

REVIEWS

HPV prophylactic vaccines: Second-generation or first-generation vaccines

Kimia Kardani^{1,2}, Golnaz Mardani^{1,2}, Azam Bolhassani¹

1. Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran. 2. Department of Food Sciences and Technology, Faculty of Advanced Sciences and Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.

Correspondence: Azam Bolhassani, PhD, Associate Professor. Address: Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran. Email: azam.bolhassani@yahoo.com; A_bolhasani@pasteur.ac.ir

Received: June 5, 2015

Accepted: July 26, 2015

Online Published: August 10, 2015

DOI: 10.5430/jst.v5n2p112

URL: <http://dx.doi.org/10.5430/jst.v5n2p112>

Abstract

High-risk genotypes of human papillomavirus (HPV) are associated with genital cancers especially cervical cancer. United State Food and Drug Administration (USFDA) has recently licensed two first-generation prophylactic vaccines (*i.e.*, Gardasil and Cervarix), for control of HPV 16 and 18 infections. Both vaccines are able to generate neutralizing antibodies against major capsid protein L1 assembled as virus-like particles (VLPs). To enhance protection against other HPV genotypes, second-generation vaccines are underway. A HPV L1-based nonavalent vaccine showed is potent and safe in prevention of precancerous lesions associated with HPV types 16/18/31/33/45/52/58, as well as anogenital warts associated with HPV types 6/11. This vaccine is in the advanced stage of phase III clinical trials. Other second-generation vaccines were based on L1-pentameric subunits and also the minor capsid protein L2 that have shown to be effective in preclinical studies. The L2 protein co-assembles with the L1 protein for VLP formation increasing virion aggregation. This mini-review describes two vaccination strategies including first-generation and second-generation vaccines against HPV infections.

Key words

Human papillomavirus, Cervical cancer, Major capsid protein, Minor capsid protein, Prophylactic vaccine

1 Introduction

Human papillomavirus (HPV) is the most prevalent sexually transmitted infection in worldwide and the main cause of cervical cancer (~11%-12% of women) ^[1-3]. HPV types were classified as high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66) and low-risk (6, 11, 40, 42, 43, 44, and 54) types consistent with the generation of squamous cell carcinomas in the uterine cervix ^[4]. HPV types 16 and 18 contribute to 70% of cervical cancer cases, while HPV types 6 and 11 associate with 96%-100% of genital warts ^[1,3]. Generally, three HPV types (16, 18 and 45) and five HPV genotypes (16, 18, 45, 31 and 33) comprised 87% and 92% of all cervical adenocarcinomas, respectively ^[5]. The genome of HPV is completely conserved among different types of papillomaviruses. HPV genome encodes six regulatory or early proteins such as E1, E2, E4, E5, E6, and E7 as well as two capsid or late proteins including L1, and L2. The late proteins are involved in the packaging of new virions ^[6]. HPV genotypes are phylogenetically classified based on the homology of their

L1 genes^[5]. The development of HPV-associated cervical cancer is due to the uncontrolled expression of E7 and E6 proteins, resulting in genomic instability and the disruption of cell cycle regulation^[6]. L1 protein makes the pentameric aggregation unit of the viral shell. L2 protein shows significant functions in intracellular encapsidation of papillomavirus genomes and virus entry into the host cells. The virus-like particles (VLPs) have been known as the best candidate for vaccine development against HPV infections. These particles can be produced by expression and assembly of the L1 protein alone or its co-expression with the L2 protein^[7]. Two prophylactic HPV vaccines have been currently approved including the quadrivalent vaccine (Gardasil) and the bivalent vaccine (Cervarix) composed of L1 VLPs. The studies indicated that the addition of L2 to L1 VLPs increases the levels of neutralizing antibodies for the L1/L2 particles than for the L1 VLPs^[7,8]. Indeed, L2 protein is a potential target for vaccination against HPV infections; because L2 elicits more cross-reactive antibodies than L1 between different HPV types^[1-3]. Herein, we briefly explain first-generation and second-generation vaccines against HPV infections.

