HPV-RELATED CANCERS: PREVENTION, VACCINES AND TREATMENT

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INTRODUCTION

Human papillomaviruses (HPVs) induce the carcinogenesis of several diseases including 5% of all cancers diagnosed worldwide (cervical, vulvar, vaginal, anal, penile, oropharyngeal…)

INTRODUCTION

• HPV is the most common sexually-transmitted infection in the world
• HPV is so common that nearly all sexually-active men and women will get at least one type of HPV at some point in their lives
• Cancer of the cervix, which affects almost 530,000 women each year, represents the “historical” model of HPV-driven carcinogenesis
• Recently, high-risk HPVs have been recognised as a risk factor responsible for an increasing number of oropharyngeal cancers (OPCs)
• These cancers represent a distinct disease from traditional head and neck cancers caused by smoking and alcohol consumption
• The objective of this presentation is to discuss several aspects of HPV-related cancers with a special focus on HPV-driven OPC, which is an emerging disease

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- Viral cycle
- Genital and oral HPV infection rates
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- Specific issues raised by HPV-driven OPC

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- Therapeutic vaccination
- New diagnostic tool to define HPV-driven OPC
PART 1

Human papillomaviruses – the basics
VIRAL STRUCTURE AND GENOME

HPVs are a family of small DNA viruses that have a tropism for the epithelia of the genital and upper respiratory tracts and for the skin.

HPVs have a 55 nm diameter capsid composed of two main proteins named L1 and L2.

VIRAL STRUCTURE AND GENOME

HPVs are non-enveloped, small DNA viruses with circular and double-stranded DNA genome of approximately 8000 base pairs.

All DNA sequences coding for proteins, called open reading frames, are restricted to one strand. The coding sequences have been classified as early (E) containing the early genes E1 to E7, and late (L) containing the late genes L1 and L2.

Roles of the HPV proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>Initiates replication of the viral genome. Activates helicase, keeps viral DNA episomal.</td>
</tr>
<tr>
<td>E3</td>
<td>Unknown.</td>
</tr>
<tr>
<td>E5</td>
<td>Interaction with EGF receptor, activates PDGF receptor. Oncoprotein, allows continuous proliferation of the host cell and delays differentiation.</td>
</tr>
<tr>
<td>E6</td>
<td>Blocks the normal regulation of host cell division. Degrades p53 in the presence of E6-AP. Interaction with several host proteins. Major oncoprotein.</td>
</tr>
<tr>
<td>E7</td>
<td>Blocks the normal regulation of host cell division. Binds to pRB-105, p107 and p130. Interaction with several host proteins. Major oncoprotein.</td>
</tr>
<tr>
<td>L1</td>
<td>Major capsid protein (target of prophylactic vaccines).</td>
</tr>
<tr>
<td>L2</td>
<td>Minor capsid protein.</td>
</tr>
</tbody>
</table>

Structure and locations of the HPV proteins

VIRAL CYCLE

Schematic representation of E6 and E7 activities in cervical cells

Approximately 150 HPV types have been discovered so far, which are classified into several genera based on their DNA sequence.

Approximately 15 high-risk (HR) mucosal HPV types are clearly associated with cancer.

HPV16 is the most carcinogenic since it is responsible for approximately 90% of all cervical cancers and more than 90% of anal and HPV-related OPC.

*HPV16, HPV18 are the most frequent type associated with cancers; other HR-HPV include HPV 31/33/35/39/45/51/52/58/59/68/83

Reprinted from Virology, 445(1-2), de Villiers EM, Cross-roads in the classification of papillomaviruses, 2-10. Copyright 2013, with permission from Elsevier.
CERVICAL HISTOLOGY WITH TRANSFORMATION ZONE

• The **ectocervix** (exocervix) is composed of a non-keratinizing squamous epithelium with multiple layers: basal, parabasal, intermediate and superficial layer.

• The **endocervix** is lined by a simple columnar epithelium that secretes mucus (HEX40).
VIRAL CYCLE

Expression of the viral genes is precisely regulated and depends on the host cell differentiation level.
Loss of E2 gene following integration leads to over-expression of E6 and E7 oncogenes.
CERVICAL INFECTION NATURAL HISTORY

Bethesda classification:¹,²

1. Low-grade squamous intraepithelial lesion (LSIL): CIN1

2. High-grade squamous intraepithelial lesion (HSIL): CIN2 and CIN3

- Most individuals will spontaneously clear HPV infection in 1 to 3 years
- <1% will have a persistent infection, among whom a minority will develop a lesion
- Most CIN1, CIN2 and a significant fraction of CIN3 lesions (up to 70%) are cleared by the immune system

Image Reprinted from Cancer Epidemiol Biomarkers Prev 2008;17:2536–45, Cuschieri K and Wentzensen N, Human Papillomavirus mRNA and p16 Detection as Biomarkers for the Improved Diagnosis of Cervical Neoplasia, with permission from AACR.
CERVICAL HPV:
INFECTION RATES AND CANCER CASES

Cervical HPV infection rates vary around the world, as does the number of infected women who go on to develop cervical cancer.

Worldwide HPV prevalence in healthy women

Government-reported age-standardized cervical cancer incidence rate per 100,000 women per year.

A number of risk factors are known to increase the risk of HPV infection progressing to cervical cancer, including HPV coinfection, smoking, younger mothers, high number of children.
# CERVICAL HPV INFECTION

HPVs are the first cause of sexually transmitted infections worldwide

<table>
<thead>
<tr>
<th></th>
<th>Sample size</th>
<th>Any HPV Prevalence (95% CI)</th>
<th>Low-risk HPV* Prevalence (95% CI)</th>
<th>High-risk HPV** Prevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>4150</td>
<td>42.5 (40.3–44.7)</td>
<td>28.5 (26.8–30.3)</td>
<td>29.0 (26.8–31.3)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–19</td>
<td>1363</td>
<td>32.9 (29.5–36.5)</td>
<td>22.2 (19.2–25.5)</td>
<td>25.3 (22.0–28.8)</td>
</tr>
<tr>
<td>20–24</td>
<td>432</td>
<td>53.8 (45.9–61.5)</td>
<td>35.5 (29.5–41.9)</td>
<td>43.4 (36.0–51.2)</td>
</tr>
<tr>
<td>25–29</td>
<td>403</td>
<td>46.8 (42.9–50.8)</td>
<td>34.1 (29.9–38.5)</td>
<td>30.8 (25.8–36.2)</td>
</tr>
<tr>
<td>30–39</td>
<td>702</td>
<td>44.2 (40.5–48.0)</td>
<td>29.6 (25.6–34.0)</td>
<td>30.4 (26.8–34.3)</td>
</tr>
<tr>
<td>40–49</td>
<td>705</td>
<td>42.4 (39.0–46.0)</td>
<td>27.9 (24.8–31.3)</td>
<td>27.3 (23.8–31.1)</td>
</tr>
<tr>
<td>50–9</td>
<td>545</td>
<td>38.8 (33.9–44.0)</td>
<td>25.7 (21.3–30.5)</td>
<td>23.5 (19.1–28.6)</td>
</tr>
<tr>
<td><strong>Age at sexual debut</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;16 years</td>
<td>1073</td>
<td>55.8 (51.2–60.3)</td>
<td>38.8 (34.5–43.3)</td>
<td>39.2 (35.0–43.6)</td>
</tr>
<tr>
<td>≥16 years</td>
<td>2089</td>
<td>40.6 (37.7–43.6)</td>
<td>26.9 (24.7–29.3)</td>
<td>27.2 (24.6–30.0)</td>
</tr>
<tr>
<td>Never had sex</td>
<td>656</td>
<td>15.0 (10.9–20.1)</td>
<td>9.6 (6.5–14.0)</td>
<td>8.7 (5.7–13.2)</td>
</tr>
<tr>
<td><strong>Total lifetime sex partners</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>656</td>
<td>15.0 (10.9–20.1)</td>
<td>9.6 (6.5–14.0)</td>
<td>8.7 (5.7–13.2)</td>
</tr>
<tr>
<td>1</td>
<td>709</td>
<td>18.2 (14.2–23.0)</td>
<td>10.9 (8.5–14.0)</td>
<td>11.2 (8.2–15.3)</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>37.8 (31.7–44.3)</td>
<td>22.4 (17.7–28.0)</td>
<td>24.6 (18.7–31.5)</td>
</tr>
<tr>
<td>3–5</td>
<td>902</td>
<td>48.1 (44.7–51.5)</td>
<td>32.6 (28.7–36.6)</td>
<td>32.3 (28.9–35.8)</td>
</tr>
<tr>
<td>≥6</td>
<td>1141</td>
<td>55.9 (52.6–59.1)</td>
<td>38.8 (35.9–41.8)</td>
<td>39.4 (35.8–43.2)</td>
</tr>
</tbody>
</table>

