Oropharyngeal HPV and cancer screening

Tim Waterboer, PhD MSc
Head, Infections and Cancer Epidemiology
Molecular Diagnostics of Oncogenic Infections Division
German Cancer Research Center (DKFZ)
What we know
Head and Neck Squamous Cell Carcinoma (HNSCC)

- worldwide: ~400,000 cases/year
- US: ~60,000 cases/year
- risk factors:
  - tobacco and alcohol
  - HPV Infection (>90% HPV16)

Risk factors for HNSCC include tobacco and alcohol consumption, as well as HPV infection, particularly with HPV16.
Head and Neck Squamous Cell Carcinoma (HNSCC)

- worldwide: ~400,000 cases/year
- US: ~60,000 cases/year
- risk factors:

Risk factor: 
HR 0.30 (95% CI 0.13-0.67)
Head and Neck Squamous Cell Carcinoma (HNSCC)

- worldwide: ~400,000 cases/year
- US: ~60,000 cases/year
- risk factors:

HPV attributable fraction

- worldwide: 3-5%
- US: <5-100%
- <1%

HR 0.30 (95% CI 0.13-0.67)
Worldwide oropharyngeal cancer incidence (Globocan 2012)
HPV attributable fraction in OPC by region

Castellsagué et al., JNCI 2016
A paradigm shift for HPV (US)

Shaturvedi et al., JCO 2011
Antibodies to HPV proteins

Capsid protein L1

- marker of past or present (transient) infection
- weak association with HPV-induced tumors
- cumulative exposure marker
Oncoproteins E6/E7

*In healthy population*
- rare (<1%)

*In HPV-positive invasive carcinomas (overexpression of oncoproteins)*
- disease (cancer) marker
- strongly associated with HPV-induced tumors at time of, or prior to diagnosis
Sensitivity and specificity of HPV16 serology for HPV-driven OPC

Inclusion criteria

- Serum samples at time of diagnosis
- Fresh-frozen tumor biopsies with ≥25% tumor-cell content
- Defined HPV DNA status of the tumor tissues
- HPV RNA as gold standard (Holzinger et al., Cancer Research 2012)
- non-HPV16 positive cases were excluded

214 HNSCC (Heidelberg n=40, Leipzig n=76, Padua n=98)

118 HPV DNA-

24 HPV16 DNA+RNA-

72 HPV16 DNA+RNA+

142 not HPV-driven

72 HPV-driven

Holzinger et al., IJC 2017
Heatmap of HPV antibodies, DNA and RNA in HNSCC patients

Holzinger et al., IJC 2017
## Sensitivity and specificity of HPV16 serology for HPV-driven OPC

<table>
<thead>
<tr>
<th>HPV seromarker</th>
<th>HPV- (n=142)</th>
<th>HPV+ (n=72)</th>
<th>Sensitivity % (95%CI)</th>
<th>Specificity % (95%CI)</th>
<th>Diagnostic accuracy % (95%CI)</th>
<th>Cohen’s kappa (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E7</td>
<td>-</td>
<td>130</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>12</td>
<td>48</td>
<td>67 (55-77)</td>
<td>92 (86-95)</td>
<td>83 (78-88)</td>
</tr>
<tr>
<td>E1</td>
<td>-</td>
<td>135</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>7</td>
<td>44</td>
<td>61 (50-72)</td>
<td>95 (90-98)</td>
<td>84 (78-88)</td>
</tr>
<tr>
<td>E2</td>
<td>-</td>
<td>127</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>15</td>
<td>58</td>
<td>81 (70-88)</td>
<td>89 (83-94)</td>
<td>87 (81-90)</td>
</tr>
<tr>
<td>L1</td>
<td>-</td>
<td>120</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>22</td>
<td>39</td>
<td>54 (43-65)</td>
<td>85 (78-90)</td>
<td>74 (68-80)</td>
</tr>
<tr>
<td>E6&gt;1000</td>
<td>-</td>
<td>141</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1</td>
<td>66</td>
<td>92 (83-96)</td>
<td>99 (96-100)</td>
<td>97 (93-98)</td>
</tr>
</tbody>
</table>
European Prospective Investigation into Cancer and Nutrition (EPIC)

- 521,330 participants from 10 European countries
- 385,747 blood samples
- Evaluation of HPV serologic biomarkers in head and neck cancer cases and controls
- Blood draw to cancer diagnosis: median 6.3 years (0.1 to 13.7 years)
# HPV16 Serology Status before Tumor Diagnosis

