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Designing Chimeric Virus-like Particle-based Vaccines for Human Papillomavirus and HIV: Lessons Learned

*Yoshiki Eto[1], Narcís Saubi[1], Pau Ferrer[2], and Joan Joseph[1]**

[1]Vall d'Hebron Research Institute, Hospital Universitari Vall d'Hebron; [2]Department of Chemical, Biological, and Environmental Engineering, School of Engineering, Autonomous University of Barcelona, Barcelona, Spain

Abstract

Virus-like particles (VLPs) are a type of subunit vaccine which resembles viruses but do not contain any genetic material so that they are not infectious. VLPs maintain the same antigenic conformation to the original virus, and they could be a better vaccine candidate than live-attenuated and inactivated vaccines. In addition, compared to other subunit vaccines such as soluble protein, VLPs can stimulate both innate and adaptive immune responses effectively and safely against several pathogens by the closer morphology to its native virus. They have already been licensed as vaccines against Hepatitis B virus, human papillomavirus (HPV), and several veterinary diseases. Moreover, it has been investigated to prevent other viral infections including HIV. While HIV VLP-based vaccines have been studied over 35 years, none of them has been successful enough to reach even Phase III clinical trials. In this review, we summarize: (i) general features of VLPs; (ii) epidemiological data and current status of vaccine research and development on HPV and HIV; and (iii) previous studies held on HPV VLPs, HIV VLPs, and chimeric HPV/HIV VLPs including production methods and different animal immunization assays. Furthermore, we review present state of human clinical trials with VLPs and consider the potential to develop a successful preventive HIV vaccine using HPV VLP models. Finally, we discuss the benefits, limitations, and challenges of developing chimeric VLP-based HPV/HIV vaccines with recent findings, critical issues to improve VLP-based vaccines, and hot topics for the next 5 years to join the global effort to fight against these two pathogens. **(AIDS Rev. 2019;21:218-232)**

Corresponding author: Joan Joseph, jjoseph@vhebron.net

Key words

Human papillomavirus. HIV. Chimeric. Vaccine. Virus-like particle.

Introduction

Since the discovery of the world's first vaccine by Edward Jenner until the present, vaccination has been controlling and preventing many infectious diseases and still regarded as the most powerful means to fight against pathogens. After several years from the appearance of the classical vaccines, in other words, live-attenuated and inactivated vaccines, progress in genetic science allowed us to develop subunit vaccines

Correspondence to:
*Joan Joseph
HIV Vaccine Research
Hospital Universitari Vall d'Hebron
Vall d'Hebron Barcelona Hospital Campus
Barcelona, Spain
E-mail: jjoseph@vhebron.net

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(SUVs). However, SUVs generally were less immunogenic than those classical vaccines and more administrations were needed to reach sufficient immunity. The advance of SUV technology led to generate virus-like particles (VLPs) with a higher immunogenic effect than previous SUV models. VLPs have very similar appearance to the original virus and are composed of only virus structural proteins, which can be displayed in a highly repetitive manner (Fig. 1)[1-3]. Therefore, VLPs are capable to effectively cross-link specific receptors on B cells and induce robust humoral responses without adjuvants[4]. Since they do not contain any viral genetic material inside, they lack capacity to replicate and are non-virulent, and composing proteins can form VLPs by self-assemble manner. VLPs are classified as enveloped or non-enveloped (Fig. 1B)[1]. Non-enveloped VLPs are categorized as single or multilayered capsid protein while enveloped VLPs generally consist of glycoproteins embedded in the lipid membrane layers (Fig. 1B). In addition, VLPs have ideal sizes for the favorable intake by dendritic cells (DCs) through micropinocytosis and endocytosis, resulting in activating both innate and adaptive immunity[5]. They have been used as Food and Drug Administration (FDA) – and European Medical Agency (EMA) – approved protein SUVs against hepatitis B virus and human papillomavirus (HPV) (Table 1)[1]. VLPs can be mass produced in cell cultures such as yeast, insect, and eukaryotic cell lines. Other large-scale protein expression systems as plant production or insect larvae have also been considered.

HPV and HIV are both sexually transmitted viral pathogens and are leading causes of death for humans. In 2018, HPV infection was one of the causing agents of cervical cancer, which is the second most common cancer among women living in less developed areas with an approximated 570,000 new cases (84% of the global new cases) and an estimated 311,000 deaths, of which more than 85% occurred in low- and middle-income nations[6]. HPV infection is also involved in oral and anal cancers[6]. While three HPV vaccines are already licensed and all of them based on VLP, they cover only major HPV genotypes (types 16, 18, 31, 33, 35, 52, and 58 are related to cervical cancer, and types 6 and 11 are related to genital warts) and still miss other less prevalent ones. Moreover, they are still prohibitively expensive for the majority of women in developing countries and cervical cancer caused by HPV is a lasting menace in those regions.

The HIV infection causes AIDS. According to UNAIDS's 2018 fact sheet, it has been estimated that roughly 36.9 million people were living with HIV in 2017, and 1.8 million new infections were diagnosed[7]. Even though there is no effective preventive vaccine against HIV or therapeutic vaccine against AIDS yet after more than 35 years of our vigorous investigations, HIV VLP has been considered as a promising vaccine candidate for its higher immunogenicity and safety compared to conventional vaccine models. Several candidates of HIV VLPs have already been produced and their results in animal models have demonstrated that VLP-based HIV vaccines can elicit a high level of HIV-1-specific neutralizing antibodies and CTL immune responses[8].

Since both HPV and HIV are important public health issues in developing and industrialized countries, and the available prevention strategies have not been able to stop the epidemics, a safe, effective, and affordable vaccine is immediately needed against both viruses. Considering that one of the mutual main access routes to human body for those pathogens is the mucosal genital tract, the development of a combined vaccine which would protect against HPV and HIV infections is a logical effort in the fight against these two major global pathogens.

VLP technology allows to combine multiple components of different viruses into a single VLP. Until today, several articles have been published regarding bovine papillomavirus (BPV)/HIV and HPV/HIV VLP-based bivalent vaccines, but those results are rather controversial and none of them elicited robust HPV- and HIV-specific humoral and T cell-mediated immune responses that could justify moving to Phase I clinical trials[9-17]. Thus, this review will discuss: (i) the current status, limitations, and challenges of VLP-based HPV, HIV, and chimeric HPV/HIV vaccine development; (ii) what has been learned in previous VLP research; and (iii) what should be taken into account to develop chimeric HPV/HIV VLPs as an effective dual vaccine, especially focused on yeast, baculovirus/insect cell, and eukaryotic expression systems.

VLP-based HPV vaccine

Current vaccines and how to improve them

Heretofore, three HPV vaccines have been licensed, and the main component of all of them is a protein subunit of HPV, capsid protein L1, which self-assembles to form VLPs. These three vaccines are multivalent. Gardasil® (2006, Merck & Co.), Cervarix® (2009, GlaxoSmithKline), and Gardasil®9 (2014, Merck & Co.) are

