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REVIEW PAPER

Virus-like Particles as Antiviral Vaccine: Mechanism, Design, and Application

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Abstract Virus-like particles (VLPs) are viral structural protein that are noninfectious as they do not contain viral genetic materials. They are safe and effective immune stimulators and play important roles in vaccine development because of their intrinsic immunogenicity to induce cellular and humoral immune responses. In the design of antiviral vaccine, VLPs based vaccines are appealing multifunctional candidates with the advantages such as self-assembling nanoscaled structures, repetitive surface epitopes, ease of genetic and chemical modifications, versatility as antigen presenting platforms, intrinsic immunogenicity, higher safety profile in comparison with live-attenuated vaccines and inactivated vaccines. In this review, we discuss the mechanism of VLPs vaccine inducing cellular and humoral immune responses. We outline the impact of size, shape, surface charge, antigen presentation, genetic and chemical modification, and expression systems when constructing effective VLPs based vaccines. Recent applications of antiviral VLPs vaccines and their clinical trials are summarized.

Keywords: virus-like particles, viral infection, immune response, antiviral vaccine, antigen presentation

1. Introduction

Virus-like particles (VLPs) are multimeric self-assembling virus capsid protein devoid of viral genetic materials that structurally resemble to their parental viruses. The size of most VLPs range from 10 to 200 nm in diameter, with repetitive viral surface epitopes self-assemble into icosahedral or rod like structures [1,2]. There are two categories of VLPs classified, the non-enveloped VLPs and enveloped VLPs. Non-enveloped VLPs are virus coat protein that normally expressed as single or multilayered protein particles without the outer lipid envelope. Enveloped VLPs represent viral coat proteins that are encapsulated within the lipid layer during virus replication process. The envelop layer itself, named as 'virosome', comprises a phospholipid bilayer membrane derived from the host cellular membrane and membrane-stabilizing glycoprotein. Currently, over 100 VLPs originated from human, animal, plant and bacteriophage viruses grouped into 35 families have been constructed for applications in drug delivery, medical imaging, bio-catalysis and vaccine [3].

Owing to the intrinsic properties inherited from their parental viruses, VLPs are recognized as ideal templates in vaccine development. VLPs interact with both innate and adaptive immune cells, inducing strong humoral immune response, and cellular immune response is commonly observed as well. In comparison with live-attenuated vaccines and inactivated vaccines, VLPs have a higher safety profile as they are non-infectious because of the lack of viral genome. Not only VLPs can elicit an immune response against the viruses from the same origin, VLPs are also ideal vaccine platforms to display foreign antigens inducing immune response for the prevention and treatment of different pathogens [4]. VLPs enhance the immunogenicity of many soluble antigens by presenting multiple copies of antigens on their exterior, resulting in a tunable immune response by controlling the antigen valence. These advantages make VLPs appealing candidate in antiviral vaccine development, and researchers highly appreciate them in the battle against emerging viral infections. Several VLPs

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vaccines have received Food and Drug Administration (FDA) approval to treat viral infection, including hepatitis B virus (HBV), hepatitis E virus (HEV), human papillomaviruses (HPV) and Influenza, and increasing number of VLPs vaccines have entered clinical trials [5].

2. Mechanism

Inherited from their parental viruses, both enveloped and non-enveloped VLPs still possess the capabilities to target and enter antigen-presenting cells (APCs) and other target cells without viral genome. Cellular uptake of VLPs into host cells uses different mechanisms, including phagocytosis, micropinocytosis, receptor-mediated endocytosis, caveolae-dependent or independent uptake [6,7]. The highly repetitive surface epitopes of VLPs are potent pathogen-associated molecular patterns (PAMPs) that engage with the pattern recognition receptors (PRRs) such as toll-like receptors (TLRs), nod-like receptors and RIG1-like receptors of both innate and adaptive immune cells enticing immunogenicity *in vivo* [8,9].

Interactions between PAMPs and the PRRs activate APCs such as dendritic cells (DCs) and elicit subsequent VLPs internalization within the APCs, followed by lysosomal digestion result in the loading of VLPs derived antigens peptide onto the MHC II molecules (Fig. 1A). The presentation of VLPs derived antigen via MHC II molecules mediates the activation of the CD4+ T cells. T cell receptors (TCRs) of the CD4+ helper T cells then recognize specific antigen epitopes on MHC II surface, inducing cytokine and co-stimulatory receptor signaling that activates the CD4+ helper T cells [6]. CD4+ T cells mediate the activation of macrophagocyte, CD8+ cytotoxic T lymphocyte (CTL) and T cell dependent B cells activation through the release of cytokines including the interleukins and interferons. Activation of the macrophagocyte provides positive feedback for amplifying the T cells activation effect owing to its enhanced function as APCs. CD8+ T cell and T cell dependent B cell activation elicit downstream cellular and humoral immunity. The repetitive VLPs surface epitopes can induce T cell independent B cell activation by cross-linking B cell receptors (BCRs), stimulating antibody production [10].

PAMPs interact with the PRRs expressed on the surface of innate immune cells such as phagocyte, NK cells, DCs, B-1 cells, mastocyte and granulocyte resulting the activation of the innate immune responses such as phagocytosis, target cell lysis and inflammatory response. Resemble to their parental virus, many VLPs can induce antigen cross presentation on the MHC I surface of innate immune cells such as DCs, mediating CTL immune response through interaction with the TCR of CD8+ T cells [6,11,12]. Signaling through co-stimulatory receptors and cytokines from CD4+ T cells is also required for the activation of CD8+ T cells. Activated CD8+ T cells induce target cells lysis and apoptosis through the release of perforin, granzymes and initiation of the Fas/FasL pathway. Although CD8+ T cell response is commonly observed, it seems VLPs rarely excel in cytotoxic T cells induction in comparison with other ways of vaccination [13].

It is noteworthy that VLPs may encounter intracellular host defense such as TRIM (Tripartite Motif) proteins post entry the membrance of target cells, resulting in the proteasomal degradation of the VLPs or changing their ability to interact with other proteins via ubiquitination process [14-16]. K48 linked ubiquitination elicits proteasomal degradation, whereas K63 linked polyubiquitination often modulates interaction of VLPs with other protein [17]. These mechnisms may potentially affect the antigen presentation and downstream immune response of VLPs vaccine. It has been reported that the intervention of TRIM with HIV-1 viral capsid is associated with enhanced the CD8+ T cells activation, however further investigation is warranted to valid the mechnism of this effect [18].

Owing to the highly organized and repetitive surface epitope, VLPs are endorsed with high valence to cross link BCRs surpassing the activation threshold of B cells, inducing strong humoral responses (Fig. 1B). This process can occur sometimes even without help from T cells [10]. Most VLPs comprise structurally identical surface epitopes that provide a stimulatory advantage over subunit vaccines in protective antibody induction, as high valence promotes binding avidity and enhances activation of unresponsive or B cells with low BCRs affinity [19,20]. CD4+ helper T cells recognise VLPs derived antigen on the MHC II surface, inducing cytokine and co-stimulatory receptor signaling, which initiates the transition of B cells into antibody producing plasma cells and memory B cells. Alternatively, some VLPs induce T cell independent B cell activation and antibody production, resulting in a rapid immune response [21]. The biological activities of the protective antibody include neutralization of pathogen, activation of complement, antibody dependent cellular cytotoxicity (ADCC) and opsonization of pathogen. In general, VLPs can stimulate both cellular and humoral immune response [22,23], hence are optimal candidates for vaccine design owing to their advantages in inducing protective immunity and as platforms for antigen display.

