JEMI-PEARLS

Rethinking Human Papillomavirus Vaccine for Oral and Oropharyngeal Cancer Prevention and Global Implementation

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BACKGROUND INFORMATION

Oral and oropharyngeal cancer (OOC) is a subtype of head and neck cancer, in which malignant cells grow in the oral cavity and the oropharynx. These two anatomical locations encompass the inside lining of the lips, the gums, the tongue, the hard and soft palates, and the side and the back wall of the throat. OOC has long be attributed to synergistic use of alcohol and tobacco [1]. However, the recent discovery that human papillomavirus (HPV) can also cause OOC is prompting a paradigm shift in the fields of OOC, HPV vaccination, and clinical treatment.

A comparison of the current trends in OOC and cervical cancer reveals the need for a change in the perspective on OOC. The incidence rate for cervical cancer has been steadily declining since the 1980s as a result of better screening (e.g. Pap test) and vaccines (e.g. 2vHPV, 4vHPV, and 9vHPV) [2]. On the other hand, HPV-positive OOC is an emerging threat. The HPV-positive oropharyngeal cancer incidence rate is projected to increase by 50% over the next 15 years [3]. Moreover, it is projected to eclipse the cervical cancer prevalence by 2020 [4]. The proportion of HPV-positive head and neck cancers has been also increasing from 18% in 1985, and is projected to increase to 90% by 2025 [3]. This is likely due to the combined result of declining alcohol and tobacco use, and the increasing prevalence of HPV. One of the reasons for the increasing prevalence of HPV-positive OOC is a change in sexual behaviours [4]: oral sex, which was previously considered a taboo, has become more common today [4]. The fact that OOC is 2 times more prevalent in men than in women yet most vaccine programs only cover women demonstrates how OOC is neglected in today's society [5].

HPV is a sexually transmitted non-enveloped double-stranded DNA virus with over 170 types. It is classified by its L1 structural protein. It infects the keratinocytes in the epithelium and can cause common skin warts or cancer [6]. It can be divided into high-risk or low-risk based on oncogenesis. High-risk types include HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58 and -59 [7].

Cervical cancer is predominantly caused by HPV-16 and -18, whereas OOC is caused by several HPV types [7]. In 2008, Dr. Harald zur Hausen was awarded the Nobel Prize for his discovery that HPV causes cervical cancer [8].

The circular genome of the virus is around 7900 base pairs (Fig. 1). It is divided into early (E) region, late (L) region, and an upstream regulatory region (URR). There are seven E genes important in the transcription, replication, and structural modification of the host cell. The L genes express the L1 and L2 capsid proteins, which are important for entry into the host cell.

> " The HPV-positive oropharyngeal cancer incidence rate is projected to increase by 50% over the next 15 years "

Currently, there are three licensed vaccines. 2vHPV is a bivalent vaccine targeting the L1 protein. It protects against HPV-16 and -18, which account for only 68% of cervical cancers and only 60% of OOC [7]. 4vHPV is a tetravalent vaccine that protects against two additional types, HPV-6 and -11. The newly introduced 9vHPV protects against 90% of HPV types that cause cervical cancer [7]. Extensive immunization programs can achieve relatively wide coverage: for example, the program in British Columbia (BC) provides free vaccines to girls in grade 6, and men 9 to 26 years of age who are infected with HIV, or have sex with men (MSM) [9]. However, such wide coverage is not the case in less economically developed countries (LEDCs), where men are not covered at all. For example, in Kenya, the vaccine is not financed by the national health authority, and it costs more than the average annual family income [10]. hypothesis.

RESEARCH QUESTIONS

The high cost of HPV vaccine is forcing immunization programs to prioritize the recipients, thereby often excluding men and neglecting the importance of OOC. This trend of primarily focusing on cervical cancer is leading to increasing incidence rates of OOC, and this will not stop unless the cost of the vaccine is significantly reduced or a novel vaccine is developed. In this context, this article will examine three key areas. First, it is valuable to study the link between HPV and OOC, and the mechanism for HPV pathogenesis. Second, it is important to assess the current vaccine, 9vHPV, as a prophylactic agent for OOC. Because a tremendous amount of time and resources have already been put into developing the current vaccine, there must be sufficient reason to justify the development of a novel vaccine. Lastly, the possibility of a next-generation HPV vaccine that overcomes the limitations of 9vHPV is explored. Recent HPV vaccine studies have been centred on the L2 protein because it could potentially be implemented on a global scale unlike the L1-based 9vHPV.

