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LEHTINEN ALEKSI: Profylaktisten ja terapeuttisten HPV-rokotteiden vaikutus pään ja kaulan alueen HPV-liitännäisiin levyepiteelikarsinoomiin

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TAUSTA. Ihmisen papilloomavirukseen (HPV) liitännäisten pään ja kaulan alueen levyepiteelikarsinoomien osuus on länsimaissa kasvanut selvästi viime vuosikymmenien aikana. Tämän kirjallisuuskatsauksen tarkoituksena oli selvittää, miten HPV-rokotukset vaikuttavat näiden syöpien kehittymiseen.

AINEISTOT JA MENETELMÄT. Suoritimme haun Yhdysvaltain Kansallisen Lääketiedekirjaston PubMed -elektronisesta arkistosta aiheeseemme liittyvillä hakusanoilla. Valikoimme hakutulosten joukosta ne, jotka täyttivät ennalta määrittämämme kriteerit.

TULOKSET. Yksikään tutkimus ei suoraan mitannut olemassa olevien profylaktisten HPV-rokotteiden vaikutusta pään ja kaulan alueen HPV-liitännäisten levyepiteelikarsinoomien esiintyvyyteen. Löytämämme suppean aineiston perusteella profylaktiset rokotteet kuitenkin mahdollisesti vähentävät pään ja kaulan alueen HPV-infektioita. Lisäksi profylaktiset rokotteet aikaansaavat valtaosalla rokotetusta väestöstä kohonneet HPV-vasta-aine –tasot sylkeen. Nämä tasot ovat kuitenkin merkittävästi alhaisemmat kuin veren vastaavat vasta-ainetasot. Terapeuttiset rokotteet puolestaan saivat aikaan vain hyvin vähäisiä vasteita olemassa olevien pään ja kaulan alueen levyepiteelikarsinoomien hoidossa. Terapeuttiset rokotteet olivat kuitenkin melko hyvin siedettyjä, ja aikaansaivat rokotetuissa henkilöissä kohonneita vasta-ainetasoja haluttuja antigeenejä kohtaan.

JOHTOPÄÄTÖKSET. HPV-rokotusten vaikutusta HPV-liitännäisten pään ja kaulan alueen levyepiteelikarsinoomiin on tutkittu vasta hyvin vähän, ja näyttö näiden syöpien mahdollisesta rokotevälitteisestä ehkäisystä on pääosin epäsuoraa. Olemassa olevat profylaktiset HPV-rokotteet saattavat ehkäistä pään ja kaulan alueen HPV-infektioita ja näin myös ehkä HPV:n aiheuttamia pään ja kaulan alueen levyepiteelisyöpiä. Jotta profylaktinen rokote olisi täysin HPV-infektiolta suojaava, se tulisi kuitenkin antaa jo ennen ensimmäistä pään ja kaulan alueen HPV-tartuntaa, jonka ajankohta on pääosin tuntematon. Terapeuttiset HPV-rokotteet ovat puolestaan vasta kehitysvaiheessa, ja tutkimusnäyttö näiden rokotteiden vaikutuksista pään ja kaulan alueen HPV-liitännäisten levyepiteelikarsinoomien hoidossa on toistaiseksi vähäistä.

Avainsanat: HPV, profylaktinen rokote, terapeuttinen rokote, pään ja kaulan alueen levyepiteelikarsinooma

ABSTRACT

1. INTRODUCTION

1.1. Overview of human papillomavirus (HPV). Papillomaviruses (PV) are a highly diverse family of viruses that are thought to infect most birds and mammals, including humans. By 2016, 205 PVs had been identified through the isolation of complete genomes. PVs are classified to five genera, of which alpha-, beta-, and gamma papillomaviruses can infect human epithelial cells. Human papillomavirus (HPV) is the shared name for the family of over 200 double-stranded DNA viruses that infect human epithelial cells. The HPV viral genome is fairly small, containing only three domains: a noncoding regulatory region, a region of six early open-reading frames (E1, E2, E4, E5, E6, and E7) responsible for the coding of six early proteins (*E*1, *E*2, *E*4, *E*5, *E*6, and *E*7), and a region of late open-reading frames (L1 and L2) responsible for the coding of two late proteins (*L*1 and *L*2), totalling to a length of approximately 8000 base pairs. Human beta-papillomaviruses infect the basal or parabasal cells of damaged cutaneous epithelium (such as that of hands, feet, and anogenital regions), while alpha-papillomaviruses infect the mucosal epithelial cells in oral cavity, pharynx, throat, respiratory tract, and anogenital regions. The alpha-HPVs can further be categorised as high-risk (HR) or low-risk (LR) in terms of their association with the development of precancerous or cancerous lesions. The most important HR HPV genotypes are 16 and 18, while the most important LR types are 6 and 11. Type 16 is the single most prominent HPV type in terms of association with the development of cancerous and precancerous lesions in infected areas, and it is also the most prevalent HPV type in cervical and head and neck regions. The viruses are transmitted upon contact between infected and uninfected epithelium via breaks in tissue, such as those caused by microtrauma. (Stanley 2010, Burd 2003, Castellsagué 2008). HPV transmission is