3. Considerations for VLPs Vaccines Design

To develop VLPs based vaccines with high efficacy and

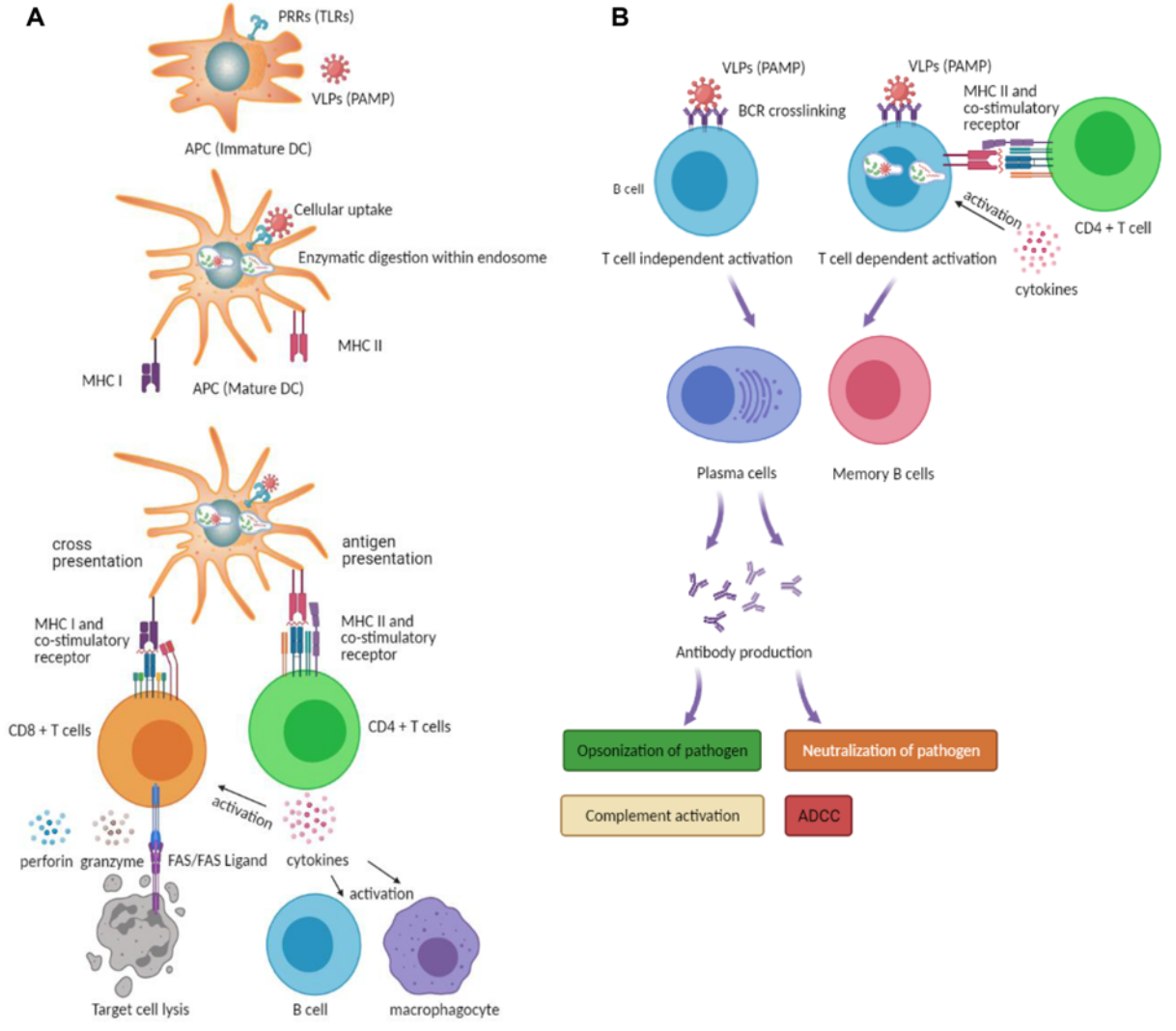

Fig. 1. Hypothesized mechanisms of virus-like particles (VLPs) inducing humoral and cellular immune responses. (A) Pattern recognition receptors (PRRs) of antigen-presenting cells (APCs) recognise pathogen-associated molecular patterns (PAMPs) epitopes of VLPs resulting in the maturation of APCs and cellular internalization of VLPs. VLPs are internally processed within the endosome of APCs, and their antigen epitopes are presented on the surface of MHC molecules. Antigen peptide presented on MHC II surface inducing costimulatory receptor signaling and the release of cytokines, mediating the activation of CD4+ T cells. CD4+ T cells induce T cell dependent B cell activation and macrophagocyte activation. Antigen epitope presented on the MHC I surface through cross-presentation pathway result in the activation of CD8+ T cell, which induces target cells lysis and apoptosis through the release of perforin, granzymes and initiation of the Fas/FasL pathway. (B) The repetitive VLPs surface epitopes cross link B cell receptors (BCRs), inducing T cell independent B cell activation. Both T cell dependent and independent activation induce the transition of B cells into memory B cells and antibody producing plasma cells. The downstream biological activities of protective antibody include neutralization of pathogens, activation of complements, antibody dependent cellular cytotoxicity (ADCC) and opsonization of pathogens. TLRs: toll-like receptors, DC: dendritic cell.

safety profiles, multiple factors related to improving internalization and interaction with immune cells need to be considered. We are discussing in below some aspects which we believe will impact the design of an effective VLPs based vaccine in the case of either using VLPs as vaccine against their parental viruses or antigen (or immune adjuvants) display platform.

3.1. Size and shape

The lymphatic vessel walls are porous, which allow particles smaller than 200 nm to diffuse through freely. As the size of most VLPs are between 10-200 nm which allow the trafficking of VLPs into lymphatic system to interact with immune cells [1]. VLPs can diffuse through the porous lymphatic vessel walls, then accumulate in the subcapsular

sinus of the lymph nodes by passive transportation [24]. VLPs have similar size with their parental virus, which facilitates direct interactions with immune cells, enabling antigen presentation via MHC molecules, antibody production and CTL response. It may also induce similar effects in the case of utilizing VLPs as antigen displaying platform. Immune system recognises the highly repetitive and organized structure of VLPs as PAMPs to elicit cellular and humoral immune response. The ordered structure pattern of VLPs conveniently cross link BCRs that serves as a strong activation signal for humoral immune response. Most viral coat proteins are 5-10 nm apart, this spacing may apply when presenting foreign antigens on VLPs surface for optimal BCR crosslinking [25]. Owing to their unique structure pattern, some VLPs can induce T cell independent B cell activation, result in a rapid release of IgM against pathogens.

Size also determines whether VLPs can be efficiently taken up by APCs. VLPs are endowed with pathogen size, charged or hydrophobic surface area, receptor binding modality, which all contribute to a better APCs induction in contrast to some small and soluble protein antigens [4]. Induction of adaptive immune response requires activation of APCs to produce cytokine and upregulation of co-stimulatory molecules. The geometrical configuration and size are important factors for the induction of DCs maturation, as we have noted that some VLPs, but not unassembled VLPs monomers can trigger this process. For example, DCs should be activated prior to induction of CTL response, as cross-presentation pathway alone is not sufficient [4,26]. The TLRs of DCs recognise the shape and repetitive surface epitopes of many VLPs as PAMPs resulting in functional maturation of DCs, releasing cytokines and over express costimulatory molecules which will regulate subsequent adaptive immune response [27].

3.2. Surface charge

Surface charge may affect the internalization of VLPs particles into immune cells and possibly altering immune response. Cationic nanoparticles tend to induce higher cellular internalization in comparison with negatively or neutrally charged nanoparticles, which may attribute to the electrostatic interaction between nanoparticles and anionic cell membrane lipid bilayer. VLPs with positive surface charge shield their negatively charged cargo such as nucleic acid, and can be readily taken up by cells [28]. However, acute inflammation and nonspecific immune stimulation are associated with positively charged nanoparticles, as they may activate the pattern-recognition receptors [29,30]. Cage like nanoparticles with high positive or negative surface charge exhibit higher nonspecific cellular delivery into murine macrophage, result in liver accumulation in a preclinical study [31]. This may attribute to the active phagocytosis of Kuffer cell within liver. The nonspecific accumulation within normal tissues including liver can be reduced when the net charge of nanoparticles is weak negative [31]. The formation of protein corona will change the surface properties of the VLPs under preclinical and clinical conditions [32], potentially affecting cellular uptake and downstream cell signaling. In a study using iron oxide based nanoparticles, cationic and neutral nanoparticles exhibit higher serum absorption in comparison with negatively charged nanoparticles [33]. Overall, predicting the outcome of immune response by altering the surface charge of VLPs still requires significant efforts in the future, in order to obtain definitive conclusions guiding the design of VLPs vaccines.

3.3. Antigen presentation

VLPs are not only validated candidates in vaccine design, they are also suitable to serve as platforms for multimeric antigen presentation to induce optimal immune response. Because VLPs themselves have immunogenicity, hence they will not only serve as a multivalent scaffold for three-dimensional antigen display, but as immunoadjuvant to enhance the immune response. The incapability of many soluble antigens to elicit sufficient immune response can be solved by attaching them to a multivalent platform such as VLPs [34]. It seems APCs have evolved to process pathogen sized (20-100 nm) antigen more effectively, hence increase the size of a soluble antigen to this range may promote antigen uptake and presentation by APCs, eventually boosting cellular and humoral immunity. The efficiency of a soluble antigen to enter MHC I cross presentation pathway in DCs is often 3-4 orders of magnitude higher after presenting on VLPs, which enhance the CTL response [4]. For VLPs presenting T cell epitopes, it is not required that the antigen has to be exposed at the exterior surface, as VLPs would be degraded within the lysosomal-endosomal system of APCs and the resulting epitope peptides will be subsequently presented to the target TCRs. In this case, antigen can also be inserted within the sites of VLPs that are normally unexposed to the environment. Attempts must be made to identify the optimal insertion site based on structural analysis and prediction, as some antigen insertion sites may endanger VLPs structural integrity or alter immunogenicity [4]. The size of antigen is usually restricted to 20-30 amino acids when inserting into VLPs, especially into the immunodominant regions, sequence longer than that often fail to fold into complete VLPs structure. Unlike T cell epitopes, direct interaction between BCR and B cell epitopes is necessary for the induction of BCR crosslinking and antibody production. This requires B cell epitopes to

be displayed at exposed sites of VLPs surface, optimally at the immunodominant regions. Insertion at the surface loop of VLPs is often a preferable choice as it is the site where many viruses interact with BCRs. Besides, exterior N or C terminus is another choice for insertion. The size tolerance could reach tenfold higher (200-300 aa) at these exposed sites of VLPs [35]. It is difficult to predict whether an antigen epitope will be successfully integrated within defined insertion site without affecting VLPs structural stability, and the general immunogenicity after the insertion. For instance, minor change of insertion site at primary sequence (such as 2-3 amino acid) could lead to a large shift at three-dimensional space where structural hindrance may occur. Antigen with a long sequence, strong positive charge, high hydrophobicity and β-strand index may cause problems to the structural integrity of VLPs [4]. It has been reported that slight variation of the insertion site could also result in a drastic change in immunogenicity of both VLPs and inserted antigen [36]. Protein-protein docking is an established technique to predict protein structures. LZerD docking server (https://lzerd.kiharalab.org/) enables full atomic modeling based on protein sequences, which requires no pre-determined structre information to be submited [37]. In the area of protein structural prediction, deep learning has become the dominant technology in analysing and modeling of the protein folding process, hence has significant impact in vaccine development [38]. *De novo* structure prediction tool Rosetta suite (https://www.rosettacommons.org/) assembles protein segments from protein strcture database and use Rosetta algorithm to form final prediction [39]. The recently released Alpha Fold 2 algorithm (https://github.com/deepmind/alphafold/) may contribute to a better prediction result when inserting antigen to VLPs, as it has achieved a very high fidelity (global distance test score 92.4) in three-dimensional structural prediction, however further validation at larger sampling scale with Alpha Fold is required.

3.4. Interior surface

The interior surface of VLPs is for the storage of genetic materials, including DNA and RNA encoding enzymes and structural protein that are essential for virus replication and structural stability. The inner surface of VLPs is normally positively charged, hence can package negatively charged nuclear acids or other immune adjuvants, inducing potent immune response to synergize with the immunogenicity of the VLPs. VLPs protect its cargo from enzymatic degradation (DNases, RNases, and proteinase) within their hollow cavity, enhancing the cellular uptake of payload within target cells, and release the immune adjuvants to boost the immunogenicity of VLPs within the endosomal-lysosomal compartment where the delivery vehicle is degraded [40]. Adjuvants including dsRNA, ssRNA, and CpGs that activate APCs can be encapsulated within interior surface of VLPs to stimulate respective TLRs receptors of APCs releasing cytokines such as interferons for the induction of APCs activation and pro-inflammatory reaction [1,41-43]. Two simple ways of packaging adjuvants within VLPs named nanoreactor or reassembly route may be employed [44]. Adjuvants can diffuse directly through pores of VLPs shell and accumulate within interior surface via the nanoreactor route, or the VLPs protein shell can be disassembled to mix with adjuvants, then reassemble the VLPs cage in the presence of adjuvants via reassembly route.