2 Biology

HPV targets the epithelial cells and its life cycle is related to the differentiation level of the infected epithelial cells. Indeed, the regulation of HPV gene transcription depends on the type and the differentiation status of infected epithelial cells as well as the episomal or chromosomally integrated state of the viral genome^[9]. Cellular and viral transcription factors regulate HPV expression by binding to specific elements within a segment of non-coding region entitled as long control region (LCR) that is variable between various HPV genotypes. The linkage of E2 protein to the LCR inhibits the transcription of E6 and E7. Indeed, in tumor cells, viral genome is integrated into the host DNA, due to interruption of the E2 gene and subsequently, high expression of the E6 and E7 oncogenes^[9]. The E6 and E7 oncoproteins bind with various affinities to host cell proteins and disturb the differentiation and apoptosis of normal epithelial cells. Altogether, both oncoproteins mediate cellular genomic instability and HPV-associated epithelial cell immortalization promoting the viral-infected cells toward a fully malignant phenotype^[9]. Figure 1 shows the progression of HPV infection.

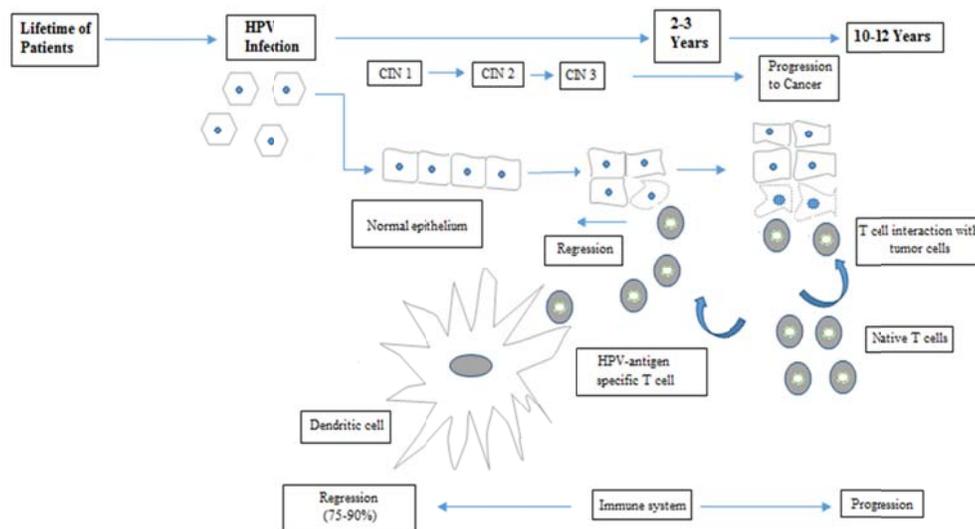


Figure 1. The progression of HPV infection: HPV infects basal epithelial cells promoting cell proliferation toward cervical intraepithelial neoplasia (CIN) with different degrees (1, 2, 3), and finally the progression to cervical carcinoma. Generally, the dendritic cells migrate to the secondary lymphoid organs for interaction with antigen-specific T cells and thus, the inhibition of the low-grade lesions. Usually, after infection, a time of 2 or 3 years is needed for development of CIN 1/2/3 and it takes about ten years for progression of cancer.

3 Immunity against HPV

3.1 Humoral immunity

HPV infection just happens in epithelial cells; thus, the presentation of viral antigens to the immune system of host is restricted. Viral load and persistence determine the violence of the antibody response. The presence of HPV antibodies is long-term but does not help to clear the established infections^[10]. The HPV capsid proteins of papillomaviruses (L1 and L2) are considered to stimulate virus-neutralizing antibody responses. The studies showed that virus-neutralizing anti-L1 antibodies are type-specific. The anti-L2-antibodies are less efficient than anti-L1 antibodies, because a small fragment of L2 protein can be exposed at the surface and be recognized by neutralizing antibodies. In contrast with the anti-L1 antibodies, anti-L2-antibodies indicated cross-reactivity to various HPV genotypes^[10].

3.2 Cellular immunity

Generally, a specific cell-mediated immunity (CMI) is generated to clear a naturally acquired HPV infection. Langerhans cells play an important role for identifying HPV-infected cells in the cervical epithelial cells. These DCs induce Th1 responses and subsequently the generation of cytotoxic T lymphocytes (CTLs) for combating the virus^[10].

4 HPV vaccination

In general, two vaccination strategies are proposed to reduce HPV-related diseases: 1) therapeutic vaccines, and 2) prophylactic vaccines. Regarding the studies, the basis of approved HPV vaccines is the recombinant proteins assembled as VLPs^[11].