regarded to occur predominantly through sexual intercourse (Stanley 2010, Burd 2003, Castellsagué 2008); however, horizontal transmission also plays a role in its dissemination (Trottier et al. 2016, Zouridis et al. 2018, Sabeena et al. 2017, Stanley 2010). In the majority of genital HPV infections, the immune system is capable of clearing the virus on its own, but 10-20% of infections persist and transform into persistent infections (Stanley 2010, Castellsagué 2008). Persistent HPV infection with HR-genotypes 16 and 18 has been established as a contributing factor in the development of cancers of both the anogenital region (Stanley 2010, Burd 2003, Castellsagué 2008, Bosch et al. 2002) and head and neck region (Stanley 2010).

1.2. Role of HPV in carcinogenesis. Of the six early open-reading frames of the HPV, *E*2, *E*6, and *E*7 are the most integral to the oncogenic potential of the HPV infection. Ordinarily, the viral protein E2 is responsible for regulating the expression of *E*6 and *E*7, while the viral protein E6 prevents host cell apoptosis by degrading p53, and the viral protein E7 promotes host cell proliferation. *E*2 is often damaged during viral genome integration into host cell genome in chronic HPV infection. Consequently, the production of E6 and E7 runs rampart, contributing to the carcinogenesis of the host cell. (Mittal 2017, Stanley 2010, Burd 2003).

1.3. Role of HPV in cervical carcinoma. The significance of HPV in the aetiology of cervical carcinoma is particularly well-established (Stanley 2010, Burd 2003, Bosch et al. 2002). Cervical HPV-infection is an essential factor in the development of cervical carcinoma (Bosch et al. 2002), the fourth most common cancer in women worldwide (World Health Organization. Human Papillomavirus (HPV). https://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/). Cervical carcinoma is therefore a preventable disease, and the most effective and

widely employed means of its prevention are prophylactic HPV vaccinations (Burd 2003, Bosch et al. 2002).

1.4. HPV vaccination. Currently, the most widely employed HPV vaccinations approved by WHO are the quadrivalent (Gardasil, Merck & Co., Inc., US) and bivalent vaccine (Cervarix, GlaxoSmithKline, UK) (World Health Organization. HPV Vaccines and Safety. https://www.who.int/immunization/hpv/vaccines/en/). Both vaccines protect against HPV types 16 and 18, while the former also protects against types 6 and 11. In addition, the nine-valent Gardasil 9, which protects against types 6, 11, 16, 18, 31, 33, 45, 52, and 58, has been approved for use in the U.S. by the FDA (The U.S. Food and Drug Administration. Gardasil 9. https://www.fda.gov/vaccines-blood-biologics/vaccines/gardasil-9). These vaccines have similar underlying mechanisms of action in terms of conferring protection against HPV: both utilize recombinant DNA technology and are prepared from the purified L1 protein, which self-assembles to form HPV type-specific empty protein shells (virus-like particles; VLPs). (The U.S. National Cancer Institute. Human Papillomavirus (HPV) Vaccines. https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-vaccine-fact-sheet). Vaccination with either the quadrivalent or bivalent vaccine has been demonstrated as an effective countermeasure against cervical HPV-infection and cervical cancer; a meta-analysis by Drolet et al. (2019) found HPV vaccination with either the bivalent or quadrivalent vaccine to significantly reduce the prevalence of cervical HPV, anogenital warts, and cervical intraepithelial neoplasia grade 2+ (CIN2+) among women aged 13-29 years roughly half a decade after vaccination. Prophylactic vaccines should be administered before patients are exposed to the virus; in other words, the vaccination should occur prior to the patient's sexual debut (World Health Organization). It should be noted, however, that prophylactic

vaccination in this way does not protect against HPV transmission that has occurred prior to the administration of the vaccine, such as via vertical or horizontal HPV transmission from parents to fetus or nenate at birth (Koskimaa et al. 2012, Rintala et al. 2005). In addition to prophylactic HPV vaccines, therapeutic HPV vaccines are being developed to counteract already-existing infections in patients. However, no therapeutic vaccination strategy has yet been qualified for clinical use by WHO (World Health Organization. HPV vaccines and safety. https://www.who.int/immunization/hpv/vaccines/en/).