*Risk of HPV infection is also increased in smokers and in immunocompromised individuals.

HPV causes nearly half of vulvar and two-thirds of vaginal cancers

### Number of cancer cases attributable to HPV and corresponding attributable fraction (AF) by cancer site, sex and age; World, 2012

<table>
<thead>
<tr>
<th>HPV-related cancer site (ICD-10 code)</th>
<th>Number of incident cases(^1,2)</th>
<th>Number attributable to HPV</th>
<th>AF (%)</th>
<th>Number attributable to HPV by gender</th>
<th>Number attributable to HPV by age group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Males</td>
<td>&lt;50 years</td>
</tr>
<tr>
<td>Cervix uteri (C53)</td>
<td>530,000</td>
<td>530,000</td>
<td>100.0</td>
<td>0</td>
<td>250,000</td>
</tr>
<tr>
<td>Anus(^3) (C21)</td>
<td>40,000</td>
<td>35,000</td>
<td>88.0</td>
<td>17,000</td>
<td>6,600</td>
</tr>
<tr>
<td>Vulva(^3) (C51)</td>
<td>34,000</td>
<td>8,500</td>
<td>24.9</td>
<td>0</td>
<td>2,600</td>
</tr>
<tr>
<td>Vagina(^3) (C52)</td>
<td>15,000</td>
<td>12,000</td>
<td>78.0</td>
<td>0</td>
<td>2,500</td>
</tr>
<tr>
<td>Penis(^3) (C60)</td>
<td>26,000</td>
<td>13,000</td>
<td>50.0</td>
<td>13,000</td>
<td>2,700</td>
</tr>
<tr>
<td>Oropharynx(^3) (C01, C09–10)</td>
<td>96,000</td>
<td>29,000</td>
<td>30.8</td>
<td>24,000</td>
<td>5,400</td>
</tr>
<tr>
<td>Oral cavity(^3) (C02–06)</td>
<td>200,000</td>
<td>4,400</td>
<td>2.2</td>
<td>2,900</td>
<td>890</td>
</tr>
<tr>
<td>Larynx (C32)</td>
<td>160,000</td>
<td>3,800</td>
<td>2.4</td>
<td>3,300</td>
<td>420</td>
</tr>
<tr>
<td>Other pharynx(^3) (C12–C14)</td>
<td>78,000</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total HPV-related sites</td>
<td>1,200,000</td>
<td>630,000</td>
<td>54.0</td>
<td>60,000</td>
<td>270,000</td>
</tr>
</tbody>
</table>

Adapted from de Martel C, et al. Int J Cancer 2017;141(4):664–70. Reproduced under the terms of the Creative Commons Attribution License (CC BY). Available at: https://creativecommons.org/licenses/by/4.0/. Accessed January 2020.
**ANAL HPV INFECTION**

Number of cancer cases attributable to HPV and corresponding attributable fraction (AF) by cancer site, sex and age; World, 2012

<table>
<thead>
<tr>
<th>HPV-related cancer site (ICD-10 code)</th>
<th>Number of incident cases(^1,2)</th>
<th>Number attributable to HPV</th>
<th>AF (%)</th>
<th>Number attributable to HPV by gender</th>
<th>Number attributable to HPV by age group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervix uteri (C53)</td>
<td>530,000</td>
<td>530,000</td>
<td>100.0</td>
<td>0</td>
<td>530,000</td>
</tr>
<tr>
<td>Anus(^3) (C21)</td>
<td>40,000</td>
<td>35,000</td>
<td>88.0</td>
<td>17,000</td>
<td>18,000</td>
</tr>
<tr>
<td>Vagina(^3) (C52)</td>
<td>34,000</td>
<td>8,500</td>
<td>24.9</td>
<td>0</td>
<td>8,500</td>
</tr>
<tr>
<td>Penis(^3) (C60)</td>
<td>15,000</td>
<td>12,000</td>
<td>78.0</td>
<td>0</td>
<td>12,000</td>
</tr>
<tr>
<td>Oropharynx(^3) (C01, C09–10)</td>
<td>26,000</td>
<td>13,000</td>
<td>50.0</td>
<td>13,000</td>
<td>0</td>
</tr>
<tr>
<td>Oral cavity(^3) (C02–06)</td>
<td>96,000</td>
<td>29,000</td>
<td>30.8</td>
<td>24,000</td>
<td>5,500</td>
</tr>
<tr>
<td>Larynx (C32)</td>
<td>200,000</td>
<td>4,400</td>
<td>2.2</td>
<td>2,900</td>
<td>1,500</td>
</tr>
<tr>
<td>Other pharynx(^3) (C12–C14)</td>
<td>160,000</td>
<td>3,800</td>
<td>2.4</td>
<td>3,300</td>
<td>460</td>
</tr>
<tr>
<td>Total HPV-related sites</td>
<td>1,200,000</td>
<td>630,000</td>
<td>54.0</td>
<td>60,000</td>
<td>570,000</td>
</tr>
</tbody>
</table>

Adapted from de Martel C, et al. Int J Cancer 2017;141(4):664–70. Reproduced under the terms of the Creative Commons Attribution License (CC BY). Available at: https://creativecommons.org/licenses/by/4.0/. Accessed January 2020.
Risk factors:
> HIV-positive men who have sex with men
> HIV-negative men who have sex with men
> HIV-positive women
> HIV-positive men who have sex with women
> HIV-negative women
> HIV-negative men who have sex with women
In the largest study performed so far (5579 healthy individuals aged 14 to 69 years), the prevalence of oral HPV infection was 6.9% (95% CI: 5.7, 8.3) and of HPV16 was 1.0% (95% CI: 0.7, 1.3).

Oral HPV infection followed a bimodal pattern with respect to age, with peak prevalence among individuals aged 30 to 34 years (7.3%; 95% CI: 4.6, 11.4) and 60 to 64 years (11.4%; 95% CI: 8.5, 15.1).
Men had a significantly higher prevalence than women for any oral HPV infection (10.1% [95% CI: 8.3, 12.3] vs. 3.6% [95% CI: 2.6, 5.0]; p<0.001) and for oral HPV16 infection (1.6% vs. 0.3%, p<0.001; 95% CI: 2.12, 13.83)

Infection was less common among those without vs. those with a history of any type of sexual contact (0.9% [95% CI: 0.4, 1.8] vs. 7.5% [95% CI: 6.1, 9.1]; p<0.001; prevalence ratio, 8.69 [95% CI: 3.91, 19.31]), and increased with number of sexual partners (p<0.001) and cigarettes smoked per day (p<0.001)

Associations with age, sex, number of sexual partners, and current number of cigarettes smoked per day were independently associated with oral HPV infection in multivariable models
Virtually all cervical cancers and most anal cancers are HPV-driven. In other anatomical locations, the attributable fraction varies.