4.1 Therapeutic vaccines

Some therapeutic vaccines including live/killed-vector-based, peptide/protein-based, nucleic acid-based, or cell-based vaccines are in clinical trials but they need to improve their potency^[12-15]. The effects of therapeutic vaccines include treatment of pre-existing lesions and even malignant tumors by stimulating cell immunity against HPV-infected cells^[6]. The HPV early/regulatory proteins are ideal target antigens since they are constitutively expressed in the infected cells. The HPV-encoded oncoproteins, E6 and E7, have often shown potential targets for the development of therapeutic HPV vaccines^[6, 16, 17].

4.2 Prophylactic vaccines

The studies indicated that VLP-based preventive vaccination leads to generate virus-neutralizing antibodies (NAb) in serum^[10]. Intramuscular vaccination with L1 VLP was studied in clinical trials (phases I, II, and III) as a monovalent HPV16, a bivalent HPV16/18 (Cervarix) and a quadrivalent HPV6/11/16/18 (Gardasil) vaccine generating high immunogenicity, safety, and protection against HPV infections. Two licensed HPV vaccines (Gardasil and Cervarix) have globally the potency to protect approximately 70% of cervical cancer cases^[18]. Prophylactic VLP vaccines offer several advantages against other types of vaccines such as: 1) the significant proteins of some viruses are able to self-assemble into highly immunogenic VLPs, which mimic native structure of virions without viral genome. Thus, VLPs are safer than other types of vaccination^[11, 19]; 2) VLPs are produced in different expression systems including bacteria, yeast or insect cells; 3) vaccination with VLPs stimulates very high titers of neutralizing antibodies which is stable, even in the lack of adjuvants; 4) VLPs with an extremely organized structure repetitively permit the presentation of immunogens to DCs in order to stimulate cell-mediated immunity or to activate B-cells^[11, 20-24]. Table 1 demonstrates some preventive vaccines based on L1/ L2 proteins.

Table 1. The preventive vaccines based on L1/ L2 proteins

Structure	Preclinical	Animal	Type of Immunity	Approved	References
	Clinical	Human			
L1 protein	Clinical	Human	High CD4+ responses	Yes	[60]
L1 VLP	Clinical	Human	Low Interleukin/ High NAb	Yes	[61]
L2 protein	Preclinical	Mice	High NAb	No	[55]
L2 VLP	Preclinical	Mice	High NAb	No	[53]
L1/L2 VLP	Preclinical	Mice	High NAb	No	[62]

5 First- or second-generation HPV vaccines

Up to now, two commercially prophylactic HPV vaccines entitled as first-generation vaccines (Cervarix and Gardasil) are available in worldwide [1, 25-27]. These L1-based VLPs produced high-titer anti-HPV antibody responses which were 100-fold higher than that generated by natural infections [11, 28-35]. Although, L1 VLP-vaccinated animals are protected by stimulation of humoral immunity [11, 28, 36-39] but the induction of native immunity and cell-mediated immunity cannot be ruled out [11]. It was shown that L1 VLPs can activate DCs via a MyD88-dependent procedure that probably induces a potent primary acquired immune response even without the use of adjuvant [38, 40]. Generally, L1 VLPs induce a polyclonal neutralizing antibody response which demonstrates multivalent epitopes of the virus and promotes to potent neutralizing of HPV virions [38, 41]. However, the current HPV L1 VLP vaccines do not create therapeutic effects against pre-existing HPV infections; because the infected basal epithelial cells do not express detectable levels of L1 and/or L2 capsid antigens [6]. Regarding the studies, at least 110 countries have permitted Cervarix™ which induce protection against HPV 16 and 18 types and upon 120 countries have permitted Gardasil™ which induce protection against HPV 6, 11, 16 and 18 types [1, 42]. These HPV VLP vaccines induce genotype-limited protection [6, 11, 24, 36, 43-48], although partial cross-protection has been observed for closely related HPV genotypes [6]. The approved vaccines should be accessible in low-resource areas to control the incidence of cervical cancer. They need refrigeration for storage, which is an important problem in low-resource areas [1]. Therefore, it is essential to improve low-cost, effective and stable prophylactic vaccines that are able to stimulate broader protection against various HPV genotypes and also suitable for low-resource areas. The new vaccines were called as second-generation vaccines. Recently, L2-based vaccination is another approach to produce broader cross-type protective immunity and lower cost as compared to L1 VLP immunization [11, 49]. Indeed, L2 vaccines have been discovered to improve the present HPV vaccines, which are greatly type-specific. Several studies have shown that the N-terminus region of L2 induces antibodies which can neutralize infections generated by various HPV types [47, 50, 51]. However, the immunogenicity of recombinant L2 protein is poor, thus, different formulations are necessary to increase its immunogenicity such as the multivalent display of an L2 epitope on the surface of a recombinant bacteriophage PP7 coat protein VLP [47, 52]. Bacteriophage VLPs improve vaccines which direct a greatly conserved epitope from HPV L2 [53]. In addition, although the sequence of the N-terminal region of HPV L2 is relatively conserved in different HPV types, but there is some heterogeneity that can limit the reactivity of antibodies against individual HPV L2 sequences. For instance, a concatenated multimeric L2 fusion protein, containing N-terminal L2 peptides derived from 3 to 22 HPV types could induce broader neutralizing antibody responses than similar immunogens containing L2 sequence from a single HPV type [47, 54, 55]. Similarly, the studies indicated that immunization with PP7 VLPs displaying a peptide from HPV16 L2 elicits antibodies that bind extremely to HPV16 and HPV18 L2 peptides, but poorly to L2 peptides from other HPV types (HPV1, 5, and 6) in mice model. Thus, to develop the protection conferred by vaccination, mice were immunized with a mixture of eight L2-PP7 VLPs displaying L2 sequence from various HPV types. This type of vaccine elicited a broad anti-L2 immune response as well as potent protection from genital challenge with HPV pseudo-virions [47, 52]. Generally, high-titer neutralizing antibody responses have been elicited by fusion VLPs compared to the particles expressing single epitope [53]. Another study showed that the nonavalent L1-VLP vaccine is one of the second-generation preventive vaccine which developed in the clinical trial (phase III) [47, 51]. In this vaccine, five new oncogenic