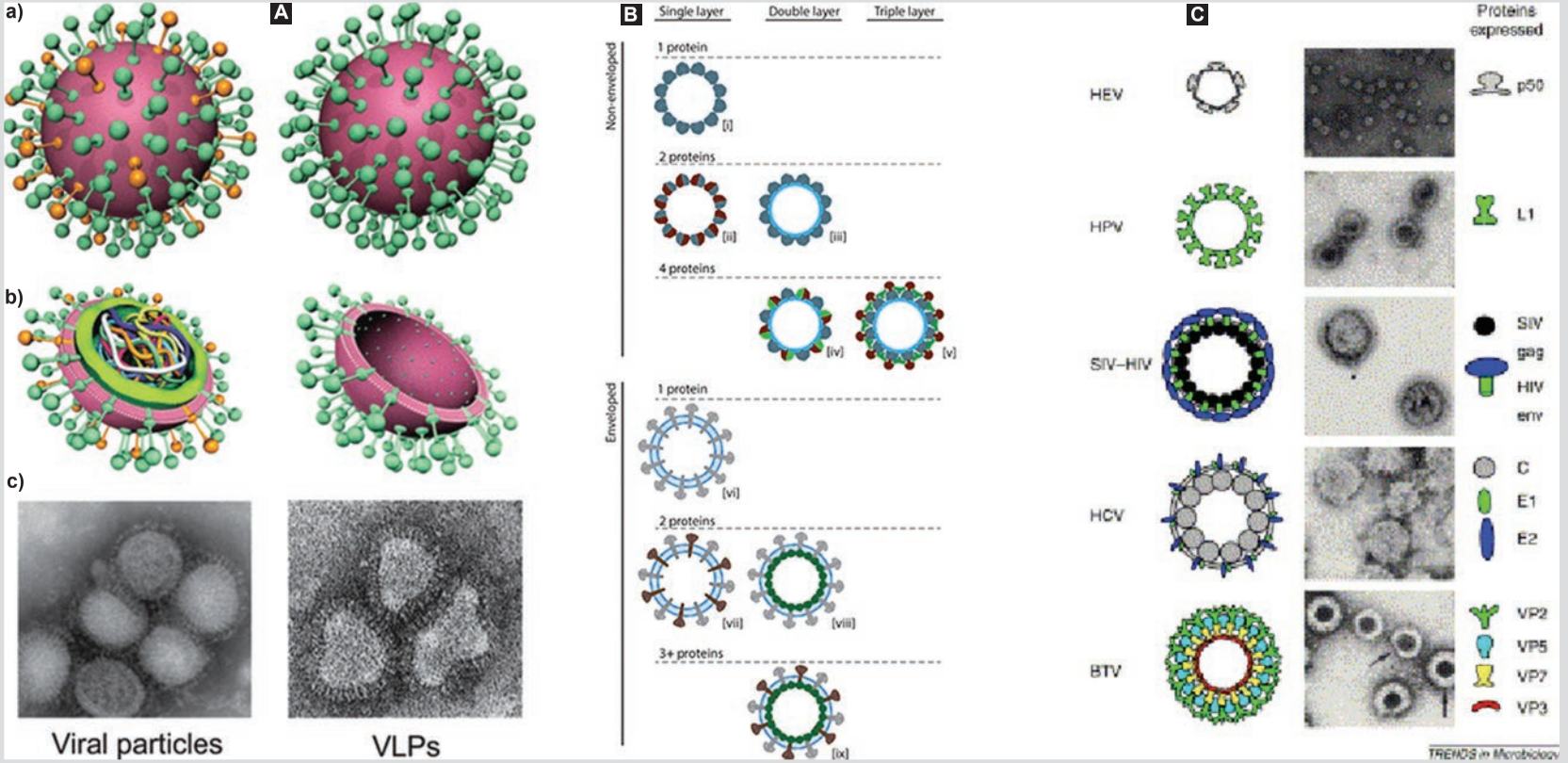

Figure 1. A: *Schematic representation of structural characteristics of viral particles and virus-like particles (VLPs). (a) Comparison of external characteristics. (b) Cross-section representation showing internal distinctions. (c) Transmission electron microscopy images of influenza viral particles and plant-produced influenza VLPs[2];* **B:** *Structural diversity of VLPs. Both capsid-based and enveloped VLPs can be single or multilayered and composed of single or multiple proteins. Single layered non-enveloped VLPs can be assembled from (i) a single protein (e.g., hepatitis B core antigen [Clarke et al., 1987; Whitacre et al., 2009]); or (ii) two proteins (e.g., cowpea mosaic virus VLPs [Saunders et al., 2009]). Double layered non-enveloped VLPs can be assembled from (iii) two proteins (e.g., papillomavirus L1 and L2 VLPs [Kirnbauer et al., 1993]); or (iv) four proteins (e.g., Foot-and-mouth disease virus [Porta et al., 2013]). Triple-layered VLPs (v) have been assembled from four coat proteins of bluetongue virus (Hewat et al., 1994) and rotavirus (Conner et al., 1996). For enveloped VLPs, expression of one (vi) or two glycoproteins (vii) will form a single layer, as demonstrated by the expression of influenza virus hemagglutinin (D'Aoust et al., 2008) and the coexpression of both hemagglutinin and neuraminidase (Chen et al., 2007), respectively. Double layered enveloped VLPs can be formed by the (viii) inclusion of structural proteins (e.g., influenza VLPs [Latham and Galarza, 2001]) and may possess (ix) multiple glycoproteins on their surface (Latham and Galarza, 2001; Pushko et al., 2011)[1];* **C:** *structure of VLPs for a range of viruses. Capsid morphology and the proteins expressed to achieve this are depicted for each virus. In each case, the electron micrograph shows the VLP produced. Abbreviations: BTV, bluetongue virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HPV, human papillomavirus 16; SIV – HIV, hybrid VLP between simian immunodeficiency virus gag and human immunodeficiency virus Env[3].*

Table 1. Compositions of commercial VLP-based vaccines (*adapted from Lua et al., 2013*)[1]

Vaccine	Composition per dose		
	Protein	Adjuvant	Others
Engerix-B hepatitis B vaccine (1 mL/dose)	HBsAg (20 µg)	Aluminum hydroxide (500 µg)	2-Phenoxyethanol (5.0 mg)
Recombivax HB hepatitis B vaccine (1 mL/dose)	HBsAg (10 µg)	Aluminum hydroxyphosphate (500 µg)	Sodium chloride (9 mg) Sodium borate (70 µg)
Gardasil human papillomavirus vaccine (0.5 mL/dose)	HPV 6 L1 (20 µg) HPV 11 L1 (40 µg) HPV 16 L1 (40 µg) HPV 18 L1 (20 µg)	Aluminum hydroxyphosphate sulfate (225 µg)	Sodium chloride (9.56 mg) L-histidine (0.78 mg) Polysorbate 80 (50 µg) Sodium borate (35 µg)
Cervarix human papillomavirus vaccine (0.5 mL/dose)	HPV 16 L1 (20 µg) HPV 18 L1 (20 µg)	AS04 adjuvant system (50 µg of 3-O-desacyl-40 monophosphoryl lipid A, 500 µg aluminum hydroxyphosphate)	Sodium chloride (4.4 mg) Sodium dihydrogen phosphate dehydrate (0.624 mg)
Hecolin hepatitis E vaccine (0.5 mL/dose)	HE antigen (30 µg)	Aluminum hydroxide (800 µg)	Buffered saline
Gardasil9 human papillomavirus vaccine (0.5 mL/dose)	HPV 6 L1 (30 µg) HPV 11 L1 (40 µg) HPV 16 L1 (60 µg) HPV 18 L1 (40 µg) HPV 31 L1 (20 µg) HPV 33 L1 (20 µg) HPV 45 L1 (20 µg) HPV 52 L1 (20 µg) HPV 58 L1 (20 µg)	Aluminum hydroxyphosphate sulfate (500 µg)	Sodium chloride (9.56 mg) L-histidine (0.78 mg) Polysorbate 80 (50 µg) Sodium borate (35 µg)

HBsAg: hepatitis B virus surface antigen; HPV: human papillomavirus.

quadrivalent (genotypes 6, 11, 16, and 18), bivalent (genotypes 16 and 18), and 9-valent (genotypes 6, 11, 16, 18, 31, 33, 45, 52, and 58), respectively (Fig. 2)[18]. The tetravalent Gardasil® has been replaced by non-avalent Gardasil®9 so that Cervarix® and Gardasil®9 are currently used to prevent HPV infections. While HPV genotypes 6 and 11 cause 90% of genital warts[19], genotypes 16 and 18, 31, 33, 45, 52, and 58 and some others cause cervical, vulvar, vaginal, and anal cancer, and genotypes 16 and 18 are responsible for nearly 70% of all the cervical cancer cases[6]. Although HPV vaccination has been effective and reduced HPV infections significantly among the vaccinees, completing all the immunization schedules of two or three doses are economically challenging even in high-income countries, and the HPV vaccines are still very expensive and not affordable in low-income countries where most HPV-related cancers occur, and the vaccination coverage is very low[20]. From these results, a cheaper and stable HPV vaccine ideally potent enough with one dose is urgently needed. In fact, preliminary data show that even one dose of bivalent vaccine would have merit for prophylactic motives[21]. Besides Gardasil®9, second-generation of VLP-based preventive HPV vaccines has been developed and tested preclinically. Regarding the production system, different species of yeast such as *Pichia pastoris*[22] and *Hansenula polymorpha*[23] have potential to produce L1 protein with lower cost and higher yields compared to *Saccharomyces cerevisiae* used for Gardasil® and Gardasil®9, or compared to the baculovirus/insect cell (Hi-5 Rix4446 cells derived from the insect Trichoplusia ni) expression system used for Cervarix®. Based on the fact that the antibodies against minor papillomavirus (PV) capsid protein L2 have a cross-neutralizing activity, monovalent L1 expressing RG1 epitope (aa17-36 of capsid protein L2) might work to protect from other HPV genotype infections[19]. Moreover, Novartis Vaccines and Diagnostics (Emeryville, CA, USA) stated a method to generate yeast-expressed mosaic VLPs composing both HPV-6 and -16 L1 proteins[24]. Furthermore, as vehicles of edible HPV vaccines, Asahi Glass (Kanazawa, Japan) pat-