PROJECT NARRATIVE

What is the link between HPV and OOC, and what is the mechanism for the pathogenesis?
Until recently, HPV has primarily been known to cause cervical cancer, and smoking and drinking have been thought to be the main risk factors in OOC. Blot *et al.* found that there was a 35-fold increase in OOC among those who consumed two or more packs of cigarettes and four or more drinks per day [1]. Moreover, quitting smoking was associated with a steep decrease in the risk of cancer [1]. A notable study by Syrjänen *et al.* demonstrated using immunohistochemistry that HPV antigens are detected in oral squamous cell lesions, raising the possibility that HPV may be responsible for more than just cervical cancer [11]. A case-control study by Schwartz *et al.* found that oral squamous cell carcinoma risk increased with an increasing number of
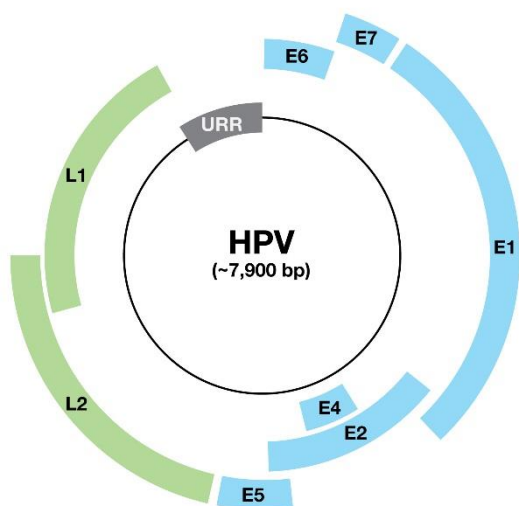

FIG. 1 HPV genome organization: HPV genome is a double-stranded circular genome of around 7900 bp. It is divided into early (E), late (L), and upper regulatory (URR) regions.

sexual partners, and suggested that HPV-16 may have an effect on oral squamous cell carcinoma [12]. At the time, they believed that HPV-16 worked in conjunction with smoking [12].

A breakthrough paper by Gillison *et al.* provided PCR-based evidence that HPV-16 was identified in 90% of HPV-positive head and neck squamous cell carcinomas [13]. Moreover, an integrated HPV-16 genome was detected using Southern blot [13]. Contrary to the findings of Schwartz *et al.*, Gillison *et al.* found that HPV-positive oropharyngeal tumours arising from lingual and palatine tonsils were distinct from HPV-negative oropharyngeal tumours, suggesting that HPV causes an etiologically different subset of head and neck tumours from tobacco and alcohol-associated tumours [13]. To further substantiate that HPV-positive OOC is molecularly distinct, the tumour suppressor protein p53 was found to be degraded by the viral E6 protein [14]. However, Ahomadegbe *et al.* demonstrated that HPV-negative OOC is actually associated with a p53 mutation rather than degradation [15]. This difference in pathogenesis is significant as the clinical treatment for HPV-positive OOC would have to change. Tinhofer *et al*. suggest that next-generation sequencing in order to optimize adjuvant chemotherapy may allow better targeting of head and neck cancers [16]. The landmark 7-year population study by D'Souza *et al.* illustrated that a high lifetime number of oral sex partners is strongly associated with oropharyngeal cancer regardless of tobacco and alcohol consumption [17]. This finding eventually led to the acknowledgement of the World Health Organization (WHO) that HPV causes oral and oropharyngeal cancers, though the occurrence rate is lower in the oral cavity compared to the oropharynx.

