A global epidemic increase of an HPV-induced tonsil and tongue base cancer – potential benefit from a pan-gender use of HPV vaccine

A. Näsman1,†, J. Du2,† & T. Dalianis1

From the 1Department of Oncology-Pathology; and 2Department of Microbiology, Tumor Biology and Cell Biology, Centre for Translational Microbiome Research (CTMR), Karolinska Institutet, Stockholm, Sweden

Abstract. Näsman A, Du J, Dalianis T (Karolinska Institutet, Stockholm, Sweden). A global epidemic increase of an HPV-induced tonsil and tongue base cancer – potential benefit from a pan-gender use of HPV vaccine (Review). J Intern Med 2020; 287: 134–152.

In 2007, human papillomavirus (HPV) type 16 was finally recognized as a risk factor, besides smoking and alcohol, for oropharyngeal squamous cell carcinoma (OPSCC), including tonsillar squamous cell carcinoma (TSCC), by the International Agency for Research against Cancer. Just before, in 2006, the Food and Drug Administration had approved Gardasil, the first vaccine against HPV16, 18, 6 and 11, for preventive vaccination women against cervical cancer. Concurrently, some Western countries, where smoking was decreasing, disclosed an epidemic increase in the incidence of OPSCC, especially of TSCC and base of tongue cancer (BOTSCC), the two main HPV-positive OPSCC sites, together accounting for 80–90% of all OPSCCs, and mainly affecting men. The epidemic was later revealed to be due to a rise in HPV-positive cases, and scientists in the field suggested HPV vaccination also of boys. Globally, there are roughly 96 000 incident OPSCC cases/year of which 20–24% are caused by HPV, thereby accounting for around 22 000 OPSCC cases annually. Of these cases, 80–90% are due to HPV16 infection and would be prevented with the presently registered HPV vaccines. In Western countries, such as Sweden (with almost 400 TSCC and BOTSCC cases per year) and the United States, HPV prevalence in OPSCC is higher and around 70%. HPV vaccination of girls has been initiated in many countries, and the vaccines have been efficient and their side effects limited. HPV vaccination of boys has, however, been the exception, but should definitely not be delayed any further. It would benefit both girls and boys directly, and result in better and more robust herd immunity. Today, we have the possibility to eliminate several high-risk HPV types in the younger generations and avoid more than 600 000 cancer cases annually worldwide, and this possibility should be embraced by offering global pan-gender HPV vaccination.

Keywords: base of tongue cancer, head and neck cancer, HPV vaccines, human papillomavirus, oropharyngeal cancer, tonsillar cancer.

Introduction

In 2007, based on reports by others and us, human papillomavirus (HPV) type 16 (HPV16) was recognized as a risk factor for oropharyngeal squamous cell carcinoma (OPSCC) and tonsillar squamous cell carcinoma (TSCC) by the International Agency for Research against Cancer (IARC) [1–3]. During the same period, an increased incidence of OPSCC, especially of TSCC and base of tongue cancer (BOTSCC), together accounting for 80–90% of all OPSCCs, and mainly affecting men. The epidemic was later revealed to be due to a rise in HPV-positive cases, and scientists in the field suggested HPV vaccination also of boys. Globally, there are roughly 96 000 incident OPSCC cases/year of which 20–24% are caused by HPV, thereby accounting for around 22 000 OPSCC cases annually. Of these cases, 80–90% are due to HPV16 infection and would be prevented with the presently registered HPV vaccines. In Western countries, such as Sweden (with almost 400 TSCC and BOTSCC cases per year) and the United States, HPV prevalence in OPSCC is higher and around 70%. HPV vaccination of girls has been initiated in many countries, and the vaccines have been efficient and their side effects limited. HPV vaccination of boys has, however, been the exception, but should definitely not be delayed any further. It would benefit both girls and boys directly, and result in better and more robust herd immunity. Today, we have the possibility to eliminate several high-risk HPV types in the younger generations and avoid more than 600 000 cancer cases annually worldwide, and this possibility should be embraced by offering global pan-gender HPV vaccination.

Keywords: base of tongue cancer, head and neck cancer, HPV vaccines, human papillomavirus, oropharyngeal cancer, tonsillar cancer.

Western countries [4–15]. The reason for this sharp rise was later found due to an increase in the incidence of the corresponding HPV-positive cases [6–10, 12–15]. Notably, patients with HPV-positive OPSCC, TSCC and BOTSCC were younger and had much better outcome (80% vs. 25–50% 5-year disease-specific survival), irrespective of treatment regime, as compared to patients with corresponding HPV-negative cancer and head–neck squamous cell carcinoma (HNSCC) in general [1, 2, 6, 12–18]. Lately, due to the poor prognosis of HNSCC, its therapy including that of TSCC and BOTSCC has been intensified with induction chemotherapy (CT)
and hyper-fractionated radiation, or concurrent chemoradiotherapy (CRT) and sometimes, epidermal growth factor receptor (EGFR) inhibitors [13, 18–21]. These treatments result in more severe side effects, for example xerostomia, difficulties in talking, and eating and may prevent the return to normal life and work [18–22]. Intensified therapy does not always improve outcome and is often not needed for the majority of patients with HPV-positive TSCC and BOTSCC, since they usually respond well to less exhaustive therapy [1–3, 18–22]. For these reasons, there is an immediate need to identify patients with HPV-positive TSCC and BOTSCC that would benefit from less aggressive treatment or targeted therapy, and attempts focus on uncovering prognostic, as well as targetable markers [23–38]. In parallel, experts in the field emphasize the importance of urgently vaccinating boys against HPV [8, 14, 15]. Below, a background and update of the field of HPV-positive TSCC and BOTSCC, and the current global HPV vaccination situation are presented.

Historic background

The presence of genital warts was described already by the ancient Greeks, and 1760–1839, in Verona, it was reported that cervical cancer was much more common in prostitutes than in nuns [39]. A hundred years later in 1941, Georgios Papanicoloas showed that cellular changes were present in the cervix before invasive cancer appeared [40]. Together, these findings suggested an infective agent being the culprit. However, in 1983 when HPV16 was detected in cervical cancer, this finding was not only complex, due to a plethora of HPV types concurrently being disclosed, but also controversial, by being a challenge to the existing opinion that Herpes simplex virus 2 (HSV-2) was liable for the development of cervical cancer [41, 42]. Finally, in 1995, HPV16 was acknowledged as being carcinogenic by IARC [43]. In 2006, the first vaccine against HPV16, 18 and 11 (Gardasil) was approved by the Food and Drug Administration (FDA) and a second vaccine (Cervarix) against HPV16 and 18 was validated in 2007 [44–47]. Soon after in 2008, Harald zur Hausen was awarded the Nobel Prize. That HPV was associated with OPSCC and TSCC, mainly affecting men, was reported already in 2000, but it took until 2007, until HPV was (besides the traditional risk factors smoking and alcohol) recognized as a risk factor for OPSCC and TSCC by IARC [1–3]. By then, reports on an epidemic increase of these diseases had already been observed in many Western countries [6–10, 12–15]. Since 2014, the nonavalent HPV vaccine (Gardasil9) (against HPV16, 18, 31, 33, 45, 52, 58, and 6 and 11) has been available, and HPV vaccination of girls is ongoing, but the vaccination of boys has still not yet been initiated in most countries [44–47].

HPV, its genome and diseases

HPV and its genome

There are >200 HPV types, where most are found in the skin, and where roughly 40 are detected in the mucosa, and with the latter often defined as low-risk, high-risk or putatively high-risk HPV types [48–50]. All HPV types have an ~8 kb double-stranded circular DNA genome surrounded by a 52–55 nm viral capsid [50]. The HPV genome is arbitrarily divided into a noncoding regulatory region, and an early and a late coding region (coding for the E1-E2, E4-E7 regulatory proteins and the L1 and L2 structural proteins) (Fig. 1) [48, 50]. The E1–E2, E4–E7 regulatory proteins are responsible for gene regulation, replication, pathogenesis and transformation [48–50]. In high-risk HPVs, E6 and E7 are regarded as oncogenes and deregulate cell cycle control. E6 binds and degrades p53, permitting cells with mutated or damaged DNA to enter the cell cycle without undergoing DNA repair and allowing the cells to harbour more mutations [49, 50]. E7 binds to the retinoblastoma protein (Rb) and disrupts its binding to the E2F transcription factor, resulting in E2F activation and progression of the cell cycle into synthesis phase (S-phase) [50]. This leads to over-expression of the cyclin-dependent kinase inhibitor p16INK4a (p16), often used as surrogate marker of HPV infection [49–53]. E5 is also regarded as an oncogene and is supportive of transformation [54, 55]. Furthermore, both E5 and E7 down-regulate major histocompatibility (MHC) class I antigens, thereby enhancing the virus to avoid immune recognition [54–56]. The late region encodes the major L1 and minor L2 viral capsid proteins [48–50]. L1 contributes with 360 molecules on the viral capsid surface, whilst L2 contributes with 12–20 molecules on the inside of the capsid [48–50]. L1 can under specific circumstances self-assemble into virus-like particles (VLPs), which constitute today’s HPV vaccines, but also L2 has been suggested to be useful for vaccine production [44–47].

HPV and diseases

Most HPVs are found asymptomatically in the skin, and some HPVs induce skin warts, but if HPV induces
skin cancer is not resolved, except in patients with epidermodysplasia verruciformis, an autosomal recessive disease, where some HPV infections are not cleared [50]. Mucosal low-risk HPV types, for example HPV6 and 11 can induce genital warts, condylomas and laryngeal papillomas [50]. High-risk HPV types (e.g. HPV16 and 18) are associated with virtually all cervical cancer cases and a considerable proportion of vulvar cancer, vaginal cancer, penile cancer and anal cancer, as well as OPSCC, more specifically TSCC and BOTSCC [50]. Together, HPV-positive tumours account for more than 600 000 cancer cases annually worldwide (Table 1) [50, 57, 58]. In TSCC and BOTSCC, the proportion of HPV-positive cases varies depending on geographical region, and the time periods when the investigations were performed [14]. HPV16 dominates and is responsible for 80–90% of all HPV-positive cases, whilst HPV18 frequently observed in cervical cancer is rarely found and less common as compared to, for example, HPV33 [6, 14, 16, 48, 50].