1.5. Role of HPV in head and neck squamous cell carcinomas (HNSCCs). As previously mentioned, HPV infection has long since been established as a risk factor in the development of head and neck squamous cell carcinomas (Ndiaye et al. 2014, Saulle et al. 2015, Syrjänen et al. 1983), which make up 90% of all head and neck cancers (HANC) (Vigneswaran et al. 2014). Oropharyngeal carcinomas are the most strongly associated with HPV infection, with 25-70% of all oropharyngeal carcinomas associated with HPV infection (Ndiaye et al. 2014, Mehanna et al. 2013, Chaturvedi et al. 2008, Chaturvedi et al. 2011). The incidence of HPV positive HNSCCs has increased considerably in European and North American populations in recent decades while the incidence of non-HPV positive HNSCCs has remained largely unchanged (Mehanna et al. 2013, Simard et al. 2014, Chaturvedi et al. 2008). This increase is particularly sharp in male populations (Jemal et al. 2013).

1.6. Aim of the review. Considering the efficient and reliable protection existing prophylactic HPV vaccines provide against cervical carcinoma, it seems reasonable to extrapolate that the bivalent and quadrivalent vaccines could impede the development of other HPV-associated carcinomas as well – namely those of the oropharynx and oral

cavity. So far, comparatively little research has been published on the effects of vaccinations on the incidence of oral HPV infection and HPV positive HNSCCs. This undergraduate thesis was conducted in order to assess whether HPV vaccinations could prove a useful tool in combating oral HPV infection and HPV positive HNSCCs in addition to their cervical counterparts.

2. MATERIALS AND METHODS

2.1. Search strategy and selection criteria. National Institutes of Health PubMed electronic databases were searched for studies on the effects of HPV vaccines on HPV positive HNSCCs. The search phrases used were: 1) ("head and neck" OR mouth OR tongue OR lingual OR larynx OR laryngeal OR *pharynx OR *pharyngeal OR throat OR nose OR nasal OR sinus* OR salivary gland*) AND (cancer OR neoplasm OR "squamous cell carcinoma" OR adenocarcinoma) AND (HPV OR human papillomavirus) AND (vaccine OR vaccination) and 2) ("head and neck" OR mouth OR tongue OR lingual OR larynx OR laryngeal OR *pharynx OR *pharyngeal OR throat OR nose OR nasal OR sinus* OR salivary gland*) AND (cancer OR neoplasm OR "squamous cell carcinoma" OR adenocarcinoma) AND (Gardasil OR Cervarix). Studies published prior to 13.6.2018 were included. The searches with aforementioned entry terms yielded a total of 596 records (Figure 1).

2.2. Study selection. Each search result was assessed to identify studies that met the inclusion criteria. The inclusion criteria were the following: the studies reported the effects of any HPV vaccine(s) on HPV positive HNSCCs, and/or the vaccine efficacy of any HPV vaccine(s) against oral HPV infection, and/or the effects of any HPV vaccine(s) on HPV antibody levels in the oral cavity. Studies were excluded if they were duplicates, and/or unavailable in English, and/or reviews, meetings abstracts, single-case studies, or animal studies, and/or discussed recurrent respiratory papillomatosis. The selection process is presented in further detail in Figure 1.

2.3. Data extraction. The search through the PubMed database yielded 559 results. Of these, 84 were either duplicates or unavailable in English and were discarded. The

remaining 512 were screened by title and/or abstract by two persons. Of these 512, 446 did not meet the inclusion criteria and were discarded. The remaining 66 were assessed in full. Of these, 59 did not meet the inclusion criteria and were discarded. The search thus yielded a total of 7 studies qualified for use in this review.