Understanding HPV pathogenesis is essential when exploring potential vaccine and therapeutic targets. As illustrated in Figure 2, micro-abrasion or wounding of oropharynx mucosal lining exposes the cells of the basement membrane to HPV. The L1 major capsid protein of HPV attaches to stratum basal keratinocytes by binding to cellular heparan-sulfate proteoglycans (HSPG), specifically syndecan-1 [6]. It is thought that α6 integrins are a secondary cellular receptor [6]. The HPV virion then enters the cell via actin-dependent endocytosis [18]. The HPV genome can remain as an episome in the cytoplasm, or it can integrate into the cellular genome for a latent infection [6]. HPV has two oncogenes, E6 and E7, which express oncoproteins [6]. E6 first binds to E6AP, a cellular protein, in order to bind to its targets: p53, PDZ, a domain found in multiple cellular proteins, and NFX-1, a transcriptional repressor [6]. When the E6/E6AP complex binds to the targets,
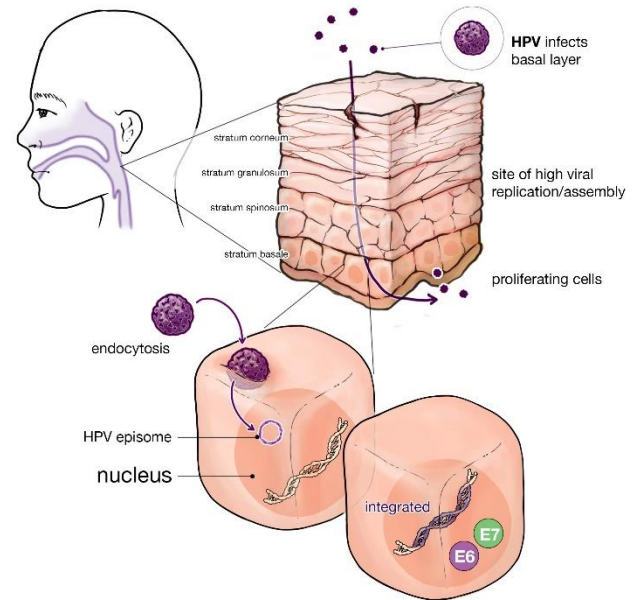
FIG. 2 Site of oropharyngeal cancer and HPV infection of the mucosal membrane: When there is a microabrasion or tear in the outer membrane, HPV virion can enter the mucosa and infect the proliferating cells in the basal layer. The virus enters the cell by endocytosis, and the genome can remain as an episome in the cytoplasm or enter the nucleus and integrate with the cellular DNA. When integrated, the oncoproteins, E6 and E7, can be expressed at stratum spinosum or stratum granulosum, where high viral replication occurs.

degradation of these proteins is induced via the ubiquitin pathway [14]. Degradation of p53 leads to inhibition of apoptosis, PDZ degradation leads to cellular transformation, and NFX1 degradation leads to activation of telomerase [14]. The E7 protein promotes the cleavage of the tumour suppressor pRb, preventing regulation of E2F1, which activates genes that facilitate G1/S phase [14].

How effective is the current vaccine, 9vHPV, as a prophylactic agent for OOC?
To discuss the need for an alternative vaccine target, effectiveness of current vaccines must be evaluated. There are currently three FDA-approved vaccines: 2vHPV, 4vHPV, and 9vHPV. 2vHPV includes HPV-16 and -18, 4vHPV adds HPV-6 and -11, and 9vHPV adds HPV-31, -33, -45, -52 and -58. Kash *et al.* reported no serious vaccine-related side effects to 4vHPV in multiple double-blind, placebo-controlled trials with females aged 18-30 in the United States [19]. 2vHPV, which contains an aluminum hydroxide adjuvant, was mostly well-tolerated except for dose-dependent local injection site reactions [19]. This study also demonstrated a 98.1% efficacy against cervical

intraepithelial neoplasia [19]. The new 9vHPV was shown to be 97% effective against the additional five HPV types [19]. Although the 9vHPV theoretically should prevent 65.9% of total oropharyngeal cancers (94% of HPV-positive oropharyngeal cancers), there have been no trials that assessed this efficacy [7, 2]. The lack of trials for vaccine efficacy on OOC again highlights how OOC is often an unacknowledged consequence of HPV infection. Fleury and Touzé generated mutations in HPV-16 L1capsid protein surface-exposed FG loop and found that the mutated self-assembled virus-like particles (VLP) were only weakly neutralized by sera from wild-type VLP-immunized mice [20]. This suggests that only a few point mutations in the FG loop of L1 would generate a new serotype, rendering the current vaccines useless [20].