3.5. Genetic and chemical modification of VLPs platform

Genetic modification is commonly used when introducing foreign antigens into VLPs platform. Antigen gene can be inserted at specific sites of VLPs DNA sequence, result in a chimeric display of antigens on each subunit of VLPs scaffold. The antigen and VLPs chimeric gene normally go through codon optimization according to the preference of the eukaryotic or prokaryotic expression systems, then the fusion gene is artificially synthesized and subjected to the chosen protein expression system to produce the recombinant chimeric protein. However, the drawback of this technique is as previously mentioned, that the size of antigen, insertion site will affect the structural integrity and the overall immunogenicity of the final product. Such problem can be circumvented by chemical modification on the preformed VLPs platform. VLPs and antigens can be synthesized separately and chemically attached via covalent or non-covalent bonds. The covalent attachment of antigens to VLPs relies on the presence of functional moieties on the surface of VLPs, which are naturally presented or artificially introduced to the VLPs scaffold. Commonly used VLPs surface function groups are amino groups, carboxylic groups, sulfhydryl groups, and hydroxyl groups [1]. Chemical conjugation via acylation of amino groups of lysine, alkylation of the sulfhydryl group of cysteine and amine coupling with activated carboxylic acid residues can be employed to attach antigens to VLPs [4]. The covalent and non-covalent chemical modifications of VLPs platform are listed in Table 1. EDC-NHS-amino coupling is widely used to attach drugs including folic acid and doxorubicin to the amino group on the exterior surface of VLPs such as cowpea mosaic virus (CPMV) for cancer theraputic applications [45,46]. Cysteine residues of VLPs provides a useful handle to perform maleimide-thiol conjugation, however they often form disulfide bonds rather than exsit as free cysteine. Recently disulfide-bridging maleimide derivatives such as

Table 1. Chemical functionalization of VLPs

Method	VLPs	Ligands	Reference
EDC-NHS-Amide group	CPMV	Folic acid, Doxorubicin	[45,46]
Maleimide-thiol group	Bacteriophage Qbeta	Dibromo-maleimide (Fluoresent)	[47,49]
Click chemistry	Bacteriophage Qbeta	Tn antigen (GalNAc-α-O-Ser/Thr)	[50]
Non covalent streptavidin-biotin interaction	HPV16 L1	Monovalent streptavidin (mSA)-fused malaria antigen (VAR2CSA)	[51]

VLPs: virus-like particles, CPMV: cowpea mosaic virus, HPV: human papillomaviruses.

dibromo-maleimide are developed to attach functional group to cystine without affecting the covalent disulfide bonds [47]. The fluoresent dibromo-maleimide is attached to 180 cysteine residues on the surface of bacteriophage Qbeta VLPs for the application of intracellular imaging [48,49]. Unnatural amino acids can be artificially introduced to VLPs, which enable chemical modification such as 'click chemistry' to conjugate antigens at specific site of VLPs. Alkyne group of unnatural amino acids is introduced to the surface of Qbeta VLPs, which enables copper-catalyzed azide-alkyne cycloaddition reaction to covalently attach azide functionalized Tn antigen (GalNAc-α-O-Ser/Thr) [50]. In addition, non-covalent interaction betwenn streptavidin and biotin can be used to functionalize VLPs with antigens. HPV16 L1 VLPs is genetically fused with biotin acceptor Avitag, monovalent streptavidin (mSA)-fused malaria antigen (VAR2CSA) can be attached to the biotinylated Avitag-VLPs forming a VLPs based anti-malaria vaccine [51]. Chemical modification provides substantial benefits compared to genetic modification, however, it is not without its own disadvantages. Although it avoids steric hindrance when inserting antigens to VLPs, the modification rate of chemical conjugation is often less than 100% in comparison with genetic modification, and the reaction process is not as easy to control and reproduce [4]. The complexity and cost of chemical modification will also increase, which can hamper the industrialization process from a manufacturing point of view.

3.6. Expression platforms

To overcome the barriers to market entry, producing VLPs vaccines in a timely, reproducible, low cost, high yield and safe manners should be critically considered. Hence, the significance of expression platforms is seriously appreciated in the manufacturing process of VLPs vaccine. Currently, a few expression hosts have been developed for VLPs vaccine production, including bacteria, yeast, insect cells, plants, mammalian cells, cell-free expression systems and living animal [4,52-54].

Bacterial system (especially *Escherichia coli* expression system) is probably the most widely used and well characterized in VLPs expression. About 30% of VLPs are cloned and codon optimized for expression in this system under strong bacterial promotors [1]. This host provides high expression yield, short cultivation time, low-cost and simple process control in comparison with other expression platforms. However, disadvantages of this host may be encountered during expression process, which include incapable of performing posttranslational modifications (PTMs), solubility issue, and introduction of endotoxin [55]. In some cases, the introducing glycosylation will not affect the immunogenicity of a target protein, hence the inability of performing PTMs may not be a major issue when producing VLPs vaccine in bacterial host [56]. The solubility issue could be eased by lowering the cultivation temperature, clone the genes sequence to a low temperature plasmid vector (such as pCold vector) or genetically fuse the target gene with a highly soluble protein tag (such as GST tag). In the case of forming the inclusion body, denaturing and refolding steps could be introduced to help the target protein forming native structure. The contamination of endotoxin can also be reduced to an acceptable level by polishing the protein product with endotoxin binding matrix.

Since the FDA approval of the first HBV S protein VLPs vaccine against HBV and the HPV L1 VLPs vaccine against HPV, yeast expression system (especially *Pichia pastoris*, *Hansenula polymorpha*, and *Saccharomyces cerevisiae* strains) has been proved as a validated host for VLPs vaccine production. Although the overall production yield is often less than the bacterial system and the PTMs is different compared to mammalian cells, yeast host can provide PTMs such as glycosylation or phosphorylation, which affects the surface charge, hydrophobicity, stability and function of the VLPs vaccine. Yeast expression host is also used to express more complexed structures, such as enveloped VLPs [52]. Insect cell-baculovirus expression system is gaining its popularity as it offers advantages such as mammalian-like PTMs, high yield and fast cultivation time compared to the bacterial and yeast host. We may need to pay attention to the contamination issue when using this host as an expression platform [53].

Mammalian cell is one of the most widely used hosts for

the manufacturing of biopharmaceutics, including VLPs vaccine. Mammalian system not only offers more complete PTMs, it can also be used to co-express multiple protein which allow construction of more complicated VLPs vaccine structure. However, the production cost is higher and downstream processing is more complex in comparison with the bacterial expression system. Plant based expression system is a cost effective and scalable platform for vaccine production, but it may require strict regulation according to local ecological status. Take advantage of the highly polyploid property of the plant chloroplast genomes (up to 10,000 copies per cell), integrate exogenous VLPs genes into chloroplast genomes resulting in high level of protein expression [57,58]. Cell-free system can be an attractive alternative when expressing proteins that are toxic to living cells, or in the case of introducing unnatural amino acids to VLPs vaccines [53].

Living animal is another source for the expression of VLPs vaccines. Protozoan *Leishmania tarentolae* (*L. tarentolae*) expression system offers mammalian-like post-translational modification machinery and is non-pathogenic for humans. Protozoan derived Norovirus GII.4 VP1 VLPs is immunogenic and can stimulate the production of neutralizing antibodies [59]. Norovirus GII.4 VP1 is cloned into the *Bombyx mori* nucleopolyhedrovirus vector and expressed in silkworm larva. The silkworm fat body lysate goes through a two-step purification of the sucrose gradient centrifugation and immobilized metal affinity chromatography, and the final purity of Norovirus VLPs is above 90% [54]. The advantages of living animal expression system include more complete PTMs, relatively low cost, robustness and ease of mass production in comparison with other systems.

4. Applications and Recent Clinical Trials of Antiviral VLPs Vaccines

VLPs are ideal candidate in vaccine development as they share many characteristics of their parental virus [60]. They preserve strong immunogenicity of virus as they display highly repetitive surface antigen epitopes which stimulate immune cells. The absence of viral nuclear acids also enhances the safety profiles of VLPs to be developed as vaccine platforms. In this section, we have reviewed applications of antiviral VLPs vaccines, and we present the clinical trials within recent five years of the VLPs vaccines in Table 2.

Table 2. Clinical trials of VLPs based vaccines within recent five years

References	Pathogens	VLPs vaccine constructs	Phase	Trial number	Sponsor
Development of AAVLP(HPV16/31L2) Particles as Broadly Protective HPV Vaccine Candidate [71]	Human papillomavirus (HPV)	L2 peptide of HPV 16 and 31 displays on VP3 VLPs of the adeno-associated virus	I	NCT03929172	2A Pharma
Phase 1 randomized trial of a plant-derived virus-like particle vaccine for COVID-19 [107]	SARS-CoV-2	CoVLP, SARS-CoV-2 S protein (with S2P, GSAS substitution) fused with the transmembrane and cytoplasmic domain of influenza A hemagglutinin (H5)	II/III	NCT04636697	Medicago
An enveloped virus-like particle vaccine expressing a stabilized prefusion form of the SARS-CoV-2 spike protein elicits highly potent immunity [108]	SARS-CoV-2	VBI-2902a, transmembrane cytoplasmic domain of the vesicular stomatitis virus (VSV) displaying prefusion state S protein	I/II	NCT04773665	VBI vaccines
Capsid-like particles decorated with the SARS-CoV-2 receptor-binding domain elicit strong virus neutralization activity [109]	SARS-CoV-2	ABNCoV2, S RBD fused to bacteriophage scaffold surface	I/II	NCT04839146	AdaptVac
A modular protein subunit vaccine candidate produced in yeast confers protection against SARS-CoV-2 in non-human primates [110]	SARS-CoV-2	RBD SARS-CoV-2 HBsAg VLP vaccine, S RBD display on the surface of HBV surface antigen VLPs	I/II	ACTRN12620000817943	Accelagen Pty Ltd
Study of a Severe Acute Respiratory Syndrome CoV-2 (SARS-CoV-2) Vrus-like Particle (VLP) Vaccine in Healthy Adults (COVID-19)	SARS-CoV-2	VLP vaccine harboring M, N, E, and hexapro modified S proteins of SARS-CoV-2 virus	I	NCT04818281	Ihsan GURSEL, PhD, Prof.