Prevalence (%) of different HPV high-risk types among women with cancer

HPV16 is responsible for almost 90% of anal cancer and HPV-driven OPC contrasting with cervical cancer, in which the genotype distribution is wider.

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Table from Kreimer AR. Oral Oncol 2014;50(6):555-9;
Figure adapted from Muñoz N, et al. Int J Cancer 2004;111(2):278-85.
## HPV-DRIVEN CANCERS

### Relative contribution of HPV16/18 or HPV6/11/16/18/31/33/45/52/58 to HPV-associated cancers by site and by sex; World 2012

<table>
<thead>
<tr>
<th>HPV-related cancer site (ICD-10 code)</th>
<th>Number attributable to HPV(^1)</th>
<th>Relative contribution of HPV16/18(^2)</th>
<th>Relative contribution of HPV6/11/16/18/31/33/45/52/58(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent</td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>Cervix uteri (C53)</td>
<td>530,000</td>
<td>70.8</td>
<td>89.5</td>
</tr>
<tr>
<td>Anus (C21)</td>
<td>35,000</td>
<td>87.0</td>
<td>95.9</td>
</tr>
<tr>
<td>Vulva (C51)</td>
<td>8,500</td>
<td>72.6</td>
<td>87.1</td>
</tr>
<tr>
<td>Vagina (C52)</td>
<td>12,000</td>
<td>63.7</td>
<td>85.3</td>
</tr>
<tr>
<td>Penis (C60)</td>
<td>13,000</td>
<td>70.2</td>
<td>84.6</td>
</tr>
<tr>
<td>Head and neck (C01-06, C09-10, C32)</td>
<td>38,000</td>
<td>84.9</td>
<td>89.7</td>
</tr>
<tr>
<td>Total HPV-related sites in women</td>
<td>570,000</td>
<td>71.4</td>
<td>89.6</td>
</tr>
<tr>
<td>Total HPV-related sites in men</td>
<td>60,000</td>
<td>82.3</td>
<td>90.4</td>
</tr>
<tr>
<td>Total HPV-related sites</td>
<td>630,000</td>
<td>72.4</td>
<td>89.7</td>
</tr>
</tbody>
</table>

\(^1\) Derived from Plummer, de Martel et al.\(^8\); numbers are rounded to two significant digits.

\(^2\) Derived from Serrano et al.\(^1\), Alemany et al.\(^1\) and Castellsague et al.\(^1\).

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It takes 15 to 20 years for cervical cancer to develop in women with normal immune systems.

It can take only 5 to 10 years in women with weakened immune systems, such as those with untreated HIV infection.

Squamous cell carcinomas and adenocarcinomas are due to HPV infection.

Risk factors for HPV persistence and development of cervical cancer:
- Early first sexual intercourse
- Multiple sexual partners
- Tobacco use
- Immune suppression (HIV-infected individuals are at higher risk of HPV infection and are infected with a broader range of HPV types)
Age standardised (world) incidence rates (per 100,000) of cervical cancer attributable to HPV in 2012

ASR: Age-standardized rate

Age-standardised (world) incidence rates (per 100,000) of cancer cases attributable to HPV in 2012, both sexes.

Anogenital cancer cases (vulvar, vaginal, anal and penile)
HPV TESTING METHODS

There is a wide range of assays to identify HPV infection and HPV-driven cancers

HPV TESTING METHODS

There is a wide range of assays to identify HPV infection and HPV-driven cancers. The choice will depend on several factors including the advantages and drawbacks of each test.

<table>
<thead>
<tr>
<th>Detection technique</th>
<th>Advantages</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>E6/E7 mRNA RT-PCR</td>
<td>• High sensitivity, high specificity</td>
<td>• Requires fresh or frozen material</td>
</tr>
<tr>
<td></td>
<td>• Detects clinically relevant infections</td>
<td>• Risk of RNA degradation</td>
</tr>
<tr>
<td></td>
<td>• Considered as the “gold standard”</td>
<td>• Inadequate for routine screening</td>
</tr>
<tr>
<td>p16 immunostaining</td>
<td>• High sensitivity, feasible on FFPE</td>
<td>• Low specificity</td>
</tr>
<tr>
<td></td>
<td>• Indirect proof of E7 activity</td>
<td>• Surrogate marker of viral activity</td>
</tr>
<tr>
<td></td>
<td>• Cost effective and adequate for routine screening</td>
<td>• No exclusive link</td>
</tr>
<tr>
<td>In situ hybridisation</td>
<td>• High specificity, feasible on FFPE</td>
<td>• Reduced sensitivity</td>
</tr>
<tr>
<td></td>
<td>• Provides visual detection of infected cells</td>
<td>• No information about viral transcription</td>
</tr>
<tr>
<td></td>
<td>• Distinction between episomal and integrated</td>
<td>• Technically difficult to interpret</td>
</tr>
<tr>
<td>Consensus HPV PCR</td>
<td>• High sensitivity, feasible on FFPE</td>
<td>• No information about viral transcription</td>
</tr>
<tr>
<td>(Endpoint PCR [qualitative])</td>
<td>• Targets numerous oncogenic HPV stains</td>
<td>• Risk of contamination and detection of HPV from surrounding healthy tissue</td>
</tr>
</tbody>
</table>

PART 2
HPV-driven oropharyngeal cancers (OPCs)
The oropharynx is the middle part of the pharynx located behind the oral cavity. The tonsils and the tongue base are part of Waldeyer’s lymphoid tissue ring.
Each year, approximately 100,000 new cases of OPC are diagnosed worldwide. Tobacco and alcohol consumption are the traditional risk factors.
OROPHARYNGEAL CANCER (OPC) EPIDEMIOLOGY

Since the 1960s, the incidence of HNSCC is decreasing in most Western countries, thanks to a drop in tobacco and alcohol consumption caused by effective public health policies.

However, unlike most HNSCC the incidence of OPCs is increasing.

<table>
<thead>
<tr>
<th>Country</th>
<th>Reference</th>
<th>Years</th>
<th>Incidence trends for oropharyngeal cancers</th>
<th>Incidence trends for other head and neck cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MEN</td>
<td>WOMEN</td>
</tr>
<tr>
<td>Australia</td>
<td>Hocking, 2011</td>
<td>1982–2005</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Canada</td>
<td>Auluck, 2010</td>
<td>1980–2006</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Denmark</td>
<td>Blomberg, 2011</td>
<td>1978–2007</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Japan</td>
<td>Ioka, 2005</td>
<td>1965–1999</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>Braakhuı́ıs, 2009</td>
<td>1989–2006</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Sweden</td>
<td>Hammarstedt, 2006</td>
<td>1970–2002</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>USA</td>
<td>Chaturvedi, 2008</td>
<td>1973–2004</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
</tbody>
</table>

HNSCC, head and neck squamous cell carcinoma; NR, not reported.
OROPHARYNGEAL CANCER (OPC) EPIDEMIOLOGY

The rise of OPC is driven by high-risk HPVs

60 to 80% of OPCs are caused by HR-HPVs in Northern America and Northern Europe. Rates vary widely in other countries (30% in France\(^1\); <10% in India\(^2\)…)

Denmark


USA


OROPHARYNGEAL CANCER (OPC) EPIDEMIOLOGY
At the current rhythm, HPV-driven OPC could become the predominant type of HNSCC*