Methods to detect HPV in HNSCC, with EMPHASIS on OPSCC, TSCC AND BOTSCC

HPV DNA is detected in 0–100% in OPSCC, TSCC and BOTSCC [1, 2, 6–10, 12–17, 50]. This variation can depend on, for example, OPSCC subsite, since HPV DNA is mainly detected in TSCC and BOTSCC as compared to other OPSCC subsites [59, 60]. In addition, the time period and the country from which the samples were obtained both influence HPV prevalence, due to that in some countries, there has been an epidemic increase in HPV-positive cases and a varying decrease of smoking [6–15]. The material and the methodology used also influence HPV prevalence. Fresh-frozen and newly formalin-fixed paraffin-embedded (FFPE) tumours are superior to FFPE material stored for decades, since the latter is degraded and suboptimal to test for HPV DNA and RNA [14, 61]. Detection of HPV DNA is generally done using FFPE tumour tissue, but attempts have also been made to test for HPV DNA
in fine needle aspirate cytology [FNAC] [61, 62]. In the 1980s, Southern blot techniques or in situ hybridization was used to assay for HPV DNA, but their sensitivity was generally lower than that of polymerase chain (PCR)-based technology, where the elaboration of different general primers further

| Region                        | Total All cancer sites | Total HPV-related cancer sites | Total attributable to HPV | PAF (%) | Cervix uteri | Anus | Penis | Vulva/ Vagina | Oropharynx |
|------------------------------|------------------------|-------------------------------|---------------------------|---------|--------------|------|------|---------------|------------|
| **AFRICA**                   |                        |                               |                           |         |              |      |      |                |            |
| Sub-Saharan Africa           | 550,000                | 82,000                        | 78,000                    | 14.2    | 75,000       | 1,500| 330  | 940           | 390        |
| Northern Africa and Western Asia | 390,000                | 12,000                        | 11,000                    | 2.8     | 9,200        | 900  | <100 | 620           | 110        |
| **ASIA**                     |                        |                               |                           |         |              |      |      |                |            |
| India                        | 950,000                | 170,000                       | 150,000                   | 15.5    | 130,000      | 2,800| 3,500| 3,400         | 3,200      |
| Other Central Asia           | 470,000                | 48,000                        | 43,000                    | 9.0     | 39,000       | 1,800| <100 | 500           | 780        |
| China                        | 2,800,000              | 85,000                        | 80,000                    | 2.8     | 75,000       | 1,500| 1,200| 1,100         | 440        |
| Japan                        | 620,000                | 12,000                        | 11,000                    | 1.8     | 8,900        | 630  | 120  | 360           | 950        |
| Other Eastern Asia           | 1,000,000              | 62,000                        | 55,000                    | 5.4     | 51,000       | 1,500| 1,000| 1,200         | 710        |
| **AMERICA**                  |                        |                               |                           |         |              |      |      |                |            |
| Central and Southern America | 910,000                | 84,000                        | 75,000                    | 8.3     | 68,000       | 2,300| 1,400| 2,000         | 780        |
| Northern America             | 1,600,000              | 35,000                        | 26,000                    | 1.6     | 12,000       | 3,900| 670  | 2,900         | 6,200      |
| **EUROPE**                   |                        |                               |                           |         |              |      |      |                |            |
| Europe                       | 3,200,000              | 110,000                       | 80,000                    | 2.5     | 55,000       | 6,800| 2,400| 7,400         | 8,100      |
| **OCEANIA**                  |                        |                               |                           |         |              |      |      |                |            |
| Australia/ New Zealand       | 130,000                | 2,100                         | 1,600                     | 1.2     | 800          | 280  | <100 | 190           | 230        |
| Other Oceania                | 8,800                  | 920                           | 840                       | 9.4     | 800          | <100 | <100 | <100          | <100       |
| Less developed regions       | 7,100,000              | 550,000                       | 490,000                   | 6.9     | 450,000      | 12,000| 7,600| 9,800         | 6,400      |
| More developed regions       | 5,600,000              | 150,000                       | 120,000                   | 2.1     | 77,000       | 12,000| 3,200| 11,000        | 15,000     |
| **WORLD**                    | 12,700,000             | 700,000                       | 610,000                   | 4.8     | 530,000      | 24,000| 11,000| 21,000        | 22,000     |

PAF, population attributable fraction.

"HPV-associated cancer sites are as follows: cervix uteri, vulva, vagina, anus, penis and oropharynx including base of tongue and tonsils. Based on de Martel et al.[58]"
improved detection rates [32, 63–69]. These PCR techniques have been improved continuously [32, 67–69]. Still, the presence of HPV DNA cannot determine biological activity of HPV. Here, assaying for E6 and E7 mRNA expression is regarded as the golden standard and can be done by, for example, RT-PCR [61]. The combined presence of HPV DNA and p16 overexpression by immunohistochemistry (IHC) has, however, been shown to be almost as sensitive as the golden standard, and this approach is now used in many laboratories [60]. p16 overexpression (strong diffuse cytoplasmic and nuclear staining in >70% tumour cells) alone is nevertheless still frequently used as a surrogate marker for HPV, due to its correlation to the presence of HPV [70, 71]. This is unfortunate, since 5–10% of the p16 overexpressing TSCC and BOTSCC cases are not HPV DNA-positive and vice versa, and for OPSCC at other subsites, this discrepancy is higher [52, 59, 60].

HPV-Positive OPSCC, TSCC AND BOTSCC differ from corresponding HPV-negative cancer and have better clinical outcome

HPV-positive OPSCC, TSCC and BOTSCC, and characteristics of patients and their tumours

In 2000, the association between HPV and OPSCC and TSCC and that patients with HPV-positive cancer were younger and had much better clinical outcome than those with corresponding HPV-negative cancer was reported (Fig. 2a) [1, 2, 13, 14]. In 2004, a similar association of HPV to BOTSCC was found [70]. HPV-positive and HPV-negative OPSCC, TSCC and BOTSCC were suggested to be separate entities and have different characteristics, with the former due to HPV infection (often never smokers) and the latter due to smoking and alcohol [1–3, 13, 14]. Similar to cervical cancer, HPV-positive TSCC and BOTSCC generally possessed normal (and not mutated) p53 and overexpressed 16\(^{\text{INK4a}}\) contrary to corresponding HPV-negative cases [1–3]. HPV-positive TSCC was more frequently aneuploid, less differentiated and had similar to cervical and vulvar cancer chromosome 3q amplification, and independent of stage, differentiation or ploidy, patients had better prognosis than those with HPV-negative tumours [1–3, 72–75]. HPV was often episomal in the tumours, but whether the genome was integrated or episomal did not correlate to prognosis, whilst having a high viral load was a prognostic favourable factor [74, 75]. Viral genome integration into cancer cell genomes could, however, affect DNA methylation and viral and host gene expression [76]. Furthermore, when defining HPV-positive status only by the presence of HPV DNA, or only by p16 overexpression, never smokers with HPV-positive cancer had better clinical outcome than smokers [16, 52]. Later, defining HPV-positive cancer by the combined presence of HPV DNA and p16 overexpression, the influence on smoking on outcome was not as prominent [19]. Still, similar to cervical cancer, it was later disclosed that smoking also increased the risk of acquiring an HPV-positive OPSCC [77].

There are important differences between OPSCC sites and tumour location matters. In a meta-analysis, covering >50 studies, HPV was more frequently found in TSCC and BOTSCC compared

Fig. 2 Patients with HPV-positive tonsillar squamous cell carcinoma (TSCC) and patients with HPV-positive base of tongue squamous cell carcinoma (BOTSCC), respectively, have better disease-specific survival than patients with corresponding HPV-negative carcinomas (a and b, respectively). Patients with HPV-positive oropharyngeal squamous cell carcinoma of nontonsillar and base of tongue sites do not have a better disease-specific survival as compared to those with corresponding HPV-negative carcinoma (c). Published with permission, or according to the conditions required from the publishers, for a, b and c, respectively, from Lindquist et al., [16], Dahlgren et al., [70] and Marklund et al.,[59], respectively.
to other OPSCC sites (56% vs. 19%, respectively) [60]. This is also reflected by the epidemic increase of HPV-positive TSCC and BOTSCC, and not of other OPSCC subsites, and that HPV is a prognostic factor for TSCC and BOTSCC, and not for other OPSCC subsites (Fig. 2) [59, 78]. Notably, TSCC and BOTSCC arise in the tonsil and the base of tongue, two subsites that share a distinct histological appearance with a reticulated epithelium that invaginates into lymphatic tissue and forms crypts (Fig. 3) [60]. The importance of lymphatic tissue is also emphasized in that HPV-positive status occurs more frequently in specified, than nonspecified tonsillar tissue, and has better prognostic significance in specified TSCC as compared to that in nonspecified TSCC [79, 80].

The Union for International Cancer Control 8th edition (UICC8) staging of OPSCC an improvement, but with some disadvantages

p16 overexpressing and p16 non-overexpressing OPSCC are classified separately in the Union for International Cancer Control 8th edition (UICC8) [81, 82], In the earlier UICC 7th edition, all OPSCC were staged together, resulting in that small primary HPV-positive OPSCC with lymph node metastasis were classified as high-stage tumours, although their prognosis was better compared to that of corresponding stages of HPV-negative OPSCC [81, 82]. Despite p16-overexpressing OPSCC and p16 non-overexpressing OPSCC are classified separately in UICC8, treatment of these patients in the Nordic countries has not changed, partly due to the ambiguity of OPSCC subsite and the definition of HPV-positive status. The new staging system does namely not use TSCC and BOTSCC location, and optimal determination of HPV-positive status, since it includes all OPSCC and only evaluates p16 overexpression [81, 82]. This results in the risk of de-escalating treatment for a considerable number of OPSCC patients with a higher risk of disease relapse [59, 60]. In our opinion, the next UICC staging edition should classify only TSCC and BOTSCC, and not all OPSCC, and define HPV-positive status as tumours expressing either HPV E6 and/or E7 mRNA, or displaying presence of HPV DNA combined with p16 overexpression.