<table>
<thead>
<tr>
<th>HPV16 Serology Status</th>
<th>Controls (n=1599)</th>
<th>Oral Cavity (n=180)</th>
<th>Oropharynx (n=135)</th>
<th>Larynx (n=247)</th>
<th>Oesophagus (n=300)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>OR (95%CI)</td>
<td>N (%)</td>
<td>OR (95%CI)</td>
</tr>
<tr>
<td><strong>E6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seronegative</td>
<td>1590 (99.4)</td>
<td>178 (98.9)</td>
<td>1.0</td>
<td>88 (65.2)</td>
<td>1.0</td>
</tr>
<tr>
<td>Seropositive</td>
<td>9 (0.6)</td>
<td>2 (1.1)</td>
<td>1.3 (0.3 - 6.9)</td>
<td>47 (34.8)</td>
<td><strong>270 (110 - 680)</strong></td>
</tr>
<tr>
<td><strong>E7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seronegative</td>
<td>1421 (88.9)</td>
<td>155 (86.1)</td>
<td>1.0</td>
<td>108 (80.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Seropositive</td>
<td>178 (11.1)</td>
<td>25 (13.9)</td>
<td>1.2 (0.7 - 1.9)</td>
<td>27 (20.0)</td>
<td><strong>2.4 (1.5 - 3.9)</strong></td>
</tr>
<tr>
<td><strong>E1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seronegative</td>
<td>1536 (96.1)</td>
<td>165 (91.7)</td>
<td>1.0</td>
<td>113 (83.7)</td>
<td>1.0</td>
</tr>
<tr>
<td>Seropositive</td>
<td>63 (3.9)</td>
<td>15 (8.3)</td>
<td>2.1 (1.1 - 3.9)</td>
<td>22 (16.3)</td>
<td><strong>5.7 (3.2 - 10)</strong></td>
</tr>
<tr>
<td><strong>E2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seronegative</td>
<td>1527 (95.5)</td>
<td>170 (94.4)</td>
<td>1.0</td>
<td>102 (75.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>Seropositive</td>
<td>72 (4.5)</td>
<td>10 (5.6)</td>
<td>1.1 (0.5 - 2.1)</td>
<td>33 (24.4)</td>
<td><strong>9.5 (5.7 - 16)</strong></td>
</tr>
<tr>
<td><strong>E4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seronegative</td>
<td>1437 (89.9)</td>
<td>165 (91.7)</td>
<td>1.0</td>
<td>120 (88.9)</td>
<td>1.0</td>
</tr>
<tr>
<td>Seropositive</td>
<td>162 (10.1)</td>
<td>15 (8.3)</td>
<td>0.8 (0.5 - 1.5)</td>
<td>15 (11.1)</td>
<td>1.3 (0.7 - 2.4)</td>
</tr>
<tr>
<td><strong>L1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seronegative</td>
<td>1270 (79.4)</td>
<td>138 (76.7)</td>
<td>1.0</td>
<td>79 (58.5)</td>
<td>1.0</td>
</tr>
<tr>
<td>Seropositive</td>
<td>329 (20.6)</td>
<td>42 (23.3)</td>
<td>1.2 (0.8 - 1.7)</td>
<td>56 (41.5)</td>
<td><strong>3.1 (2.1 - 4.5)</strong></td>
</tr>
</tbody>
</table>

All OR adjusted for sex, age at enrolment (in five-year age categories), country, tobacco (never, former, current) and alcohol use (never/ever, and continuous values in grams per day) at recruitment

Kreimer et al., J Clin Oncol 2013
HPV16 E6 Seroprevalence in OPSCC and risk by time from blood draw to diagnosis

Kreimer et al., J Clin Oncol 2013
**Replication: HPV16 E6 and risk of OPC in US PLCO cohort**

<table>
<thead>
<tr>
<th></th>
<th>Controls n= 924</th>
<th>Oral cavity n= 62</th>
<th>Larynx n= 88</th>
<th>Oropharynx n= 47</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N (%)</strong></td>
<td>5 (0.5)</td>
<td>5 (7.9)*</td>
<td>0 (0)</td>
<td>22 (46.8)**</td>
</tr>
<tr>
<td><strong>Adj. OR</strong></td>
<td></td>
<td>31</td>
<td>N/A</td>
<td>172</td>
</tr>
<tr>
<td><strong>95% CI</strong></td>
<td></td>
<td>(6.0 to 161)</td>
<td>(48 to 610)</td>
<td></td>
</tr>
</tbody>
</table>