HPV genotypes, including 31, 33, 45, 52, and 58 as well as the genotypes in the approved quadrivalent vaccine decreased a further 15%-30% in the incidence of cervical cancer cases^[18]. The nonavalent HPV vaccine V503 appeared to be safe and effective in preventing persistent infection and precancerous lesions^[56]. Generally, the nonavalent HPV vaccine is promising to have a broader protection compared to cervical cancer vaccines which aim conserved epitopes in the HPV L2 coat peptide. L2 is antigenically less important than HPV L1 major capsid peptide. In an experiment, for eliciting a potent anti-L2 antibody response to another mucosal genotypes, VLPs were produced by fusion of HPV16 L2 neutralizing epitopes (e.g., L2 residues 69-81 or 108-120) into an immunodominant surface loop (among the residues 133 and 134 of the L1 coat peptide of bovine papillomavirus type 1)^[57, 58]. Regarding to the cost-effectiveness of the nonavalent against the quadrivalent vaccine, and also most cervical cancers are caused by HPV types of 16 and 18, it is improbable that the nonavalent vaccine would be used if its efficacy against these two types is lower than current HPV vaccines^[59].

6 Conclusion

Two prophylactic vaccines containing the recombinant HPV L1 VLPs have been already approved. The effects of prophylactic HPV vaccine are still unclear for several years after vaccination. Boosting is potential approach if these vaccines do not generate stable protection. Regarding the incidence of cervical adenocarcinoma (ADC) is growing, thus the current HPV vaccines should be highly efficient in prevention of cervical ADC. The chimeric L1-L2 VLP showed a promising strategy to improve more potent HPV vaccines. The HPV16 L1-HPV16 L2 17-36 VLP vaccine may provide broad cross-protection to heterotypic HPV types. However, the vaccine potency particularly is affected by HPV genotypes in cancers, *i.e.*, the level of effect would be attenuated by vaccine cross-protective results. The recent studies suggest the second generation vaccination including the mixture of L1 epitopes of different HPV genotypes, the use of L2 conserved epitopes, and L1-L2 chimeric VLPs, as compared to L1 VLP vaccines. However, these vaccines were further used in preclinical tests and will be needed to study in clinical trials.

Acknowledgements

This study was supported by Iran National Science Foundation (INSF) (ID#91060229). Both Kimia Kardani and Golnaz Mardani are first authors.

Conflict of interest

The authors declare that there is no conflict of interest statement.