HPV in cancer of unknown primary (CUP) of the head and neck region and similarities with HPV-positive TSCC and BOTSCC

In the above context, some information on cancer of unknown primary (CUP) of the head and neck region could be relevant. CUP of the head and neck region has better outcome (35–50% 5-year survival) than CUP in general, with a mostly very dismal outcome [83–85]. Moreover, location of a squamous cell carcinoma (SCC) metastasis in level I or II of the neck can be due to an OPSCC [86]. The prevalence of HPV in CUP of the head–neck region was reported 18–52% depending on, for example, if tonsillectomy or biopsies were, or were not done, as well as geographical region, since HPV prevalence in TSCC and BOTSCC (OPSCC) can vary considerably between different countries [86–90]. Notably,

Fig. 3 Representative haematoxylin-eosin staining of specified tonsillar squamous cell carcinoma (a) and nonspecified tonsillar squamous cell carcinoma (b). Published according to the conditions required from the publisher from Haeggblom et al., [80].
patients with HPV-positive (HPV DNA and p16 overexpression) CUP of the head–neck region tend to have a better clinical outcome than those with corresponding HPV-negative CUP, with some data reaching statistical significance (80.0% vs. 36.7% 5-year overall survival (OS), respectively, \( P = 0.004 \)) [87, 89, 90]. Moreover, assessing HPV status in FNAC in head–neck masses indicated that an HPV-positive lymph node metastasis likely had an HPV-positive OPSCC, TSCC or BOTSCC origin, irrespective if the primary tumour was disclosed or not [62, 91–96]. Patients with HPV-positive CUP with SSC cytology should therefore likely receive similar treatment as those with HPV-positive TSCC and BOTSCC and be spared neck dissection and receive more limited radiation fields.

**HPV in OPSCC, TSCC and BOTSCC and an epidemic increase of these two latter tumour sub-types**

An increased incidence of TSCC/BOTSCC and OPSCC and the association with HPV

Soon after the first reports of HPV being causative of OPSCC and TSCC, several studies from, for example, Scotland, Sweden, Finland, the Netherlands, the United Kingdom and the United States, reported an increased incidence of OPSCC, especially TSCC [4–15]. The reason for this was initially obscure and caused considerable confusion, especially since the rise in incidence was occurring in countries, where smoking was declining. Furthermore, HPV prevalence in OPSCC, TSCC and BOTSCC varied between countries depending on, for example, the proportion of smokers in that country, the quality of the material, the methodology used and the time period at which the analysed samples were acquired [6–10, 12–15]. Eventually, it was revealed that some of the variation especially within countries, where an increased incidence of TSCC and BOTSCC was described, was due to an epidemic rise of HPV-positive cases [6–10, 12–15]. A report from Sweden by us was likely the first in its kind [6]. In 2006, using the Swedish Cancer Registry, which covers virtually all cancer cases in Sweden since 1953, we disclosed an increase in the incidence of TSCC (2.6-fold for men and 3.5-fold for women) for 1970–2002 in the Stockholm region, where around 25% of all Swedish TSCC patients are treated [6]. Moreover, when analysing 237 obtained TSCC samples from the 515 reported patients during the above period, a parallel rise in the percentage (23% to 68%) of HPV-positive TSCC was observed [6]. Similar developments were later reported from, for example, the United States, Greece and Denmark [13, 15, 79, 97–99]. In addition, in 2009 and 2010, studies from Sweden on TSCC and BOTSCC, respectively, and in 2011, on OPSCC from the United States, confirmed the increased incidences of HPV-positive cases and decreased incidences of HPV-negative cases, where the latter was ascribed to a decline in smoking (Fig. 4) [10, 12, 15].

**Reasons for the increased incidence of HPV-positive cases**

The reason for the rise in incidence of HPV-positive OPSCC was in due course assigned to changes in sexual habits, since a relation between HPV-positive OPSCC, early sex debut and numbers of oral or vaginal partners was observed [100]. The fact that HPV-positive OPSCC, TSCC and BOTSCC were all more frequently occurring in men than in women was enigmatic and has not entirely been resolved yet. Still, when analysing different factors, men generally had more lifetime sexual partners, which could be one contributing factor, whilst smoking also more frequent in men – at least in some countries – could be another [101]. Smoking has namely, as mentioned above, been shown to increase the risk of acquiring an HPV-positive OPSCC [77]. It has also been hypothesized that women develop a better immune response than men, after a corresponding genital HPV infection,
and are thereby more resistant to developing an OPSCC [102]. That the increased incidences of TSCC and BOTSCC were due to changes in the numbers of tonsillectomies per decade or to different ways of removing the tonsils has, however, been excluded [103, 104]. Irrespective of the reason for the observed epidemic increase of OPSCC, TSCC and BOTSCC, if these trends continue, it has been calculated that the number of cases in these categories will surpass the numbers of cervical cancer cases in, for example, both the United States and Denmark (Fig. 5) [15, 99].

Reflections regarding HPV tumour location, the epidemic and impact on survival

In the context of an epidemic due to HPV infection, emphasis on HPV tumour location and whether HPV has impact on outcome is of importance [78–80]. It must be highlighted that HPV is mainly found in TSCC and BOTSCC and not in other OPSCC subsites, and the rises in incidence of OPSCC are attributed to increases in TSCC (i.e. specified TSCC) and BOTSCC [10, 12, 60, 78–80]. The influence of HPV on survival is as stated above, limited to TSCC and BOTSCC, and not OPSCC at other subsites (Fig. 2) [59]. Historically, due to an original report on HPV and OPSCC, and because TSCC and BOTSCC account for 80–90% of all OPSCC, the distinction between OPSCC, and TSCC and BOTSCC is not always made [1, 79, 80]. To cover the scientific field, one has to follow reports on OPSCC, TSCC and BOTSCC. In the future, as mentioned above, being more specific, and using specified TSCC and BOTSCC location, with an improved definition of HPV-positive status, will likely be of benefit for diagnosis and prognostication, patient treatment, survival and quality of life.

New trends in the epidemic and changes in the age of the patients

In general, there is still an increase in incidence of HPV-positive OPSCC, TSCC and BOTSCC in many countries, but some variations have been reported, both with regard to the age groups affected, and as to whether one studies urban or rural areas [14, 15, 79, 98, 99, 105–111]. Following the incidences of TSCC and BOTSCC in Stockholm, more specifically, for the years 2000–2012, we noted a stabilization of the increased incidence of TSCC in Stockholm for some years, but not for BOTSCC, or for TSCC and BOTSCC in Sweden as a whole [105]. Possible differences in trends of OPSCC, TSCC and BOTSCC depending on whether one is covering rural or urban areas were also recorded by others, but the data were not concordant over time, which is very likely due to the complexity of geographical areas [106, 107, 110]. In some cases, rural areas had more HPV-associated cancers than urban areas, and in some cases, the nonmetropolitan counties initially had less HPV-associated TSCC and BOTSCC, but encountered increased incidences later in time [106, 107, 110].

The incidences of TSCC and BOTSCC were followed in more detail for the period 2000–2016 in Stockholm, and we noted they had continued to rise especially between 2008 and 2016 [108]. The less steep increased incidence curves between the earlier time periods 2000–2004 and 2005–2008, we assume may have been due to changes in sexual behaviour in the early 1990s due to the HIV epidemic. This assumption was supported by a documented decrease in the number of chlamydia cases in the Stockholm region from 1988, with a major dip 1992–1998, just before the introduction of HIV medication, a period followed by an increase of Chlamydia cases [112]. This would hypothetically indicate a lag period of roughly 20-30 years between HPV infection and the development of TSCC.

Fig. 5 Observed and projected incidence rates and bootstrap 95% CIs (30 to 84 years of age), for oropharyngeal cancers overall (solid squares), oropharyngeal cancers amongst men (solid circles), oropharyngeal cancers amongst women (open circles) and cervical cancers (open squares). Adapted from Carlander et al., [99] and printed with permission from the publisher.
Notably, it has also been observed that in both the United States and in Stockholm, the median age for patients with HPV-positive OPSCC, TSCC and BOTSCC has gone up over the years [6, 10, 105, 108]. In our studies from Stockholm, during the period 1970–2002, the median age of patients with HPV-positive cancer was 55 years of age as compared to those with HPV-negative cancer, which was 65 years of age [6]. In a later study in Stockholm, patients with HPV-positive cancer diagnosed 2013–2016 were on average 61.2 years of age and those with HPV-negative cancer were 66.7 years of age [108]. We suspect that we are encountering older patients from a cohort that was infected approximately 30 years ago. However, the numbers of our patients are limited and one should not draw too many conclusions. Nevertheless, in a very recent study on different populations in the United States, the authors suggest that the rise in incidence is increasing in older men over 65 years of age and that in younger men the increase in incidence has ebbed for the time being [109]. The reason for the decrease in younger men was suggested to be due to a decrease of sexual activity in this group, as indicated by a decline in this group of the seroprevalence of HSV-2, a surrogate for high-risk sexual behaviour [113]. This should be important information with regard to the planning of treatment of an older cohort [22, 109]. On the other hand, trends in Stockholm and with increases in Chlamydia infections, since 1998, suggest a more recent increase in sexual activity and may point towards the risk for a new sharp rise in incidence of TSCC and BOTSCC in younger men within the next decade or two [108]. It is also likely that the same shifts will be seen also in other countries.