Table 2. Continued

References	Pathogens	VLPs vaccine constructs	Phase	Trial number	Sponsor
Effect of a Chikungunya Virus–Like Particle Vaccine on Safety and Tolerability Outcomes [117]	Chikungunya virus (CHIKV)	CHIKV VLP (VRC-CHIKVLP059-00-VP)	II	NCT02562482	National Institute of Allergy and Infectious Diseases (NIAID)
Immunogenicity and safety of a quadrivalent plant-derived virus like particle influenza vaccine candidate-Two randomized Phase II clinical trials in 18 to 49 and ≥ 50 years old adults [118] & Efficacy, immunogenicity, and safety of a plant-derived, quadrivalent, virus-like particle influenza vaccine in adults (18-64 years) and older adults (65 years): two multicentre, randomised phase 3 trials [119]	Influenza A viruses	Hemagglutinin-bearing quadrivalent virus-like particle (QVLP)	II&III	NCT02233816 NCT02236052 NCT03301051 NCT03739112	Medicago
Safety and Immunogenicity of a Respiratory Syncytial Virus Fusion (F) Protein Nanoparticle Vaccine in Healthy Third-Trimester Pregnant Women and Their Infants [120]	Respiratory syncytial virus (RSV)	Recombinant RSV fusion glycoprotein nanoparticles (Genbank Accession No. U63644)	II	NCT02247726	Novavax
A ten-year study of immunogenicity and safety of the AS04-HPV-16/18 vaccine in adolescent girls aged 10-14 years [121]	Human papillomavirus (HPV)-16, 18, 31, 45	HPV-16/18 L1 virus-like particle (L1 VLP)	III	NCT00196924 NCT00316706 NCT00877877	GlaxoSmithKline
Persistence of Antibodies to 2 Virus-Like Particle Norovirus Vaccine Candidate Formulations in Healthy Adults: 1-Year Follow-up With Memory Probe Vaccination [122]	Noroviruses (NoVs)	GI.1/GII.4 bivalent virus-like particle (VLP) vaccine	II	NCT02142504	Takeda
Safety and immunogenicity of a plant-produced Pfs25 virus-like particle as a transmission blocking vaccine against malaria: A Phase 1 dose-escalation study in healthy adults [123]	Malaria	Chimeric non-enveloped virus-like particle (VLP)-Pfs25 fused to the Alfalfa mosaic virus coat protein (Pfs25 VLP-FhCMB)	I	NCT02013687	Fraunhofer, Center for Molecular Biotechnology
Induction of Human T-cell and Cytokine Responses Following Vaccination with a Novel Influenza Vaccine [124]	Influenza A viruses	gH1-Qbeta VLP vaccine-bacteriophage Qbeta VLP covalently linked with influenza HA globular head domain (gH1) Trivalent Influenza Vaccine (TIV)- (A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2), and B/Brisbane/60/2008)	I	#CIRB2010/720/E #CIRB2012/906/E Approval from Singapore Health Sciences Authority (HSA)	Bio Medical Research Council
Development of an Interleukin-1β Vaccine in Patients with Type 2 Diabetes [125]	Type 2 diabetes	hIL1bQb VLP vaccine-chemically cross-linking the IL-1β proteins to bacteriophage Qbeta VLP	I	NCT00924105	Cytos Biotechnology AG
Immunogenicity of a novel Clade B HIV-1 vaccine combination: Results of phase 1 randomized placebo controlled trial of an HIV-1 GM-CSF-expressing DNA prime with a modified vaccinia Ankara vaccine boost in healthy HIV-1 uninfected adults [126]	HIV-1	Boost: recombinant modified vaccinia Ankara poxvirus vaccine (MVA/HIV62B) encodes HIV-1 VLP Prime: GEO-D03 DNA vaccine co-expresses GM-CSF and non-infectious VLPs from clade B HIV-HXB2/BH10 (Gag-Pol) and HIV-ADA sequences (gp120/gp41 cleavage site intact)	I	NCT01571960	National Institute of Allergy and Infectious Diseases (NIAID)

4.1. HBV vaccine

Intense interest and research have been intrigued since the FDA approval of the VLPs based Hepatitis B vaccine Engerix-B (GSK) and Recombivax (Merck) in 1986. These HBV surface antigen HBsAg (S protein, 226 aa) based vaccines form a 22 nm spherical shell with host cell (yeast, mammalian) glycosylation [61]. They provoke long lasting immune protection although their response rate is only 90-95% because of the lack of Pre-S epitopes on the surface of the vaccines [62]. The risk of central nervous system-inflammatory demyelination in infant boys is threefold higher during vaccination using Engerix-B [63]. To overcome these issues, the VLPs based HBV vaccine Sci-B-Vac which incorporates S, Pre-S1 and Pre-S2 surface antigens has been licensed [1,64]. This vaccine induces high titer of antibody against S antigen, also additional Pre S1 and Pre S2 antibodies, that can be applied as a therapeutic vaccine in the treatment of chronic HBV disease [65]. A long term follow up study indicates that the vaccine induced antibody titers against HBV surface antigen is strongly enhanced with a single booster vaccination by Engerix-B or Sci-B-Vac in Palestinian children [66].

4.2. HPV vaccine

The first prophylactic HPV vaccine Gardasil developed by Merck is approved by FDA in 2006 in the battle against human HPV infection. Gardasil is a recombinant protein consist of the L1 major capsid protein (VLPs) of HPV type 6, 11, 16, and 18. In comparison with Gardasil, Cervarix (GSK) comprises L1 protein of HPV type 16 and 18 is approved by FDA in 2007. Although this vaccine generates only 2 serotypes compare to 4 serotypes of Gardasil, Cevarix induces stronger and longer immune response benefit from the monophosphoryl lipid A within its formulation [67]. Gardasil9, marketed by Merck in 2014, offers broad-spectrum inducing 9 serotypes including HPV type 31, 33, 45, and 58 in addition to Gardasil's [1]. For adolescents below the aga of 15, the initial 3 dose prime-boost schedule has been changed to 2 does at birth and 6 or 12 months by World Health Organization in 2014. For licenced vaccines, limited evidence indicates that after priming doses, boost vaccinations after 3,6, and 8 year intervals will elict a strong anamnestic response [68]. As an alternative to Gardasil9, equal molar stoichiometry of L1 mutants (C175A and C428A) from 9 HPV genotypes co-assemble into a capsomere-hybrid virus-like particle is able to generate comparable neutralization antibody titers in mice [69]. Although L1 VLPs vaccines are highly immunogenic, L1 protein is not conserved hence offer little cross protection among different HPV types [70]. In the meanwhile, the minor capsid protein L2 is highly conserved across different HPV types, which makes it an appealing candidate when developing broadly protective HPV vaccine. Comparing with L1 protein, L2 is less immunogenic and cannot form VLPs on its own, displaying L2 protein on VLPs platform may enhance its immunogenicity as a cross protective HPV vaccine. The AAVLP-HPV (2A Pharma) is comprised of L2 peptides of HPV 16 and 31 display on VP3 VLPs of the adeno-associated virus [71]. This vaccine has shown protection against more than 20 HPV types in pre-clinical tests and has completed clinical phase I trials (NCT03929172). Similarly, displaying HPV31/16 L2 peptides on the surface of bacteriophage MS2 VLPs offers similar level of protection to Gardasil9 in pre-clinical model [72].

4.3. Influenza A vaccine

Hemagglutinin (HA), neuraminidase (NA) and matrix protein M1 are considered as the primary antigen source when developing a recombinant influenza vaccine. Antibody targeting the surface glycoproteins such as HA and NA can neutralize viral infection and paralyze its invading mechanism, stagnating viral progression at its initial phase. FluBlok is the first recombinant influenza vaccine approved by FDA in 2013, which uses the envelope glycoproteins HA as its primary target [73]. Vaccines targeting the HA head may require updates annually because of the antigenic drifting nature in this region. However, the HA stem region is evolutionally more stable across different influenza stains hence may provide a solution for constructing 'universal' influenza vaccines that eliciting broad immune protection and do not need to be updated yearly [74,75]. NA based VLPs vaccines have not reached the market yet and are under intense research and testing [76,77]. Influenza M1 protein structurally resembles to its parental virion and can induce both humoral and cellular immune responses in mice challenged with homologous and heterologous influenza viruses [78]. M1 protein has also been employed as VLPs scaffold protein to display different influenza antigens, such as HA [79], NA [80], and M2 [81] on its surface. Influenza ion channel protein M2 is another genetically conserved target for universal influenza vaccine design, especially the M2 ectodomain (M2e, 23 aa) is highly conserved across different human influenza subtypes [61]. Tandem repeat of heterologous M2e from different species (including human, swine, and avian) has been presented on the surface of influenza VLPs (transmembrane and cytoplasmic domains of HA), which induced cross protective antibodies against different influenza A H1 and H3 subtypes in mice [82]. Transdermal vaccination of this construct (AS04 adjuvanted) induce both cellular and humoral immune response in mice challenged with H3N2 influenza A virus [83]. In another study, both influenza M2e and HA are attached to the

surface of norovirus VLPs carrier (VP1 capsid) via SpyTag-SpyCatcher conjugation [84]. Interestingly, *in vitro* data indicates high antibody response against HA rather than M2e is induced. Co-expression of NA and M1 in insect cells produce NA VLPs (NA and M1 hybrid cage), which can be used to induce protective immunity against influenza A virus infection [85,86]. Simultaneous vaccination with NA1 and NA2 VLPs results in the production of anti-NA serum IgG, which protect mice against lethal challenge with H1N1 and H3N2 viruses [87]. In a preclinical study, two does of intramuscular prime-boost imunization at day 0 and 21 with unadjuvanted H6N1-VLPs hybrid cage (consist of viral HA, NA, M1, and M2 proteins) elicits long-lasting antibody immunity in mice [88].