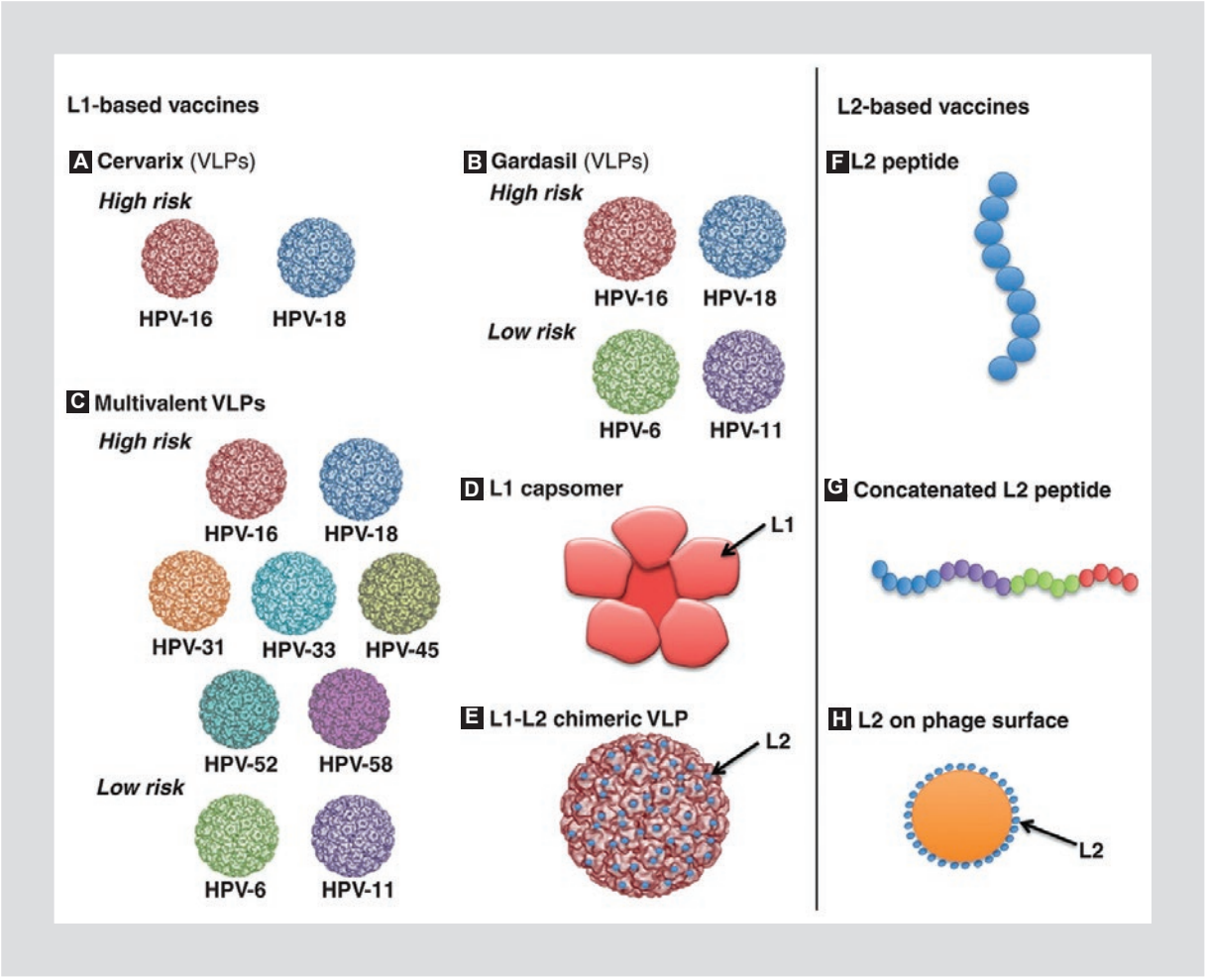

Figure 2. *Schematic diagram to depict the next generation of preventive human papillomavirus (HPV) vaccines based on HPV capsid proteins (L1 and/or L2)[18].* **A:** *Cervarix composed of HPV-16 and HPV-18 virus-like particles (VLPs);* **B:** *Gardasil composed of HPV-6, HPV-11, HPV-16, and HPV-18 VLP;* **C:** *Multivalent VLP vaccines composed of HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58 VLPs (V503 Merck);* **D:** *L1 capsomer vaccine;* **E:** *Chimeric L1-L2 VLP vaccine with L2 on the surface;* **F:** *L2 peptide vaccine;* **G:** *Concatenated L2 peptide vaccine;* **H:** *L2 peptides displayed on the surface of bacteriophage VLP.*

ented a recombinant yeast *Schizosaccharomyces pombe* expressing HPV-16 L1 protein, and Genome, Inc. (Pohang, Korea) patented transgenic plants expressing recombinant HPV L1[24]. These edible vaccines were developed with the intention of lowering the cost of HPV vaccine and improve its availability regardless of regions and poverty. Ultimately, an ideal preventive HPV vaccine should protect vaccinees from all 15 of the high-risk HPV genotypes[24].

HPV VLP production systems: yeast, baculovirus, and other eukaryotic cells

HPV VLPs have been produced previously using different expression systems such as *Escherichia coli*, yeast, baculovirus/insect cells, and plants. Among yeast strains, *S. cerevisiae*[25], *P. pastoris*[22], and *H. polymorpha*[23], were used to produce several types of HPV VLPs. Furthermore, the VLPs were generated by baculovirus in Sf9, Sf21, Hi5 insect cells[26-28], and *Leishmania*[29]. The antigenic conformation of HPV is most accurately retained by mammalian cells followed by baculovirus/insect, plant, yeast, and *E. coli* protein expression systems[24]. In addition, the codon-optimization for host cells plays an important role to improve the yield of HPV L1 protein[30]. Although there are differences in heterologous protein productivity, regardless of expression systems, appropriate HPV L1 proteins were generated, and the VLPs were self-assembled properly. Furthermore, since 1998, the disassembly and reassembly of HPV VLPs by exchanging buffers have been considered as an important process to improve the quality and uniformity of VLP because the L1 proteins could stick together improperly during the pu-

rification process[31]. In addition, the new expression system, *Leishmania*, has a great potential to produce VLPs massively, 2-3 mg/500 mL media[29], which is roughly 10 times of amount compared to one of baculovirus/insect cell systems generating 0.25 mg of VLP from 0.5 L of insect cells in 1993[26].

HPV VLP immunogenicity

All immunogenicity studies have been focused on neutralizing antibodies rather than T-cell mediated immunity because the neutralizing antibodies have been shown to have the main role in the prevention of HPV infection as considered in the commercial vaccines[32,33].

Interestingly, both Gardasil® (genotypes 6, 11, 16, and 18) and Cervarix® (genotypes 16 and 18) showed cross-protection against HPV-31 and HPV-45 due to their similarity to HPV-16 and HPV-18[4,34]. This suggests that Gardasil® 9 and the next generation of HPV VLP vaccines should be evaluated if they can reduce the incidence of infection with other HPV genotypes besides the targeted ones by cross-protection.

Recently, in 2017, Huber et al.[35] created HPV L1-L2-based VLP targeting cutaneous HPV. Minor capsid protein L2 was considered to extend the genotype-restricted protection generated by the current HPV L1-based vaccine. It showed not only humoral immunity against HPV genotypes included in the VLPs, also had cross protections against other HPV genotypes, and this could be a promising next-generation HPV vaccine candidate.

In favor of facilitating vaccine administration, non-needle injection routes such as nasal and oral administration should be considered. In addition, further studies of dose-route responses should be performed and compared with three-dose immunization schedule.

VLP-based HIV vaccine

Current status and its challenge

Although VLP-based HIV vaccine studies have been held over 30 years, none of the HIV VLP vaccine candidates has shown to be protective enough. A few of them have successfully reached clinical trials but were not assessed in efficacy trials due to low immunogenicity and safety properties[36-38]. However, several important factors have been found regarding its immunogenicity in comparison to subunit proteins. In general, HIV VLPs could induce stronger humoral and cellular immunity than HIV recombinant proteins or other SUVs. As HIV-1 polyprotein precursor can form 100 nm to 120 nm VLP, Gag-based and Env-based VLPs were studied separately first[39,40], and then chimeric Env-Gag HIV VLPs have been targeted as a vaccine model[41] to improve immunogenicity. Furthermore, chimeric VLPs of HIV and other viruses such as influenza virus, Bovine/HPV (B/HPV), and hepatitis E virus have also been studied[9-17,42,43]. Nowadays, for the sake of enhancing VLP-derived immune responses, especially T-cell mediated immunity, delivering VLPs with different adjuvants such as toll-like receptors have been assessed, and it is known that nonmethylated CG motifs could generate higher T-cell mediated cytotoxicity when they were codelivered with VLPs[44]. Moreover, recent studies suggest that the exposure of highly conserved epitopes of HIV Env by removing glycosylation sites could improve the production of broadly neutralizing antibodies (bNAbs) by HIV Env-based VLPs[8]. In addition, natural killer cell immune responses were mediated by Gag-VLP through activating and maturating DCs[45]. Furthermore, although DC-based immunization is still intricate, DCs loaded with HIV-1 VLP was suggested to be used as a supplementary boost regimen for HIV-1 VLP vaccination because DCs are potent and indispensable antigen-presenting cells to induce HIV-1 specific humoral immunity[8].

What is more, a few independent non-human primate immunization studies with SIV Gag VLPs demonstrated cross-protection between SIV Env and Gag (reviewed in[8]). All the research groups detected elevated Env-specific or neutralizing antibody responses after SIV or SHIV challenge in rhesus macaques which were immunized with only SIV Gag VLPs.

Another series of studies in small animal models demonstrated that membrane-anchored flagellin and CD40 ligand have been shown as effective adjuvants to enhance HIV-1-specific immune responses after incorporating various adjuvants into HIV-1 VLPs (reviewed by Zhao et al[8]).

While bNAbs against HIV are considered as a crucial factor to prevent HIV infection, it does not seem sufficient and the induction of HIV-specific T-cell-mediated immune responses is also essential to develop a prophylactic vaccine against HIV. In other words, an optimal HIV vaccine should induce innate mucosal, humoral, and cellular immunity specific for HIV. Another difficulty in developing preventive HIV vaccines is HIV's high mutation rates and genetic diversity so that designing a universal and cross-clade HIV vaccine is extremely challenging. Therefore, several researchers have been currently aiming to select and target more conserved regions/epitopes to their HIV VLP mod-

els[8,46]. Moreover, finding perfect animal models to evaluate protective effect of vaccine candidates is one of other big issues. Evident immunogenicity data have been described by many authors after non-human primate immunization with several HIV-1 vaccine candidates[47], but these had disappointing results in humans[48]. Nowadays, the humanized mouse model has also been considered as an alternative animal model to assess immunogenicity and protection as it can mimic the human immune system[49]. Furthermore, the conformational changes and glycan shield of the HIV envelope are other challenges for the development of an effective HIV-1 vaccine[50,51]. Finally, understanding the immune correlates of protection against HIV-1 would be an important key to develop an efficacious HIV-1 vaccine[52].