Attempts to screen for OPSCC, TSCC and BOTSCC

Today, there is no screening for OPSCC, TSCC and BOTSCC, since in contrast to cervical cancer corresponding prestages including cellular abnormalities are not readily observed in tumour patients [114, 115]. The presence of HPV DNA in mouthwashes as indicative of HPV infection, or HPV-positive TSCC and BOTSCC was also explored [115–120]. HPV prevalence in mouthwashes varied between 1% and 20.7% in healthy individuals, sometimes with and sometimes without gender differences [115–117, 119–122]. The quantity of HPV DNA in mouthwashes was generally lower than that obtained in cervical samples, likely due to the daily production of 0.5–1.5 litre of saliva, and subsequently, it is more complicated to identify an HPV infection in the oral cavity as compared to the cervix [115, 120]. When examining HPV prevalence in tonsillar swabs and mouthwashes from patients with TSCC, BOTSCC and HNSCC and benign conditions, an HPV-positive mouthwash was indicative of an HPV-positive TSCC or BOTSCC [115]. More specifically, HPV DNA was present in 82% and 50%, respectively, of mouthwash from respective TSCC and BOTSCC patients, as compared to 14% in those with other HNSCC and benign conditions [115]. The HPV DNA signal was generally stronger in mouthwashes from TSCC and BOTSCC patients compared to that in healthy youth, but still often weaker than that in cervical samples [115, 120]. HPV prevalence in mouthwashes was also studied in patients undergoing tonsillectomy, but there was no association between presence of HPV in their oral samples and in the tonsillectomies, where HPV was not found [121–123]. Antibodies to HPV16 E6 and E7 have, however, been observed in patient sera 10 years prior to the development of OPSCC, but so far there is no optimal strategy for using this approach for individual screening [124, 125].

Treatment of OPSCC, TSCC and BOTSCC

Patients with HPV-positive TSCC and BOTSCC often have nodal spread and generally seek help when their tumours are spread, and are therefore mainly given the aggressive treatment offered to other HNSCC patients [13–22]. Radiotherapy (RT) is delivered at high doses for 6–7 weeks. Patients with advanced stage (III–IV), according to UICC7, are treated with chemotherapy (CT) or chemoradiotherapy (CRT) and sometimes epidermal growth factor receptor (EGFR) blockers, for example cetuximab [20]. Treatment is, however, not only determined by the tumour burden, but also by whether the patients are fit medically [20]. Intensified therapy may result in complications with regard to swallowing and eating due to radiation, as well as nausea, mucositis and local and/or systemic infections and fatigue. Patients with lymph node metastases persisting after RT are managed with neck dissection. CRT and surgery cause more side effects than RT alone, with more fibrosis, stiffness of the neck, less mobility and worsening of the possibility to swallow due to RT. Later adverse effects are, for example, xerostomia, taste alterations, swallowing problems, trismus and diminishing of hearing, and some present radio-osteonecrosis needing reconstruction surgery.
Prognostication focusing on quality of life and not only survival is essential for the patients. Notably, in parallel, to therapy intensification, patient numbers with HPV-positive TSCC and BOTSCC are growing, with patients of varying ages, and most do not need aggressive therapy [126]. However, to personalize medicine, better means to identify patients that will respond well clinically or could benefit by targeted therapy are needed [126]. There are clinical trials decreasing RT, or replacing cisplatin by cetuximab, using p16 overexpression as surrogate marker for HPV in OPSCC [21]. These trials allow for suboptimal selection of the patients included in the trials, because 10–15% of their patients with a p16-positive cancer will not have a truly HPV-driven cancer, and the data obtained can be false, since recurrences are rare.

Studies on different biomarkers and clinical markers and re-staging of HPV-positive TSCC and BOTSCC for personalized medicine

Attempts have been made to find TSCC and BOTSCC patients that responded well to current therapy or need alternative, for example targeted therapy [126]. Early studies disclosing differences in outcome depending on HPV status resulted in, for example, UPCCI8, classifying OPSCC based on p16 overexpression, separately [81, 82]. In parallel, other markers were examined [23–38, 127–145]. Notably, HPV-positive TSCC and BOTSCC usually have more CD8+ T lymphocytes than HPV-negative cancer, and high CD8+ counts correlated to better prognosis regardless of HPV status [31–34]. In one of those studies, in HPV-negative tumours, increased CD68+ macrophage counts and expressing PD-L1 were favourable [34]. Surprisingly, absent/low major histocompatibility complex (MHC) class I expression had a positive prognostic influence in HPV-positive TSCC and BOTSCC, whilst the opposite occurred in HPV-negative cancer, and the presence of HLA-A*02 also influenced outcome [25–27]. MHC class I, antigen peptide transporter 2 (TAP2), proteasome units LMP2 and LMP7 expression were often reduced in HPV-positive cases, and decreased LMP7 and LMP10 expression correlated to better survival [28, 29]. Down-regulation of MHC class I by HPV E5 and E7 was assumed to be up-regulated upon RT and partly shown experimentally, whilst the absence of HPV 16 E2 mRNA correlated to worse prognosis [19, 127]. Alongside, low CD44 or low CD98 expression was advantageous regardless of HPV status, whilst high leucine-rich repeats and immunoglobulin-like domain 1 (LRIG1) suppressor gene expression were associated with improved clinical outcome in HPV-positive cancer [16, 24, 30, 35]. In addition, different algorithms or nomograms have been used to combine different markers for better prognostication [19, 146, 147].

Molecularly, next-generation sequencing (NGS) and sequencing in general showed differences depending on HPV status [36–38, 128–131]. HPV-positive OPSCC, TSCC and BOTSCC had increased mutation rates in, for example, phosphatidylinositol 3-kinase, catalytic subunit alpha (PIK3CA), notch homolog 1 (NOTCH1) and the fibroblast growth factor 3 (FGFR3) [37, 38]. HPV-negative tumours had mutations in tumour protein (TP53 and cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B) [37, 38]. Associations of these genes with outcome were shown, but the data were inconsistent [37, 38]. However, a number of drugs that target the PI3K pathway (including PI3KCA) and FGFR (including FGFR3), as well as p53, are available indicating that targeted therapy could be of use for HNSCC, including HPV-positive TSCC and BOTSCC [148–152].

Other studies have investigated RNA expression. MicroRNA expression has been explored in OPSCC, TSCC and BOTSCC with regard to both HPV and outcome, but the obtained data with regard to their prognostic value have been inconsistent between studies [134–140, 153]. RNA expression according to HPV status and also heterogeneity in transcription between HPV-positive tumours and outcome have been described [141–145]. Using transcriptional analysis, CD8a was notably correlated to HPV status and superior clinical outcome [145]. Reverse-phase protein array profiling on a limited set of total and phosphorylated proteins was attempted and the proteomic analysis disclosed differences in, for example, PI3Kserine-threonine protein kinase (AKT)/mammalian target of rapamycin (mTOR) [133]. Likewise, proteomic profiling by mass spectrometry showed differences in pathways in HPV-positive and HPV-negative OPSCC [132].

Research on the microbiome and HPV infection is still limited. Significant variations in microbiota have been reported in oral squamous cell carcinoma (OSCC) patients [154]. However, no specific species were showed consistently to be associated with OSCC. Such data in other sites of HNSCC are very preliminary and need to be pursued further.
The HNSCC-related shifts in the variations of the microbiome may allow for an alternative potential explanation for HNSCC progression as well as a therapeutic potential.

**HPV vaccines**

**Background and present HPV vaccines**

Already in 1978, it was shown that purified major capsid protein VP1 of murine polyomavirus (previously belonging to the papovavirus family, where HPV was also included in before) could, upon renaturation, form pentameric subunits, which could assemble into VLPs [155]. Knowledge regarding the potential possibility to form HPV VLPs was therefore available early on. However, large-scale production was not resolved at the time, despite that it was known that VP1 or L1 pentameric subunits could be produced in mammal and yeast cells, insect cells using baculoviruses, *Escherichia coli* or tobacco chloroplasts [156–160]. The choice finally depended on the ease of production, safety and economical issues. The present HPV VLP vaccines have been established on an industrial scale in yeast (Gardasil®, Merck, NJ, USA) and baculovirus (Cervarix®, GlaxoSmithKline, UK). It is more than a decade that the tetravalent Gardasil vaccine against HPV 16, 18, 6 and 11 and Cervarix directed against HPV16 and 18 were FDA-approved, and this has in 2014 been followed by approval of the nonavalent Gardasil 9 vaccine against HPV16, 18, 31, 33, 45, 6 and 11 [44–47]. All vaccines induce high titres of neutralizing antibodies that protect against HPV infection with the corresponding HPV types, included in the respective vaccines [44–47, 161–165].

Several studies have since then examined the potential effects of reducing the numbers of doses given, and within which time frames they should be offered in order to prevent prestages of cervical cancer [44–47]. The World Health Organization (WHO) has presently recommended two doses for either Gardasil, Gardasil 9 or Cervarix for those up to 15 years of age and three doses for women 15 years or older [44]. Different doses are not specified specifically for males according to age, but one should assume that the recommended doses should be similar to those offered to women and depend on the age of the individual. A number of high-income countries have introduced these vaccines quite successfully, but from a global perspective, the vaccination of young girls is still very low and the uptake of HPV vaccinations across the world was in 2017 <2% in females 9–45 years of age [44]. More details regarding the HPV vaccines, mainly from high-income countries, are presented below.