* 5 HPV16 E6 positive oral cavity cases: C029 Tongue, NOS (N=3), C140 Pharynx, NOS (N=1), C148 Overlapping lesions of the lip, oral cavity and pharynx (N=1)

** HPV16 E6 positivity in anatomic subsites: tonsil 50%, base of tongue 50%, other OP 0%
Replication: Lead time analysis among 22 HPV16 E6+ OPC cases in US PLCO cohort

Kreimer et al., JNCI 2017
Serial sample analysis among HPV16 E6+ individuals in US PLCO cohort

Kreimer et al., JNCI 2017
Cumulative 10-year risk of OPC by gender and HPV16 E6 in PLCO
### HPV16 E6 Antibodies and Risk of Subsequent Anogenital Cancer (EPIC)

<table>
<thead>
<tr>
<th>Anatomic site</th>
<th>Total cancer cases</th>
<th>% HPV16 E6 seropositive among cases</th>
<th>adjOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix</td>
<td>273</td>
<td>3.3</td>
<td>9.5 (2.4 to 37.1)</td>
</tr>
<tr>
<td>Anus</td>
<td>24</td>
<td>29</td>
<td>75.9 (17.9 to 321)</td>
</tr>
<tr>
<td>Penis</td>
<td>24</td>
<td>8.3</td>
<td>5.4 (0.5 to 63.4)</td>
</tr>
<tr>
<td>Vagina</td>
<td>12</td>
<td>8.3</td>
<td>24.1 (2.1 to 277)</td>
</tr>
<tr>
<td>Vulva</td>
<td>67</td>
<td>1.5</td>
<td>4.0 (0.4 to 46.0)</td>
</tr>
</tbody>
</table>

Kreimer et al., JCO 2015
Lead time analysis, HPV16 E6+ anogenital cancer cases in EPIC

Kreimer et al., JCO 2015
Conclusions

• Patients with HPV-driven oropharyngeal cancer almost always develop strong antibody responses to HPV early proteins, especially E6 (specificity >95%, sensitivity >90%)

• Much weaker association with other head and neck (oral cavity, larynx) and anogenital sites (anus), and not (as) prospective

• Antibodies to HPV 16 E6 and other early proteins can develop more than 10 years before cancer diagnosis, indicating the existence of yet unidentified HPV-specific lesions many years prior to oropharyngeal cancer diagnosis

• Very low population background (~1%) leads to extremely high NPV, yet PPV ~1% per year
What we don‘t know
Diagnostic window of HPV16 E6 antibodies in cervical and oropharyngeal cancer

CIN, cervical intraepithelial neoplasia
CIS, carcinoma in situ
ICC, invasive cervical cancer

Tonsillar crypt infection? Early serovonversion based on Waldeyer's ring epithelium?
The questions

- Natural history of oral HPV
- The role of specific antibody signatures in disease prediction
- The role of HPV early antigen serology in non-OPC head and neck tumors
- The role of non-16 HPV types in head and neck tumors
- Can we use serology for prediction of recurrence?
- Can we screen for HPV-OPC?
The questions

- Natural history of oral HPV
- The role of specific antibody signatures in disease prediction
- The role of HPV early antigen serology in non-OPC head and neck tumors
- The role of non-16 HPV types in head and neck tumors
- Can we use serology for prediction of recurrence?
- Can we screen for HPV-OPC?
Can we use serology for prediction of recurrence?

- What happens to HPV antibody titers after therapy?
- Are antibody titers at the time of diagnosis and/or therapy (‘pre-treatment’) associated with recurrence?
- Are antibody kinetics during follow-up (‘post-treatment’) informative for prediction of recurrence?
HPV post-OPC treatment antibody kinetics

HPV16 E6

HPV16 E7

Fakhry et al., Cancer Prev Res (Phila) 2016
HPV post-OPC treatment antibody kinetics

HPV16 E6

Lang Kuhs et al., Cancer 2017
HPV post-CxCa treatment antibody kinetics

HPV 16 E6

HPV 16 E7

Piontek et al., in preparation
The questions

• What happens to HPV antibody titers after therapy?
• Are antibody titers at the time of diagnosis and/or therapy (‘pre-treatment‘) associated with recurrence?
• Are antibody kinetics during follow-up (‘post-treatment‘) informative for prediction of recurrence?
Association of pre-treatment antibodies with recurrence

- **Dahlstrom et al., Clin Cancer Res 2015**
  E antibody pos versus neg patients with HPV DNA+ tumors: HR 0.3 (95%CI 0.1–0.8) for PFS