HIV VLP production in yeast, baculovirus, and other eukaryotic cells

HIV VLPs have been also generated in several expression systems. Yeast expression system was used to produce HIV VLPs for the 1st time in 1987 by Adams et al. using the yeast retrotransposon, Ty, that encodes a set of proteins that are assembled into VLPs, Ty-VLPs. They showed that the major structural components of Ty-VLPs were proteolytic products of the primary translation product, p1, and such protein p1 alone can form Ty-VLPs by itself. Moreover, they demonstrated that p1 fusion proteins, consisting of most of p1 and part of HIV-1 gp120 could form hybrid HIV: Ty-VLPs[53]. In 2002, Sakuragi et al. produced HIV-1 p55 (Gag) VLPs by budding from yeast spheroplasts, in other words, yeast cells without cell wall[54]. However, to get spheroplasts, first, the cell walls needed to be gently enzymatically digested by Zymolyase-100T because thick yeast cell walls were considered as one difficulty to secret proper HIV VLP[54]. Baculovirus/insect cell system was mainly chosen to produce HIV VLPs in 1990s and 2000s, especially because of higher yields compared to yeast system[40,55]. Recently, human embryonic kidney cells (HEK) 293 cells have relatively more efficient protein production than baculovirus/insect cell expression system and it has been the main expression system of HIV VLP production. HEK 293 cells have been adapted and can produce 1 mg/L of VLP or more in a few days and can produce gag-env VLPs. For example, Cervera et al. showed HIV-1 VLP production of 2.8 µg of recombinant Gag-GFP/ml of HEK 293 cell culture[56].

HIV VLP immunogenicity studies

Tsunetsugu-Yokota et al.[57] separated DCs and T cells from fresh peripheral blood mononuclear cells of non-infected and HIV-infected individuals and observed that pulsing DCs with 1 or 10 µg/mL HIV-1 Gag p55 VLP for 2 days induced perforin expression in Gag-specific CD8 T cells. Furthermore, BALB/c mice were inoculated intradermally with 20 µg of HIV-1 Gag p55 VLPs twice at 3-week interval, and statistically significant anti-Gag humoral responses were detected[58].

Immunogenicity data with three types of HIV-1 Env-based VLPs were demonstrated by Crooks et al.[59] Guinea pigs were immunized with (i) “naked VLPs”, which do not bear Env, (ii) “SOS-VLPs” bearing disulfide-shackled functional trimers, and (iii) “UNC-VLPs” that present uncleaved nonfunctional Env or soluble monomeric gp120 3 times on days 0, 43, and 97 by a combination of intradermal and intramuscular (i.m.) routes in two locations each. UNC- and SOS-VLPs immediately induced anti-gp120 antibodies while in naked VLP case, humoral immunity was barely detected after third dose. Ultimately, low level of neutralizing activity was found in all candidates.

Tagliamonte et al.[60] administered 20 µg of HIV Gag p55-based VLPs presenting trimeric HIV-1 gp140 spikes in BALB/c mice subcutaneously twice at the 3-week interval and acquired statistically significant anti-gp140 activity while anti-Gag activity was not analyzed.

Benen et al.[46] assessed HIV Gag VLP-based immunogen presenting membrane proximal external region (MPER) of HIV-1 gp41. They demonstrated in an immunization study in rabbits that priming with DNA and boosting with VLPs generated that low titers of anti-MPER antibodies and low neutralizing activity.

Poteet et al.[61] inoculated HIV-1 Gag/Env VLPs with monophosphoryl lipid A (MPLA) adjuvant to C57BL/6 mice through different routes including a novel oral, buccal cheek subcutaneous administration. After trying several combinations of injection routes, they concluded that an intranasal prime sub-cheek boost regimen of HIV-1 Gag/Env VLPs with MPLA adjuvant has a strong potential to induce Env-specific Th1-oriented HIV-specific immune responses.

Vzorov et al.[62] specifically modified the transmembrane spanning (TMS) and cytoplasmic tail (CT) domains of HIV-1 Env. They demonstrated that the immunization of guinea pigs by a construct containing a short version of the TMS domain induced the highest titers of anti-Env IgG immune responses. In addition,

Table 2. Chimeric B/HPV-S/HIV VLP production and immunogenicity in small animal and non-human primate models

Year	Expression system	Cells	Recombinant proteins	Animal model	Route	Dose	Schedule	Immunogenicity	References
1998	Baculovirus/ insect cells	Sf-9 cells	L1 (BPV-1) + P18-I10(HIV-1)	BALB/c mice	SC	20 μg	0, 2, 4 weeks	Anti-BPV1 VLP Ab and CTL	Peng et al.[14]
1998	Baculovirus/ insect cells	Sf-9 cells	L1 (BPV-1) + P18-I10(HIV-1)	C57BL/6J mice	IN, IM	50 μg × 2	Day 0, 21	IgG, IgA, CTL	Liu et al.[15]
1999	Baculovirus/ insect cells	Sf-9 cells	L1 (BPV-1) + mCCR5	C57BL/6 mice	ID	10 μg × 3	0, 2, 4 weeks	IgG, anti-CCR5 Ab, chemokine	Chackerian et al.[17]
2000	Baculovirus/ insect cells	Sf-9 cells	L1 (BPV-1) + P18-I10 (HIV-1) + RT (HIV-1) + Nef (HIV-1)	BALB/c mice C57BL/6J mice HLA-A2.1/K^b transgenic ($H\text{-}2^b$) mice	IM	20 μg × 2	Day 0, 21	IgG, CTL	Liu et al.[16]
2002	Baculovirus/ insect cells	Sf-9 cells	L1 (BPV-1) + V3/ P18-I10 (HIV-1)	BALB/c mice	IM, IVA, IR	50 μg × 2	Day 0, 14	IgG, IgA, CTL	Liu et al.[9]
2002	Baculovirus/ insect cells	Sf-9 cells	L1 (HPV6b) + P27 gag (SIV)/ tat (HIV-1)/ rev (HIV-1)	Pigtailed macaques	IM, IR	IM 20 μg × 7 as prime IR 20 μg × 3 as boost	8, 11, 14 weeks	AntiHPV L1 ab, IFN-Y, SHIV challenge	Dale et al.[10]
2004	Baculovirus/ insect cells	Sf-9 cells	L1 (BPV-1) + ptCCR5	C57BL/6 mice, pig-tailed macaques	Mice: SC, Macaques: IM	mice: 5 μg × 3 macaques: 20-25 μg × 9	Mice: 0, 2, 4 weeks Macaques: 9 times during>2 years	IgG, Challenge with S/HIV	Chackerian et al.[11]
2009	293 GPR HIV-1 inducible packaging cells	293 GPR HIV-1 inducible packaging cells	HIV-1 Nef+HPV16 E7	C57BL/6 mice	SC	10 μg × 3	0, 2, 4 weeks	IFN-Y	Di Bonito et al.[12]
2013	Baculovirus/ insect cells	Sf-9 cells	L1 (BPV-1) + gp41 (HIV-1)	BALB/c mice	Oral	10 μg × 3	0, 2, 4 weeks	mAb, IgG, IgA, HIV neutralizing assay	Zhai et al.[13]

SC: subcutaneous, ID: intradermal, IM: intramuscular, IN: intranasal, IP: intraperitoneal, BPV: bovine papillomavirus, HPV: human papillomavirus, SIV: simian immunodeficiency virus.