References

- [1] Wigle J, Coast E, Watson-Jones D. Human papillomavirus (HPV) vaccine implementation in low and middle-income countries (LMICs): Health system experiences and prospects. *Vaccine*. 2013; 31(37): 3811-7. PMID:2377795. <http://dx.doi.org/10.1016/j.vaccine.2013.06.016>
- [2] WHO/ICO. Human papillomavirus and related cancers in world. Summary report; 2010.
- [3] Forman D, de Martel C, Lacey CJ, *et al.* Global burden of human papillomavirus and related diseases. *Vaccine*. 2012; 30S: F12-F23. PMID:23199955. <http://dx.doi.org/10.1016/j.vaccine.2012.07.055>
- [4] Schiffman M, Clifford G, Buonaguro FM. Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. *Infect Agent Cancer*. 2009; 4: 8. PMID:19486508. <http://dx.doi.org/10.1186/1750-9378-4-8>
- [5] Pimenta MJ, Galindo C, Jenkins D, *et al.* Estimate of the global burden of cervical adenocarcinoma and potential impact of prophylactic human papillomavirus vaccination. *BMC Cancer*. 2013; 13: 553. PMID:24261839. <http://dx.doi.org/10.1186/1471-2407-13-553>
- [6] Hung C, Ma B, Monie A, *et al.* Therapeutic human papillomavirus vaccines: current clinical trials and future directions. *Expert Opin Biol Ther*. 2008; 8(4): 421-39. PMID:18352847. <http://dx.doi.org/10.1517/14712598.8.4.421>