There are also logistical challenges to the licensed vaccines. Immunological memory has to persist for at least 15 years to prevent cervical cancer and to be cost-effective [21]. Long-term efficacy studies have demonstrated that all 2vHPV, 4vHPV, and 9vHPV continue to be immunogenic for over 8 years [22]. At present, the HPV vaccine is administered at 0-, 2-, and 6-month time points. However, Widdice et al. found that 50% of the required doses were completed late [23]. In another study, Schluterman et al. found that only 11% of those who initiated the vaccination completed all three doses [24]. Such a low vaccine completion rate in more economically developed countries is worrisome as it would most likely translate to minimal completion rate in LEDCs. This low vaccine completion rate is encouraging researchers to consider the possibility of reducing the number of doses from to three to two. Dobson et al. demonstrated in a clinical trial that the immunogenicity of girls given two doses at 0- and 6-month time points was noninferior for both HPV-16 and HPV-18 than the girls given three doses, when measured 1 month after the final dose [25]. However, the efficacy of the two-dose regimen is still unknown, and requires more research. Other downsides of licensed HPV vaccines are the price, the need for a cold chain to transport the vaccine, and type-restricted immunity. For global implementation, the $360 USD cost is a major deterrent [26]. VLP are not stable at a wide range of temperatures, so delivery is a challenge. The requirement for a three-dose regimen would bring logistic challenges to countries where healthcare workers who can perform the vaccination are limited. Moreover, due to the type-restricted nature of licensed vaccines, there is a need for continuous screening for cervical cancer [27]. Moreover, an oropharyngeal cancer

screening method would have to be developed, since an effective method currently does not exist. Thus, it may be more cost-effective to develop a new vaccine that is cheap to manufacture, easy to transport, and not type-restricted.

How can we develop a novel HPV vaccine that will address the shortcomings of 9vHPV?

The main arguments against the current licensed vaccines are that they are too costly for global implementation and not protective against all relevant HPV genotypes. In order to overcome these shortcomings, the next-generation HPV vaccine should be cheap to manufacture, and be protective against a broad range of genotypes. Figure 3 illustrates the composition of VLPs, the full virus, and a possible peptide-based vaccine. One target that is currently being explored is the L2 minor capsid. Unlike the L1 protein, the L2 protein is normally concealed inside the L1 capsomere. When the L1 protein binds to HSPG and a conformational change occurs, L2 is then transiently exposed to be cleaved by furin [28]. This furin cleavage is necessary for infection, and is also necessary for endosomal escape [28]. Richards et al. found that the L2 cleavage site for furin is highly conserved among multiple genotypes [28].

The difference between the L1 and L2 proteins with respect to vaccine development is the L2 protein's highly conserved regions. In a pivotal paper, Gambhira et al. discovered a broadly cross-neutralizing epitope of L2 by generating a monoclonal antibody [29]. The RG-1
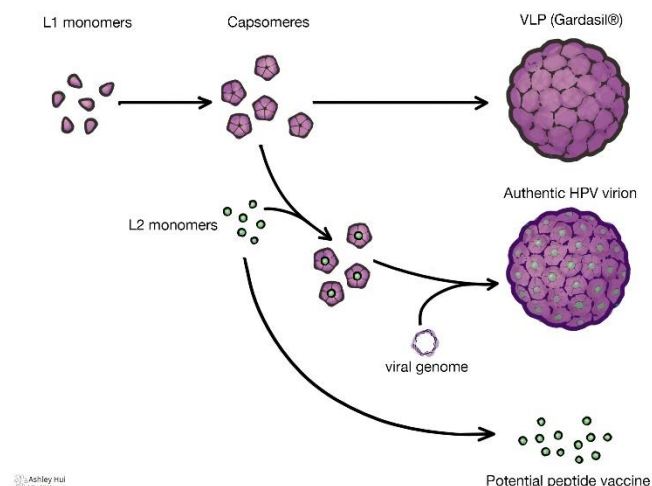
FIG. 3 Composition of VLP, authentic HPV virion, and potential peptide vaccine: Five L1 monomers form a capsomere. These capsomeres can spontaneously form a VLP. When L2 monomer is embedded in the capsomeres surrounding a viral genome, authentic HPV virion is formed. L2 monomer may be enough to function as a potential peptide vaccine.

epitope comprises residues 17 to 36, and it is highly conserved among different genotypes. One drawback is that L2 is subdominant to L1, and elicited lower titres of antibodies [29]. It was later found that there are other conserved epitopes. Tyler *et al.* used a mouse genital challenge model to show that mice vaccinated with peptide residues 65–85 elicited strongly neutralizing antibody response against an HPV-16 pseudovirus, but not broadly neutralizing antibody response against other HPV types [30]. However, when they vaccinated the mice with VLPs displaying a peptide that represented a consensus sequence from multiple HPV types, the mice elicited a strong and broadly neutralizing antibody response [30]. A possible alternative to L1- or L2-based vaccines is the use of L1/L2 VLPs. However, this method applied in a rabbit model again did not improve the immunogenicity of L2 as it is only transiently exposed during infection [31].