*North America and Northern Europe
## TYPICAL CLINICAL PRESENTATION

<table>
<thead>
<tr>
<th></th>
<th>HPV-positive OPC</th>
<th>HPV-negative OPC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td>Mainly men</td>
<td>Mainly men</td>
</tr>
<tr>
<td><strong>Primary tumour</strong></td>
<td>Small</td>
<td>Large (main symptom)</td>
</tr>
<tr>
<td><strong>Neck nodes</strong></td>
<td>Large (main symptom)</td>
<td>Small</td>
</tr>
<tr>
<td><strong>Throat pain</strong></td>
<td>Little or absent</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td>Tonsil, tongue base</td>
<td>All oropharyngeal anatomical sub sites</td>
</tr>
<tr>
<td><strong>2nd upper aero digestive tract cancer</strong></td>
<td>Exceptional</td>
<td>10–15% of cases</td>
</tr>
<tr>
<td><strong>Socio-economic level</strong></td>
<td>Medium to high</td>
<td>Low to medium</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td>No or moderate</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>Medical condition</strong></td>
<td>Good</td>
<td>Numerous comorbidities</td>
</tr>
<tr>
<td><strong>Relationship with sexual practices</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Table by Prof. H. Mirghani
A non-smoking 50-year-old man with a painless left neck mass
The physical examination identifies an asymptomatic small left tonsil lesion

2. Image from Prof H. Mirghani’s personal collection.
The risk of death of HPV-positive patients is cut in half compared with HPV-negative patients.

This prognosis differences led to the adoption of a new staging system for OPC patients, which includes two distinct TNM classifications according to the aetiology.

HPV+

>75% were staged IVa !!!

Stade I-IVa: comparable survival !!!

HPV–

PROGNOSIS

For HPV-driven OPC, substantial changes were adopted particularly for nodal staging

| N0       | No regional lymph node metastasis |
| N1       | Metastasis in a single ipsilateral lymph node, <3 cm in greatest dimension |
| N2a      | Metastasis in a single ipsilateral lymph node >3 cm but <6 cm in greatest dimension |
| N2b      | Metastasis in a multiple ipsilateral lymph node <6 cm in greatest dimension |
| N2c      | Metastasis in bilateral or contralateral lymph node <6 cm in greatest dimension |
| N3       | Metastasis in lymph node >6 cm in greatest dimension |

8th edition of the TNM classification

Positive p16 immunostaining

Non-keratinising oropharyngeal squamous cell carcinoma

Images from Prof C. Badoual’s personal collection
Multistep carcinogenesis\(^1\) and field cancerisation\(^2\) concepts

All the mucosal lining is genetically altered by the genotoxic substances released by tobacco and alcohol

---


Oncogenic addiction concept

E6/E7 oncoproteins are the key players in cancer initiation and progression.
OPC’s MUTATIONAL LANDSCAPE

Comprehensive genomic characterization of head and neck squamous cell carcinomas

Candidate therapeutic targets and driver oncogenic events

PENDING ISSUES
HPV-driven OPCs raise several questions including:

What is the best way to determine tumoural HPV status?
Can HR-HPV induce cancer outside the oropharynx?
Can treatment be personalised according to HPV status?
What is the risk of developing a second HPV-related cancer?
Accurate screening of HPV-driven OPCs is a critical issue

HPV status is currently used as a prognostic marker, but in the near future it may have a more important role in deciding treatment and follow-up of OPCs

Expert groups have issued recommendations regarding the optimal way to determine HPV status\(^1\-^3\)

HPV TESTING

IHC, immunohistochemistry.

HPV TESTING

HPV TESTING

IHC, immunohistochemistry.


Strong nuclear and cytoplasmic staining in more than 70% of tumoural cells.\(^1\)

HPV TESTING

p16-IHC

- Negative
- Positive

HPV-driven
	but 5–20\% false positives

HPV DNA identification

ISH Assay

- ISH– : HPV–
- ISH+ : HPV+

PCR Assay

- PCR+ : HPV+
- PCR– : HPV–

Klussmann J, et al. 2003

Smeets, et al. 2007

Singhi, et al. 2010

Thavaraj, et al. 2011

IHC, immunohistochemistry; ISH, in situ hybridization.
HPV TESTING

- **p16-IHC**
  - Negative
  - Positive

- **HPV DNA identification**
  - HPV-driven
    - Level of keratinisation
      - but 5–20% false positives

- **ISH Assay**
  - ISH− : HPV−
  - ISH+ : HPV+

- **PCR Assay**
  - PCR+ : HPV+
  - PCR− : HPV−

**Knockout Controls**

- Singhi et al. 2010
- Smeets et al. 2007
- Thavaraj et al. 2011

**Additional Information**

- Klussmann J, et al. 2003
- Cancer Care Ontario College of American Pathologists (2013)

**IHC, immunohistochemistry; ISH, in situ hybridization.**
HPV TESTING

p16-IHC

Negative

HPV-driven

Level of keratinisation

HPV DNA identification

ISH Assay

PCR Assay

ISH

PCR

ISH− : HPV−

ISH+ : HPV+

PCR− : HPV−

PCR+ : HPV+

ASCOS Clinical Practice Guideline JCO 2018
Additional testing at the discretion of physicians/pathologists

Cancer Care Ontario
College of American Pathologists
(2013)

Singhi, et al. 2010

Smeets, et al. 2007

Thavaraj, et al. 2011

IHC, immunohistochemistry; ISH, in situ hybridization.
HPV TESTING

Human Papillomavirus Testing in Head and Neck Carcinomas: ASCO Clinical Practice Guideline Endorsement of the College of American Pathologists Guideline

**Recommendation 1:** Pathologists should perform HR-HPV testing on all patients with newly diagnosed OPC either on the primary tumour or from cervical nodal metastases only if an oropharyngeal primary tumour is present.

**Recommendation 2:** For oropharyngeal tissue specimens, pathologists should perform HR-HPV testing by surrogate marker p16 IHC. Additional HPV-specific testing may be done at the discretion of the pathologist and/or treating clinician, or in the context of a clinical trial.

**Recommendations 3 & 4:** Pathologists should not routinely perform HR-HPV testing on patients with non-SCCs of the oropharynx and on patients with non-oropharyngeal primary tumours.

**Recommendations 5 & 6:** Pathologists should routinely perform p16 IHC on patients with metastatic SCC of unknown primary in a cervical lymph node. In this situation, it is also recommended to perform an HPV-specific test to account for frequent p16 overexpression in non-HPV–related cervical metastases from skin, salivary gland, or lung primaries.

HR-HPV, high-risk human papillomavirus; IHC, immunohistochemistry; SCC, squamous cell carcinoma.
## SIMILARITIES AND DIFFERENCES BETWEEN CERVICAL AND OROPHARYNGEAL CANCER

<table>
<thead>
<tr>
<th></th>
<th>Cervical cancer</th>
<th>Oropharyngeal cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aetiology</strong></td>
<td>Oncogenic HPV infection only with or without contravening factors</td>
<td>Multifactorial: tobacco, alcohol, HPV (among others)</td>
</tr>
<tr>
<td><strong>Cases worldwide (2008)</strong></td>
<td>530,000</td>
<td>85,000</td>
</tr>
<tr>
<td><strong>Evidence for HPV role</strong></td>
<td>Large, robust, diverse</td>
<td>Less strong and consistent</td>
</tr>
<tr>
<td><strong>Aetiological HPV fraction</strong></td>
<td>100%</td>
<td>26%</td>
</tr>
<tr>
<td><strong>Trends</strong></td>
<td>Decreasing in most, but not all, countries</td>
<td>Sharp increase in US and some North-European countries</td>
</tr>
<tr>
<td><strong>Geographical variability in HPV DNA detection</strong></td>
<td>None</td>
<td>Substantial (4-fold): (North America: 56%; Japan: 52%; Australia: 45%; Northern &amp; Western Europe: 39%; Eastern Europe: 38%; Southern Europe: 17%; rest of world: 13%)</td>
</tr>
<tr>
<td><strong>Evidence for type-specific carcinogenicity</strong></td>
<td>For all high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59)</td>
<td>Only for HPV16</td>
</tr>
</tbody>
</table>
HR-HPV IN NON-OPC

HPVs are the cause of laryngeal papillomatosis, a benign but severe condition. This demonstrates that these viruses can infect and induce disease in different anatomical sites within the head and neck region, but can they cause cancer outside the oropharynx (oral cavity, larynx...)?