4.4. HIV-1 vaccine

Because of the incapability of generating sufficient neutralizing antibodies against HIV-1 and simian immunodeficiency virus (SIV), production and purification obstacles and difficulties in maintaining the conformational authenticity of native HIV-1 surface glycoprotein epitopes, there is no clinically approved prophylactic HIV-1 vaccine currently available in the market. The first VLPs based HIV-1 vaccine entered clinical trial 25 years ago [13,89]. The HIV-1 p24-VLP derived from Gag (group antigen) capsid is the first VLPs vaccine that reached phase I/II clinical trial, although p24-VLP has showed a good safety profile, its low immunogenicity do not elicit significant enhancement in both humoral and cellular immune response [90,91]. Another HIV-1 vaccine RV144 combines a recombinant canarypox vector (ALVAC) expressing HIV Gag, protease and gp120 as a prime, and a gp120 secreted on Gag capsid protein based VLPs (AIDSVAX) as a boost [92]. This clinical trial has achieved partial protection, inducing a low protective efficacy of 31.2% against HIV-1 infection in Thailand [89,92]. The P18I10 peptide (RGPGRAFVTI) form the V3 loop of HIV-1 gp120 is inserted to the D-E loop of the HPV L1 capsid, the resulting chimeric VLPs vaccine named L1P18 is expressed in yeast aiming to elicit protective effect against both HPV and HIV [93]. Further efficiency test of this construct will be conducted in future study. In another study, a VLPs expressing mRNA vaccine consist of the SIV Gag capsid and HIV-1 Env (envelope) proteins is able to generate neutralizing antibodies and achieve 79% per-exposure risk reduction in rhesus macaques [94]. Sublingual immunization is known for its efficiency of inducing mucosal and systemic immune responses. The C1q (CD91 ligand) surface conjugated CD40L/HIV VLPs vaccine binds and cross the epithelial layer eliciting IgA salivary antibody production and cellular immune response when sublingually administrated to mice [95]. Owing to the efficient Ebola envelope glycoprotein (EboGP) mediated viral entry into human macrophages and DCs, replacement of the mucin-like domain of EboGP with HIV envelope C2-V3-C3 (134 aa) region generates a chimeric VLPs capable of inducing higher titers of HIV-1 antibody than GP120 alone in mice [96]. A chimeric VLPs vaccine is constructed by replacing the transmembrane and cytoplasmic regions of HIV-1 Env with the coresponding domains of influenza H5 HA [97]. However, this resulting in a decreased neutralizing antibody response against HIV-1, indicating full-length gp120 may be required in this case. Strong cellular immune responses against HIV-1 is induced in mice when using Pr55Gag based chimeric HIV-1 VLPs with reverse transcriptase or a Tat-Nef protein fused at their C terminal as a boost, and a HIV-1 DNA vaccine as a prime [98]. The elicited cellular immunity is 2-3 fold higher than HIV-1 DNA vaccination alone. When using HIV-1 envelope VLPs functionalized with V3-glycan immunogen as a prime, and native-like Env-VLPs as a boost, heterologous neutralizing antibodies is induced in non-human primates (NHPs), and this effect is more potent than Env-VLPs vaccination alone [99].

4.5. Norovirus vaccine

Takeda's bivalent norovirus VLPs vaccine (TAK-214) is currently undergoing phase II clinical trial. This vaccine consists of norovirus GI.1 and GII.4c major capsid protein VLPs, which elicit neutralizing antibodies 8 days after intramuscular vaccination and the antibody level stays above the baseline for at least one year [100,101]. Cross genotype protection is also observed against the heterotypic GII.2 genotype [102]. Another bivalent vaccine combines the norovirus GII.4 VLPs and enterovirus 71 (EV71) VLPs has showed neutralizing effects against EV71 infection and GII.4-VLP binding to mucin in a preclinical study [103]. A major capsid protein VLPs of norovirus GII.17 has been expressed in baculovirus insect cells system. The preclinical study demonstrates this vaccine can generate protective antibody against NoV GII.17 in immunized mice [104]. In another study, a recombinant GII.P16-GII.2 VLPs vaccine expressed in baculovirus system polarize the macrophages to M1 type for the induction of CD4+ Th1 oriented immune response [105]. A preclinical study in mice indicates that prime boost immunization of genetically close norovirus VLPs antigens may induce strong blocking antibody response, and vaccination using the mixure of these VLPs antigens simultaneously is superior to sequential immunization in this study [106].

4.6. SARS-CoV-2 vaccine

The CoVLP from Medicago can generate high titer of

neutralizing antibody and has entered phase III clinical trials. The transmembrane and cytoplasmic domain of the SARS-CoV-2 S protein (with S2P, GSAS substitution) of the CoVLP are replaced with that of the influenza A HA (H5), forming a heterologous VLPs vaccine [107]. Another chimeric SARS-CoV-2 VLPs vaccine named VBI-2902a consists of the S protein (in prefusion state) and the transmembrane cytoplasmic domain of the vesicular stomatitis virus. This vaccine generate protective immunity in SARS-CoV-2 challenged hamsters at a single dose, and is currently undergoing phase I/II clinical trials [108]. In another study, the receptor binding domain (RBD) of S protein is fused on bacteriophage scaffold (Acinetobacter phage AP205 coat protein) using SpyTag-SpyCatcher technology [109]. This VLPs vaccine named ABNCoV2 (AdaptVac) elicits high titers of neutralizing antibody in a preclinical study and is undergoing phase I/II clinical trials at present. The SpyTag-SpyCatcher linkage also enables the display of SARS-CoV-2 RBD on the surface of HBV surface antigen VLPs [110]. This construct induces high titers of neutralizing antibodies that protect against SARS-CoV-2 challenge in NHPs and has recently entered phase I/II clinical trials. The capsid of tobacco mosaic virus is used to display the SARS-CoV-2 RBD fused with a human IgG1 Fc domain [111]. This vaccine candidate triggers neutralizing antibody production in a preclinical study, the non-adjuvanted VLPs vaccine induced a balanced Th1 and Th2 response, whereas the CPG 7909 adjuvanted formulation elicits a Th1-oriented response. In order to synergize both BCR signaling and TLR signaling, a phage (acinetobacter phage AP205) derived VLPs contains host cell derived RNA as TLRs ligands and display SARS-CoV-2 RBD on its surface is developed [112]. This VLPs construct induces a Th1-biased response and high titers of neutralizing antibodies with durable memory in NHPs. The S1 modified AP205 can generate neutralizing antibody against SARS-CoV-2 variants (Wuhan and UK/B.1.1.7) in mice prime-boost with 0.5 μg of this agent [113]. Both receptor binding motif and fusion peptide (S2 domain, AA 817-855) of SARS-CoV-2 spike protein are displayed on CuMVTT VLPs (cucumber mosaic virus with a universal Th activating tetanus toxin epitope) for the induction of neutralizing antibody [114]. It is worth noting that this vaccine candidate along with its fusion peptide only version elicit neutralizing antibodies in mice, indicating the potential application of S2 fusion peptide as immune stimulating agent. A dodecahedric adenovirus VLP is modified with 60 copy of SARS-CoV-2 RBD, the prime vaccination with this vaccine induces neutralizing humoral response which is enhanced by a single boost with the same construct in mice [115]. In another *in vitro* study, CPMV or Qβ VLPs are modified with antigen epitope peptides from S protein of SARS-CoV-2, prime-boost administration of these constructs induces neutralizing antibodies [116].

5. Future Perspective and Conclusion

VLPs based vaccines present a promising future in the battle against the urgent viral infections and other diseases. Despite the progresses have been achieved so far, barriers still exist in the development of VLPs based antiviral vaccines. The technical difficulty in the production of the VLPs vaccines, especially the enveloped VLPs vaccines need to be stressed, as the structural complexity of VLPs may cause decreased immunogenicity during the expression and purification process using current expression platforms. New strategy and expression system using stable transgenic cell lines need be developed to express proteins with complex structures and PTMs. Substantial improvement in term of inducing CD8+ T cell response is required in VLPs vaccine development. Modifying the exterior or interior surfaces of VLPs with immunoadjuvants that could induce cross presentation on MHC I surface may help researchers resolve this issue. To control the strength of immune response, bioinformatics and protein structural predication will shine a light on tuning interactions between VLPs vaccines and the receptors of immune cells, as well as the process of screening large number of VLPs assemblies for optimal immune responses. We hope this review will provide general background and useful guidance when designing VLPs as antiviral vaccine.

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Author's Contributions

Conceptualization, L.Z. and Y.W.; investigation, L.Z., X.M., JB.F., and XJ.S.; writing-original draft preparation, L.Z. and Y.W.; writing-review and editing, W.X. and Y.W.; final approval of the version to be submitted, Y.W. All authors have read and agreed to the published version of the manuscript.

Ethical Statements

The authors declare no conflict of interest.
Neither ethical approval nor informed consent was required for this study.