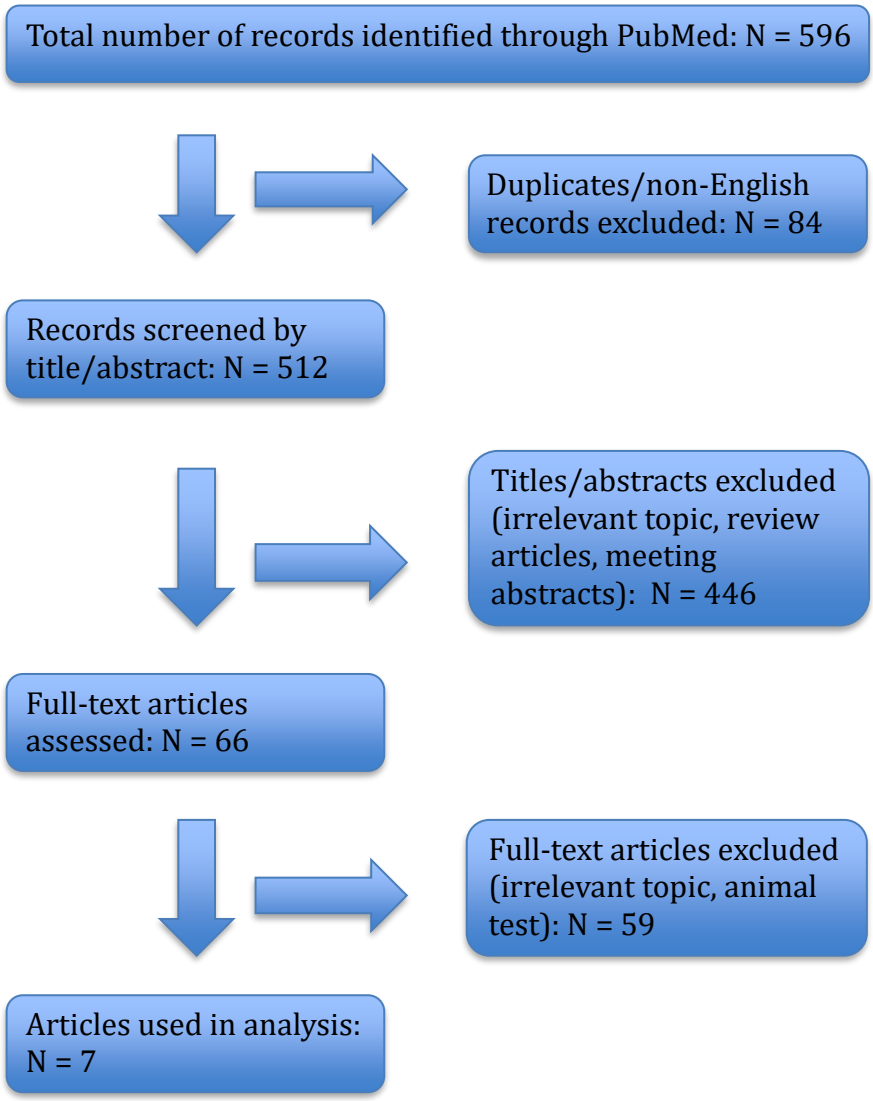

Figure 1. Summary of the study selection process.

3. RESULTS

3.1. Prophylactic vaccines

3.1.1. Studies. Four studies were available on the effects of the prophylactic HPV vaccines. The composition and results of these studies are presented in Table 1. Three of the studies were prospective in nature (Pinto et al. 2016, Handisurya et al. 2016, Herrero et al. 2013) and one was retrospective (Hirth et al. 2017). Two of the studies were specifically designed to determine the effects of vaccination on the levels of HPV antibodies in oral fluids (i.e. whole saliva) (Pinto et al. 2016, Handisurya et al. 2016), while the other two examined vaccine efficacies against oral HPV infection (Herrero et al. 2013, Hirth et al. 2017).

3.1.2. Vaccines. Three of the studies (Pinto et al. 2016, Handisurya et al. 2016, Hirth et al. 2017) observed the effects of the quadrivalent HPV vaccine (Gardasil, Merck & Co., Inc., US), administered according to the licensed schedule (0,5 mL dose each at day 1, month 2, and month 6) (Centers for Disease Control and Prevention. Administering HPV Vaccine. https://www.cdc.gov/vaccines/vpd/hpv/hcp/administration.html). The fourth study (Herrero et al. 2013) observed the bivalent HPV vaccine (Cervarix, GlaxoSmithKline, UK) administered according to licensed schedule (0,5 mL dose each at day 1, month 1, and month 6) (The U.S. Food & Drug Administration. Package Insert & Patient Information, Cervarix. https://www.fda.gov/vaccines-blood-biologics/vaccines/cervarix, 20.02.2020). In the three prospective studies, the vaccine was administrated as a part of the study procedure (Pinto et al. 2016, Handisurya et al. 2016, Herrero et al. 2013). In the retrospective study, the vaccine was administered prior to the beginning of the study (Hirth et al. 2017)

3.1.3. Patients. The combined population between all four studies (Pinto et al. 2016, Handisurya et al. 2016, Herrero et al. 2013, Hirth et al. 2017).) amounted to 9058, with two of the studies (Herrero et al. 2013, Hirth et al. 2017) contributing 98% of this number. The studies included patients between the ages of 18 and 45, with mean age being 23 years. Of the combined population between all four studies, 86% were female.

3.1.4. Samples. Oral fluid samples were collected in three studies (Pinto et al. 2016, Handisurya et al. 2016, Herrero et al. 2013). The fourth study examined pre-collected oral fluid samples (Hirth et al. 2017). The methods of sample collection were either a 15-second rinse and 15-second gargle using mouthwash (Herrero et al. 2013, Hirth et al. 2017), oral pads (Handisurya et al. 2016), or both mouthwash and Merocel-sponges (Pinto et al. 2016).