Although L2 protein elicits the desired broadly neutralizing antibodies, a recurring challenge is its inferior immune response compared to that from L1 protein [29]. Jiang *et al.*, however, argue that the current assays being used to test neutralizing antibody titres are more sensitive to L1 than L2 [32]. Thus, new assays to measure L2-specific antibodies are needed to see whether L2-based vaccines are really inferior to L1-based vaccines [32]. There are several possible approaches to improve the immunogenicity of L2-based vaccines. L2 polypeptide or peptide protein may elicit high titres of broadly-neutralizing antibodies if provided with an adjuvant to overcome their poor immunogenicity. A follow-up study by Schellenbacher *et al.* demonstrated that RG1-VLP, which is a chimeric VLP presenting the L2 residues 17–36 (RG1 epitope) within a surface loop of HPV16 L1, along with an alum-MPL (aluminum hydroxide and monophosphoryl lipid A) adjuvant provided a robust antibody response in mice to 20 HPV pseudovirus types [33]. Moreover, the protection lasted at least 1 year [33]. This RG1-VLP is currently in an early phase clinical development. Another way to improve RG1-VLP is to incorporate the concatenated multi-type L2 fusion protein, which combines cross-protective L2 epitopes of several HPV types, instead of a single RG1 epitope. Vaccination with L2 fusion protein derived from multiple epitopes resulted in high titres of neutralizing antibodies against all HPV types tested [34]. One major disadvantage of the current licensed vaccine is the requirement for multiple doses, which is a logistical impediment in developing countries lacking qualified healthcare personnel that can administer the vaccine. Thus, oral

vaccine is also being explored to circumvent this issue [35].

SUMMARY AND CONCLUSION

Over the last 30 years, there have been incredible achievements in HPV research—from the discovery of oncogenic HPV types to working vaccines. The efficacy of these vaccines is demonstrated in the 64% decrease in the prevalence of the four vaccine-preventable HPV types among girls aged 14 to 19 in the United States [36]. However, the current solution mostly benefits more economically developed countries (MEDCs) and females, as the cost of vaccination is too high to allow full global implementation or inclusion of males in vaccine programs. Therefore, OOC, which is more prevalent in men than in women, is projected to overtake cervical cancer prevalence by 2020 [4]. There are steps being taken to make the vaccine more cost-effective, such as reducing vaccine doses from three to two, but this will not solve other inherent limitations of currently licensed HPV vaccines.

As summarized in Figure 4, there are 5 key limitations of 9vHPV. It requires multiple doses; this results in low adherence, and low vaccine program completion rate [24]. The use of a VLP vaccine necessitates a cold chain for storage and transportation. Newly introduced 9vHPV still does not protect against 6% of HPV-positive OOC and 10% of HPV-positive cervical cancer. Type-restricted immunity would require continuous screening of cervical cancer as well as the development of an OOC screening method as it currently does not exist. High cost is a major drawback as it limits the vaccine coverage to mostly women, and hinders global implementation. This could all change with the introduction of an L2-based vaccine.