From: http://ent4students.blogspot.com/2013/05/blog-post.html
Accessed May 2020

### p16 expression and DNA detection in oral cavity cancers (OCC)

<table>
<thead>
<tr>
<th>Authors</th>
<th>N° of cases</th>
<th>p16 over-expression</th>
<th>Detection HPV DNA/mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harris, et al</td>
<td>25</td>
<td>11/25 (44%)</td>
<td>0/11 (0%)</td>
</tr>
<tr>
<td>Pooling, et al</td>
<td>78</td>
<td>9/78 (11.5%)</td>
<td>1/9 (11%)</td>
</tr>
<tr>
<td>Lingen, et al</td>
<td>409</td>
<td>46/409 (11.2%)</td>
<td>19/46 (41.3%)</td>
</tr>
<tr>
<td>Upile, et al</td>
<td>102</td>
<td>8/102 (7.8%)</td>
<td>4/8 (50%)</td>
</tr>
<tr>
<td>Chung, et al</td>
<td>79</td>
<td>21/79 (26.6%)</td>
<td>8/21 (38%)</td>
</tr>
</tbody>
</table>

**p16 is a poor surrogate marker outside the oropharynx to identify potentially HPV-driven cancers**
HR-HPV IN NON-OPC

No obvious survival differences according to p16/HPV status outside the oropharynx

Laryngeal cancer¹

Oral cavity cancer²

The role of HR-HPV is still controversial outside the oropharynx

Non-OPC potentially caused by HR-HPV (those with HPV DNA and mRNA) are rare

They do not seem to correspond to a clear clinical, pathological and biological entity contrary to the situation in the oropharynx

Further research is warranted
TREATMENT AND HPV STATUS

As HPV-positive and negative OPCs represent distinct diseases, treatment personalisation according to the aetiology is a relevant issue.

Indeed, these two diseases have different prognostic profiles and biological basis.

Currently, OPC treatment choices should not vary according to HPV status and all OPC should be treated following the same guidelines.

However, the situation should change as several clinical trials are assessing this fundamental question.

Most ongoing trials are assessing de-escalation strategies* while others evaluate new treatment options.

* Decreasing toxicities while maintaining good oncological outcomes
TREATMENT AND HPV STATUS

Traditional therapies of HNSCC are generally associated with serious side effects (xerostomia, swallowing disorder, pain …)

As HPV-positive patients have a high likelihood of surviving their disease, post-treatment quality of life becomes of paramount importance.

This led to the treatment de-escalation concept: “less intensive treatment regimens could achieve similar efficacy with less toxicity and improved quality of life”
**TREATMENT AND HPV STATUS**

Several de-escalation strategies have been/are being assessed (non-exhaustive list)⁴

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Phase</th>
<th>Population</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Substitution of cisplatin by cetuximab</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01302834</td>
<td>3</td>
<td>N=987</td>
<td>RT (70 Gy) with cisplatin (100 mg/m² X2) or weekly cetuximab</td>
</tr>
<tr>
<td>RTOG 1016</td>
<td></td>
<td>Stage III-IV</td>
<td></td>
</tr>
<tr>
<td>NCT01874171</td>
<td>3</td>
<td>N=304</td>
<td>RT (70 Gy) with cisplatin (100 mg/m² X3 ) or weekly cetuximab</td>
</tr>
<tr>
<td>De Escalate HPV</td>
<td></td>
<td>Stage III-IVA</td>
<td></td>
</tr>
<tr>
<td><strong>Induction chemotherapy followed by lower radiation dose or volume in good responders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01084083</td>
<td>2</td>
<td>N=80</td>
<td>Paclitaxel, cisplatin and cetuximab followed by low (54 Gy) or standard dose IMRT with cetuximab depending on the response to IC</td>
</tr>
<tr>
<td>ECOG 1308</td>
<td></td>
<td>Stage III, IV</td>
<td></td>
</tr>
<tr>
<td>NCT01706939</td>
<td>3</td>
<td>N=365</td>
<td>3 cycles TPF followed by low (56 Gy) or standard dose (70 Gy) IMRT with weekly cetuximab + carboplatin or carboplatin only, depending on the response to IC</td>
</tr>
<tr>
<td>Quaterback trial</td>
<td></td>
<td>Stage III, IV</td>
<td></td>
</tr>
<tr>
<td><strong>Induction chemotherapy followed by reduced (chemo)radiation dose and volume in good responders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT02258659</td>
<td>2</td>
<td>N=62</td>
<td>All patients receive 3 cycles of carboplatin and nab-paclitaxel and dose/volume-adapted radiotherapy</td>
</tr>
<tr>
<td>OPTIMA trial</td>
<td></td>
<td>Stage III-IV</td>
<td></td>
</tr>
<tr>
<td>1) Low-risk patients with 50% response received low-dose radiotherapy alone to 50 Gy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Low-risk patients with 30–50% response or high-risk patients with 50% response received low-dose CRT to 45 Gy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) All other patients, i.e., poor responders, receive regular-dose CRT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients also received de-escalated RT volumes limited to the first echelon of uninvolved nodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Radiation therapy alone (standard or reduced dose)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT02254278</td>
<td>2</td>
<td>N=295</td>
<td>Reduced dose IMRT (60 Gy) with or without cisplatin (40 mg/m²)</td>
</tr>
<tr>
<td>NRG HN002</td>
<td></td>
<td>Stage III, IV</td>
<td></td>
</tr>
<tr>
<td><strong>Upfront surgery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT02215265</td>
<td>3</td>
<td>N=1100</td>
<td>Transoral surgery followed by pathological risk stratification:</td>
</tr>
<tr>
<td>PATHOS Trial⁵</td>
<td></td>
<td>Stage III-IVA</td>
<td></td>
</tr>
<tr>
<td>(no T1–2 N1)</td>
<td></td>
<td></td>
<td>- Low-risk patients do not have adjuvant therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Intermediate-risk patients are randomised between 50 and 60 Gy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- High-risk patients are randomised between RT-CT and RT alone</td>
</tr>
</tbody>
</table>

CRT, cardiac resynchronisation therapy; IMRT, intensity-modulated radiation therapy; RT-CT, radiotherapy and chemotherapy.

TREATMENT AND HPV STATUS

Therapy de-escalation – The ECOG 1308 trial

Oncological outcomes in patients with CR treated with 54 Gy (n=51):

- 2-year PFS: 80% (95% CI: 0.65, 0.89)
- Low-risk group (Smoker <10 PY and <T4/N2c) had a 2-year PFS of 95% (0.71, 0.99)
- High-risk group composed of smokers >10 PY (2-year PFS: 65% [0.41, 0.82]), T4 (2-year PFS: 50% [0.11, 0.80]), N2c (2-year PFS: 73% [0.44, 0.89])

CR, clinical response; CT, chemotherapy (paclitaxel, cisplatin, cetuximab); NE, not evaluable; OPSCCs, oropharyngeal squamous cell carcinomas; PR, partial response; PY, pack years; SD, stable disease.

NE: tonsillectomy or extensive biopsy after baseline tumour measurement; CR/PR/SD: based on Recist criteria.