**HPV vaccine efficacy against cervical cancer and other HPV-associated tumours**

All three vaccines have been produced to inhibit infection with HPV types involved in cervical cancer, affecting more than 500 000 women every year, and the end-point of the vaccines, which was prevention of prestages to cervical cancer, was efficiently accomplished [42, 44–47, 161–165]. These three vaccines can thereby inhibit roughly 70–90% of all cervical cancer cases, but they can also potentially also prevent other associated cancers such as other anogenital cancer as well as HPV-positive TSCC and BOTSCC [42, 47, 166–172]. The two Gardasil vaccines can, in addition, also abrogate a substantial number of condylomas and papillomas [44–47, 172]. In the Future I/II study, prestages of vulvar and vaginal cancer as well as anogenital warts were prevented after HPV vaccination [166]. Furthermore, early experimental models, in the related canine oral papillomavirus (COPV), demonstrated that it was possible to protect against the development of oral cancer through COPV L1 VLP vaccination [167]. In addition, the preliminary utilities of HPV vaccination for prevention of oral HPV infection, as well as the acquisition of antibodies in the saliva, have been demonstrated [168–171]. Notably, since HPV16 is present in roughly 80–90% of all HPV-positive OPSCC, TSCC and BOTSCC, and all today’s vaccines are directed against HPV16, they should all be extremely efficient to prevent the development of these tumours [6, 14, 16, 48, 50].

**Annual global contribution of HPV to cancer and benefits with the introduction of the HPV vaccine**

The global attribution of HPV to cancer is 630 000 cases per year according to Globocan 2012 [57]. Of these, 570 000 affect women and 60 000 affect men, and for details of these cancer cases please, see Table 1 [57, 58]. Cervical cancer followed by anal cancer dominates in women, whilst OPSCC followed by penile cancer dominates in men. The relative contribution of HPV16/18 and HPV6/11/16/18/31/33/45/52/58 was estimated with 73% and 90%, respectively. Global HPV vaccination within decades result in avoiding most HPV-attributable cancer cases [57, 173, 174].
fact, today we have the means within a few generations to wipe out a great fraction of HPV-associated cancer cases worldwide. This would decrease the need of screening, as well as the medical treatments necessary to take care of all these cancer patients and take a considerable burden of the health system. Furthermore, it would save the lives of relatively young people that contribute socio-economically.

**Vaccination coverage today and different hurdles**

Since 2007, almost 100 countries and territories have introduced HPV vaccination programmes and approximately 270 million doses have been distributed; however, in the vast majority of these countries, vaccination is only offered to girls [172–174]. Very few side effects have been documented, and these have been limited to local reactions [173]. Some countries have a high HPV vaccine coverage (≥80%) such as Australia of young girls and boys, and the UK and Sweden for girls [174–176]. Others like Denmark and Japan have dropped from a high coverage to <40% and <1%, respectively, due to different unconfirmed worrisome negative reports on, for example, side effects regarding the HPV vaccines [177, 178]. A low socio-economic background is often associated with lower vaccine uptake [174]. In Norway, a lower vaccine initiation was observed in girls, with mothers with lower education and in the lowest income bracket [179]. Likewise, in Denmark, a lower HPV vaccine introduction and completion was found in immigrant girls, where much, but not all, could also be due to socio-economic differences [180]. Ethnicity and cultural norms also affected HPV vaccination [180, 181].

In spite of the documented successful effect of these vaccines, their worldwide presentation has been hampered not only due to economic resources, or to concern for potential physical side effects or efficiency of the vaccines, but also due to anxiety including risk for changes in the behaviour of vaccinated youth [182–189]. It was suggested that the vaccines would allow youth to lose their moral and become sexually adventurous, and that HPV vaccination should not be necessary for youth with higher principles [186, 187].

In addition, youth have limited knowledge about HPV and the link between sexual behaviour and HPV-associated cancer, and in general boys know less than girls [182]. Boys often believed HPV only affected girls and would accept being vaccinated for the sake of girls, but upon being aware that an HPV infection also could result in a cancer affecting them, their awareness and will to be vaccinated have increased [190]. To summarize, the introduction of HPV vaccination has clearly differed in many respects from the establishment of other vaccines [172–174, 182–189].

**Herd immunity and gender-neutral HPV vaccination**

Unfortunately, currently a large part of the human population is still sceptical to many types of vaccines, and for HPV vaccination, there is an even greater reluctance to accept its need from a moral point of view [181, 183]. This has together with socio-economic reasons, ethnicity and cultural norms in some respects hindered the efficient introduction of this vaccine to young girls (and boys) in order to reach stable high vaccine coverage and herd immunity that could spill off to also protecting young boys [174, 191–195]. Instead, when scientific reports or other media of dubious quality are spread, immediate serious dips in the compliance to HPV vaccination may occur as mentioned above in, for example, Denmark and Japan [177, 178]. This immediately abolishes the possibility to achieve a ≥80% HPV vaccine coverage amongst girls and efficient herd immunity for unvaccinated girls and boys [177, 178]. The efficacy of HPV vaccines by using gender-neutral vaccination is well documented [191–195]. Furthermore, when setting the horizon to 100 years, this was also the case when having a vaccine coverage ≥80% amongst girls [194]. Gender-neutral HPV vaccination would benefit both girls and boys directly, and also be more robust in that it also can reduce the effects of potential acutely occurring dips in HPV vaccination coverage, which in turn wipe out the prospect of herd immunity [177, 178]. As mentioned above, some surveys have been conducted about adolescent boys’ attitudes to HPV vaccinations. Many have focused on male college students, or men who have sex with men, but also young boys’ knowledge and beliefs regarding HPV, as well as HPV vaccination, have been studied [190, 196–199]. With increasing information, present knowledge suggests that also boys want to be vaccinated [190].

In some countries, for example Australia and the United States, gender-neutral vaccination has been introduced, and recently, additional some European countries, for example Denmark and the UK, have decided to follow this policy [172].
Including boys in the national vaccination programme is an approach towards which the Public Health Agency of Sweden is also in favour, but unfortunately so far this has not yet been applied [195]. We believe in the long run, it is of great benefit for both health care and government to vaccine both genders. Finally, last but not least, one should recollect, that importantly, it is amongst human rights to be treated equally [200].

Conclusion

Patients with HPV-positive TSCC and BOTSCC have much better prognosis than those with corresponding HPV-negative cancers, and the incidence of HPV-positive cancers has been increasing epidemiologically the past decades. Tailoring therapy for this growing group of patients is crucial, and efforts in finding markers useful for tapering and targeting therapy are on the way and will hopefully be of benefit for some patients. For the younger generations, however, it is feasible to inhibit most HPV-associated tumours, and for this purpose, the introduction of pan-gender HPV vaccination is urgently encouraged.

Acknowledgements

This authors of this work have been supported by grants from the Swedish Research Council, the Swedish Cancer Foundation, the Swedish Allergy Foundation, the Stockholm Cancer Society, the Stockholm City Council and the Karolinska Institutet, Sweden.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

References

1 Gillison ML, Koch WM, Capone RB et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000; 92: 709–20.
2 Mellin H, Friesland S, Lewensohn R, Dalianis T, Munk-Wikland E. Human papillomavirus (HPV) DNA in tonsillar cancer: clinical correlates, risk of relapse, and survival. Int J Cancer 2000; 89: 300–4.
3 IARC. A review of human carcinogens. Monograph on the evaluation of carcinogenic risks to humans. 2007.
4 Robinson KL, Macfarlane GJ. Oropharyngeal cancer incidence and mortality in Scotland: are rates still increasing? Oral Oncol 2003; 39: 31–6.
5 Conway DJ, Stockton DL, Warnakulasuriya KA, Ogden G, Macpherson LM. Incidence of oral and oropharyngeal cancer in United Kingdom (1990–1999) – recent trends and regional variation. Oral Oncol 2006; 42: 586–92.
6 Hammarstedt L, Lindquist D, Dahlstrand H et al. Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. Int J Cancer 2006; 119: 2620–3.
7 Hammarstedt L, Dahlstrand H, Lindquist D et al. The incidence of tonsillar cancer in Sweden is increasing. Acta Otolaryngol 2007; 127: 988–92.
8 Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? Cancer 2007; 110: 1429–35.
9 Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. J Clin Oncol 2008; 26: 612–9.
10 Nasman A, Attner P, Hammarstedt L et al. Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? Int J Cancer 2009; 125: 362–6.
11 Braakhuis BJ, Visser O, Leemans CR. Oral and oropharyngeal cancer in The Netherlands between 1989 and 2006: Increasing incidence, but not in young adults. Oral Oncol 2009; 45: e85–9.
12 Attner P, Du J, Nasman A et al. The role of human papillomavirus in the increased incidence of base of tongue cancer. Int J Cancer 2010; 126: 2879–84.
13 Marur S, D’Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol 2010; 11: 781–9.
14 Ramqvist T, Dalianis T. Oropharyngeal cancer epidemic and human papillomavirus. Emerg Infect Dis 2010; 16: 1671–7.
15 Chaturvedi AK, Engels EA, Pfeiffer RM et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011; 29: 4294–301.
16 Lindquist D, Romainian M, Hammarstedt L et al. Human papillomavirus is a favourable prognostic factor in tonsillar cancer and its oncogenic role is supported by the expression of E6 and E7. Mol Oncol 2007; 1: 350–5.
17 Attner P, Du J, Nasman A et al. Human papillomavirus and survival in patients with base of tongue cancer. Int J Cancer 2011; 128: 2892–7.
18 Fakhry C, Westra WH, Li S et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst 2008; 100: 261–9.
19 Bersani C, Mints M, Tertipis N et al. A model using concomitant markers for predicting outcome in human papillomavirus positive oropharyngeal cancer. Oral Oncol 2017; 68: 53–9.
20 Licitra L, Bernier J, Grandi C, Merlino M, Bruzzi P, Lefebvre JL. Cancer of the oropharynx. Crit Rev Oncol Hematol 2002; 41: 107–22.
21 Marur S, Li S, Cmelak AJ et al. E1308: Phase II Trial of Induction Chemotherapy Followed by Reduced-Dose Radiotherapy and Weekly Cetuximab in Patients With HPV-Associated Resectable Squamous Cell Carcinoma of the Oropharynx-ECOG-ACRIN Cancer Research Group. J Clin Oncol 2017; 35: 490–7.
Pan-gender use of HPV vaccine / A. Nasman et al.