Current research on an L2-based vaccine is promising, and it could improve on almost all aspects of the current vaccine. The L2 protein is the minor capsid protein that is only exposed during an infection. As it is the site of furin cleavage, it requires highly conserved regions, unlike the L1 proteins. Thus, it could potentially lead to a pan-genotype vaccine that would fully protect people from HPV. Targeting these highly-conserved regions for vaccine development resulted in a robust antibody response when combined with adjuvant [33]. Combining multiple highly-conserved epitopes yielding an L2 fusion protein elicited high titres of neutralizing antibodies against all HPV types tested [34]. With this peptide or protein-based vaccine, only a single dose may be required [37]. This would eliminate adherence problem associated with licensed vaccine,
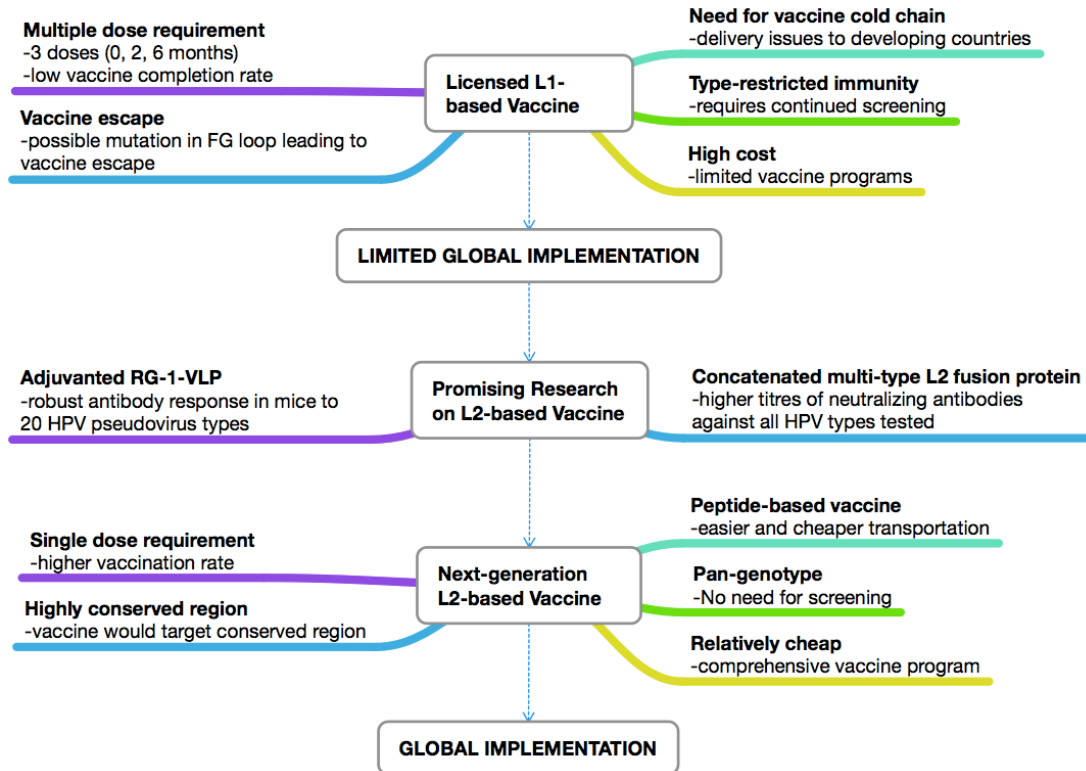
FIG. 4 Summary of 9vHPV evaluation and next-generation L2-based vaccine. The currently licensed L1-based vaccine has many disadvantages that lead to limited global implementation. Promising research on L2-based vaccine may use adjuvanted RG-1-VLP or concatenated multi-type L2 fusion protein, which improves upon the L1-based vaccine, and is more suitable for global implementation.

and increase the vaccination rate. A peptide vaccine would also eliminate the transportation and storage issues. Ultimately, a cheap manufacturing cost associated with the peptide vaccine would result in a more comprehensive vaccine program that includes males as well as the older population. Moreover, it could be globally implemented.

Although the next-generation HPV vaccine is promising, there is still a long way to go. One major obstacle it will face is the direct comparison with currently available vaccines. It will be difficult to justify and push for phase 1 clinical trials when there already is an imperfect but working solution. There needs to be more data on the efficacy of the HPV vaccine on non-cervical cancer. There also needs to be more effort to eliminate the stigma related to HPV in order to improve the vaccination rate, because many misinformed parents worry that the vaccine will increase teenage sexual activity [38]. The next-generation HPV vaccine, together with improvements on the current vaccines, may be the key to finally putting an end to the HPV epidemic in not only developed countries but also developing countries.

REFERENCES
1. Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, Bernstein L, Schoenberg JB, Stemhagen A, Fraumeni JF. 1988. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 48:3282–7.
2. Giuliano A, Kreimer A, Sanjose S. 2015. The Beginning of the end: vaccine prevention of HPV-driven cancers. J Natl Cancer I 107:djv128.
3. Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, Jiang B, Goodman MT, Sibug-Saber M, Cozen W, Liu L, Lynch CF, Wentzensen N, Jordan RC, Altekruse S, Anderson WF, Rosenberg PS, Gillison ML. 2011. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 29:4294–301.

4. Moore KA, Mehta V. 2015. The growing epidemic of HPV-positive oropharyngeal carcinoma: A clinical review for primary care providers. J Am Board Fam Med 28:498–503.

5. Wu X. 2015. Human papillomavirus–associated cancers — United States, 2004–2008. Morbidity and Mortality Weekly Report. 2012;61(15):258-61.