3.1.5. HPV genotypes, antibodies, and detection methods. Two of the studies examined HPV types 16 and 18 (Pinto et al. 2016, Herrero et al. 2013), while another also examined HPV 6 (Handisurya et al. 2016), and the fourth examined 37 HPV types (though primarily types 16 and 18) (Hirth et al. 2017). In two studies, the presence of HPV in samples was confirmed by detecting HPV DNA via either MagNAPure LC DNA isolation procedure (Roche Diagnostics) and the HPV SPF10 PCR-DEIA (DNA enzyme immunoassay)-LiPA25 (Line probe assay) version 1 system (Labo Biomedical Products, Rijswijk, The Netherlands) (Herrero et al. 2013), or using Roche Linear Array HPV Genotyping Test and Roche Linear Array Detection Kit (Hirth et al. 2017). In the two other studies (Pinto et al. 2016, Handisurya et al. 2016), the presence of HPV in samples was not measured; rather, only anti-HPV IgG antibodies (both neutralising and non-neutralising) were detected via VLP-ELISA.

3.1.6. Follow-up. Of the prospective studies, the two that examined the effects of Gardasil (Pinto et al. 2016, Handisurya et al. 2016) had a follow-up time of seven months, whereas the one that examined the effects of Cervarix (Herrero et al. 2013) had no follow-up time, as the HPV status of the gargle samples was assessed only once (four years after the vaccination). The sole retrospective study (Hirth et al. 2017) examined repeated cross-sectional data from the National Health and Nutrition Examination Survey and medical examinations conducted between years 2009-2014.

3.1.7. HPV-antibody induction in oral fluids. In both of the prospective studies examining Gardasil (Pinto et al. 2016, Handisurya et al. 2016), the majority of the study population (96% and 60%, respectively) developed detectable HPV 16 antibody responses in oral fluids. The responses reported for detectable oral HPV 18 antibodies were similar, though somewhat lesser in scope (72% and 35%, respectively). The levels of vaccine-induced oral HPV 16 antibodies were found to correlate with levels in sera when normalised for IgG levels. One of these studies (Pinto et al. 2016) found the levels of HPV 18 antibodies to correlate with the IgG levels in the same way, although the other (Handisurya et al. 2016) did not find a statistically significant correlation. The HPV 16 antibody levels in oral fluids were reported to be 262-495 –fold lower in comparison to those of sera (Pinto et al. 2016).

3.1.8. Vaccine efficacy (VE). Two studies (Herrero et al. 2013, Hirth et al. 2017) sought to determine the efficacy of vaccines against oral HPV 16 and 18 infections. For Cervarix, VE against oral HPV 16 was 91.6% (95% CI 0.625 – 0.997) and VE against oral HPV 18 was 100% (95% CI -0.120 – 1.00) (Herrero et al 2013). For Gardasil, oral HPV 16 prevalence in the vaccinated group was 0.09 weighted percentage (w%) (95% CI

0.01 – 0.69) and the same prevalence in the control group was 0.84w% (CI 0.45 – 1.55), while oral HPV 18 prevalence in the vaccinated group was 0.07w% (CI 0.01 – 0.50) and the same prevalence in the control group was 0.29w% (CI 0.11 – 0.75) (using percentages weighted according to standard NHANES instructions) (Hirth et al. 2017).

Reference and publication year	Location	Study type	HPV types	Confirmation method of oral HPV or HPV antibody status; time of confirmation	Population size; sex; age range	Cases; controls	Vaccine type; dosage	Follow-up	Results: prevalence of oral HPV antibodies in vaccinated population in the beginning and end of study	Results: vaccine efficacy against oral HPV infection
Pinto et al. 2016	USA, Mexico	Single-arm intervention trial	16, 18	Oral rinse and gargle, Merocel sponges; 1 d, 7 m	150; male; 27-45 y	150; 0	Gardasil; 1 d, 2 m, 6 m	7 m	**1 d**: <9% for HPV 16 and HPV 18. **7 m**: 96% for HPV 16 and 72% for HPV 18.	N/A
Handisurya et al. 2016	Austria	Case-control study	6, 16, 18	Mouth pads; 1 d, 7 m	34; female; 18-26	20; 14	Gardasil; 1 d, 2 m, 6 m	7 m	**1 d**: 0% for HPV 16 and HPV 18. **7 m**: 60% for HPV 16 and 35% for HPV 18.	The induced antibody responses in oral fluids were sufficient to neutralize HPV-particles in vitro
Herrero et al. 2013	Costa Rica	Randomised and blinded case-control study	16, 18	Oral rinse and gargle; 4 y	5834; female; 18-25	2910; 2924	Cervarix; 1 d, 1 m, 6 m	N/A	N/A	HPV 16: 91,6% (CI 0,625 - 0,997) HPV 18: 100% (CI -0,120 – 1,00)
Hirth et al. 2017	USA	Cross-sectional study	37 types; primarily types 16 and 18	Oral rinse and gargle; prior to study onset	3040; male and female; 18-30	668; 2372	Gardasil; N/A	N/A	N/A	HPV 16 prevalence Vaccine group: 0,09w% (CI 0,01 - 0,69) Controls: 0,84w% (CI 0,45 - 1,55)

Table 1. Summary of the composition and results of the studies discussing prophylactic HPV vaccines.

