043V</td>
<td>0</td>
<td>1 (2.9%)</td>
</tr>
<tr>
<td>R975S</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>V344G</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>W328S</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>45 (18.4%)</strong></td>
<td><strong>13 (37.1%)</strong></td>
</tr>
</tbody>
</table>

**Biologically validated mutations:**

- HPV+ 34.3%
- HPV- 11.7%
Mutational Spectrum in HPV(-) vs HPV(+) 

HPV(-): Mostly tumor suppressors – TP53, CDKN2A, NOTCH1

HPV(+) More oncogenes – PIK3CA, FGFR2/3

Kech, et al ASCO 2013
Detecting PIK3CA mutation

Mutation Hotspot Genotyping
FFPE FNA cell block

Entire protein coding region of PIK3CA gene
FFPE core biopsy
Detecting PIK3CA mutation

Targeted sequencing
PIK3CA + 50-250 most commonly cancer-related genes
FFPE 3-4 mm³ biopsy or surgical specimen

Whole exome sequencing
PIK3CA + protein coding regions of ~20,000 genes (~1.5%)
Frozen tumors + Normal DNA
or
Whole genome sequencing
Entire genome
Frozen tumors + Normal DNA
(3 billion base pairs X 2)
What to tell the patient about the testing

• Genomic testing is NOT a standard of care
• Yes, there are scientific evidence that results of genomic testing MAY help the outcome in HN cancer
• But there is NO data to support that the treatment based on the testing results prolongs survival in HN cancer
• Expensive and no cost-benefit analysis is available
• While the technology is there, clinical research, health care policy, insurance policy and ethics guidelines have not caught up yet
What to do as a clinician

• Educate yourself about potential benefits and current limitations of genomics – It’s here to stay
• DO NOT order the test if you cannot provide comprehensive pre- and post-test counseling for patients or if you don’t have time.
• Refer the patients early to centers with comprehensive programs for trial participation. Trials may be the only way to access expensive but promising medications
• Whenever you can, please do your best to put patients on clinical trials
Conclusions

• The incidence of HPV+ HNSCC is increasing and current treatment paradigm needs to be re-evaluated.

• Data from molecular characterization of HNSCC need to be applied in clinical trials.

• Activation of the PI3K pathway is common and PI3K/mTOR inhibitors may have a role in HPV+ HNSCC.

• Improving treatments for the HPV-/p16- patients should be an important clinical research focus.
Conclusions

• While DNA sequencing data reveal a complex genome, the biological and clinical significance of genetic aberrations are largely unknown.

• New genomic analysis technology allows comprehensive analyses of pathways rather than individual genes, and they are promising tools to identify biomarkers and potential therapeutic targets.

• While they are powerful discovery tools, each finding must be vigorously validated before broad clinical application.
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• Sidransky/Califano Laboratory

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