13/80 protocol deviations (5 CR had 70 Gy and 8 PR/CR had 54 Gy).

TREATMENT AND HPV STATUS

Therapy de-escalation – The ECOG 1308 trial

Tolerance and toxicity:

- The IC regimen was well tolerated as 96% of patients received all planned cycles.
- At 1 year, significantly fewer patients treated with 54 Gy of RT had difficulty swallowing solids (40% vs. 89%; p=0.011) or had impaired nutrition (10% vs. 44%; p=0.025).

Primary endpoint: 2-year PFS in the low-dose arm.

CR, clinical response; CT, chemotherapy (paclitaxel, cisplatin, cetuximab); NE, not evaluable; OPSCCs, oropharyngeal squamous cell carcinomas; PR, partial response; SD, stable disease; NE: tonsillectomy or extensive biopsy after baseline tumour measurement; CR/PR/SD: based on Recist criteria. 13/80 protocol deviations (5 CR had 70 Gy and 8 PR/CR had 54 Gy)

TREATMENT AND HPV STATUS

Therapy de-escalation

Replacement of cisplatin by cetuximab

What is the rational?

- More benefit for “HPV-like” patients
- Cetuximab is “supposed to be less” toxic than cisplatin
- HPV+ patients do well independently of treatment choice, as long as this conforms to current standard of care

Reprinted from The Lancet Oncology 11, Bonner JA, et al. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survivalle, 21–8, Copyright 2010, with permission from Elsevier.
Toxicities did not differ significantly between the two treatment regimens.
**PATHOS TRIAL DESIGN**

1082 patients
COP HPV-positive
T1-3, N0-N2b

Transoral surgery + neck dissection

**Histopathological assessment**

- **Low risk**
  - No adjuvant treatment

- **Intermediate risk**

- **High risk**

**Randomisation**

- **A**
  - B1 60 Gy (control arm)
  - B2 50 Gy (test arm)

- **B**
  - C1 60 Gy* + cisplatin (control arm)
  - C2 60 Gy* (test arm)

**Co-primary endpoints:**
- Swallowing function (MDADI) at 12 months (superiority)
- Overall survival (non-inferiority)

**Group A:** no pathological risk factors
**Group B:** close (1–5 mm) margins, T3 tumours (or T1–T2 tumours with additional risk factors, N2a–b (>1 malignant lymph node and/or a single lymph node >3 cm), perineural or vascular invasion
**Group C:** ‘positive’ (<1 mm) primary tumour margins or nodal extracapsular spread

*Centres may opt to boost high-risk sub-volume(s) for patients in Group C to 66 Gy in 30# → they should declare whether they will be doing so prior to recruiting to the trial

TREATMENT AND HPV STATUS

Therapy escalation for the subset of aggressive HPV-driven OPC (T4, N2/3, smokers >10 PY) – IMMUNEBOOST Trial

- 61 untreated HPV-driven OPSCC* at high-risk of relapse
- 41 OPC
- 20 OPC

CT-scan, MRI, PET-CT
Tumoural and blood samples
HPV-testing
Randomisation

Nivolumab

RT-CT<sup>a</sup> Follow up

D1 D15 D30

3 months after RT-CT completion:
CT-scan, MRI, PET-CT, blood samples

MRI, magnetic resonance imaging; PET-CT, Positron Emission Tomography – Computed Tomography; PY, pack years; RT-CT, radiotherapy and chemotherapy.

*Non metastatic patients amenable to curative treatment with chemoradiation.

a. In the experimental Arm, RT-CT starts 2 to 3 weeks after the second nivolumab infusion.
b. In the control arm, RT-CT starts after randomisation, once preparations are completed (dental care, RT-planning…)

1. Feasibility and Tolerance of Nivolumab Neoadjuvant Immunotherapy in High Risk HPV Driven Oropharynx Cancer (IMMUNEBOOST); ClinicalTrials.gov Identifier: NCT03838263; Principal Investigator: H. Mirghani
RISK OF SECOND HPV CANCER

In recent years, several authors\(^1\)–\(^5\) have questioned whether patients treated for a pre-invasive or invasive HPV-associated cancer may be at increased risk of a second HPV malignancy.

This question is relevant, as individuals who develop an HPV-related malignancy are less capable of clearing HPV infection compared with the general population* and therefore could have a second infection in a different anatomical site at the same time as the index infection** or later.

Most studies have concluded to an increased risk of second HPV-related cancers – although the risk remains low, this raises the question of screening.

* Most people get infected but only a small number will develop a disease;
** Concurrent HPV-infections at different anatomical sites are not rare.

RISK OF SECOND HPV CANCER

Long-lasting increased risk of human papillomavirus-related carcinomas and premalignancies after cervical intraepithelial neoplasia Grade 3: a population-based Cohort Study

- The aim of this study was to determine the risk of HPV-related carcinomas and premalignancies in women diagnosed with cervical intraepithelial neoplasia grade 3 (CIN3). Knowledge of this risk is important to preventing the development and progression of other HPV-related premalignancies and carcinomas, by considering prophylactic HPV vaccination and/or by paying increased attention to other HPV-related carcinomas and premalignancies when CIN3 is identified.

- Women diagnosed with a CIN3 between 1990 and 2010 were identified from the Dutch nationwide registry of histopathology and cytopathology (PALGA) and matched with a control group of women without CIN3. Subsequently, all cases of high-risk (hr) HPV–associated high-grade lesions and carcinomas in the anogenital region and oropharynx between 1990 and 2015 were extracted. Incidence rate ratios were estimated for carcinomas and premalignancies of the vulva, vagina, anus, and oropharynx.

- A total of 178,036 women were identified: 89,018 with a previous diagnosis of CIN3 and 89,018 matched control subjects without a history of CIN3. Women with a history of CIN3 showed increased risk of HPV-related carcinomas and premalignancies, with incidence rate ratios of 3.85 (95% CI, 2.32 to 6.37) for anal cancer, 6.68 (95% CI, 3.64 to 12.25) for anal intraepithelial neoplasia grade 3, 4.97 (95% CI, 3.26 to 7.57) for vulvar cancer, 13.66 (95% CI, 9.69 to 19.25) for vulvar intraepithelial neoplasia grade 3, 86.08 (95% CI, 11.98 to 618.08) for vaginal cancer, 25.65 (95% CI, 10.50 to 62.69) for vaginal intraepithelial neoplasia grade 3, and 5.51 (95% CI, 1.22 to 24.84) for oropharyngeal cancer. This risk remained significantly increased, even after long-term follow-up of up to 20 years.

PART 3

Prevention of HPV-related diseases and therapeutic vaccination
PRIMARY PREVENTION

Goal: To avoid the disease

Anti-HPV prophylactic vaccination was introduced in 2006/2007
Vaccines are safe – several millions of doses were administered worldwide\(^1-4\)
Efficacy against anogenital HPV infections* and pre-cancers is well documented\(^1-4\)

Cervarix, Gardasil, Gardasil-9*

*Genotypes included in the vaccine: Cervarix: HPV16/18; Gardasil: HPV6/11/16/18; Gardasil-9: HPV6/11/16/18/31/33/45/52/58

PRIMARY PREVENTION

Goal: To avoid the disease

An increasing literature supports the efficacy of vaccination to prevent oral HPV infection and related disease

Cervarix, Gardasil, Gardasil-9*

*Genotypes included in the vaccine: Cervarix: HPV16/18; Gardasil: HPV6/11/16/18; Gardasil-9: HPV6/11/16/18/31/33/45/52/58

PRIMARY PREVENTION
HPV vaccination integration in the national immunisation schedules in Europe

PROPHYLACTIC VACCINATION

Significant reduction of oral HPV infection¹⁻⁴

Effect of prophylactic human papillomavirus (HPV) vaccination on oral HPV infections among young adults in the United States