6. Miller DL, Puricelli MD, Stack MS. 2012. Virology and molecular pathogenesis of HPV (human papillomavirus) associated oropharyngeal squamous cell carcinoma. Biochem J 443:339–353.

7. Saraiya M, Unger ER, Thompson TD, Lynch CF, Hernandez BY, Lyu CW, Steinau M, Watson M, Wilkinson EJ, Hopenhayn C, Copeland G, Cozen W, Peters ES, Huang Y, Saber MS, Altekruse S, Goodman MT. 2015. US assessment of HPV types in cancers: implications for current and 9-valent HPV vaccines. J. Natl Cancer Inst 107:djv086.

8. Weiss RA. 2008. On viruses, discovery, and recognition. Cell 135(6):983-986.

9. Ogilvie G, Anderson M, Marra F, McNeil S, Pielak K, Dawar M, McIvor M. 2010. A population-based evaluation of a publicly funded, school-based HPV vaccine program in British Columbia, Canada. PLoS Med 7(5):e1000270.

10. Friedman AL, Oruko KO, Habel MA, Ford J, Kinsey J, Odhiambo F, Phillips-Howard PA, Wang SA, Collins T, Laserson KF, Dunne EF. 2014. Preparing for human papillomavirus vaccine introduction in Kenya: implications from focus-group and interview discussions with caregivers and opinion leaders in Western Kenya. BMC Public Health 14:855.

11. Syrjänen KJ, Pyrhönen S, Syrjänen SM, Lamberg MA. 1983. Immunohistochemical demonstration of human papilloma virus (HPV) antigens in oral squamous cell lesions. Br J Oral Surg 21:147–53.

12. Schwartz SM, Daling JR, Doody DR, Wipf GC, Carter JJ, Madeleine MM, Mao EJ, Fitzgibbons ED, Huang S, Beckmann AM, McDougall JK, Galloway DA. 1998. Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. J Natl Cancer Inst 90:1626–36.

13. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. 2000. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 92:709–20.

14. Faridi R, Zahra A, Khan K, Idrees M. 2011. Oncogenic potential of Human Papillomavirus (HPV) and its relation with cervical cancer. Virol J 8:269.

15. Ahomadegbe JC, Barrois M, Fogel S, Bihan ML Le, Douc-Rasy S, Duvillard P, Armand JP, Riou G. 1995. High incidence of p53 alterations (mutation, deletion, overexpression) in head and neck primary tumors and metastases; absence of correlation with clinical outcome. Frequent protein overexpression in normal epithelium and in early non-invasive lesions. Oncogene 10:1217–27.

16. Tinhofer I, Budach V, Saki M, Konschak R, Niehr F, Jöhrens K, Weichert W, Linge A, Lohaus F, Krause M, Neumann K, Endris V, Sak A, Stuschke M, Balermpas P, Rödel C, Avlar M, Grosu AL, Abdollahi A, Debus J, Belka C, Pigorsch S, Combs SE, Mönnich D, Zips D, Baumann M. 2016. 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 57:78–86.

17. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, Westra WH, Gillison ML. 2007. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 356:1944–56.

18. Schelhaas M, Shah B, Holzer M, Blattmann P, Kühling L, Day PM, Schiller JT, Helenius A. 2012. Entry of human papillomavirus type 16 by actin-dependent, clathrin- and lipid raft-independent endocytosis. PLoS Pathog 8:e1002657.

19. Kash N, Lee MA, Kollipara R, Downing C, Guidry J, Tyring SK. 2015. Safety and efficacy data on vaccines and immunization to human papillomavirus. J Clin Med 4:614–33.

20. Fleury MJJ, Touzé A, Coursaget P. 2014. Human papillomavirus type 16 pseudovirions with few point mutations in L1 major capsid protein FG loop could escape actual or future vaccination for potential use in gene therapy. Mol Biotechnol 56:479–86.

21. Barnabas RV, Laukkanen P, Koskela P, Kontula O, Lehtinen M, Garnett GP. 2006. Epidemiology of HPV 16 and cervical cancer in Finland and the potential impact of vaccination: mathematical modelling analyses. PLoS Med 3:e138.

22. Vincenzo R, Conte C, Ricci C, Scambia G, Capelli G. 2014. Long-term efficacy and safety of human papillomavirus vaccination. International journal of women's health 6:999.

23. Widdice LE, Bernstein DI, Leonard AC, Marsolo KA, Kahn JA. 2011. Adherence to the HPV vaccine dosing intervals and factors associated with completion of 3 doses. Pediatrics 127:77–84.