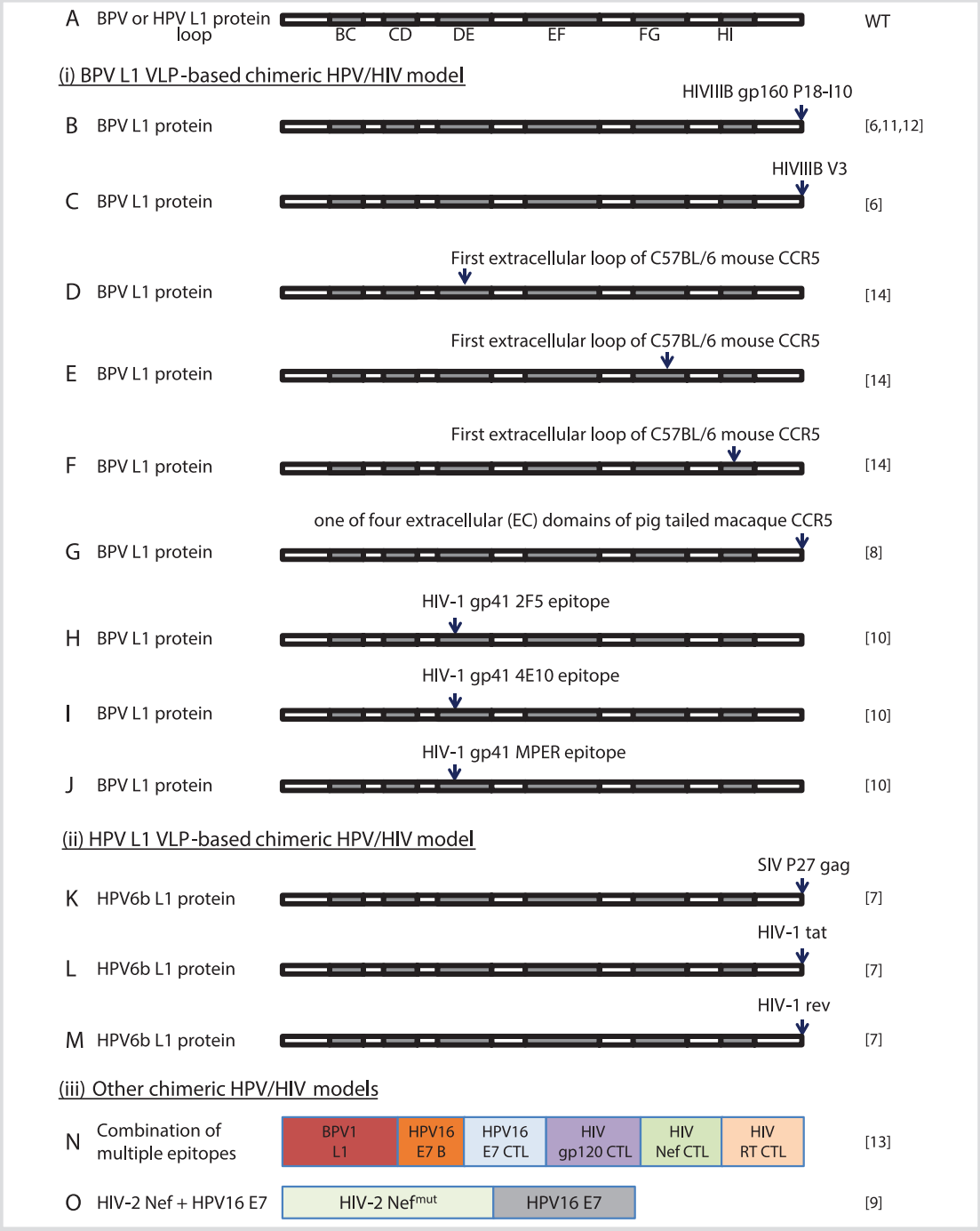

Figure 3. *Schematic representation of chimeric B/HPV and S/HIV-1 proteins for virus-like particles (VLP)-based vaccine development (adapted from Sadeyen et al.[64]).* **A:** *Wild type model of B/HPV L1 protein.* **B-M:** *The insertion point of HIV protein in B/HPV L1 protein is indicated.* *360 copies of each protein form a VLP. (i) BPV L1 VLP-based chimeric HPV/HIV model.* **B and C:** *HIV protein was inserted at C-terminal.* **D,H,J:** *D-E loop 130-136 of BPV1 L1 protein was replaced by mCCR5 or HIV-1 epitope.* **E:** *FG loop 275-285 of BPV1 L1 protein was replaced by mCCR5.* **F:** *HI loop 344-350 of BPV1 L1 protein was replaced by mCCR5.* **G:** *Purified BPV1L1 VLPs were biotinylated and EC domains were bound to those biotins. (ii) HPV L1 VLP-based chimeric HPV/HIV model.* **K-M:** *SIV or HIV protein was inserted at C-terminal. (iii) Other chimeric HPV/HIV model.* **N:** *BPV1L1, HPV16E7 B, HPV16E7 CTL, HIV1 P18-I10, HIV1 Nef CTL, and HIV RT CTL are combined as seen above.* **O:** *gag-pol-nef HIV VLPs with modification at nef c-terminal by HPV16 E7 were produced. BPV: bovine papillomavirus; HPV: human papillomavirus; SIV: simian immunodeficiency virus; WT: wild type.*

Table 3. Ongoing clinical trials of HPV VLP and other other VLP models[65]

HPV VLP	Estimated study completion date
A study of V503 (a multivalent HPV L1 VLP vaccine) in preadolescents and adolescents (V503-002)	December 3, 2020
Long-term follow-up of broad-spectrum HPV vaccine study in women (V503-021)	January 1, 2024
A PMS study to monitor the safety of GSK Biologicals' HPV vaccine in female Chinese subjects	December 4, 2020
Immunobridging Study of 9vHPV vaccine (V503) in Chinese females 9-45 years of age (V503-024)	September 17, 2024
Other VLP models	**Estimated study completion date**
VRC 313: a trivalent VLP encephalitis vaccine (WEVEE) in healthy adults	February 2021
Trial of a chikungunya vaccine, PXVX0317 CHIKV-VLP, in healthy adults	December 2020
Venezuelan equine encephalitis monovalent virus-like particle vaccine (VEEV)	January 2021
Long-term immunogenicity of the norovirus GI.1/GII.4 bivalent VLP vaccine (NoV vaccine) in adults	October 26, 2021
A Phase 2 open-label study to assess the safety and immunogenicity of an alum-adjuvanted chikungunya virus-like particle vaccine (PXVX0317) in prior recipients of other alphavirus vaccines (WRAIR)	December 15, 2020

HPV: human papillomavirus, VLP: virus-like particle, HPV: human papillomavirus, PMS: postmarketing surveillance, GSK: GlaxoSmithKline, 9vHPV: 9-valent human papillomavirus.

VLPs with high Env content and containing the CT trimerization sequence improved neutralization activity and antibody avidity in guinea pig.

Beltran-Pavez et al.[63] demonstrated that rabbit immunization with HIV-1-Gag VLPs containing 4E10-selected envelope variant (LR1-C1), induced humoral responses to 4E10-proximal region.

Taken as a whole, HIV VLPs can be safe and immunogenic using different immunization routes regardless of coadministration of adjuvants and should be continuously pursued to acquire an effective HIV vaccine.

Chimeric VLP-based HPV/HIV vaccine

Current status and vaccine production

To the best of our knowledge, the production of chimeric PV/HIV VLP was first described by Peng et al. in 1998[14], using BPV/HIV VLP to present HIV epitopes. Afterward, Chackerian et al.[17] published a research article regarding chimeric BPV VLP with CCR5 coreceptor, which is required for HIV entry (Table 2 and Fig. 3)[9-17,64]. Henceforth, the production and immunogenicity data of BPV/HIV were introduced by Liu et al.[9,15] and Liu et al[16] and those data of HPV/HIV VLP by Dale et al.[10] In all cases, VLPs were produced by baculovirus/insect sf9 cell expression system. In 2013, Zhai et al.[13] constructed BPV-1 L1 VLP harboring (i) B/T cell conserved epitopes from MPER of HIV-1 gp41 and (ii) the linear epitopes recognized by neutralizing monoclonal antibodies 2F5 and 4B10, which were inserted in D-E loop of L1 protein. While all the previous VLPs were designed to add HIV epitopes into B/HPV L1-based VLPs, in 2009, the incorporation of HPV protein into HIV-1-based VLPs produced in HEK 293 cells was first reported by Bonito et al.[12]

Immunogenicity of chimeric HPV/HIV VLP-based vaccines

Liu et al.[9] investigated in BALB/c mice whether mucosal administration of chimeric BPV/HIV VLP could elicit mucosal cellular and humoral immune responses to BPV VLP and incorporated HIV epitopes. They detected specific antibodies for BPV-1 VLP and HIV-1 CTL epitope P18 from gp120 in serum by i.m. administration but not intrarectal (i.r.) or intravaginal (i.va) immunization. Regarding VLP specific IgA, it was higher in the intestine by i.r. than i.va administration, and higher in vaginal by i.m. than i.r. or i.va. administration. CTL precursor cells specific for HIV P18 were found in

spleen from all three routes of immunization but in Peyer's patches only from i.m. or i.r. immunization.

Dale et al.[10] designed HPV genotype 6b L1 VLPs incorporating SIV Gag p27 and HIV-1 tat and vaccinated pigtailed macaques with DNA encoding SIV gag, HIV-1 tat, and HIV-1 rev or HPV/SHIV VLP prime intramuscularly and with three VLP boosters intrarectally, comparing DNA prime/HPV VLP boost regimen versus all HPV/SHIV VLPs. However, they could detect only weak antibody or T cell responses to the chimeric SHIV antigen in DNA prime/HPV VLP boost regimen, but not in the all HPV/SHIV VLP group.

Di Bonito et al.[12] fused HPV genotype 16 E7 to Nef-mutant, inserted into HIV-1 gag-pol VLP, and vaccinated C57BL/6 mice subcutaneously 3 times over 4 weeks at 2-week interval. The culture of the murine splenocytes demonstrated an anti-E7 CTL activity. Furthermore, the vaccinated mice were challenged with tissue culture number one (TC-1) tumor cells causing HPV-related tumor 2 weeks after the last VLP inoculum. The mice inoculated with the chimeric VLPs were protected after tumor challenge. Furthermore, effective Nef-specific CTL activity was detected.

BPV L1 VLPs presenting HIV-1 epitopes from MPER of gp41 constructed by Zhai et al.[13] and were inoculated to BALB/c mice orally, and induced strong vaginal IgG responses against BPV while only weak vaginal HIV-specific secretory IgA responses were detected. They confirmed that IgG and mucosal secretory IgA were elicited against 2F5 and MPER. The induced antibodies recognized native MPER in HIV-1 infected cells and were able to partially neutralize infectivity from HIV-1 viruses of clade B and C.

Human clinical trials using VLPs

In the near future, more VLP-based vaccine candidates will enter human clinical trials. After the licensure of three VLP-based HPV vaccines, four clinical trials are currently on-going to follow-up the safety and long-term efficacy (Table 3)[65]. Regarding HIV VLP, only a few candidates have been tested in clinical trials. The first candidate, p17/p24:Ty, where yeast-derived Ty protein containing 25% HIV-1 p17 and 79% HIV-1 p24, was tested in Phases I and II[36]. They only induced low level of HIV-specific humoral and cell-mediated immune responses and were discontinued. Another candidate, p24-VLP, which is HIV VLP composed of Gag p24, was reached to Phase II[37]. The VLPs were administered with or without zidovudine, a nucleoside reverse transcriptase inhibitor to asymptomatic HIV-1 infected patients but it could not improve HIV-specific immune responses in them. Finally, DNA and recombinant modified vaccinia virus Ankara (MVA) vaccines expressing HIV-1 VLP were assessed in Phase I trial for safety and immunogenicity[38]. The MVA vaccine producing HIV-1 VLPs of HIV-1 Gag, PR, RT, and Env was well tolerated and elicited different patterns of T cell and Ab responses when administered alone or in combination with the DNA vaccine producing HIV-1 VLPs composed of HIV-1_{HXB-2} Gag, HIV-1_{BH10} PR and RT, and Env, Tat, Rev, and Vpu derived from a recombinant of the HXB-2 and ADA strains of HIV-1.