22 Zumsteg ZS, Lok BH, Ho AS et al. The toxicity and efficacy of concomitant chemoradiotherapy in patients aged 70 years and older with oropharyngeal carcinoma in the intensity-modulated radiotherapy era. Cancer 2017; 123(1): 1345–53.

23 Lindquist D, Ahlund-Richter A, Tarjan M, Tot T, Dalénäs T. Intense CD44 expression is a negative prognostic factor in tonsillar and base of tongue cancer. Anticancer Res 2012; 32: 153–61.

24 Nasman A, Nordfors C, Grun N et al. Absent/weak CD44 intensity and positive human papillomavirus (HPV) status in oropharyngeal squamous cell carcinoma indicates a very high survival. Cancer Med 2013; 2: 507–18.

25 Nasman A, Andersson E, Nordfors C et al. MHC class I expression in HPV positive and negative tonsillar squamous cell carcinoma in relation to tumor HPV status and clinical outcome. PLoS ONE 2013; 8: e77025.

27 Tertipis N, Villabona L, Nordfors C et al. HLA-A*02 in relation to outcome in human papillomavirus positive tonsillar and base of tongue cancer. Anticancer Res 2014; 34: 2369–75.

29 Tertipis N, Haeggbloem L, Nordfors C et al. Correlation of LMP10 expression and clinical outcome in Human Papillomavirus (HPV) positive and HPV-Negative tonsillar and base of tongue cancer. PLoS ONE 2014; 9: e95624.

31 Nasman A, Andersson E, Marklund L et al. HLA class I and II expression in oropharyngeal squamous cell carcinoma in relation to tumor HPV status and clinical outcome. Int J Cancer 2013; 133(2): 72–81.

33 Nasman A, Andersson E, Marklund L et al. CD8+ and Foxp3+ lymphocytes correlate to clinical outcome. Transl Oncol 2015; 8: 10–7.

35 Lindquist D, Ahlund-Richter A, Tarjan M, Tot T, Dalénäs T. Distinct patterns of infiltrating CD8+ T cells in HPV+ and CD68 macrophages in HPV- oropharyngeal carcinoma identifies novel genetic alterations in HPV+ and HPV- tumors. Genome Med 2013; 5: 49.

37 Tinhofer I, Budach V, Saksi M et al. Targeted next-generation sequencing of locally advanced squamous cell carcinomas of the head and neck reveals druggable targets for improving adjuvant chemoradiation. Eur J Cancer 2016; 57: 78–86.

38 Berhani S, Sivas L, Haeggbloem L et al. Targeted sequencing of tonsillar and base of tongue cancer and human papillomavirus positive unknown primary of the head and neck reveals prognostic effects of mutated FGFR3. Oncotarget 2017; 8: 35339–50.

39 Rigoni-Stern DA. Fatti Statistici relativi alla malattie cancerose che Serviranno di Base Alle Poche Cose dal Dott. G Servire Progr Path Terra. 1842; 2: 507–17.

40 Panpaticolaou GN, Traut HF. The diagnostic value of vaginal smears in carcinoma of the uterus. Am J Ob Gynecol 1941; 42: 193.

41 Durst M, Gissmann L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc Natl Acad Sci USA 1983; 80: 3812–5.

42 DiMaio D. Nuns,warts, viruses, and cancer. Yale J Biol Med 2015; 88: 127–9.

43 Oguejiofor K, Hall JS, Mani N et al. Human papillomavirus and survival of patients with oropharyngeal cancer. J Obst Gynecol 2019; 44: 193.

44 Barra F, Leone Roberti Maggiore U, Bogani G et al. New prophylactics human papilloma virus (HPV) vaccines against cervical cancer. J Obst Gynecol 2019; 39(1): 1–10.

45 Qendi V, Schurink-Van ’t Klooster TM, Bogaards JA, Berkhof J. Ten years of HPV vaccination in the Netherlands: current evidence and future challenges in HPV-related disease prevention. Expert Rev Vaccines 2018; 17: 1093–104.

46 de Villiers EM, Fauquet C, Broker T, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology 2004; 324: 17–27.

47 Doorbar J, Quint W, Banks L et al. The biology and life-cycle of human papillomaviruses. Vaccine 2012; 30(Suppl 5): F55–70.

48 Tommasino M. The human papillomavirus family and its role in carcinogenesis. Semin Cancer Biol 2014; 26: 13–21.

49 Klussmann JP, Gultekin E, Weissenborn SJ et al. Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. Am J Pathol 2003; 162: 747–53.

50 Ang KK, Harris J, Wheeler R et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010; 363: 24–35.

51 Oguejiofor KK, Hall JS, Mani N et al. The prognostic significance of the biomarker p16 in oropharyngeal squamous cell carcinoma. Clin Oncol (R Coll Radiol) 2013; 25: 630–8.

52 Venuti A, Paolini F, Nasir L et al. Papillomavirus E5: the smallest oncoprotein with many functions. Mol Cancer 2011; 10: 140.

53 Ashraf GH, Haghshehars MR, Marchetti B, O’Brien PM, Campo MS. E5 protein of human papillomavirus type 16
selectively downregulates surface HLA class I. Int J Cancer 2005; 113: 276–83.
56 Li W, Deng XM, Wang CX et al. Down-regulation of HLA class I antigen in human papillomavirus type 16 E7 expressing HaCaT cells: correlate with TAP-1 expression. Int J Gynecol Cancer 2010; 20: 227–32.
57 Forman D, de Martel C, Lacey CJ et al. Global burden of human papillomavirus and related diseases. Vaccine 2012; 30(Suppl 5): F12–23.
58 de Martel C, Ferlay J, Franceschi S et al. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol 2012; 13: 607–15.
59 Marklund L, Nasman A, Ramqvist T, Dalianis T, Munck-Wikland E, Hammarstedt L. Prevalence of human papillomavirus and survival in oropharyngeal cancer other than tonsil or base of tongue cancer. Cancer Med 2012; 1: 82–8.
60 Haeggblom L, Ramqvist T, Tommasino M, Dalianis T, Nasman A. Time to change perspectives on HPV in oropharyngeal cancer. A systematic review of HPV prevalence per oropharyngeal sub-site the last 3 years. Papillomavirus Res 2017; 4: 1–11.
61 Smets SJ, Hesselink AT, Speel EJ et al. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. Int J Cancer 2007; 121: 2465–72.
62 Sivars L, Landin D, Haeggblom L et al. Human papillomavirus DNA detection in fine-needle aspirates as indicator of human papillomavirus-positive oropharyngeal squamous cell carcinoma: A prospective study. Head Neck 2017; 39: 419–26.
63 Manos MMTY, Wright DK, Lewis J, Broker TR, Wolinsky SM. The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. Cancer Cells 1989; 7: 209–14.
64 de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3’ ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J Gen Virol 1995; 76(Pt 4): 1057–62.
65 Tieben LM, ter Schegget J, Minnaar RP et al. Detection of cutaneous and genital HPV types in clinical samples by PCR using consensus primers. J Virol Methods 1993; 42: 265–79.
66 van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ. GP5+6/ 6 PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol 2002; 40: 779–87.
67 Clavel C, Masure M, Bory JP et al. Hybrid Capture II-based human papillomavirus detection, a sensitive test to detect in routine high-grade cervical lesions: a preliminary study on 1518 women. Br J Cancer 1999; 80: 1306–11.
68 Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol 1998; 36: 3020–7.
69 Schmitt M, Bravo IG, Snijders PJ, Gissmann L, Pawlita M, Waterboer T. Bead-based multiplex genotyping of human papillomaviruses. J Clin Microbiol 2006; 44: 504–12.
70 Dahlgren L, Dahlstrand HM, Lindquist D et al. Human papillomavirus is more common in base of tongue than in mobile tongue cancer and is a favorable prognostic factor in base of tongue cancer patients. Int J Cancer 2004; 112: 1015–9.
71 Mellin Dahlstrand H, Lindquist D, Bjornestal L et al. P16 (INK4a) correlates to human papillomavirus presence, response to radiotherapy and clinical outcome in tonsillar carcinoma. Anticancer Res 2005; 25: 4375–83.
72 Mellin H, Friesland S, Auer G, Dalianis T, Munck-Wikland E. Human papillomavirus and DNA ploidy in tonsillar cancer—correlation to prognosis. Anticancer Res 2003; 23: 2821–8.
73 Dahlgren L, Mellin H, Wangaard C et al. Comparative genomic hybridization analysis of tonsillar cancer reveals a different pattern of genomic imbalances in human papillomavirus-positive and -negative tumors. Int J Cancer 2003; 107: 244–9.
74 Mellin H, Dahlgren L, Munck-Wikland E et al. Human papillomavirus type 16 is episomal and a high viral load may be correlated to better prognosis in tonsillar cancer. Int J Cancer 2002; 102: 152–8.
75 Koskinen WJ, Chen RW, Leivo I et al. Prevalence and physical status of human papillomavirus in squamous cell carcinomas of the head and neck. Int J Cancer 2003; 107: 401–6.
76 Parfenov M, Pedamallu CS, Gahlenborg N et al. Characterization of HPV and host genome interactions in primary head and neck cancers. Proc Natl Acad Sci USA 2014; 111: 15544–9.
77 Chaturvedi AK, D’Souza G, Gillison ML, Katki HA. Burden of HPV-positive oropharynx cancers among ever and never smokers in the U.S. population. Oral Oncol 2016; 60: 61–7.
78 samverkan Rcci. Nationellt vårdprogram Huvud- och. Halscancer.2015.
79 Garnaes E, Kiss K, Andersen L et al. A high and increasing HPV prevalence in tonsillar cancers in Eastern Denmark, 2000–2010: the largest registry-based study to date. Int J Cancer 2015; 136: 2196–203.
80 Haeggblom L, Attoff T, Hammarstedt-Nordenvall L, Nasman A. Human papillomavirus and survival of patients per histological subsite of tonsillar squamous cell carcinoma. Cancer Med 2018; 7: 1717–22.
81 Huang SH, Xu W, Waldron J et al. Refining American Joint Committee on Cancer/Union for International Cancer Control TNM stage and prognostic groups for human papillomavirus-related oropharyngeal carcinomas. J Clin Oncol 2015; 33: 836–45.
82 O’Sullivan B, Huang SH, Su J et al. Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-5): a multicentre cohort study. Lancet Oncol 2016; 17: 440–51.
83 Varadhachary GR, Raber MB. Carcinoma of unknown primary site. N Engl J Med 2014; 371: 2040.
84 Economopoulou P, Mountzios G, Pavlidis N, Pentheroudakis G. Cancer of Unknown Primary origin in the genomic era: Elucidating the dark box of cancer. Cancer Treat Rev 2015; 41: 598–604.
85 Tothill RW, Li J, Milesklin L et al. Massively-parallel sequencing assists the diagnosis and guided treatment of cancers of unknown primary. J Pathol 2013; 231: 413–23.
86 Sivars L, Bersani C, Grun N et al. Human papillomavirus is a favourable prognostic factor in cancer of unknown primary in the head and neck region and in hypopharyngeal cancer. Mol Clin Oncol 2016; 5: 671–4.
87 Sivars L, Nasman A, Tertipis N et al. Human papillomavirus and p53 expression in cancer of unknown primary in the head and neck region in relation to clinical outcome. Cancer Med 2014; 3: 376–84.
88 Park GC, Lee M, Roh JL et al. Human papillomavirus and p16 detection in cervical lymph node metastases from an unknown primary tumor. Oral Oncol 2012; 48: 1250–6.
89 Compton AM, Moore-Medlin T, Herman-Ferdinandez L et al. Human papillomavirus in metastatic lymph nodes from unknown primary head and neck squamous cell carcinoma. Otolaryngol Head Neck Surg 2011; 145: 51–7.
90 Tribius S, Hoffmann AS, Bastrop S et al. HPV status in patients with head and neck cancer of unknown primary site: HPV, tobacco smoking, and outcome. Oral Oncol 2012; 48: 1178–84.
91 Faguin WC. Human papillomavirus (HPV) assays for testing fine-needle aspiration specimens in patients with head and neck squamous cell carcinoma. Cancer Cytopathol 2014; 122: 92–5.
92 Begum S, Gillison ML, Nicol TL, Westra WH. Detection of human papillomavirus-16 in fine-needle aspirates to determine tumor origin in patients with metastatic squamous cell carcinoma of the head and neck. Clin Cancer Res 2007; 13: 1186–91.
93 Zhang MQ, El-Moffy SK, Davila RM. Detection of human papillomavirus-related squamous cell carcinoma cytologically and by in situ hybridization in fine-needle aspiration biopsies of cervical metastasis: a tool for identifying the site of an occult head and neck primary. Cancer 2008; 114: 118–23.
94 Ummudum H, Rezanko T, Dag F, Dogruluk T. Human papillomavirus genome detection by in situ hybridization in fine-needle aspirates of metastatic lesions from head and neck squamous cell carcinomas. Cancer 2005; 105: 171–7.
95 Lastra RR, Pramick MR, Nakashima MO et al. Adequacy of fine-needle aspiration specimens for human papillomavirus infection molecular testing in head and neck squamous cell carcinoma. Cytojournal 2013; 10: 21.
96 Smith DF, Maleki Z, Coughlan D et al. Human papillomavirus status of head and neck cancer as determined in cytologic specimens using the hybrid-capture 2 assay. Oral Oncol 2014; 50: 600–4.
97 Romanitan M, Nasman A, Ramqvist T et al. Human papillomavirus frequency in oral and oropharyngeal cancer in Greece. Anticancer Res 2008; 28: 2077–80.
98 Svahn MF, Munk C, von Buchwald C, Frederiksen K, Kjaer SK. Burden and incidence of human papillomavirus-associated cancers and precancerous lesions in Denmark. Scand J Public Health 2016; 44: 551–9.
99 Carlander AF, Gronhov Larsen C, Jensen DH et al. Continuing rise in oropharyngeal cancer in a high HPV prevalence area: A Danish population-based study from 2011 to 2014. Eur J Cancer 2017; 70: 75–82.
100 D’Souza G, Agraval Y, Halpern J, Bodison S, Gillison ML. Oral sexual behaviors associated with prevalent oral human papillomavirus infection. J Infect Dis 2009; 199: 1263–9.
101 Chaturvedi AK, Graubard BI, Broutian T et al. NHANES 2009–2012 Findings: Association of Sexual Behaviors with Higher Prevalence of Oral Oncogenic Human Papillomavirus Infections in U.S. Men. Cancer Res 2015; 75: 2468–77.
102 Windom MJ, Waterboer T, Hillel AT et al. Sex differences in HPV immunity among adults without cancer. Hum Vaccin Immunother 2019; 15: 1935–41.
individuals: a systematic review of the literature. Sex Transm Dis 2010; 37: 586–91.