**Population:** 2627 individuals (18–33 years) enrolled in NHANES*

**Method:** Detection of HPV in oral rinses (PGMY09/11 – Linear Array) and assessment according to vaccination

**Results:** Oral HPV16/18/6/11 infections was significantly reduced in vaccinated vs. unvaccinated individuals (0.11% vs. 1.61%; p=0.008). This corresponded to an estimated 88.2% (95% CI: 5.7%, 98.5%) reduction in vaccine-type infections among vaccinated individuals

---

* National Health and Nutrition Examination Survey

PROPHYLACTIC VACCINATION

Induction of salivary anti-HPV antibodies¹,²

**Population:** 150 men (27–45 years)*

**Method:** Detection of anti-HPV16/18 Ab (IgG) in serum and saliva prior to vaccination and 7 months after

**Results:** Baseline: detectable anti-HPV16/18 Ab in sera of 19–21% individuals* and in <9% of salivary samples

After vaccination: All participants seroconverted and anti-HPV16/18 Ab were detectable in the saliva of most cases (92.3% and 72.1%, respectively)

*Baseline oral HPV infection: HPV-16: 2.7% HPV-18 DNA: 0.7%

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¹ Pinto AL, et al. J Infect Dis 2016;214(8):1276–83. Reproduced under the terms of the Creative Commons Attribution IGO License (http://creativecommons.org/licenses/by/3.0/igo/legalcode. Accessed January 2020);

PROPHYLACTIC VACCINATION

Significant reduction of laryngeal papillomatosis

Goal: Assessment of RRP incidence in a population of children following the introduction of a HPV vaccination programme

Method: Monitoring by the APSU and 28 specialised ENT

Results: Among 15 incident cases, no mothers were vaccinated pre-pregnancy. Over the period of surveillance, the rate declined from 0.16 per 100,000 in 2012 to 0.022 per 100,000 in 2016 (Fisher's exact test, p=0.034)

RRP decreased by a factor of 0.614 (95% CI: 0.540, 0.698; p<0.001)

APSU, *Australian paediatric surveillance unit (National network of 1500 paediatricians that monitor rare paediatric diseases); ENT, ear, nose, and throat;

RRP, recurrent respiratory papillomatosis

PROPHYLACTIC VACCINATION

Goal: To avoid the disease

Several limiting factors:
1. Insufficient vaccine coverage†
2. Most programmes target only women
3. Vaccine introduction dates back to 2006

*Genotypes included in the vaccine: Cervarix: HPV16/18; Gardasil: HPV6/11/16/18; Gardasil-9: HPV6/11/16/18/31/33/45/52/58
† Excepted in some countries where vaccine coverage is >70–80% (Canada, Australia, UK…)
# PROPHYLACTIC VACCINATION

Evaluation of HPV vaccine refusal and parental reasons for this refusal

<table>
<thead>
<tr>
<th>Study</th>
<th>Survey period, location</th>
<th>Participants</th>
<th>% Refusal HPV immunization</th>
<th>Parental reasons for refusal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darden <em>et al.</em> (2013) [6]</td>
<td>2008–2010, US</td>
<td>$N = 98,000$ parents of boys or girls age 13–17</td>
<td>40–44% ‘had ever refused’</td>
<td>‘Safety concerns/side effects’ tripled as the main reason cited for refusal, from 4% to 16%</td>
</tr>
<tr>
<td>Kester <em>et al.</em> (2013) [7]</td>
<td>2010, US</td>
<td>$N = 501$ parents of girls age 14–17</td>
<td>51% had not vaccinated</td>
<td>36% cited ‘Concern for vaccine side effect’ as one of the reasons</td>
</tr>
<tr>
<td>Dorell <em>et al.</em> (2014) [5]</td>
<td>2010, US</td>
<td>$N = 4103$ parents of girls age 13–17</td>
<td>20% refused</td>
<td>55% cited ‘Concern about short-term problems like fever or discomfort’ as one of the reasons</td>
</tr>
<tr>
<td>Gilbert <em>et al.</em> (2016) [4]</td>
<td>2013, Canada</td>
<td>$N = 5720$ parents of girls age 12–14</td>
<td>14% refused</td>
<td>36% ‘concerned about the potential side effects of vaccines’ as one of the reasons</td>
</tr>
<tr>
<td>Gilkey <em>et al.</em> (2017) [8*]</td>
<td>2014–2015, US</td>
<td>$N = 1484$ parents of boys or girls age 11–17</td>
<td>29% refused</td>
<td>18% cited ‘Concern for short-term health problems’ as one of the reasons</td>
</tr>
<tr>
<td>Dayal <em>et al.</em> (2017) [9]</td>
<td>2015, US (Texas)</td>
<td>$N = 60$ parents of girls age 9–18</td>
<td>23% refused</td>
<td>‘Perceived HPV vaccine harm’ was the most predictive of parental refusal</td>
</tr>
</tbody>
</table>

PROPHYLACTIC VACCINATION

In 2017, 1 girl in 5 was vaccinated in France (3 doses)

**PROPHYLACTIC VACCINATION**

Goal: To avoid the disease

Several limiting factors:

1. Insufficient vaccine coverage†
2. Most programmes target only women
3. Vaccine introduction dates back to 2006

*Cervarix, Gardasil, Gardasil-9*

†Genotypes included in the vaccine: Cervarix: HPV16/18; Gardasil: HPV6/11/16/18; Gardasil-9: HPV6/11/16/18/31/33/45/52/58

†Excepted in some countries where vaccine coverage is >70–80% (Canada, Australia, UK…)
PROPHYLACTIC VACCINATION

Goal: To avoid the disease

Countries with gender neutral anti-HPV vaccination programme*
(updated in November 2018)

*UK, Germany and Denmark are underway
PROPHYLACTIC VACCINATION

Goal: To avoid the disease

Several limiting factors:
1. Insufficient vaccine coverage†
2. Most programmes target only women
3. Vaccine introduction dates back to 2006

Genotypes included in the vaccine: Cervarix: HPV16/18; Gardasil: HPV6/11/16/18; Gardasil-9: HPV6/11/16/18/31/33/45/52/58
†Excepted in some countries where vaccine coverage is >70–80% (Canada, Australia, UK…)
SECONDARY PREVENTION

Goal: Early identification and treatment of the disease (pre-cancer +++)

Conventional (Pap) test

Liquid-based cytology

Papanicolaou staining
SECONDARY PREVENTION
Goal: Early identification and treatment of the disease (pre-cancer +++)

Pap smear screening has revolutionised cervical cancer care

In the “1930s”: Cervical cancer was the first cause of cancer death in the US … 90% reduction in 2000s

Image courtesy of Prof Cécile Badoual
SECONDARY PREVENTION

Goal: Early identification and treatment of the disease (pre-cancer +++)

Cervical cancer screening:

Despite the undeniable contribution of cytology, it is limited by a lack of sensitivity (<70%). Consequently, cytological screening must be repeated regularly, from the onset of sexual activity to the age of 65 years. Identification of HR-HPV cervical infection (HPV testing) represents a promising screening strategy that is much more sensitive than cytology. A negative test almost completely eliminates the risk of development of a precancerous or cancerous lesion within the next 5 years (NPV >99%). However, its specificity is weak; a positive result can simply reflect a transient infection, which is a frequent situation with no consequence, particularly in young women. Screening policies varies from a country to another and relies on cytology or a combination of cytology and HPV testing.