- [7] Wakabayashi AMT, Da Silva DM, Potkul RK, *et al.* Comparison of human papillomavirus type 16 L1 chimeric virus-like particles versus L1/L2 chimeric virus-like particles in tumor prevention. *Intervirology*. 2002; 45: 300-7. PMID:12566713. <http://dx.doi.org/10.1159/000067921>
- [8] Hernandez BBY, Ton T, Shvetsov YB, *et al.* Human papillomavirus (HPV) L1 and L1-L2 virus-like particle-based multiplex assays for measurement of HPV virion antibodies. *Clinical and Vaccine Immunology*. 2012; 19: 1348-52. PMID:22761294. <http://dx.doi.org/10.1128/CVI.00191-12>
- [9] Betiol J, Villa L, Sichero L. Impact of HPV infection on the development of head and neck cancer. *Braz J Med Biol Res*. 2013; 46(3): 217-26. PMID:23532264. <http://dx.doi.org/10.1590/1414-431X20132703>
- [10] Dillner J, Arbyn M, Unger E, *et al.* Monitoring of human papillomavirus vaccination. *Clin Exp Immunol*. 2011; 163(1): 17-25. PMID:21062269. <http://dx.doi.org/10.1111/j.1365-2249.2010.04268.x>
- [11] Wang WJ, Roden BSR. Virus-like particles for the prevention of human papillomavirus-associated malignancies. *Expert Rev Vaccines*. 2013; 12(2): 129-41. PMID:23414405. <http://dx.doi.org/10.1586/erv.12.151>
- [12] Asiaf A, Ahmad ST, Mohammad SO, *et al.* Review of the current knowledge on the epidemiology, pathogenesis, and prevention of human papillomavirus infection. *European Journal of Cancer Prevention*. 2014; 23(3): 206-24. PMID:24129107. <http://dx.doi.org/10.1097/CEJ.0b013e328364f273>
- [13] Bolhassani A, Mohit E, Rafati S. Different spectra of therapeutic vaccine development against HPV infections. *Human Vaccines*. 2009; 5(10): 671-89. PMID:19684468. <http://dx.doi.org/10.4161/hv.5.10.9370>
- [14] Bolhassani A, Zahedifard F. Therapeutic live vaccines as a potential anti-cancer strategy. *Int J Cancer*. 2012; 131 (8): 1733-43. PMID:22610886. <http://dx.doi.org/10.1002/ijc.27640>
- [15] Bolhassani A, Muller M, Roohvand F, *et al.* Whole recombinant *Pichia pastoris* expressing HPV16 L1 antigen is superior in inducing protection against tumor growth as compared to killed transgenic *Leishmania*. *Hum Vaccin Immunother*. 2014; 10(12): 3499-508. PMID:25668661. <http://dx.doi.org/10.4161/21645515.2014.979606>
- [16] Bolhassani A, Zahedifard F, Taghikhani M, *et al.* Enhanced immunogenicity of HPV16E7 accompanied by Gp96 as an adjuvant in two vaccination strategies. *Vaccine*. 2008; 26(26): 3362-70. PMID:18471945. <http://dx.doi.org/10.1016/j.vaccine.2008.03.082>
- [17] Daemi A, Bolhassani A, Rafati S, *et al.* Different domains of glycoprotein 96 influence HPV16 E7 DNA vaccine potency via electroporation mediated delivery in tumor mice model. *Immunology Letters*. 2012; 148(2): 117-25. PMID:23085605. <http://dx.doi.org/10.1016/j.imlet.2012.10.003>
- [18] Kiatpongsan S, Gastineau Campos N, Kim J. Potential benefits of second-generation human papillomavirus vaccines. *PLoS One*. 2012; 7(11): e48426. PMID:23144879. <http://dx.doi.org/10.1371/journal.pone.0048426>
- [19] Roldao A, Mellado MC, Castilho LR, *et al.* Virus-like particles in vaccine development. *Expert Review of Vaccines*. 2010; 9(10): 1149-76. PMID:20923267. <http://dx.doi.org/10.1586/erv.10.115>
- [20] Chackerian B, Lenz P, Lowy DR, *et al.* Determinants of autoantibody induction by conjugated papillomavirus virus-like particles. *Journal of Immunology*. 2002; 169(11): 6120-6. <http://dx.doi.org/10.4049/jimmunol.169.11.6120>
- [21] Lenz P, Day PM, Pang YY, *et al.* Papillomavirus-like particles induce acute activation of dendritic cells. *Journal of Immunology*. 2001; 166(9): 5346-55. <http://dx.doi.org/10.4049/jimmunol.166.9.5346>
- [22] Lenz P, Thompson CD, Day PM, *et al.* Interaction of papillomavirus virus-like particles with human myeloid antigen-presenting cells. *Clinical Immunology*. 2003; 106(3): 231-7. [http://dx.doi.org/10.1016/S1521-6616\(02\)00039-6](http://dx.doi.org/10.1016/S1521-6616(02)00039-6)
- [23] GlaxoSmithKline Vaccine HPV-007 Study Group. Sustained efficacy and immunogenicity of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine: analysis of a randomised placebo-controlled trial up to 6.4 years. *Lancet*. 2009; 374(9706): 1975-85. [http://dx.doi.org/10.1016/S0140-6736\(09\)61567-1](http://dx.doi.org/10.1016/S0140-6736(09)61567-1)
- [24] 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(9518): 1247-55. [http://dx.doi.org/10.1016/S0140-6736\(06\)68439-0](http://dx.doi.org/10.1016/S0140-6736(06)68439-0)
- [25] WHO: Department of Immunization, Vaccines and Biologicals, World Health Organization; Geneva: Human papillomavirus and HPV vaccines: technical information for policymakers and health professionals. 2007.
- [26] Onon TS. History of human papillomavirus, warts and cancer: what do we know today? *Best Pract Res Clin Obstet Gynaecol*. 2011; 25(5): 565-74. PMID:21708487. <http://dx.doi.org/10.1016/j.bpobgyn.2011.05.001>
- [27] Mcneil C. Who invented the VLP cervical cancer vaccines? *Journal of the National Cancer Institute*. 2006; 98(7): 433. PMID:16595773. <http://dx.doi.org/10.1093/jnci/djj144>
- [28] Einstein MH, Baron M, Levin MJ, *et al.* Comparison of the immunogenicity and safety of Cervarix and Gardasil human papillomavirus (HPV) cervical cancer vaccines in healthy women aged 18-45 years. *Human Vaccines*. 2009; 5(10): 705-19. PMID:19684472. <http://dx.doi.org/10.4161/hv.5.10.9518>