Various chimeric VLP-based vaccine candidates entered clinical trials such as the anti-influenza A M2–HBcAg VLP vaccine, the anti-HIV p17/p24:Ty VLP, two anti-malaria vaccines, the nicotine-Qb VLP and the anti-Ang II Qb VLP, and other chimeric VLP vaccine candidates went on preclinical trials[66].

Moreover, several clinical trials for other VLP-based vaccines are on-going: *Venezuelan Equine Encephalitis*, *Western Equine Encephalitis*, *Eastern Equine Encephalitis*, Chikungunya virus, and *Norovirus* (Table 3)[65].

Expert commentary

Chimeric VLP technique has brought us to develop safer, broader, and more potent vaccine candidates with higher protective effect to fight against high prevalent and emerging diseases such as malaria, dengue virus, chikungunya virus, HPV and HIV as well as to treat lifestyle diseases such as nicotine addiction and hypertension. Three HPV VLP vaccines are already licensed to prevent HPV infection. Thus, the generation of chimeric HPV/HIV vaccine holding HIV antigens would be a logical effort to fight against both pathogens. In Table 2,[9-17], we summarize preclinical development of previous chimeric BPV/HIV or HPV/HIV VLP vaccine candidates tested in small animal and non-human primate models, but none of the immunogenicity results obtained in those studies were satisfactory enough to reach human clinical trials. One reason could be the insertion point of the HIV immunogen into HPV protein to elicit HPV/HIV-specific immune responses. Many of HPV/HIV chimeric designs fused the HIV epitope to the C- terminal of HPV L1 protein. According to Sadeyen et al.[64], the insertion of Hepatitis B virus epitope into the loops located in HPV L1 protein could increase the immunogenicity. Thus, the inserted epitope would be exposed outside when they form VLP. Furthermore, it would be important to incorporate conserved HIV-1 epitopes from HIV-1 V2 loop region of the envelope into

VLPs, which recent data[67] suggest is more promising to induce humoral immunity against the virus. On the other hand, we always must keep in mind that the size of HIV epitopes inserted into HPV VLP would be an important issue to obtain the antigen conformation and VLP morphology. The inclusion of epitopes to induce bNAbs directed against lineal sequences such as MPER might be worth considering. Besides that, as for the design of chimeric HIV/HPV VLPs, most research groups used HPV VLP as a backbone to insert HIV epitopes, but HIV VLPs could also be a base to add HPV epitopes as done by Bonito et al.[12] In chimeric vaccine models, it is crucial to choose highly immunogenic antigens from interested pathogens and to design ideal chimera conformations which can induce protective immune responses for all of them. Furthermore, it cannot be ignored that the contamination of VLPs with the residual components from host protein-expressing cells may cause undesired activation of innate and adaptive immune responses[5] and denatured VLPs tend to induce lower immunogenicity than properly formed ones[68]. Thus, the purity and stable formulation of VLPs is an important factor to induce expected immunity efficiently. Overall, to reduce the cost of vaccines and improve the coverage of vaccines, scientific community is working to improve VLP-based vaccine addressing these critical issues: (i) formulation level (e.g., types of adjuvants and stabilizers), (ii) administration route (e.g., oral, i.va or i.m.), (iii) delivery vehicles (e.g., liposome, polylactide-glycolide microparticles, or alginates), and (iv) modes of delivery (e.g., needle, spray, or potable liquid).

Five-year view and concluding remarks

VLP technology will achieve great progress in production methods and efficiency, structure morphology as well as chimeric VLP designing in the next 5 years. VLPs are structurally stable and can be manipulated to display heterologous protein on their surface or to carry nanoparticles inside by disassembly and reassembly process with chemical environment changes that greatly broadened the possibility of VLPs as prophylactic and therapeutic vaccines.

In July 2015, a chimeric vaccine RTS,S (hybrid VLP of *P. falciparum* CS protein and Hepatitis B surface antigen) with adjuvant AS01, called RTS,S/AS01 (commercial name Mosquirix) was approved by EMA and WHO launched a pilot project for vaccination on April 23, 2019, in Malawi, planning to perform this project also in Ghana and Kenya later in 2019. This success and results of clinical trials for other VLP models including influenza virus, encephalitis virus, and Chikungunya virus will foster the advances in chimeric VLP technology for HPV and HIV.

In addition, effective adjuvants for multiple VLPs will be assessed to improve the efficacy and immunogenicity. As a result, more chimeric VLP vaccines will be tested in Phase I clinical trials and a few will enter in Phase II trials in the next 5 years.

These days, not only baculovirus/insect cell expression system, and yeast expression system, but also other expression systems such as *E. coli* and plant have been investigated to produce VLPs more effectively. Moreover, other VLP-based influenza virus vaccine candidates are currently tested in human clinical trials. In addition, although the immunogenicity of HPV L2 itself is known to be low, various amino acid regions of HPV-16 L2 protein hold highly conserved neutralizing epitopes that have potential to improve the current HPV L1 based vaccine to prevent other HPV genotypes[24,30]. These new designs might improve our understanding and be applicable to chimeric HPV/HIV model. Furthermore, HIV-1 vaccine candidates containing HIV envelope glycoprotein gp120 (bivalent) or gp140 (trimeric) are now being tested in human vaccine efficacy trials[65] and they have great potential to be applied to develop chimeric HPV/HIV VLP-based vaccines by incorporating them into the HPV L1 VLP. In addition, disulfide-stabilized, cleaved trimeric form of HIV-1 gp140, SOSIP[69], which displays conformational epitopes recognized by bnAbs is going to be tested in Phase I clinical trial.

For T-cell immunogens against HIV-1, mosaic immunogens, which were designed to provide maximum coverage of conserved regions of HIV-1, have been studied[70]. Another candidate, the "HIVACAT T-cell immunogen" (HTI), which was designed to cover T-cell targets, against which T-cell responses are predominantly observed in HIV-1-infected individuals with low HIV-1 viral loads, has also been investigated[71].

Recently, a new concept, called virus-like-vaccines (VLVs), of merging VLPs and replication-deficient virus vectors has been suggested for HIV vaccine development[72]. VLPs can robustly yield antibody and helper T cell responses, whereas the virus vectors can induce potent cytotoxic T cell responses. VLVs first infect cells and then produce VLPs *in situ,* but due to the replication deficiency, the infection cannot propagate. Therefore, VLVs can lead both humoral and cell-mediated immunity safely and effectively, and this method could be applied to chimeric VLP model.

Classically, chimeric VLP was designed by genetically inserting foreign antigen DNA sequence into another which was the base for VLP-forming protein, but, nowadays, target antigens and base VLPs can be produced separately, and then they could be linked using either covalent or noncovalent binding[73]. Through this method, the exposure of target antigens on the surface of base VLPs would be more secure than the classical inserting method, which could cause malconformation of VLPs and non-exposure of the target antigen by the immune cells of vaccinees. It would be very optimistic to say we would successfully have chimeric HPV/HIV VLP-based vaccine within the next 5 years, but there is a great possibility that new VLP production methods and other vaccine models for chimeric VLP would be discovered during the period.

HPV can infect basal epithelial (skin or mucosal) cells and high-risk genotypes, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66, can cause cervical cancer and other mucosal cancers (e.g., anogenital, head and neck). While most HPV infections of the cervix are asymptomatic and more than 90% are normally cleared within 2 years by host immune system, HIV-infected women are more likely to have simultaneous infection with multiple HPV genotypes and are at high risk for HPV-related cancers than women without HIV[55,74].

Both HPV and HIV are sexually transmitted pathogens and delivering chimeric HPV/HIV VLP vaccine mucosally to induce local and systemic immunity against these two pathogens would definitely help to protect humans from prevalent HPV/HIV infections, especially among the young generation. For example, currently, we need to inject the licensed HPV vaccines 3 times for a separated period. Moreover, HIV vaccines, up to the present, also are being designed to introduce a few times over months. The globalization has become much bigger and people have been moving around all over the world more often. Considering this fact, vaccinating people with chimeric vaccines would increase the number of vaccinees by less doses, and we will be more protected from those pathogens through herd immunity. Furthermore, one of the biggest facts, especially in developing countries, is that the people who had the first or second dose often do not come back for the following dose due to geographical, social, and logistic reasons[75]. In addition, in Africa, there are more HIV-infected people who are at higher risk for HPV-related malignancies so that clinical trials for chimeric HPV/HIV vaccines should be held not only in North America, Latin America, and Europe but also in Africa where any HPV VLP vaccine trial has never been conducted yet.

Therefore, a safe, affordable, effective chimeric vaccine against HPV and HIV which is ideally easy to deliver and administer, require less doses, and stay stable at room temperature would ultimately be the best solution. Even if we had acquired promising preclinical data from vaccine candidates, it would not be realistically feasible to administer the vaccines to the most needed population if the manufacturing cost is not financially acceptable. We must put all our effort into the development by designing new vaccine models, proposing cheaper production methods to make vaccines less expensive, more stable, and finding optimal HIV immunogens.