- **Fakhry et al., Cancer Prev Res (Phila) 2016**
  A \( \log_{10} \)-unit increase in pre-treatment HPV16 E6 antibody levels was associated with increased recurrence risk (HR 5.4, 95%CI 1.1-25.7)

- **Lang Kuhs et al., Cancer 2017**
  Pre-treatment HPV16 E6 seropositivity associated with reduced risk of local/regional recurrence (HR 0.14, 95%CI 0.03-0.68)

- **Zhang et al., Oral Oncology 2017; Spector et al., Clin Cancer Res 2017** – no significant association between HPV16 E6 antibody levels and risk of recurrence

- **Huang et al., Oncotarget 2017**
  E7 antibodies independent risk factor, E6 antibodies independent protective factor for local recurrence
The questions

• What happens to HPV antibody titers after therapy?
• Are antibody titers at the time of diagnosis and/or therapy (‘pre-treatment’) associated with recurrence?
• Are antibody kinetics during follow-up (‘post-treatment’) informative for prediction of recurrence?
Kinetics of post-treatment antibodies and recurrence

Broglie et al., in preparation
Kinetics of post-treatment antibodies and recurrence

- **Koslabova et al., Int J Cancer 2013**
  In 5 of 6 patients with recurrence who were positive at enrolment for HPV16 E6 and/or E7 antibodies, no decrease in antibody levels was observed during follow-up

- **Fakhry et al., Cancer Prev Res (Phila) 2016**
  In the first 3 months, a log_{10}-unit increase in E6 antibody levels was associated with risk of recurrence (HR 6.9, 95%CI 0.5-95.9).

- **Lang Kuhs et al., Cancer 2017**
  Changes in HPV16 E6 levels after treatment were not associated with recurrence of any type
Conclusions

• **Pre-diagnostic HPV early antibodies** can be detected 10+ years prior to HPV-OPC diagnosis; tumor sensitivity >90%, specificity >95%; population specificity >99% (E6)

• **Post-treatment HPV early antibodies** show a **slow decay** (Rubenstein et al., Infect Agent Cancer 2011; Koslabova et al., Int J Cancer 2013; Fakhry et al., Cancer Prev Res (Phila) 2016; Zhang et al., Oral Oncology 2017; Spector et al., Clin Cancer Res 2017; Hanna et al., Cancer Biomark 2017)

• **Association of pre-treatment antibodies with recurrence** inconclusive, needs bigger studies; comparability of serology methods and molecular markers to define HPV+ tumors!

• **Follow-up antibody kinetics and recurrence prediction** same as above; epidemiologically interesting versus clinical relevance (slow decay)
What does it take for effective screening?

- A sufficiently high incidence rate, either in the general population or an enriched high-risk population
- A sensitive and specific screening tool or biomarker
- Appropriate methods for accurately diagnosing the cancer (or pre-cancer) among individuals who screen positive
- Effective treatment for early-stage lesions
- Evidence of mortality and/or morbidity reductions
What does it take for effective screening?

- A sufficiently high incidence rate, either in the general population or an enriched high-risk population
- A sensitive and specific screening tool or biomarker
- Appropriate methods for accurately diagnosing the cancer (or pre-cancer) among individuals who screen positive
- Effective treatment for early-stage lesions
- Evidence of mortality and/or morbidity reductions
• Incidence rate now ≤50/100,000 PY
• High-risk population?

Kreimer et al., Cancer 2018
What does it take for effective screening?

- A sufficiently high incidence rate, either in the general population or an enriched high-risk population
- A sensitive and specific screening tool or biomarker
- Appropriate methods for accurately diagnosing the cancer (or pre-cancer) among individuals who screen positive
- Effective treatment for early-stage lesions
- Evidence of mortality and/or morbidity reductions
Biomarker (HPV16 E6)

- Tumor sensitivity >90%
- Tumor specificity >95%
- Population specificity >99%

<table>
<thead>
<tr>
<th>UK cancer</th>
<th>incidence per 100,000 person-years 40-70 yo (2014, CRUK)</th>
<th>attributable to HPV16 [%]</th>
<th>estimated maximum lead time [years]</th>
<th>expected to develop HPV16 driven cancer [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>oropharyngeal</td>
<td>9.0</td>
<td>70</td>
<td>20</td>
<td>0.126</td>
</tr>
<tr>
<td>penile</td>
<td>3.5</td>
<td>50</td>
<td>5</td>
<td>0.008</td>
</tr>
<tr>
<td>anal</td>
<td>2.9</td>
<td>90</td>
<td>5</td>
<td>0.013</td>
</tr>
<tr>
<td>cervical</td>
<td>13.8</td>
<td>50</td>
<td>1</td>
<td>0.007</td>
</tr>
<tr>
<td>vulva</td>
<td>5.0</td>
<td>40</td>
<td>5</td>
<td>0.010</td>
</tr>
<tr>
<td>vaginal</td>
<td>12.5</td>
<td>60</td>
<td>5</td>
<td>0.038</td>
</tr>
</tbody>
</table>