References

1. Mohsen, M. O., L. Zha, G. Cabral-Miranda, and M. F. Bachmann (2017) Major findings and recent advances in virus-like particle (VLP)-based vaccines. *Semin. Immunol.* 34: 123-132.
2. Shoeb, E. and K. Hefferon (2019) Future of cancer immunotherapy using plant virus-based nanoparticles. *Future Sci. OA* 5: FSO401.
3. Zeltins, A. (2013) Construction and characterization of virus-like particles: a review. *Mol. Biotechnol.* 53: 92-107.
4. Bárcena, J. and E. Blanco (2013) Design of novel vaccines based on virus-like particles or chimeric virions. *Subcell. Biochem.* 68: 631-665.
5. Kitazawa, M., V. Anantharam, A. Kanthasamy, and A. G. Kanthasamy (2004) Dieldrin promotes proteolytic cleavage of poly(ADP-ribose) polymerase and apoptosis in dopaminergic cells: protective effect of mitochondrial anti-apoptotic protein Bcl-2. *Neurotoxicology* 25: 589-598.
6. Donaldson, B., Z. Lateef, G. F. Walker, S. L. Young, and V. K. Ward (2018) Virus-like particle vaccines: immunology and formulation for clinical translation. *Expert Rev. Vaccines* 17: 833-849.
7. Sieczkarski, S. B. and G. R. Whittaker (2002) Dissecting virus entry via endocytosis. *J. Gen. Virol.* 83: 1535-1545.
8. Grönwall, C., A. Sjöberg, M. Ramström, I. Höidén-Guthenberg, S. Hober, P. Jonasson, and S. Ståhl (2007) Affibody-mediated transferrin depletion for proteomics applications. *Biotechnol. J.* 2: 1389-1398.
9. Zepeda-Cervantes, J., J. O. Ramírez-Jarquín, and L. Vaca (2020) Interaction between virus-like particles (VLPs) and pattern recognition receptors (PRRs) from dendritic cells (DCs): toward better engineering of VLPs. *Front. Immunol.* 11: 1100.
10. Mohsen, M. O., A. C. Gomes, M. Vogel, and M. F. Bachmann (2018) Interaction of viral capsid-derived virus-like particles (VLPs) with the innate immune system. *Vaccines (Basel)* 6: 37.
11. Peacey, M., S. Wilson, R. Perret, F. Ronchese, V. K. Ward, V. Young, S. L. Young, and M. A. Baird (2008) Virus-like particles from rabbit hemorrhagic disease virus can induce an anti-tumor response. *Vaccine* 26: 5334-5337.
12. Schwarz, K., E. Meijerink, D. E. Speiser, A. C. Tissot, I. Cielens, R. Renhof, A. Dishlers, P. Pumpens, and M. F. Bachmann (2005) Efficient homologous prime-boost strategies for T cell vaccination based on virus-like particles. *Eur. J. Immunol.* 35: 816-821.
13. Andersson, A. C., M. Schwerdtfeger, and P. J. Holst (2018) Virus-like-vaccines against HIV. *Vaccines (Basel)* 6: 10.
14. Valerdi, K. M., A. Hage, S. van Tol, R. Rajsbaum, and M. I. Giraldo (2021) The role of the host ubiquitin system in promoting replication of emergent viruses. *Viruses* 13: 369.
15. van Gent, M., K. M. J. Sparrer, and M. U. Gack (2018) TRIM proteins and their roles in antiviral host defenses. *Annu. Rev. Virol.* 5: 385-405.
16. Foss, S., M. Bottermann, A. Jonsson, I. Sandlie, L. C. James, and J. T. Andersen (2019) TRIM21-from intracellular immunity to therapy. *Front. Immunol.* 10: 2049.
17. Davis, M. E. and M. U. Gack (2015) Ubiquitination in the antiviral immune response. *Virology* 479-480: 52-65.
18. Jimenez-Moyano, E., A. Ruiz, H. N. Kløverpris, M. T. Rodriguez-Plata, R. Peña, C. Blondeau, D. L. Selwood, N. Izquierdo-Useros, A. Moris, B. Clotet, P. Goulder, G. J. Towers, and J. G. Prado (2016) Nonhuman TRIM5 variants enhance recognition of HIV-1-infected cells by $CD8^+$ T cells. *J. Virol.* 90: 8552-8562.
19. Chackerian, B., M. R. Durfee, and J. T. Schiller (2008) Virus-like display of a neo-self antigen reverses B cell anergy in a B cell receptor transgenic mouse model. *J. Immunol.* 180: 5816-5825.
20. Bessa, J., F. Zabel, A. Link, A. Jegerlehner, H. J. Hinton, N. Schmitz, M. Bauer, T. M. Kündig, P. Saudan, and M. F. Bachmann (2012) Low-affinity B cells transport viral particles from the lung to the spleen to initiate antibody responses. *Proc. Natl. Acad. Sci. U. S. A.* 109: 20566-20571.
21. Yang, R., F. M. Murillo, M. J. Delannoy, R. L. Blosser, W. H. Yutzy 4th, S. Uematsu, K. Takeda, S. Akira, R. P. Viscidi, and R. B. Roden (2005) B lymphocyte activation by human papillomavirus-like particles directly induces Ig class switch recombination via TLR4-MyD88. *J. Immunol.* 174: 7912-7919.
22. Peek, L. J., C. R. Middaugh, and C. Berkland (2008) Nanotechnology in vaccine delivery. *Adv. Drug Deliv. Rev.* 60: 915-928.
23. Grgacic, E. V. and D. A. Anderson (2006) Virus-like particles: passport to immune recognition. *Methods* 40: 60-65.
24. Manolova, V., A. Flace, M. Bauer, K. Schwarz, P. Saudan, and M. F. Bachmann (2008) Nanoparticles target distinct dendritic cell populations according to their size. *Eur. J. Immunol.* 38: 1404-1413.
25. Nygren, P. A. (2008) Alternative binding proteins: affibody binding proteins developed from a small three-helix bundle scaffold. *FEBS J.* 275: 2668-2676.
26. Larsson, M., A. S. Beignon, and N. Bhardwaj (2004) DC-virus interplay: a double edged sword. *Semin. Immunol.* 16: 147-161.
27. Morón, G., G. Dadaglio, and C. Leclerc (2004) New tools for antigen delivery to the MHC class I pathway. *Trends Immunol.* 25: 92-97.
28. Shahrivarkevishahi, A., L. M. Hagge, O. R. Brohlin, S. Kumari, R. Ehrman, C. Benjamin, and J. J. Gassensmith (2022) Virus-like particles: a self-assembled toolbox for cancer therapy. *Mater. Today Chem.* 24: 100808.
29. Boland, B. and V. Campbell (2003) beta-Amyloid (1-40)-induced apoptosis of cultured cortical neurones involves calpain-mediated cleavage of poly-ADP-ribose polymerase. *Neurobiol. Aging* 24: 179-186.
30. Vaculová, A., J. Hofmanova, K. Soucek, M. Kovariková, and A. Kozubík (2002) Tumor necrosis factor-alpha induces apoptosis associated with poly(ADP-ribose) polymerase cleavage in HT-29 colon cancer cells. *Anticancer Res.* 22: 1635-1639.
31. Yang, Y., S. Zhao, and J. Song (2004) Caspase-dependent apoptosis and -independent poly(ADP-ribose) polymerase cleavage induced by transforming growth factor beta1. *Int. J. Biochem. Cell Biol.* 36: 223-234.
32. Ching, J. C., N. L. Jones, P. J. Ceponis, M. A. Karmali, and P. M. Sherman (2002) *Escherichia coli* shiga-like toxins induce apoptosis and cleavage of poly(ADP-ribose) polymerase via *in vitro* activation of caspases. *Infect. Immun.* 70: 4669-4677.
33. D'Amours, D., F. R. Sallmann, V. M. Dixit, and G. G. Poirier (2001) Gain-of-function of poly(ADP-ribose) polymerase-1 upon cleavage by apoptotic proteases: implications for apoptosis. *J. Cell Sci.* 114: 3771-3778.
34. Liu, W. and Y. H. Chen (2005) High epitope density in a single protein molecule significantly enhances antigenicity as well as immunogenicity: a novel strategy for modern vaccine development and a preliminary investigation about B cell discrimination of monomeric proteins. *Eur. J. Immunol.* 35: 505-514.
35. Plummer, E. M. and M. Manchester (2011) Viral nanoparticles and virus-like particles: platforms for contemporary vaccine design. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 3:

174-196.
36. Rueda, P., A. Hurtado, M. del Barrio, J. L. Martínez-Torrecuadrada, S. Kamstrup, C. Leclerc, and J. I. Casal (1999) Minor displacements in the insertion site provoke major differences in the induction of antibody responses by chimeric parvovirus-like particles. *Virology* 263: 89-99.
37. Christoffer, C., V. Bharadwaj, R. Luu, and D. Kihara (2021) LZerD protein-protein docking webserver enhanced with *de novo* structure prediction. *Front. Mol. Biosci.* 8: 724947.
38. Suh, D., J. W. Lee, S. Choi, and Y. Lee (2021) Recent applications of deep learning methods on evolution- and contact-based protein structure prediction. *Int. J. Mol. Sci.* 22: 6032.
39. Leman, J. K., B. D. Weitzner, S. M. Lewis, J. Adolf-Bryfogle, N. Alam, R. F. Alford, M. Aprahamian, D. Baker, K. A. Barlow, P. Barth, B. Basanta, B. J. Bender, K. Blacklock, J. Bonet, S. E. Boyken, P. Bradley, C. Bystroff, P. Conway, S. Cooper, B. E. Correia, B. Coventry, R. Das, R. M. De Jong, F. DiMaio, L. Dsilva, R. Dunbrack, A. S. Ford, B. Frenz, D. Y. Fu, C. Geniesse, L. Goldschmidt, R. Gowthaman, J. J. Gray, D. Gront, S. Guffy, S. Horowitz, P. S. Huang, T. Huber, T. M. Jacobs, J. R. Jeliazkov, D. K. Johnson, K. Kappel, J. Karanicolas, H. Khakzad, K. R. Khar, S. D. Khare, F. Khatib, A. Khramushin, I. C. King, R. Kleffner, B. Koepnick, T. Kortemme, G. Kuenze, B. Kuhlman, D. Kuroda, J. W. Labonte, J. K. Lai, G. Lapidoth, A. Leaver-Fay, S. Lindert, T. Linsky, N. London, J. H. Lubin, S. Lyskov, J. Maguire, L. Malmström, E. Marcos, O. Marcu, N. A. Marze, J. Meiler, R. Moretti, V. K. Mulligan, S. Nerli, C. Norn, S. Ó'Conchúir, N. Ollikainen, S. Ovchinnikov, M. S. Pacella, X. Pan, H. Park, R. E. Pavlovicz, M. Pethe, B. G. Pierce, K. B. Pilla, B. Raveh, P. D. Renfrew, S. S. R. Burman, A. Rubenstein, M. F. Sauer, A. Scheck, W. Schief, O. Schueler-Furman, Y. Sedan, A. M. Sevy, N. G. Sgourakis, L. Shi, J. B. Siegel, D. A. Silva, S. Smith, Y. Song, A. Stein, M. Szegedy, F. D. Teets, S. B. Thyme, R. Y. Wang, A. Watkins, L. Zimmerman, and R. Bonneau (2020) Macromolecular modeling and design in Rosetta: recent methods and frameworks. *Nat. Methods* 17: 665-680.
40. Nord, K., O. Nord, M. Uhlén, B. Kelley, C. Ljungqvist, and P. A. Nygren (2001) Recombinant human factor VIII-specific affinity ligands selected from phage-displayed combinatorial libraries of protein A. *Eur. J. Biochem.* 268: 4269-4277.
41. Akira, S., S. Uematsu, and O. Takeuchi (2006) Pathogen recognition and innate immunity. *Cell* 124: 783-801.
42. Hemmi, H., O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, K. Hoshino, H. Wagner, K. Takeda, and S. Akira (2000) A Toll-like receptor recognizes bacterial DNA. *Nature* 408: 740-745.
43. Utaisincharoen, P., W. Kespichayawattana, N. Anuntagool, P. Chaisuriya, S. Pichyangkul, A. M. Krieg, and S. Sirisinha (2003) CpG ODN enhances uptake of bacteria by mouse macrophages. *Clin. Exp. Immunol.* 132: 70-75.
44. Hennequin, B., L. Turyanska, T. Ben, A. M. Beltrán, S. I. Molina, M. Li, S. Mann, A. Patanè, and N. R. Thomas (2008) Aqueous near-infrared fluorescent composites based on apoferritin-encapsulated PbS quantum dots. *Adv. Mater.* 20: 3592-3596.
45. Destito, G., R. Yeh, C. S. Rae, M. G. Finn, and M. Manchester (2007) Folic acid-mediated targeting of cowpea mosaic virus particles to tumor cells. *Chem. Biol.* 14: 1152-1162.
46. Aljabali, A. A., S. Shukla, G. P. Lomonossoff, N. F. Steinmetz, and D. J. Evans (2013) CPMV-DOX delivers. *Mol. Pharm.* 10: 3-10.
47. Jones, M. W., R. A. Strickland, F. F. Schumacher, S. Caddick, J. R. Baker, M. I. Gibson, and D. M. Haddleton (2012) Polymeric dibromomaleimides as extremely efficient disulfide bridging bioconjugation and pegylation agents. *J. Am. Chem. Soc.* 134: 1847-1852.
48. Chen, Z., N. Li, S. Li, M. Dharmarwardana, A. Schlimme, and J. J. Gassensmith (2016) Viral chemistry: the chemical functionalization of viral architectures to create new technology. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 8: 512-534.
49. Chen, Z., S. D. Boyd, J. S. Calvo, K. W. Murray, G. L. Mejia, C. E. Benjamin, R. P. Welch, D. D. Winkler, G. Meloni, S. D'Arcy, and J. J. Gassensmith (2017) Fluorescent functionalization across quaternary structure in a virus-like particle. *Bioconjug. Chem.* 28: 2277-2283.
50. Yin, Z., M. Comellas-Aragones, S. Chowdhury, P. Bentley, K. Kaczanowska, L. Benmohamed, J. C. Gildersleeve, M. G. Finn, and X. Huang (2013) Boosting immunity to small tumor-associated carbohydrates with bacteriophage qβ capsids. *ACS Chem. Biol.* 8: 1253-1262.
51. Thrane, S., C. M. Janitzek, M. Agerbæk, S. B. Ditlev, M. Resende, M. A. Nielsen, T. G. Theander, A. Salanti, and A. F. Sander (2015) A novel virus-like particle based vaccine platform displaying the placental malaria antigen VAR2CSA. *PLoS One* 10: e0143071.
52. Jeong, H. and B. L. Seong (2017) Exploiting virus-like particles as innovative vaccines against emerging viral infections. *J. Microbiol.* 55: 220-230.
53. Ding, X., D. Liu, G. Booth, W. Gao, and Y. Lu (2018) Virus-like particle engineering: from rational design to versatile applications. *Biotechnol. J.* 13: e1700324.
54. Boonyakida, J., D. I. S. Utomo, F. N. Soma, and E. Y. Park (2022) Two-step purification of tag-free norovirus-like particles from silkworm larvae (*Bombyx mori*). *Protein Expr. Purif.* 190: 106010.
55. Wikman, M., A. C. Steffen, E. Gunneriusson, V. Tolmachev, G. P. Adams, J. Carlsson, and S. Ståhl (2004) Selection and characterization of HER2/neu-binding affibody ligands. *Protein Eng. Des. Sel.* 17: 455-462.
56. Wacker, M., D. Linton, P. G. Hitchen, M. Nita-Lazar, S. M. Haslam, S. J. North, M. Panico, H. R. Morris, A. Dell, B. W. Wren, and M. Aebi (2002) N-linked glycosylation in *Campylobacter jejuni* and its functional transfer into *E. coli*. *Science* 298: 1790-1793.
57. Nakahira, Y., K. Mizuno, H. Yamashita, M. Tsuchikura, K. Takeuchi, T. Shiina, and H. Kawakami (2021) Mass production of virus-like particles using chloroplast genetic engineering for highly immunogenic oral vaccine against fish disease. *Front. Plant Sci.* 12: 717952.
58. Daniell, H., V. Rai, and Y. Xiao (2019) Cold chain and virus-free oral polio booster vaccine made in lettuce chloroplasts confers protection against all three poliovirus serotypes. *Plant Biotechnol. J.* 17: 1357-1368.
59. Panasiuk, M., K. Zimmer, A. Czarnota, K. Grzyb, M. Narajczyk, G. Peszyńska-Sularz, S. Żołędowska, D. Nidzworski, L. Hovhannisyan, and B. Gromadzka (2021) Immunization with *Leishmania tarentolae*-derived norovirus virus-like particles elicits high humoral response and stimulates the production of neutralizing antibodies. *Microb. Cell Fact.* 20: 186.
60. Kushnir, N., S. J. Streatfield, and V. Yusibov (2012) Virus-like particles as a highly efficient vaccine platform: diversity of targets and production systems and advances in clinical development. *Vaccine* 31: 58-83.
61. Chroboczek, J., I. Szurgot, and E. Szolajska (2014) Virus-like particles as vaccine. *Acta Biochim. Pol.* 61: 531-539.
62. Yap, I., R. Guan, and S. H. Chan (1992) Recombinant DNA hepatitis B vaccine containing Pre-S components of the HBV coat protein--a preliminary study on immunogenicity. *Vaccine* 10: 439-442.
63. Mikaeloff, Y., G. Caridade, S. Suissa, and M. Tardieu (2009) Hepatitis B vaccine and the risk of CNS inflammatory demyelination in childhood. *Neurology* 72: 873-880.
64. Zuckerman, J. N., A. J. Zuckerman, I. Symington, W. Du, A.

Williams, B. Dickson, M. D. Young, and UK Hepacare Study Group (2001) Evaluation of a new hepatitis B triple-antigen vaccine in inadequate responders to current vaccines. *Hepatology* 34: 798-802.
65. Pol, S., F. Driss, M. L. Michel, B. Nalpas, P. Berthelot, and C. Brechot (1994) Specific vaccine therapy in chronic hepatitis B infection. *Lancet* 344: 342.
66. Qawasmi, M., M. Samuh, D. Glebe, W. H. Gerlich, and M. Azzeh (2015) Age-dependent decrease of anti-HBs titers and effect of booster doses using 2 different vaccines in Palestinian children vaccinated in early childhood. *Hum. Vaccin. Immunother.* 11: 1717-1724.
67. Macartney, K. K., C. Chiu, M. Georgousakis, and J. M. Brotherton (2013) Safety of human papillomavirus vaccines: a review. *Drug Saf.* 36: 393-412.
68. Stanley, M. (2019) HPV vaccines: alternative dosage schedules. *Expert Rev. Vaccines* 18: 1309-1316.
69. Wang, D., X. Liu, M. Wei, C. Qian, S. Song, J. Chen, Z. Wang, Q. Xu, Y. Yang, M. He, X. Chi, S. Huang, T. Li, Z. Kong, Q. Zheng, H. Yu, Y. Wang, Q. Zhao, J. Zhang, N. Xia, Y. Gu, and S. Li (2020) Rational design of a multi-valent human papillomavirus vaccine by capsomere-hybrid co-assembly of virus-like particles. *Nat. Commun.* 11: 2841.
70. Yadav, R., L. Zhai, and E. Tumban (2019) Virus-like particle-based L2 vaccines against HPVs: where are we today? *Viruses* 12: 18.
71. Nieto, K., M. Weghofer, P. Sehr, M. Ritter, S. Sedlmeier, B. Karanam, H. Seitz, M. Müller, M. Kellner, M. Hörer, U. Michaelis, R. B. Roden, L. Gissmann, and J. A. Kleinschmidt (2012) Development of AAVLP(HPV16/31L2) particles as broadly protective HPV vaccine candidate. *PLoS One* 7: e39741.
72. Zhai, L., J. Peabody, Y. S. Pang, J. Schiller, B. Chackerian, and E. Tumban (2017) A novel candidate HPV vaccine: MS2 phage VLP displaying a tandem HPV L2 peptide offers similar protection in mice to Gardasil-9. *Antiviral Res*. 147: 116-123.
73. Woo, E. J., P. L. Moro, M. Cano, and C. Jankosky (2017) Postmarketing safety surveillance of trivalent recombinant influenza vaccine: reports to the vaccine adverse event reporting system. *Vaccine* 35: 5618-5621.
74. Pushko, P. and I. Tretyakova (2020) Influenza virus like particles (VLPs): opportunities for H7N9 vaccine development. *Viruses* 12: 518.
75. Mallajosyula, V. V., M. Citron, F. Ferrara, N. J. Temperton, X. Liang, J. A. Flynn, and R. Varadarajan (2015) Hemagglutinin sequence conservation guided stem immunogen design from influenza A H3 subtype. *Front. Immunol.* 6: 329.
76. Smith, G. E., X. Sun, Y. Bai, Y. V. Liu, M. J. Massare, M. B. Pearce, J. A. Belser, T. R. Maines, H. M. Creager, G. M. Glenn, D. Flyer, P. Pushko, M. Z. Levine, and T. M. Tumpey (2017) Neuraminidase-based recombinant virus-like particles protect against lethal avian influenza A(H5N1) virus infection in ferrets. *Virology* 509: 90-97.
77. Kingstad-Bakke, B., A. Kamlangdee, and J. E. Osorio (2015) Mucosal administration of raccoonpox virus expressing highly pathogenic avian H5N1 influenza neuraminidase is highly protective against H5N1 and seasonal influenza virus challenge. *Vaccine* 33: 5155-5162.
78. Xie, H., T. M. Liu, X. Lu, Z. Wu, J. A. Belser, J. M. Katz, T. M. Tumpey, and Z. Ye (2009) A live attenuated H1N1 M1 mutant provides broad cross-protection against influenza A viruses, including highly pathogenic A/Vietnam/1203/2004, in mice. *J. Infect. Dis.* 200: 1874-1883.
79. Schwartzman, L. M., A. L. Cathcart, L. M. Pujanauski, L. Qi, J. C. Kash, and J. K. Taubenberger (2015) An intranasal virus-like particle vaccine broadly protects mice from multiple subtypes of influenza A virus. *mBio* 6: e01044.
80. Wiersma, L. C., G. F. Rimmelzwaan, and R. D. de Vries (2015) Developing universal influenza vaccines: hitting the nail, not just on the head. *Vaccines (Basel)* 3: 239-262.
81. Song, J. M., B. Z. Wang, K. M. Park, N. Van Rooijen, F. S. Quan, M. C. Kim, H. T. Jin, A. Pekosz, R. W. Compans, and S. M. Kang (2011) Influenza virus-like particles containing M2 induce broadly cross protective immunity. *PLoS One* 6: e14538.
82. Kim, M. C., J. S. Lee, Y. M. Kwon, E. O, Y. J. Lee, J. G. Choi, B. Z. Wang, R. W. Compans, and S. M. Kang (2013) Multiple heterologous M2 extracellular domains presented on virus-like particles confer broader and stronger M2 immunity than live influenza A virus infection. *Antiviral Res.* 99: 328-335.
83. Braz Gomes, K., S. D'Sa, G. L. Allotey-Babington, S. M. Kang, and M. J. D'Souza (2021) Transdermal vaccination with the matrix-2 protein virus-like particle (M2e VLP) induces immunity in mice against influenza A virus. *Vaccines (Basel)* 9: 1324.
84. Heinimäki, S., V. Lampinen, K. Tamminen, M. M. Hankaniemi, M. Malm, V. P. Hytönen, and V. Blazevic (2022) Antigenicity and immunogenicity of HA2 and M2e influenza virus antigens conjugated to norovirus-like, VP1 capsid-based particles by the SpyTag/SpyCatcher technology. *Virology* 566: 89-97.
85. Quan, F. S., M. C. Kim, B. J. Lee, J. M. Song, R. W. Compans, and S. M. Kang (2012) Influenza M1 VLPs containing neuraminidase induce heterosubtypic cross-protection. *Virology* 430: 127-135.
86. Kim, K. H., Y. T. Lee, S. Park, Y. J. Jung, Y. Lee, E. J. Ko, Y. J. Kim, X. Li, and S. M. Kang (2019) Neuraminidase expressing virus-like particle vaccine provides effective cross protection against influenza virus. *Virology* 535: 179-188.
87. Menne, Z., V. C. Pliasas, R. W. Compans, S. Glover, C. S. Kyriakis, and I. Skountzou (2021) Bivalent vaccination with NA1 and NA2 neuraminidase virus-like particles is protective against challenge with H1N1 and H3N2 influenza A viruses in a murine model. *Virology* 562: 197-208.
88. Yang, J. R., C. Y. Chen, C. Y. Kuo, C. Y. Cheng, M. S. Lee, M. C. Cheng, Y. C. Yang, C. Y. Wu, H. S. Wu, M. T. Liu, and P. W. Hsiao (2016) A novel H6N1 virus-like particle vaccine induces long-lasting cross-clade antibody immunity against human and avian H6N1 viruses. *Antiviral Res.* 126: 8-17.
89. Chen, C. W., N. Saubi, and J. Joseph-Munné (2020) Design concepts of virus-like particle-based HIV-1 vaccines. *Front. Immunol.* 11: 573157.
90. Peters, B. S., R. Cheingsong-Popov, D. Callow, R. Foxall, G. Patou, K. Hodgkin, and J. N. Weber (1997) A pilot phase II study of the safety and immunogenicity of HIV p17/p24:VLP (p24-VLP) in asymptomatic HIV seropositive subjects. *J. Infect.* 35: 231-235.
91. Veenstra, J., I. G. Williams, R. Colebunders, L. Dorrell, S. E. Tchamouroff, G. Patou, J. M. Lange, I. V. Weller, J. Goeman, S. Uthayakumar, I. R. Gow, J. N. Weber, and R. A. Coutinho (1996) Immunization with recombinant p17/p24:Ty virus-like particles in human immunodeficiency virus-infected persons. *J. Infect. Dis.* 174: 862-866.
92. Rerks-Ngarm, S., P. Pitisuttithum, S. Nitayaphan, J. Kaewkungwal, J. Chiu, R. Paris, N. Premsri, C. Namwat, M. de Souza, E. Adams, M. Benenson, S. Gurunathan, J. Tartaglia, J. G. McNeil, D. P. Francis, D. Stablein, D. L. Birx, S. Chunsuttiwat, C. Khamboonruang, P. Thongcharoen, M. L. Robb, N. L. Michael, P. Kunasol, J. H. Kim, and MOPH-TAVEG Investigators (2009) Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N. Engl. J. Med.* 361: 2209-2220.
93. Eto, Y., N. Saubi, P. Ferrer, and J. Joseph-Munné (2021) Expression of chimeric HPV-HIV protein L1P18 in *Pichia pastoris*; purification and characterization of the virus-like particles. *Pharmaceutics* 13: 1967.
94. Zhang, P., E. Narayanan, Q. Liu, Y. Tsybovsky, K. Boswell, S.