Key issues

- VLPs have very similar appearance to the original virus and are normally composed of only virus structural proteins, which can be displayed in a highly repetitive manner.
- Since VLPs do not contain any viral genetic material inside, they lack the capacity to replicate and are non-virulent, and composing proteins can form VLPs by self-assemble manner and can be enveloped or non-enveloped.
- VLPs are structurally stable and can be manipulated to display heterologous protein on their surface or to carry nanoparticles.
- Cervarix® (2009, GlaxoSmithKline) and Gardasil®9 (2014, Merck & Co.) are bivalent (genotypes 16 and 18) and 9-valent (genotypes 6, 11, 16, 18, 31, 33, 45, 52, and 58), respectively, and are currently used to prevent HPV infections.
- HPV VLP vaccines are still under investigation to cover more genotypes and improve immunogenicity and cross-protection.
- Based on several positive results in preclinical studies with animal models, HIV VLPs can be safe and immunogenic in various immunization routes regardless of coadministration of adjuvants and should be continuously researched to acquire an effective HIV vaccine.
- The insertion of heterologous protein into the loops located in HPV L1 protein could improve the specific immunogenicity of the heterologous protein compared to the insertion to other locations of L1 protein.
- Critical issues to improve VLP-based vaccine: (i) formulation level, (ii) administration route, (iii) delivery vehicles, and (iv) modes of delivery.

- A new concept, called VLVs, of merging VLPs and replication-deficient virus vectors can lead both humoral and cell-mediated immunity safely and effectively, and this method could be applied to chimeric VLP model.
- In July 2015, chimeric VLP vaccine RTS,S (hybrid VLP of *P. falciparum* CS protein and Hepatitis B surface antigen) with adjuvant AS01, called RTS,S/AS01 (commercial name Mosquirix), was approved by EMA, and WHO launched a pilot project for vaccination on April 23, 2019, in Malawi, planning to perform this project also in Ghana and Kenya later in 2019.

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References

1. Lua LH, Connors NK, Sainsbury F, Chuan YP, Wibowo N, Middelberg AP, et al. Bioengineering virus-like particles as vaccines. Biotechnol Bioeng. 2014;111:425-40.
2. D'Aoust MA, Couture MM, Charland N, Trépanier S, Landry N, Ors F, et al. The production of hemagglutinin-based virus-like particles in plants: a rapid, efficient and safe response to pandemic influenza. Plant Biotechnol J. 2010;8:607-19.
3. Noad R, Roy P. Virus-like particles as immunogens. Trends Microbiol. 2003;11:438-44.
4. Smith JF, Brownlow MK, Brown MJ, Esser MT, Ruiz W, Brown DR. Gardasil Antibodies Cross-neutralize Pseudovirion Infection of Vaccine-related HPV Types. Prague: 23rd International Papillomavirus Conference and Clinical Workshop Abstract PL 1-6; 2006.
5. Ludwig C, Wagner R. Virus-like particles-universal molecular toolboxes. Curr Opin Biotechnol. 2007;18:537-45.
6. World Health Organization. Fact Sheet: Human Papillomavirus (HPV) and Cervical Cancer. Geneva: World Health Organization; 2019.
7. UNAIDS Global HIV and AIDS Statistics-2018 Fact Sheet. https://www.unaids.org/en/resources/fact-sheet. Cited on june 2 2019.
8. Zhao C, Ao Z, Yao X. Current advances in virus-like particles as a vaccination approach against HIV infection. Vaccines (Basel). 2016;4:E2.
9. Liu XS, Liu WJ, Zhao KN, Liu YH, Leggatt G, Frazer IH, et al. Route of administration of chimeric BPV1 VLP determines the character of the induced immune responses. Immunol Cell Biol. 2002;80:21-9.
10. Dale CJ, Liu XS, De Rose R, Purcell DF, Anderson J, Xu Y, et al. Chimeric human papilloma virus-simian/human immunodeficiency virus virus-like-particle vaccines: immunogenicity and protective efficacy in macaques. Virology. 2002;301:176-87.
11. Chackerian B, Briglio L, Albert PS, Lowy DR, Schiller JT. Induction of autoantibodies to CCR5 in macaques and subsequent effects upon challenge with an R5-tropic simian/human immunodeficiency virus. J Virol. 2004;78:4037-47.
12. Di Bonito P, Grasso F, Mochi S, Petrone L, Fanales-Belasio E, Mei A, et al. Anti-tumor CD8+ T cell immunity elicited by HIV-1-based virus-like particles incorporating HPV-16 E7 protein. Virology. 2009;395:45-55.
13. Zhai Y, Zhong Z, Zariffard M, Spear GT, Qiao L. Bovine papillomavirus-like particles presenting conserved epitopes from membrane-proximal external region of HIV-1 gp41 induced mucosal and systemic antibodies. Vaccine. 2013;31:5422-9.
14. Peng S, Frazer IH, Fernando GJ, Zhou J. Papillomavirus virus-like particles can deliver defined CTL epitopes to the MHC class I pathway. Virology. 1998;240:147-57.
15. Liu XS, Abdul-Jabbar I, Qi YM, Frazer IH, Zhou J. Mucosal immunisation with papillomavirus virus-like particles elicits systemic and mucosal immunity in mice. Virology. 1998;252:39-45.
16. Liu WJ, Liu XS, Zhao KN, Leggatt GR, Frazer IH. Papillomavirus virus-like particles for the delivery of multiple cytotoxic T cell epitopes. Virology. 2000;273:374-82.
17. Chackerian B, Lowy DR, Schiller JT. Induction of autoantibodies to mouse CCR5 with recombinant papillomavirus particles. Proc Natl Acad Sci U S A. 1999;96:2373-8.
18. Ma B, Maraj B, Tran NP, Knoff J, Chen A, Alvarez RD, et al. Emerging human papillomavirus vaccines. Expert Opin Emerg Drugs. 2012;17:469-92.
19. Schellenbacher C, Kwak K, Fink D, Shafti-Keramat S, Huber B, Jindra C, et al. Efficacy of RG1-VLP vaccination against infections with genital and cutaneous human papillomaviruses. J Invest Dermatol. 2013;133:2706-13.
20. Bruni L, Diaz M, Barrionuevo-Rosas L, Herrero R, Bray F, Bosch FX, et al. Global estimates of human papillomavirus vaccination coverage by region and income level: a pooled analysis. Lancet Glob Health. 2016;4:e453-63.
21. Safaeian M, Porras C, Pan Y, Kreimer A, Schiller JT, Gonzalez P, et al. Durable antibody responses following one dose of the bivalent human papillomavirus L1 virus-like particle vaccine in the costa rica vaccine trial. Cancer Prev Res (Phila). 2013;6:1242-50.
22. Bazan SB, de Alencar Muniz Chaves A, Aires KA, Cianciarullo AM, Garcea RL, Ho PL, et al. Expression and characterization of HPV-16 L1 capsid protein in *Pichia pastoris*. Arch Virol. 2009;154:1609-17.
23. Liu C, Yao Y, Yang X, Bai H, Huang W, Xia Y, et al. Production of recombinant human papillomavirus type 52 L1 protein in *Hansenula polymorpha* formed virus-like particles. J Microbiol Biotechnol. 2015;25:936-40.
24. Cho HJ, Oh YK, Kim YB. Advances in human papilloma virus vaccines: a patent review. Expert Opin Ther Pat. 2011;21:295-309.
25. Kim SN, Jeong HS, Park SN, Kim HJ. Purification and immunogenicity study of human papillomavirus Type 16 L1 protein in *Saccharomyces cerevisiae*. J Virol Methods. 2007;139:24-30.
26. Rose RC, Bonnez W, Reichman RC, Garcea RL. Expression of human papillomavirus type 11 L1 protein in insect cells: *in vivo* and *in vitro* assembly of viruslike particles. J Virol. 1993;67:1936-44.
27. Dupuy C, Buzoni-Gatel D, Touze A, Le Cann P, Bout D, Coursaget P, et al. Cell mediated immunity induced in mice by HPV 16 L1 virus-like particles. Microb Pathog. 1997;22:219-25.
28. Senger T, Schädlich L, Gissmann L, Müller M. Enhanced papillomavirus-like particle production in insect cells. Virology. 2009;388:344-53.
29. Bolhassani A, Shirbaghaee Z, Agi E, Davoudi N. VLP production in *Leishmania tarentolae*: a novel expression system for purification and assembly of HPV16 L1. Protein Expr Purif. 2015;116:7-11.
30. Hatfield GW, Roth DA. Optimizing scaleup yield for protein production: computationally optimized DNA assembly (CODA) and translation engineering. Biotechnol Annu Rev. 2007;13:27-42.
31. McCarthy MP, White WI, Palmer-Hill F, Koenig S, Suzich JA. Quantitative disassembly and reassembly of human papillomavirus Type 11 viruslike particles *in vitro*. J Virol. 1998;72:32-41.
32. Perez G, Lazcano-Ponce E, Hernandez-Avila M, García PJ, Muñoz N, Villa LL, et al. Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (Types 6, 11, 16, 18) L1 virus-like-particle vaccine in Latin American women. Int J Cancer. 2008;122:1311-8.
33. Monie A, Hung CF, Roden R, Wu TC. Cervarix™: a vaccine for the prevention of HPV 16, 18-associated cervical cancer. Biologics. 2008:107-13.
34. Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet. 2006;367:1247-55.
35. Huber B, Schellenbacher C, Shafti-Keramat S, Jindra C, Christensen N, Kirnbauer R, et al. Chimeric L2-based virus-like particle (VLP) vaccines targeting cutaneous human papillomaviruses (HPV). PLoS One. 2017;12:e0169533.
36. Peters BS, Cheingsong-Popov R, Callow D, Foxall R, Patou G, Hodgkin K, et al. A pilot phase II study of the safety and immunogenicity of HIV p17/p24: vlp (p24-VLP) in asymptomatic HIV seropositive subjects. J Infect. 1997;35:231-5.
37. Kelleher AD, Roggensack M, Jaramillo AB, Smith DE, Walker A, Gow I, et al. Safety and immunogenicity of a candidate therapeutic vaccine, p24 virus-like particle, combined with zidovudine, in asymptomatic subjects. Community HIV research network investigators. AIDS. 1998;12:175-82.
38. Goepfert PA, Elizaga ML, Sato A, Qin L, Cardinali M, Hay CM, et al. Phase 1 safety and immunogenicity testing of DNA and recombinant modified *Vaccinia* Ankara vaccines expressing HIV-1 virus-like particles. J Infect Dis. 2011;203:610-9.
39. Zhang WH, Hockley DJ, Nermut MV, Jones IM. Functional consequences of mutations in HIV-1 gag p55 selected by CTL pressure. Virology. 1994;203:101-5.
40. Yamshchikov GV, Ritter GD, Vey M, Compans RW. Assembly of SIV virus-like particles containing envelope proteins using a baculovirus expression system. Virology. 1995;214:50-8.