- Expectedly, ~20% of those HPV16 E6 seropositive will eventually develop HPV-driven cancer
What does it take for effective screening?

- A sufficiently high incidence rate, either in the general population or an enriched high-risk population
- A sensitive and specific screening tool or biomarker
- **Appropriate methods for accurately diagnosing the cancer (or pre-cancer) among individuals who screen positive**
- Effective treatment for early-stage lesions
- Evidence of mortality and/or morbidity reductions
Diagnosing (pre-)cancer

- Diagnostic work-up of screen positives
  - oral cancer exam
  - non-invasive imaging of the head and neck (transcervical ultrasound? NBI? PET-CT? PET-MRI?)
  - external genital exam (to rule out HPV-driven genital cancers)
    - cervical cancer screening (females)
    - anal specimen collection (to rule out anal cancers)
  - pan-endoscopy?
  - host genetic risk stratification markers?
What does it take for effective screening?

- A sufficiently high incidence rate, either in the general population or an enriched high-risk population
- A sensitive and specific screening tool or biomarker
- Appropriate methods for accurately diagnosing the cancer (or pre-cancer) among individuals who screen positive
- **Effective treatment for early-stage lesions**
- Evidence of mortality and/or morbidity reductions
Treating (pre-)cancer

- Treatment of screen positives
  - If diagnostic work-up is positive
    - Treatment according to guidelines/local standards
  - If diagnostic work-up is negative
    - Preventive tonsillectomy? TORS? (BOT?)
    - Therapeutic HPV vaccination?
    - Watch and wait (e.g. annual monitoring) versus neck metastases (unknown primaries)
What does it take for effective screening?

- A sufficiently high incidence rate, either in the general population or an enriched high-risk population
- A sensitive and specific screening tool or biomarker
- Appropriate methods for accurately diagnosing the cancer (or pre-cancer) among individuals who screen positive
- Effective treatment for early-stage lesions
- **Evidence of mortality and/or morbidity reductions**
**Morbidity and mortality**

- **Post-treatment QoL**
  - Speech and swallowing dysfunction, impaired taste, decreased salivary flow/xerostomia, feeding tube dependence, pain, disfigurement/body image alterations, physiologic distress, depression, trismus, nutrition/resumption of normal diet, mucositis, infectious complications, etc.

- **HPV vaccination**
  - Males versus females (herd immunity)
  - Clinical trial design: missing surrogate endpoints
  - Effective no earlier than 2040
What needs to be done?

- **Description of precancerous lesions**
  - Enrich the study population!
  - Screen 100,000 individuals
    (e.g., white males >45 years, non-smokers?)
  - Follow-up of ~1000 seropositives
  - ~100 HPV-OPC cases expected in 10 years
- **Develop clinical work-up protocols**
  - Imaging not sufficiently sensitive to detect precursors
  - Invasive methods (e.g. tonsillectomy) acceptable for healthy individuals?
  - Study CUP patients?
Conclusions

- Anticipate an **increased burden of HPV-driven OPC** over the coming decades
- **Prophylactic HPV vaccination** should work, but not for decades
- We have identified a **strong predictive biomarker** for a disease that is typically diagnosed at late stage, and a major knowledge gap in between
- Should we **screen for HPV-OPC** now – not as long as our clinical options aren’t better
- Should we **intensify research on secondary prevention** of HPV-OPC – yes!
Thanks for your attention!

DKFZ
Michael Pawlita
Lea Schröder
Dana Holzinger

IARC
Paul Brennan
Mattias Johansson

NCI
Aimée Kreimer

Vanderbilt
Krystle Lang-Kuhs

Johns Hopkins
Amber D‘Souza

Univ. Hospital Leipzig
Gunnar Wichmann
Andreas Dietz

Univ. Padua
Paolo Boscolo-Rizzo
Annarosa Del Mistro

Univ. Spital Zürich
Martina Broglie
Vittoria Guarda