24. Schluterman NH, Terplan M, Lydecker AD, Tracy JK. 2011. Human papillomavirus (HPV) vaccine uptake and completion at an urban hospital. Vaccine 29:3767–72.

25. Dobson SR, McNeil S, Dionne M, Dawar M, Ogilvie G, Krajden M, Sauvageau C, Scheifele DW, Kollmann TR, Halperin SA, Langley JM, Bettinger JA, Singer J, Money D, Miller D, Naus M, Marra F, Young E. 2013. Immunogenicity of 2 doses of HPV vaccine in younger adolescents vs 3 doses in young women: a randomized clinical trial. JAMA 309:1793–802.

26. Malik H, Khan FH, Ahsan H. 2014. Human papillomavirus: current status and issues of vaccination. Arch Virol 159:199–205.

27. Malagón T, Drolet M, Boily M-CC, Franco EL, Jit M, Brisson J, Brisson M. 2012. Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. Lancet Infect Dis 12:781–9.

28. Richards, Lowy, Schiller, Day. 2006. Cleavage of the papillomavirus minor capsid protein, L2, at a furin

consensus site is necessary for infection. Proceedings of the National Academy of Sciences 103:1522–1527.

29. Gambhira R, Karanam B, Jagu S, Roberts J, Buck C, Bossis I, Alphs H, Culp T, Christensen N, Roden R. 2007. A protective and broadly cross-neutralizing epitope of human papillomavirus L2. J Virol 81:13927–31.

30. Tyler M, Tumban E, Dziduszko A, Ozbun MA, Peabody DS, Chackerian B. 2014. Immunization with a consensus epitope from human papillomavirus L2 induces antibodies that are broadly neutralizing. Vaccine 32:4267–74.

31. Breitburd F, Kirnbauer R, Hubbert NL, Nonnenmacher B, Trin-Dinh-Desmarquet C, Orth G, Schiller JT, Lowy DR. 1995. Immunization with virus-like particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. J Virol 69:3959–63.

32. Jiang RT, Schellenbacher C, Chackerian B, Roden RB. 2016. Progress and prospects for L2-based human papillomavirus vaccines. Expert Rev Vaccines

33. Schellenbacher C, Kwak K, Fink D, Shafti-Keramat S, Huber B, Jindra C, Faust H, Dillner J, Roden RB, Kirnbauer R. 2013. Efficacy of RG1-VLP vaccination against infections with genital and cutaneous human papillomaviruses. J Invest Dermatol 133:2706–13.

34. Jagu S, Karanam B, Gambhira R, Chivukula SV, Chaganti RJ, Lowy DR, Schiller JT, Roden RB. 2009. Concatenated multitype L2 fusion proteins as candidate prophylactic pan-human papillomavirus vaccines. J Natl Cancer Inst 101:782–92.

35. Gerber S, Lane C, Brown DM, Lord E, DiLorenzo M, Clements JD, Rybicki E, Williamson AL, Rose RC. 2001. Human papillomavirus virus-like particles are efficient oral immunogens when coadministered with Escherichia coli heat-labile enterotoxin mutant R192G or CpG DNA. J Virol 75:4752–60.

36. Markowitz LE, Liu G, Hariri S, Steinau M, Dunne EF, Unger ER. 2016. Prevalence of HPV after introduction of the vaccination program in the United States. Pediatrics

37. Hitzeroth II, Passmore JAS, Shephard E, Stewart D, Müller M, Williamson AL, Rybicki EP, Kast WM. 2009. Immunogenicity of an HPV-16 L2 DNA vaccine. Vaccine 27:6432-6434.

38. Zimet GD. 2005. Improving adolescent health: focus on HPV vaccine acceptance. J Adol Health 37:17-23

ACRONYMS

Human papillomavirus (HPV), Food and Drug Administration (FDA), oral and oropharyngeal cancer (OCC), Early (E), Late (L), more economically developed countries (MEDC), less economically developed countries (LEDC), world health organization (WHO), heparin sulfate proteoglycan (HSPG), post synaptic density protein + drosophila disc large tumour suppressor + zonula occludens-1 protein (PDZ), E6-associated protein (E6AP), nuclear transcriptional factor x-box binding 1 (NFX1), retinoblastoma protein (pRb), virus-like particle (VLP), aluminum hydroxide and monophosphoryl lipid A (alum-MPL)