- [29] Koutsky LA, Ault KA, Wheeler CM, *et al.* A controlled trial of a human papillomavirus type 16 vaccine. *The New England Journal of Medicine.* 2002; 347(21): 1645-51. PMID:12444178. <http://dx.doi.org/10.1056/NEJMoa020586>
- [30] Poland GA, Jacobson RM, Koutsky LA, *et al.* Immunogenicity and reactogenicity of a novel vaccine for human papillomavirus 16: a two-year randomized controlled clinical trial. *Mayo Clinic Proceedings Mayo Clinic.* 2005; 80(5): 601-10. PMID:15887427. <http://dx.doi.org/10.4065/80.5.601>
- [31] 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. *The Lancet Oncology.* 2005; 6(5): 271-8. [http://dx.doi.org/10.1016/S1470-2045\(05\)70101-7](http://dx.doi.org/10.1016/S1470-2045(05)70101-7)
- [32] FUTURE I/II Study Group. 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. PMID:20647284. <http://dx.doi.org/10.1136/bmj.c3493>
- [33] HPV PATRICIA Study Group. Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *The Lancet Oncology.* 2012; 13(1): 89-99. [http://dx.doi.org/10.1016/S1470-2045\(11\)70286-8](http://dx.doi.org/10.1016/S1470-2045(11)70286-8)
- [34] Kaufmann AM, Nitschmann S. Vaccine against human papillomavirus: PATRICIA Study (PApillomaTRial against Cancer. In young Adults). *Internist.* 2010; 51410(3): 412-3.
- [35] Mao C, Koutsky LA, Ault KA, *et al.* Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia: a randomized controlled trial. *Obstetrics and Gynecology.* 2006; 107(1): 18-27. PMID:16394035. <http://dx.doi.org/10.1097/01.AOG.0000192397.41191.fb>
- [36] Breitburd F, Kimbauer R, Hubbert NL, *et al.* Immunization with virus like particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. *Journal of Virology.* 1995; 69(6): 3959-63. PMID:7745754.
- [37] Day PM, Kines RC, Thompson CD, *et al.* In vivo mechanisms of vaccine-induced protection against HPV infection. *Cell Host & Microbe.* 2010; 8(3): 260-70. PMID:20833377. <http://dx.doi.org/10.1016/j.chom.2010.08.003>
- [38] Tumban E, Peabody J, Peabody D, *et al.* A universal virus-like particle-based vaccine for human papillomavirus: Longevity of protection and role of endogenous and exogenous adjuvants. *Vaccine.* 2013; 31(41): 4647-54. PMID:23933337. <http://dx.doi.org/10.1016/j.vaccine.2013.07.052>
- [39] 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(4): 284-92. PMID:11181775. <http://dx.doi.org/10.1093/jnci/93.4.284>
- [40] Yang R, Wheeler CM, Chen X, *et al.* Papillomavirus capsid mutation to escape dendritic cell-dependent innate immunity in cervical cancer. *J Virol.* 2005; 79(11): 6741-50. PMID:15890912. <http://dx.doi.org/10.1128/JVI.79.11.6741-6750.2005>
- [41] Carter JJ, Wipf GC, Madeleine MM, *et al.* Identification of human papillomavirus type 16 L1 surface loops required for neutralization by human sera. *J Virol.* 2006; 80(10): 4664-7462. PMID:16641259. <http://dx.doi.org/10.1128/JVI.80.10.4664-4672.2006>
- [42] Garland SM, Smith JS. Human papillomavirus vaccines: current status and future prospects. *Drugs.* 2010; 70(9): 1079-98. PMID:20518577. <http://dx.doi.org/10.2165/10898580-000000000-00000>
- [43] Roden R, Monie A, Wu TC. The impact of preventive HPV vaccination. *Discovery Medicine.* 2006; 6(35): 175-81. PMID:17234138.
- [44] Buck CB, Trus BL. The papillomavirus virion: a machine built to hide molecular Achilles' heels. *Advances in Experimental Medicine and Biology.* 2012; 726: 403-22. PMID:22297524. http://dx.doi.org/10.1007/978-1-4614-0980-9_18
- [45] Schiller JT, Day PM, Kines RC. Current understanding of the mechanism of HPV infection. *Gynecologic Oncology.* 2010; 118: S12-S17. PMID:20494219. <http://dx.doi.org/10.1016/j.ygyno.2010.04.004>
- [46] Schiller JT, Lowy DR. Immunogenicity testing in human papillomavirus virus-like-particle vaccine trials. *The Journal of Infectious Diseases.* 2009; 200(2): 166-71. PMID:19519255. <http://dx.doi.org/10.1086/599988>
- [47] Tumban E, Peabody J, Tyler M, *et al.* VLPs displaying a single L2 epitope induce broadly cross-neutralizing antibodies against human papillomavirus. *PLoS One.* 2012; 7(11): e49751. PMID:23185426. <http://dx.doi.org/10.1371/journal.pone.0049751>
- [48] Schellenbacher C, Roden R, Kimbauer R. Chimeric L1-L2 virus-like particles as potential broad-spectrum human papillomavirus vaccines. *J Virol.* 2009; 83: 10085-95. PMID:19640991. <http://dx.doi.org/10.1128/JVI.01088-09>
- [49] Karanam B, Jagu S, Huh WK, *et al.* Developing vaccines against minor capsid antigen L2 to prevent papillomavirus infection. *Immunol Cell Biol.* 2009; 87(4): 287-99. PMID:19421199. <http://dx.doi.org/10.1038/icb.2009.13>
- [50] Roden RB, Yutzy WH, Fallon R, *et al.* Minor capsid protein of human genital papillomaviruses contains subdominant, cross-neutralizing epitopes. *Virology.* 2000; 270: 254-7. PMID:10792983. <http://dx.doi.org/10.1006/viro.2000.0272>
- [51] Christensen ND, Kreider JW, Kan NC, *et al.* The open reading frame L2 of cottontail rabbit papillomavirus contains antibody-inducing neutralizing epitopes. *Virology.* 1991; 181: 572-9. [http://dx.doi.org/10.1016/0042-6822\(91\)90890-N](http://dx.doi.org/10.1016/0042-6822(91)90890-N)