3.2. Therapeutic vaccines

3.2.1. Studies. Three studies were available on the effects of therapeutic vaccines on HPV positive HNSCCs (Voskens et al. 2012, Reuschenbach et al. 2016, Zandberg et al. 2015) (Table 2). All of these were single-arm intervention trials. The study by Voskens et al. (2012) was a pilot for the study by Zandberg et al. (2015). All studies aimed to assess the safety and immunogenicity of their respective vaccines, as well as tumour response to vaccination.

3.2.2. Vaccines. The tested vaccines were a Trojan vaccine containing HPV-16 HLA-I and HLA-II restricted peptides (Voskens et al. 2012), a p16(INK4a)-based peptide vaccine (Reuschenbach et al. 2016), and a peptide immunomodulatory vaccine GL-0810 (Zandberg et al. 2015). Two of the studies (Voskens et al. 2012, Zandberg et al. 2015) tested vaccines developed specifically for HPV 16 positive HNSCC. The third study (Reuschenbach et al. 2016) tested a vaccine developed for any HPV positive HNSCC.

3.2.3. Patients. In each of the three included studies (Voskens et al. 2012, Reuschenbach et al. 2016, Zandberg et al. 2015), a portion of the study population had HPV positive HNSCC. The size of this portion ranged from two to up to nine patients per study. The combined number of patients with HPV positive HNSCC amounted to 17 across the studies. The patients within this portion were between the ages of 43 to 68, with the mean age being 56 years. The patients were predominantly male.

3.2.4. Samples. In all the three included studies, the histopathological diagnosis of HNSCC was confirmed via biopsy.

3.2.5. HPV genotypes and detection methods. Two of the studies examined vaccine efficacy on HPV 16 positive HNSCCs (Voskens et al. 2012, Zandberg et al. 2015), while the third examined vaccine efficacy on HPV associated HNSCCs regardless of HPV type (Reuschenbach et al. 2016). In two of the studies (Voskens et al. 2012, Zandberg et al. 2015), the presence of HPV in biopsies was confirmed via extracting RNA with Qiagen RNeasy Mini kit, performing cDNA synthesis with an iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA), amplifying HPV-16 cDNA with PCR using primers E7_F GCT CAG AGG AGG AGG ATG AA and E7_R GCC CAT TAA CAG GTC TTC CA10, and verifying PCR products with direct sequencing. In the third study (Reuschenbach et al. 2016), the presence of HPV in biopsies was confirmed via PCR and hybridization using a kit for multiplex HPV genotyping (DiaMex GmbH, Heidelberg, Germany).

3.2.6. Follow-up. The length of the follow-up period was 24 months in two of the studies (Voskens et al. 2012, Zandberg et al. 2015). In the third (Reuschenbach et al. 2016), it was 6 months.

3.2.7. Vaccine efficacy. Nearly all patients (80-100%) who completed their respective vaccination cycle developed antigen-specific immune responses to the tested vaccines. However, no complete or partial HPV positive HNSCCs tumour responses could be observed according to RECIST criteria (Voskens et al. 2012, Reuschenbach et al. 2016, Zandberg et al. 2015). Of the total 17 subjects across studies, 4 (23%) developed stable disease, 11 (65%) developed progressive disease, and the condition of 2 (12%) could not be assessed. Among the total study population, the vaccines were overall well-

tolerated, but a single incidence of serious adverse effect was recorded in a patient with pre-existing stable brain metastases (Voskens et al. 2012).

Reference and publication date	Location	Study type	HPV types	Confirmation of tumour HPV status at study onset	Total population	Population with HPV positive HNSCC: size; sex; age range	HPV positive HNSCCs type	Vaccine type and dosage	Follow-up	Incidence of antigen-specific immune response to vaccine in HPV+ HNSCC population	Tumour response by RECIST criteria
Voskens et al. 2012	USA	Pilot study, single-arm intervention trial	16	Biopsy	5 patients with advanced HNSCC	2; male; 47 and 55	Unspecified	Trojan vaccine containing HPV-16 HLA-I and HLA-II restricted peptides 1 d, 1 m, 2 m, and 3 m	Up to 24 m	100%	No complete or partial clinical response could be measured.
Reuschenbach et al. 2016	USA	Open-label, single-arm phase 1/2a study	Any	Biopsy	26 patients with advanced, p16(INK4a)-overexpressing HPV positive SCC	6; not reported; 49-66	Oral, pharyngeal, tonsillar, neck	p16(INK4a)-based peptide vaccine 1 d, 1 w, 2 w, 3w Repeated up to thrice for a maximum of 12 vaccinations.	Up to 6 m	100%	No complete or partial clinical response could be measured.
Zandberg et al. 2015	USA	A single center phase 1 dose escalation study	16	Biopsy	16 patients with progressive and recurrent/ metastatic HNSCC	9; male; 43-68	8 oro-pharyngeal, 1 laryngeal	Peptide immunomodulatory vaccine GL-0810 1 d, 2 w, 4 w, and 6 w	Up to 24 m	80% (among the 5 patients who received all 4 vaccination doses)	No complete or partial clinical response could be measured.