41. Truong C, Brand D, Mallet F, Roingeard P, Brunet S, Barin F, et al. Assembly and immunogenicity of chimeric gag-env proteins derived from the human immunodeficiency virus Type 1. AIDS Res Hum Retroviruses. 1996;12:291-301.
42. Guo L, Lu X, Kang SM, Chen C, Compans RW, Yao Q, et al. Enhancement of mucosal immune responses by chimeric influenza HA/SHIV virus-like particles. Virology. 2003;313:502-13.
43. Jariyapong P, Xing L, van Houten NE, Li TC, Weerachatyanukul W, Hsieh B, et al. Chimeric hepatitis E virus-like particle as a carrier for oral-delivery. Vaccine. 2013;31:417-24.
44. Storni T, Ruedl C, Schwarz K, Schwendener RA, Renner WA, Bachmann MF, et al. Nonmethylated CG motifs packaged into virus-like particles induce protective cytotoxic T cell responses in the absence of systemic side effects. J Immunol. 2004;172:1777-85.
45. Chang MO, Suzuki T, Suzuki H, Takaku H. HIV-1 gag-virus-like particles induce natural killer cell immune responses via activation and maturation of dendritic cells. J Innate Immun. 2012;4:187-200.
46. Benen TD, Tonks P, Kliche A, Kapzan R, Heeney JL, Wagner R, et al. Development and immunological assessment of VLP-based immunogens exposing the membrane-proximal region of the HIV-1 gp41 protein. J Biomed Sci. 2014;21:79.
47. Garcia-Tellez T, Huot N, Ploquin MJ, Rascle P, Jacquelin B, Müller-Trutwin M, et al. Non-human primates in HIV research: achievements, limits and alternatives. Infect Genet Evol. 2016;46:324-32.
48. Day TA, Kublin JG. Lessons learned from HIV vaccine clinical efficacy trials. Curr HIV Res. 2013;11:441-9.
49. Tager AM, Pensiero M, Allen TM. Recent advances in humanized mice: accelerating the development of an HIV vaccine. J Infect Dis. 2013;208 Suppl 2:S121-4.
50. Yang M, Huang J, Simon R, Wang LX, MacKerell AD Jr. Conformational heterogeneity of the HIV envelope glycan shield. Sci Rep. 2017;7:4435.
51. Doores KJ. The HIV glycan shield as a target for broadly neutralizing antibodies. FEBS J. 2015;282:4679-91.
52. Tomaras GD, Plotkin SA. Complex immune correlates of protection in HIV-1 vaccine efficacy trials. Immunol Rev. 2017;275:245-61.
53. Adams SE, Dawson KM, Gull K, Kingsman SM, Kingsman AJ. The expression of hybrid HIV: ty virus-like particles in yeast. Nature. 1987; 329:68-70.
54. Sakuragi S, Goto T, Sano K, Morikawa Y. HIV Type 1 gag virus-like particle budding from spheroplasts of *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A. 2002;99:7956-61.
55. Quan FS, Sailaja G, Skountzou I, Huang C, Vzorov A, Compans RW, et al. Immunogenicity of virus-like particles containing modified human immunodeficiency virus envelope proteins. Vaccine. 2007;25:3841-50.
56. Cervera L, Gutiérrez-Granados S, Martínez M, Blanco J, Gòdia F, Segura MM, et al. Generation of HIV-1 gag VLPs by transient transfection of HEK 293 suspension cell cultures using an optimized animal-derived component free medium. J Biotechnol. 2013;166:152-65.
57. Tsunetsugu-Yokota Y, Morikawa Y, Isogai M, Kawana-Tachikawa A, Odawara T, Nakamura T, et al. Yeast-derived human immunodeficiency virus Type 1 p55(gag) virus-like particles activate dendritic cells (DCs) and induce perforin expression in gag-specific CD8(+) T cells by cross-presentation of DCs. J Virol. 2003;77:10250-9.
58. Tagliamonte M, Visciano ML, Tornesello ML, De Stradis A, Buonaguro FM, Buonaguro L, et al. Constitutive expression of HIV-VLPs in stably transfected insect cell line for efficient delivery system. Vaccine. 2010;28:6417-24.
59. Crooks ET, Moore PL, Franti M, Cayanan CS, Zhu P, Jiang P, et al. A comparative immunogenicity study of HIV-1 virus-like particles bearing various forms of envelope proteins, particles bearing no envelope and soluble monomeric gp120. Virology. 2007;366:245-62.
60. Tagliamonte M, Visciano ML, Tornesello ML, De Stradis A, Buonaguro FM, Buonaguro L, et al. HIV-gag VLPs presenting trimeric HIV-1 gp140 spikes constitutively expressed in stable double transfected insect cell line. Vaccine. 2011;29:4913-22.
61. Poteet E, Lewis P, Li F, Zhang S, Gu J, Chen C, et al. A novel prime and boost regimen of HIV virus-like particles with TLR4 adjuvant MPLA induces th1 oriented immune responses against HIV. PLoS One. 2015; 10:e0136862.
62. Vzorov AN, Wang L, Chen J, Wang BZ, Compans RW. Effects of modification of the HIV-1 env cytoplasmic tail on immunogenicity of VLP vaccines. Virology. 2016;489:141-50.
63. Beltran-Pavez C, Ferreira CB, Merino-Mansilla A, Fabra-Garcia A, Casadella M, Noguera-Julian M, et al. Guiding the humoral response against HIV-1 toward a MPER adjacent region by immunization with a VLP-formulated antibody-selected envelope variant. PLoS One. 2018; 13:e0208345.
64. Sadeyen JR, Tourne S, Shkreli M, Sizaret PY, Coursaget P. Insertion of a foreign sequence on capsid surface loops of human papillomavirus Type 16 virus-like particles reduces their capacity to induce neutralizing antibodies and delineates a conformational neutralizing epitope. Virology. 2003;309:32-40.
65. NIH U.S. National Library of Medicine ClinicalTrials.gov. Available at https://clinicaltrials.gov/ Cited on July 1 2019.
66. Roldão A, Mellado MC, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. Expert Rev Vaccines. 2010;9:1149-76.
67. Karasavvas N, Billings E, Rao M, Williams C, Zolla-Pazner S, Bailer RT, et al. The Thai phase III HIV Type 1 vaccine trial (RV144) regimen induces antibodies that target conserved regions within the V2 loop of gp120. AIDS Res Hum Retroviruses. 2012;28:1444-57.
68. Zhao Q, Li S, Yu H, Xia N, Modis Y. Virus-like particle-based human vaccines: quality assessment based on structural and functional properties. Trends Biotechnol. 2013;31:654-63.
69. Sanders RW, Moore JP. Native-like env trimers as a platform for HIV-1 vaccine design. Immunol Rev. 2017;275:161-82.
70. Barouch DH, Stephenson KE, Borducchi EN, Smith K, Stanley K, McNally AG, et al. Protective efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. Cell. 2013; 155:531-9.
71. Mothe B, Hu X, Llano A, Rosati M, Olvera A, Kulkarni V, et al. A human immune data-informed vaccine concept elicits strong and broad T-cell specificities associated with HIV-1 control in mice and macaques. J Transl Med. 2015;13:60.
72. Andersson AC, Schwerdtfeger M, Holst PJ. Virus-like-vaccines against HIV. Vaccines (Basel). 2018;6:E10.
73. Jennings GT, Bachmann MF. The coming of age of virus-like particle vaccines. Biol Chem. 2008;389:521-36.
74. Palefsky JM, Gillison ML, Strickler HD. Chapter 16: hpv vaccines in immunocompromised women and men. Vaccine. 2006;24 Suppl 3:S3/140-6.
75. Malande OO, Munube D, Afaayo RN, Annet K, Bodo B, Bakainaga A, et al. Barriers to effective uptake and provision of immunization in a rural district in Uganda. PLoS One. 2019;14:e0212270.