NPV, negative predictive value.
SECONDARY PREVENTION

Goal: Early identification and treatment of the disease (pre-cancer +++)

For cervical cancer, secondary prevention is classically based on cytology. Unfortunately, this method is not effective in the oropharynx because early lesions occur in the depths of the tonsillar crypts, a site that is not reachable by brushing.
SECONDARY PREVENTION

Goal: Early identification and treatment of the disease (pre-cancer +++)

Anal cancer

Cytology

Normal

ASC-US

LSIL

HSIL (or ASC-H)

Repeat in 12 months (HIV+)
Repeat in 2–3 years (HIV-)

Anoscopy with biopsy

No lesion seen

AIN I

Follow-up in 6 months or treat if minimal potential for morbidity

AIN II or III

Treat

One of the protocols for screening of anal intraepithelial neoplasia (AIN)

ASC-H, atypical squamous cells, cannot rule out HSIL; ASC-US, atypical squamous cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.
## TERTIARY PREVENTION

**Goal:** Early identification of recurrences

### Specific biomarkers for HPV-driven cancers: Fact of fiction?

Prognostic implication of persistent human papillomavirus Type 16 DNA detection in oral rinses for human papillomavirus-related oropharyngeal carcinoma

<table>
<thead>
<tr>
<th>Persistent oral HPV16 DNA detected</th>
<th>N (%)</th>
<th>Participants with recurrent disease</th>
<th>Recurrences including distant disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Isolated local or local + regional recurrences</td>
<td>Locoregional + distant</td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>Yes: 5 (100) Local only: 1 (20) Local + regional: 1 (20)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>No</td>
<td>119</td>
<td>No: 9 (8) Local only: 1 (11) Local + regional: 0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>Total: 14</td>
<td>4 (28) Local only: 1 (7) Local + regional: 1 (7)</td>
</tr>
</tbody>
</table>

PART 4

HPV therapeutic vaccination and Perspectives
HPV THERAPEUTIC VACCINATION

HPV THERAPEUTIC VACCINATION

Therapeutic vaccines contain specific proteins that trigger an immune system (cytotoxic T lymphocyte) response to attack high-risk HPV types.

The E6 and E7 genes are ideal targets for vaccine therapy due to their role in the cell cycle and their constitutive expression in premalignant and malignant tissues.

Different strategies have been tried for therapeutic vaccines: protein-based and cell-based vaccines, nucleic acid, peptide and even live vectors. Combination approaches are also developed.
## HPV THERAPEUTIC VACCINATION

Several vaccine candidates are progressing to clinical trials

<table>
<thead>
<tr>
<th>Platform</th>
<th>Treatment</th>
<th>Adjuvant</th>
<th>Route of Injection</th>
<th>Phase</th>
<th>Patient Population</th>
<th>Immune Response</th>
<th>Clinical Response</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide</td>
<td>Four HPV16-E6 peptides</td>
<td>Yeast extract (Candin®)</td>
<td>IL</td>
<td>1</td>
<td>300 HSIL</td>
<td>Ongoing</td>
<td>Ongoing</td>
<td>NCT01653249</td>
</tr>
<tr>
<td>Peptide</td>
<td>MAG+B10:F24 and HPV16 peptides MAGE-A3 and HPV16 peptides</td>
<td>GM-CSF and Montanide ISA 51</td>
<td>SC</td>
<td>1</td>
<td>90 recurrent, progressive or metastatic HNSCC</td>
<td>Ongoing</td>
<td>Ongoing</td>
<td>NCT00257738</td>
</tr>
<tr>
<td>Protein</td>
<td>ProCervix</td>
<td>Imiquimod</td>
<td>ID</td>
<td>2</td>
<td>220 HPV16+ and/or 18+ women with normal cervical cytology or mild cervical cellular abnormalities</td>
<td>Ongoing</td>
<td>Ongoing</td>
<td>PC10VAC02 NCT01957878</td>
</tr>
<tr>
<td>Protein</td>
<td>HPV16 E7 + adenylate cyclase (ProCervix)</td>
<td>Imiquimod</td>
<td>ID</td>
<td>1</td>
<td>47 HPV16+ and/or 18+ women with normal cervical cytology</td>
<td>Antigen-specific T cell responses</td>
<td>High viral clearance</td>
<td>PC10VAC01</td>
</tr>
<tr>
<td>DNA</td>
<td>Plasmid encoding HPV16 E7(detox) fused to calreticulin (CRT) (pNGVL-4a-CRT/E7(detox))</td>
<td>—</td>
<td>IM with electroporation and cyclophosphamide</td>
<td>1</td>
<td>21 HNSCC</td>
<td>Ongoing</td>
<td>Ongoing</td>
<td>NCT01493154</td>
</tr>
<tr>
<td>DNA</td>
<td>Mixture of 2 plasmids encoding HPV16 and HPV18 E6 and E7 (VGX-3100)</td>
<td>—</td>
<td>IM with electroporation</td>
<td>2</td>
<td>167 CIN (II-IIII)</td>
<td>CTL response</td>
<td>53/107 CR</td>
<td>Trimble CL, et al. 2015</td>
</tr>
<tr>
<td>Viral</td>
<td>Modified vaccinia virus Ankara encoding BPV E2 (MVA-E2)</td>
<td>—</td>
<td>Intracervical</td>
<td>1/2</td>
<td>36 CIN (I-IIII)</td>
<td>HPV-specific CTL responses – all patients</td>
<td>34 CR</td>
<td>Corona Gutierrez CM, et al. 2004</td>
</tr>
<tr>
<td>ACT</td>
<td>Autologous CTL generated and expanded from co-culture with partially resected tumour combined with low-dose IL2</td>
<td>—</td>
<td>IV</td>
<td>1</td>
<td>18 advanced stage cervical cancer</td>
<td>Ongoing</td>
<td>Ongoing</td>
<td>NCT01585428</td>
</tr>
<tr>
<td>Checkpoint Inhibitor</td>
<td>Ipilimumab (anti-CTLA-4) post chemo/radiation treatments</td>
<td>—</td>
<td>IV</td>
<td>1</td>
<td>28 advanced stage cervical cancer</td>
<td>Ongoing</td>
<td>Ongoing</td>
<td>NCT01711515</td>
</tr>
</tbody>
</table>

ID, intradermal; IL, intralvesional; IM, intramuscular; IV, intravenous.
HPV INFECTION

Summary

• Mucosal HPV infection is sexually transmitted
• The prevalence and the natural history of HPV infection varies from one anatomical site to another
• It is not possible to precisely date the time of infection and the vast majority of infection will spontaneously clear
• Persistent infection is very rare and depends on several factors related to the host, the virus and the environment
• The cervical mucosal lining (squamo-columnar zone) is more susceptible to HPV-induced pre-cancer and cancer than the other anatomical sites (anal canal, oropharynx…)
• No intervention is currently recommended if a high-risk HPV is identified without any high-grade lesion or carcinoma
There is a wide range of assays to identify HPV infection and HPV-driven cancers.

The choice will depend on several factors including:

- The advantages and drawbacks of each test
- The population (healthy individuals vs. pre/cancer patients)
- The anatomical location of interest (cervix, anus, oropharynx…)
CONCLUSIONS

• HPV infection is extremely frequent
  - Not one but multiple HPV
  - Not one but multiple diseases
  - Not one but multiple cancers
  - Not one but multiple patients

• Prevention: The cornerstone to fight against HPV-driven cancers, as these diseases are largely preventable

• Vaccination:
  - Primary prevention (vaccination) should be promoted to drastically reduce HPV infection and related diseases – particularly cancers. HPV vaccines are safe and effective. Fake news must be strongly denied
  - Secondary prevention (cytology, HPV testing) is a priority to identify pre- and early-stage cervical cancers to achieve a high rate of cure with limited side effects. Cervical cancers are a public health concern in most parts of the world

• Targeted therapy: Targeted therapies, including therapeutic vaccine, are promising and are currently under intensive research
THANK YOU!