Table 1. Summary of the composition and results of the studies discussing therapeutic HPV vaccines.

4. DISCUSSION

4.1. Summary. Contemporary research data indicate that both the quadrivalent (Gardasil) and bivalent (Cervarix) prophylactic HPV vaccines are effective at preventing oral infection with HPV types 16 and 18. Furthermore, the data show that while therapeutic HPV vaccines are both well-tolerated and effective at inducing desired antigen-specific immune responses, they have yet to demonstrate measurable curative or palliative effects on pre-existing HPV positive HNSCCs in human populations.

4.2. The state and quality of current research. The original aim of this review was to pool research data available through PubMed on the effects of HPV vaccines on HPV positive HNSCCs. However, little research addressing this exact topic was yet available. Due to this scarcity of studies, and considering the fact that oral HPV infection has been established as a major contributing factor in the development of HPV positive HNSCCs, the inclusion criteria were widened to accommodate for studies investigating the efficacy of HPV vaccines against oral HPV infection and/or effects of HPV vaccines on HPV antibody levels in the oral cavity. It should be noted that our use of only a single electronic database (PubMed) for material selection is a limiting factor on the quality of our results.

The available research primarily focused on the effects of vaccination on HPV types 16 and 18 and on HNSCCs associated with them. Considering that HPV 16 is found in four-fifths of all HPV-associated HNSCC worldwide (Ndiaye et al. 2014) and out of all HPV types possesses the most well-established connection to the development of HNSCC (Saulle et al. 2015), this focus aligned well with the aims of this review. In all available

research, the data collected on HPV 16 was considerably more robust than the data collected on HPV 18. This was to be expected, as the latter is identified far less frequently than the former in HPV positive HNSCCs (in only 5.9% of cases worldwide) (Ndiaye et al. 2014).

As of today, there is no one golden standard method to detect the presence and evaluate the nature of oral HPV infection. The methods of confirming the presence of oral HPV infection varied between the studies included in this review, but ultimately relied on detecting either HPV DNA (Herrero et al. 2013, Hirth et al. 2017, Reuschenbach et al. 2016) or HPV RNA (Voskens et al. 2012, Zandberg et al. 2015). Detection of HPV DNA in sample confirms the presence of the virus but does not disclose whether the virus is active or not. Rather, the activity of HPV infection is determined by observing changes in host cell gene expression, such as via HPV RNA detection. (Abreu et al. 2012).

Pinto et al. 2016 and Handisurya et al. 2016 measured the presence of anti-HPV IgG antibodies in samples in order to compare the oral anti-HPV IgG antibodies in patients pre- and post-vaccination. It is prudent to note that anti-HPV IgG antibodies might not manifest in an infected patient with low seroconversion, or the seroconversion might occur months after the immunological trigger. However, undetected anti-HPV IgG antibody seroconversion at post-vaccination would not have diminished the observed results of vaccine efficacy, but rather strengthened them. As such, considering the study endpoints, the employed detection methods did not take away from the credibility of the derived data.

While there was considerable heterogeneity in the population sizes of the studies that examined the effects of prophylactic HPV vaccines (Pinto et al. 2016, Handisurya et al. 2016, Herrero et al. 2013, Hirth et al. 2017), the studies with similar endpoints (Pinto et al. 2016 and Handisurya et al. 2016; Herrero et al. 2013 and Hirth et al. 2017) had roughly comparable population sizes (150 and 34; 5834 and 3040, respectively). As

such, the data is best considered in two categories. Herrero et al and Hirth et al. offer robust evidence that existing prophylactic vaccinations prevent oral infection with HPV 16 and 18. Pinto et al. and Handisurya et al., on the other hand, attempt to evaluate the means of protection these vaccines confer against oral HPV infection, but include considerably smaller study populations. Lastly, it should be noted that Herrero et al. did not record baseline oral HPV infection status of the study participants, and as such the accuracy of the observed vaccine efficacy is limited. However, the considerable disparity in oral HPV infection status between such large study groups at the moment of examination is unlikely to have resulted from chance alone.

The HPV positive populations of the studies that examined the effects of therapeutic vaccines on HNSCCs or HPV positive squamous cell cancers were all similarly small in size. The low number of study participants was to be expected due to the constraints imposed by the nature of the studies but should nevertheless be taken into account when considering the robustness and generalisation potential of the derived data.