119 Du J, Nordfors C, Ahlund-Richter A et al. Prevalence of oral human papillomavirus infection among youth. Sweden. Emerg Infect Dis 2012; 18: 1468–71.

120 Grun N, Mbuya W, Ternhag A et al. Human papillomavirus prevalence in mouthwash samples of patients undergoing tonsillectomy shows dominance of HPV69, without the corresponding finding in the tonsils. Infect Dis (Lond) 2017; 49: 588–93.

121 Ernst JA, Sciotto CG, O'Brien MM, Robinson LJ, Willson T. Evaluation of human papillomavirus antibodies and risk of subsequent head and neck cancer. J Clin Oncol 2013; 31: 2708–15.

122 Kreimer AR, Johansson M, Waterboer T et al. Identification of a novel insight into mutational landscape of head and neck squamous cell carcinoma. J Clin Oncol 2013; 135: 554–7.

123 Palmer E, Newcombe RG, Green AC et al. Human papillomavirus infection is rare in nonmalignant tonsillar tissue in the UK: implications for tonsil cancer precursor lesions. Int J Cancer 2014; 135: 2437–43.

124 Kreimer AR, Johansson M, Waterboer T et al. Evaluation of human papillomavirus antibodies and risk of subsequent head and neck cancer. J Clin Oncol 2013; 31: 2708–15.

125 Kreimer AR, Shiels MS, Fakhry C et al. Screening for human papillomavirus-driven oropharyngeal cancer: Considerations for feasibility and strategies for research. Cancer 2018; 124: 1859–66.

126 Nasman A, Bersani C, Lindquist D, Du J, Ramqvist T, Dalanias T. Human Papillomavirus and Potentially Relevant Biomarkers in Tonsillar and Base of Tongue Squamous Cell Carcinoma. Anticancer Res 2017; 37: S319–28.

127 Haeggblo L, Nordfors C, Tertipis N et al. Effects of irradiation on human leukocyte antigen class I expression in human papillomavirus positive and negative base of tongue and mobile tongue squamous cell carcinoma cell lines. Int J Oncol 2017; 50: 1423–30. https://doi.org/10.3892/ijo.2017.3916.

128 Rusan M, Li YY, Hamerman PS. Genomic landscape of human papillomavirus-associated cancers. Clin Cancer Res 2015; 21: 2009–19.

129 GaykaloVA DA, Mambo E, Choudhary A et al. Novel insight into mutational landscape of head and neck squamous cell carcinoma. PloS ONE 2014; 9: e93102.

130 Chung CH, Guthrie VB, Masica DL et al. Genomic alterations in head and neck squamous cell carcinoma determined by cancer gene-targeted sequencing. Ann Oncol 2015; 26: 1216–23.

131 Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 2015; 517: 576–82.

132 Siebos RJ, Jehmlich N, Brown B et al. Proteomic analysis of oropharyngeal carcinomas reveals novel HPV-associated biological pathways. Int J Cancer 2013; 132: 568–79.

133 Sewell A, Brown B, Biktasova A et al. Reverse-phase protein array profiling of oropharyngeal cancer and significance of PIK3CA mutations in HPV-associated head and neck cancer. Clin Cancer Res 2014; 20: 2300–11.

134 Mirghani H, Ugolin N, Ory C et al. Comparative analysis of micro-RNAs in human papillomavirus-positive versus -negative oropharyngeal cancers. Head Neck 2016; 38: 1634–42.

135 Miller DL, Davis JW, Taylor KH et al. Identification of a human papillomavirus-associated oncogenic miRNA panel in human oropharyngeal squamous cell carcinoma validated by bioinformatics analysis of the Cancer Genome Atlas. Am J Pathol 2015; 185: 679–92.

136 Lajer CB, Nielsen FC, Friis-Hansen L et al. Different miRNA signatures of oral and pharyngeal squamous cell carcinomas: a prospective translational study. Br J Cancer 2011; 104: 830–40.

137 Lajer CB, Garnaes E, Friis-Hansen L et al. The role of miRNAs in human papilloma virus (HPV)-associated cancers: bridging between HPV-related head and neck cancer and cervical cancer. Br J Cancer 2012; 106: 1526–34.

138 Hui AB, Lin A, Xu W et al. Potentially prognostic miRNAs in HPV-associated oropharyngeal carcinoma. Clin Cancer Res 2013; 19: 2154–62.

139 Hess AK, Muer A, Mairinger FD et al. MiR-200b and miR-155 as predictive biomarkers for the efficacy of chemoradiation in locally advanced head and neck squamous cell carcinoma. Eur J Cancer 2017; 77: 3–12.

140 Gao G, Gay HA, Chernock RD et al. A microRNA expression signature for the prognosis of oropharyngeal squamous cell carcinoma. Cancer 2013; 119: 72–80.

141 Mirghani H, Ugolin N, Ory C et al. A predictive transcriptional signature of oropharyngeal cancer according to HPV16 status exclusively. Oral Oncol 2014; 50: 1025–34.