- [52] Tumban E, Peabody J, Peabody DS, *et al.* A pan-HPV vaccine based on bacteriophage PP7 VLPs displaying broadly cross-neutralizing epitopes from the HPV minor capsid protein, L2. *PLoS One*. 2011; 6(8): e23310. PMID:21858066. <http://dx.doi.org/10.1371/journal.pone.0023310>
- [53] Tyler M, Tumban E, Peabody DS, *et al.* The use of hybrid virus-like particles to enhance the immunogenicity of a broadly protective HPV vaccine. *Biotechnol Bioeng*. 2014; 111(12): 2398-406. PMID:24917327. <http://dx.doi.org/10.1002/bit.25311>
- [54] Jagu S, Karanam B, Gambhira R, *et al.* Concatenated multitype L2 fusion proteins as candidate prophylactic pan-human papillomavirus vaccines. *J Natl Cancer Inst*. 2009; 101: 782-92. PMID:19470949. <http://dx.doi.org/10.1093/jnci/djp106>
- [55] Tyler M, Tumban E, Dziduszko A, *et al.* Immunization with a consensus epitope from human papillomavirus L2 induces antibodies that are broadly neutralizing. *Vaccine*. 2014; 32(34): 4267-74. PMID:2496274. <http://dx.doi.org/10.1016/j.vaccine.2014.06.054>
- [56] Chatterjee A. The next generation of HPV vaccines: nonavalent vaccine V503 on the horizon. *Expert Review of Vaccines*. 2014; 13 (11): 1279-90. PMID:25256262. <http://dx.doi.org/10.1586/14760584.2014.963561>
- [57] Slupetzky K, Gambhira R, Culp TD, *et al.* A papillomavirus-like particle (VLP) vaccine displaying HPV16 L2 epitopes induces cross-neutralizing antibodies to HPV11. *Vaccine*. 2007; 25(11): 2001-10. PMID:17239496. <http://dx.doi.org/10.1016/j.vaccine.2006.11.049>
- [58] Schiller JT, Nardelli-Haeftiger D. Chapter 17: Second generation HPV vaccines to prevent cervical cancer. *Vaccine*. 2006; 3: S3/147-S3/153
- [59] Drolet M, Laprise JF, Boily MC, *et al.* Potential cost-effectiveness of the nonavalent human papillomavirus (HPV) vaccine. *Int J Cancer*. 2014; 134(9): 2264-8. PMID:24174175. <http://dx.doi.org/10.1002/ijc.28541>
- [60] Hassan SW, Waheed MT, Mueller M, *et al.* Expression of HPV-16 L1 capsomeres with glutathione-S-transferase as a fusion protein in tobacco plastids: an approach for a capsomere-based HPV vaccine. *Human Vaccines & Immunotherapeutics*. 2014; 10(10): 2975-82. PMID:25483463. <http://dx.doi.org/10.4161/21645515.2014.970973>
- [61] Kemp TJ, Hildesheim A, Safaeian M, *et al.* HPV16/18 L1 VLP vaccine induces cross-neutralizing antibodies that may mediate cross-protection. *Vaccine*. 2011; 29(11): 2011-4. PMID:21241731. <http://dx.doi.org/10.1016/j.vaccine.2011.01.001>
- [62] Hernandez BY, Ton T, Shvetsov YB, *et al.* Human papillomavirus (HPV) L1 and L1-L2 virus-like particle-based multiplex assays for measurement of HPV virion antibodies. *Clin Vaccine Immunol*. 2012; 19(9): 1348-52. PMID:22761294. <http://dx.doi.org/10.1128/CVI.00191-12>