4.3. Prophylactic HPV vaccination. On the topic of prophylactic HPV vaccines, contemporary research data indicate four conclusions: 1) Prophylactic vaccination with the quadrivalent vaccine elicits detectable levels of HPV antibodies in oral fluids (Pinto et al. 2016, Handisurya et al. 2016). 2) When normalising for IgG levels, the levels of HPV 16 (and possibly 18) antibodies in oral fluids correlate significantly with those in serum (Pinto et al. 2016, Handisurya et al. 2016). 3) The oral antibody levels elicited by the quadrivalent and bivalent vaccines appear sufficient to stave off oral HPV infection *in vitro* (Handisurya et al. 2016). 4) Populations vaccinated with either the quadrivalent or bivalent vaccine demonstrate greatly reduced incidence of oral HPV infection with types 16 and 18 in comparison to nonvaccinated populations (Herrero et al. 2013, Hirth et al. 2017).

These findings suggest that the quadrivalent and bivalent HPV vaccinations protect against oral HPV infection. Considering that oral HPV infection is a major contributing factor to the development of HPV positive HNSCCs, it can be extrapolated that the prophylactic HPV vaccinations are also likely to deter the development of HPV positive HNSCCs, though no research has yet directly demonstrated this. This conclusion is further supported by the results of studies published after the material selection phase of this review (Castillo et al. 2019, Mehanna et al. 2019) and echoed in another contemporary review by Ebenezer Tumban (2019).

The effectiveness of prophylactic vaccines as tools for preventing the development of HPV positive HNSCCs ought to be further examined. The higher rate of HPV positive HNSCC incidence in male populations in comparison to female populations (Jemal et al. 2013) might warrant HPV-vaccination programs to be extended to cover men as well. Another topic of further research is the timing of vaccination. The current vaccination model only requires the vaccination to be administered prior to the patient's sexual debut, and as such does not protect from non-sexual HPV transmission prior to this time. It has been established that vertical transmission of HPV, such as from mothers to children, can occur in the very early years of life (Trottier et al. 2016, Zouridis et al. 2018, Sabeena et al. 2017, Koskimaa et al. 2012, Rintala et al. 2005). Earlier vaccine administration could prevent a portion of these cases.

4.4. Therapeutic HPV vaccination. The studies investigating the effects of therapeutic HPV-vaccines on HPV positive HNSCCs (Voskens et al. 2012, Reuschenbach et al. 2016, Zandberg et al. 2015) in human populations are the first in their field. Reflecting this, their primary endpoint was to assess the safety and immunogenicity of their respective tested vaccines, with the measuring of tumour response to vaccination a secondary endpoint. All included studies presented similar results: the various therapeutic

vaccines were well-tolerated and vaccinated subjects developed desired immune responses, but tumour progression appeared largely unaffected. In one noteworthy instance (Voskens et al. 2012), tumour biopsies conducted pre- and post-vaccination on a single patient revealed that the vaccination appeared to have considerably bolstered the presence of immune cells within the tumour (from 0.4% of cells in biopsy to 8.1%).

A major limiting factor on the quality of data from the therapeutic vaccine studies was the size of the portions of the study populations with HPV positive HNSCCs, ranging from only two to nine patients per study. Furthermore, the population of the study with the highest portion of HPV positive HNSCC patients (Zandberg et al. 2015) decreased in number from nine to five individuals over study duration as participants dropped out due to tumour progression. Also, subjects in one of the study populations (Voskens et al. 2012: two individuals, representing 12% of the total initial HPV positive HNSCC population across all studies) received other cancer treatments alongside the therapeutic HPV vaccination, muddying the causality of tumour response to the HPV-16 Trojan vaccine treatment. Put together, the total final HPV positive HNSCC population between the studies discussing therapeutic vaccines tallied to only 13 individuals, of which two (15%) received treatment other than the therapeutic vaccination alongside study conduction. The pooled results presented in this review, as well as the conclusions drawn from them, should therefore be considered directional at best.

The aforementioned findings indicate that HPV positive HNSCC therapy via vaccination presents no major roadblocks to discourage further study, but the efficacy of the treatment is still in question. Other contemporary reviews (Tumban 2019, Schneider et al. 2018) concur with the former conclusion, but present a more optimistic outlook on the latter. A review by Schneider et al. (2018) includes a study (Glisson et al. 2017) that

reports a portion (33%) of the study population showing complete or partial HPV positive HNSCC tumour responses to therapeutic HPV vaccination with ISA 101 and nivolumab. In addition, a review by Yang et al. (2017) notes cases where female patients experienced partial remission of cervical HPV positive cancer lesions after therapeutic HPV vaccination.

4.5. Conclusions. We conclude that existing prophylactic HPV vaccinations appear to provide robust protection against oral infection with HPV types 16 and 18 in individuals uninfected prior to vaccination. By extension, these vaccines are likely to impede the development of HPV positive HNSCCs in the vaccinated individuals. However, further large-scale studies are required to consolidate this conclusion.

Therapeutic HPV vaccines, on the other hand, are yet in such an early phase of testing that no valid conclusion of their efficacy against HPV positive HNSCCs can be drawn. Further study is required to better assess their validity as treatment options for HPV positive HNSCCs.

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