142 Martinez I, Wang J, Hobson AF, Ferris RL, Khan SA. Identification of differentially expressed genes in HPV-positive and HPV-negative oropharyngeal squamous cell carcinomas. Eur J Cancer 2007; 43: 415–32.

143 Wichmann G, Rosolowski M, Krohn K et al. The role of HPV RNA transcription, immune response-related gene expression and disruptive TP53 mutations in diagnostic and prognostic profiling of head and neck cancer. Int J Cancer 2015; 137: 2846–57.

144 Keck MK, Zuo Z, Khattri A et al. Integrative analysis of head and neck cancer identifies two biologically distinct HPV and three non-HPV subtypes. Clin Cancer Res 2015; 21: 870–81.

145 Jung AC, Guihad S, Krugell S et al. CD8-alpha T-cell infiltration in human papillomavirus-related oropharyngeal carcinoma correlates with improved patient prognosis. Int J Cancer 2013; 132: E26–36.

146 Gronhøj C, Jensen DH, Dehlendorff C et al. Development and external validation of nomograms in oropharyngeal cancer patients with known HPV-DNA status: a European Multicentre Study (OroGrams). Br J Cancer 2018; 118: 1672–81.

147 Tertipis N, Hammar U, Nasman A et al. A model for predicting clinical outcome in patients with human papillomavirus-positive tonsillar and base of tongue cancer. Eur J Cancer 2015; 51: 1580–7.

148 Isaacsson Velho PH, Castro G Jr, Chung CH. Targeting the PI3K Pathway in Head and Neck Squamous Cell Carcinoma. Am Soc Clin Oncol Educ Book 2015; 123–8. https://doi.org/10.14694/EdBook_AM.2015.35.123.

149 von Massenhausen A, Deng M, Billig H et al. Evaluation of FGFR3 as a Therapeutic Target in Head and Neck Squamous Cell Carcinoma. Target Oncol 2016; 11: 631–42.

150 Taberner J, Bahleda R, Dienstmann R et al. Phase I Dose-Escalation Study of JNJ-42756493, an Oral Pan-Fibroblast Growth Factor Receptor Inhibitor, in Patients With Advanced Solid Tumors. J Clin Oncol 2015; 33: 3401–8.
Harboring Genetic Alterations in Fibroblast Growth Factor Receptors: Results of a Global Phase I, Dose-escalation and Dose-Expansion Study. *J Clin Oncol* 2017; 35: 157–65.

Bykov VJ, Wiman KG. Mutant p53 reactivation by small molecules makes its way to the clinic. *FEBS Lett* 2014; 588: 2622–7.

Bersani C, Mints M, Tertipis N et al. MicroRNA-155, -185 and -193B as biomarkers in human papillomavirus positive and negative tonsillar and base of tongue squamous cell carcinoma. *Oral Oncol* 2018; 82: 8–16.

Al-Hebsi NNBS, Johnson NW. The microbiome of oral squamous cell carcinomas: a functional perspective. *Current Oral Health Reports* 2019; 6: 145–60.

Brady JN, Consigli RA. Chromatographic separation of the polyoma virus proteins and renaturation of the isolated VP1 major capsid protein. *J Virol* 1978; 27: 436–42.

Palkova Z, Adamec T, Liebl D, Stokrova J, Forstova J. Production of papovavirus structural protein VP1 in yeast cells and its interaction with cell structures. *FEBS Lett* 2000; 478: 281–9.

Forstova J, Krauzewicz N, Wallace S et al. Cooperation of structural proteins during late events in the life cycle of polyomavirus. *J Virol* 1993; 67: 1405–13.

Sakunke DM, Caspar DL, Garcea RL. Self-assembly of purified polyomavirus capsid protein VP1. *Cell* 1986; 46: 895–904.

Chen XS, Casini G, Harrison SC, Garcea RL. Papillomavirus capsid protein expression in Escherichia coli: purification and assembly of HPV11 and HPV16 L1. *J Mol Biol* 2001; 307: 173–82.

Fernandez-San Millan A, Ortigosa SM, Hervas-Stubbs S et al. Human papillomavirus L1 protein expressed in tobacco chloroplasts self-assembles into virus-like particles that are highly immunogenic. *Plant Biotechnol J* 2008; 6: 427–41.

Villa LL, Costa RL, Petta CA et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol* 2005; 6: 271–8.

Koutska LA, Ault KA, Wheeler CM et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002; 347: 1645–51.

Harro CD, Pang YY, Roden RB et al. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. *J Natl Cancer Inst* 2001; 93: 284–92.

Pinto LA, Viscidi R, Harro CD et al. Cellular immune responses to HPV-18, -31, and -53 in healthy volunteers immunized with recombinant HPV-16 L1 virus-like particles. *Virology* 2006; 353: 451–62.

Harper DM, Franco EL, Wheeler CM et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet* 2006; 367: 1247–55.

Group FIIIS, Dillner J, Kjaer SK et al. Four year efficacy of prophylactic human papillomavirus quadrivalent vaccine against low grade cervical, vulvar, and vaginal intraepithelial neoplasia and anogenital warts: randomised controlled trial. *BMJ* 2010; 341: c3493.

Suzich JA, Ghim SJ, Palmer-Hill FJ et al. Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. *Proc Natl Acad Sci USA* 1995; 92: 11553–7.

Grun N, Ahrlund-Richter A, Franzen J et al. Oral human papillomavirus (HPV) prevalence in youth and cervical HPV prevalence in women attending a youth clinic in Sweden, a follow up-study 2013–2014 after gradual introduction of public HPV vaccination. *Infect Dis (Lond)* 2015; 47: 57–61.

Herrero R, Quint W, Hildesheim A et al. Reduced prevalence of oral human papillomavirus (HPV) 4 years after bivalent HPV vaccination in a randomized clinical trial in Costa Rica. *PloS ONE* 2013; 8: e68329.
the HPV vaccination in relation to their socio-demographics and religious beliefs: A cross-sectional study in Thailand. *PLoS ONE* 2018; 13: e0193054.

184 Ferrer HB, Trotter C, Hickman M, Audrey S. Barriers and facilitators to HPV vaccination of young women in high-income countries: a qualitative systematic review and evidence synthesis. *BMC Public Health* 2014; 14: 700.

185 Tung IL, Machalek DA, Garland SM. Attitudes, Knowledge and Factors Associated with Human Papillomavirus (HPV) Vaccine Uptake in Adolescent Girls and Young Women in Victoria. *Australia. PLoS One* 2016; 11: e0161846.

186 Marek E, Dergez T, Rebek-Nagy G et al. Adolescents’ awareness of HPV infections and attitudes towards HPV vaccination 3 years following the introduction of the HPV vaccine in Hungary. *Vaccine* 2011; 29: 8591–8.

187 Gottvall M, Grandahl M, Hoglund AT et al. Trust versus concerns-how parents reason when they accept HPV vaccination for their young daughter. *Ups J Med Sci* 2013; 118: 263–70.

188 Krakow MM, Jensen JD, Carcioppolo N, Weaver J, Liu M, Guntzviller LM. Psychosocial predictors of human papillomavirus vaccination intentions for young women 18 to 26: religiosity, morality, promiscuity, and cancer worry. *Womens Health Issues* 2015; 25: 105–11.

189 Rysavy MB, Kresowik JD, Liu D, Mains L, Lessard M, Ryan GL. Human papillomavirus vaccination and sexual behavior in young women. *J Pediatr Adolesc Gynecol* 2014; 27: 67–71.

190 Grandahl M, Neveus T, Dalianis T, Larsson M, Tyden T, Stenhammar C. ‘I also want to be vaccinated!’ - adolescent boys’ awareness and thoughts, perceived benefits, information sources, and intention to be vaccinated against Human papillomavirus (HPV). *Hum Vaccin Immunother* 2019; 15: 1794–802.

191 Lehtinen M, Luostarinen T, Vanska S et al. Gender-neutral vaccination provides improved control of human papillomavirus types 18/31/33/35 through herd immunity: Results of a community randomized trial (III). *Int J Cancer* 2018; 143: 2299–310.

192 Mehanna H, Bryant TS, Babrah J et al. Human papillomavirus (HPV) vaccine effectiveness and potential herd immunity for reducing oncogenic oropharyngeal HPV16 prevalence in the UK, a cross-sectional study. *Clin Infect Dis* 2018; 69: 1296–302.

193 Prue G. Vaccinate boys as well as girls against HPV: it works, and it may be cost effective. *BMJ* 2014; 349: g4834.

194 Wolff E, Elifstrom KM, Haugen Cange H et al. Cost-effectiveness of sex-neutral HPV-vaccination in Sweden, accounting for herd-immunity and sexual behaviour. *Vaccine* 2018; 36: 5160–5.

195 (Folkhalsomyndigheten) PHAoS. Human papillomavirus vaccination of boys in the Swedish national vaccination programme - support for a governmental decision.2017.

196 Gerend MA, Madkins K, Phillips G 2nd, Mustanski B. Predictors of Human Papillomavirus Vaccination Among Young Men Who Have Sex With Men. *Sex Transm Dis* 2016; 43: 185–91.

197 Cummings T, Kasting ML, Rosenberger JG, Rosenthal SL, Zimet GD, Stupiansky NW. Catching Up or Missing Out? Human Papillomavirus Vaccine Acceptability Among 18- to 26-Year-old Men Who Have Sex With Men in a US National Sample. *Sex Transm Dis* 2015; 42: 601–6.

198 Cheung T, Lau JTF, Wang JZ, Mo PKH, Ho YS. Acceptability of HPV vaccines and associations with perceptions related to HPV and HPV vaccines among male baccalaureate students in Hong Kong. *PLoS ONE* 2018; 13: e0198615.

199 Fontenot HB, Fantasia HC, Vetters R, Zimet GD. Increasing HPV vaccination and eliminating barriers: Recommendations from young men who have sex with men. *Vaccine* 2016; 34: 6209–16.

200 UNICEF. Convention on the Rights of the Child 2015.