Ding, Z. Hu, D. Follmann, Y. Lin, H. Miao, H. Schmeisser, D. Rogers, S. Falcone, S. M. Elbashir, V. Presnyak, K. Bahl, M. Prabhakaran, X. Chen, E. K. Sarfo, D. R. Ambrozak, R. Gautam, M. A. Martin, J. Swerczek, R. Herbert, D. Weiss, J. Misamore, G. Ciaramella, S. Himansu, G. Stewart-Jones, A. McDermott, R. A. Koup, J. R. Mascola, A. Finzi, A. Carfi, A. S. Fauci, and P. Lusso (2021) A multiclade env-gag VLP mRNA vaccine elicits tier-2 HIV-1-neutralizing antibodies and reduces the risk of heterologous SHIV infection in macaques. *Nat. Med.* 27: 2234-2245.

95. Liu, D., S. Zhang, E. Poteet, C. Marin-Muller, C. Chen, and Q. Yao (2021) Sublingual immunization with chimeric C1q/CD40 ligand/HIV virus-like particles induces strong mucosal immune responses against HIV. *Vaccines (Basel)* 9: 1236.
96. Ao, Z., L. Wang, H. Azizi, T. A. Olukitibi, G. Kobinger, and X. Yao (2021) Development and evaluation of an Ebola virus glycoprotein mucin-like domain replacement system as a new dendritic cell-targeting vaccine approach against HIV-1. *J. Virol.* 95: e0236820.
97. Chapman, R., M. van Diepen, S. Galant, E. Kruse, E. Margolin, P. Ximba, T. Hermanus, P. Moore, N. Douglass, A. L. Williamson, and E. Rybicki (2020) Immunogenicity of HIV-1 vaccines expressing chimeric envelope glycoproteins on the surface of Pr55 Gag virus-like particles. *Vaccines (Basel)* 8: 54.
98. Pillay, S., E. G. Shephard, A. E. Meyers, A. L. Williamson, and E. P. Rybicki (2010) HIV-1 sub-type C chimaeric VLPs boost cellular immune responses in mice. *J. Immune Based Ther. Vaccines* 8: 7.
99. Escolano, A., H. B. Gristick, R. Gautam, A. T. DeLaitsch, M. E. Abernathy, Z. Yang, H. Wang, M. A. G. Hoffmann, Y. Nishimura, Z. Wang, N. Koranda, L. M. Kakutani, H. Gao, P. N. P. Gnanapragasam, H. Raina, A. Gazumyan, M. Cipolla, T. Y. Oliveira, V. Ramos, D. J. Irvine, M. Silva, A. P. West Jr., J. R. Keeffe, C. O. Barnes, M. S. Seaman, M. C. Nussenzweig, M. A. Martin, and P. J. Bjorkman (2021) Sequential immunization of macaques elicits heterologous neutralizing antibodies targeting the V3-glycan patch of HIV-1 Env. *Sci. Transl. Med.* 13: eabk1533.
100. Yu, H., D. Pardoll, and R. Jove (2009) STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat. Rev. Cancer* 9: 798-809.
101. DeFazio-Eli, L., K. Strommen, T. Dao-Pick, G. Parry, L. Goodman, and J. Winslow (2011) Quantitative assays for the measurement of HER1-HER2 heterodimerization and phosphorylation in cell lines and breast tumors: applications for diagnostics and targeted drug mechanism of action. *Breast Cancer Res.* 13: R44.
102. Sherwood, J., P. M. Mendelman, E. Lloyd, M. Liu, J. Boslego, A. Borkowski, A. Jackson, D. Faix, and US Navy study team (2020) Efficacy of an intramuscular bivalent norovirus GI.1/GII.4 virus-like particle vaccine candidate in healthy US adults. *Vaccine* 38: 6442-6449.
103. Pucci, B., M. Kasten, and A. Giordano (2000) Cell cycle and apoptosis. *Neoplasia* 2: 291-299.
104. Bartek, J. and J. Lukas (2001) Pathways governing G1/S transition and their response to DNA damage. *FEBS Lett.* 490: 117-122.
105. Han, J. C., Q. X. Li, J. B. Fang, J. Y. Zhang, Y. Q. Li, S. Z. Li, C. Cheng, C. Z. Xie, F. L. Nan, H. Zhang, Z. X. Li, N. Y. Jin, G. Z. Zhu, and H. J. Lu (2021) GII.P16-GII.2 recombinant norovirus VLPs polarize macrophages into the M1 phenotype for Th1 immune responses. *Front. Immunol.* 12: 781718.
106. Malm, M., T. Vesikari, and V. Blazevic (2019) Simultaneous immunization with multivalent norovirus VLPs induces better protective immune responses to norovirus than sequential immunization. *Viruses* 11: 1018.
107. Ward, B. J., P. Gobeil, A. Séguin, J. Atkins, I. Boulay, P. Y. Charbonneau, M. Couture, M. A. D'Aoust, J. Dhaliwall, C. Finkle, K. Hager, A. Mahmood, A. Makarkov, M. P. Cheng, S. Pillet, P. Schimke, S. St-Martin, S. Trépanier, and N. Landry (2021) Phase 1 randomized trial of a plant-derived virus-like particle vaccine for COVID-19. *Nat. Med.* 27: 1071-1078.
108. Fluckiger, A. C., B. Ontsouka, J. Bozic, A. Diress, T. Ahmed, T. Berthoud, A. Tran, D. Duque, M. Liao, M. McCluskie, F. Diaz-Mitoma, D. E. Anderson, and C. Soare (2021) An enveloped virus-like particle vaccine expressing a stabilized prefusion form of the SARS-CoV-2 spike protein elicits highly potent immunity. *Vaccine* 39: 4988-5001.
109. F Fougeroux, C., L. Goksøyr, M. Idorn, V. Soroka, S. K. Myeni, R. Dagil, C. M. Janitzek, M. Søgaard, K. L. Aves, E. W. Horsted, S. M. Erdoğan, T. Gustavsson, J. Dorosz, S. Clemmensen, L. Fredsgaard, S. Thrane, E. E. Vidal-Calvo, P. Khalifé, T. M. Hulen, S. Choudhary, M. Theisen, S. K. Singh, A. Garcia-Senosiain, L. Van Oosten, G. Pijlman, B. Hierzberger, T. Domeyer, B. W. Nalewajek, A. Strøbæk, M. Skrzypczak, L. F. Andersson, S. Buus, A. S. Buus, J. P. Christensen, T. J. Dalebout, K. Iversen, L. H. Harritshøj, B. Mordmüller, H. Ullum, L. S. Reinert, W. A. de Jongh, M. Kikkert, S. R. Paludan, T. G. Theander, M. A. Nielsen, A. Salanti, and A. F. Sander (2021) Capsid-like particles decorated with the SARS-CoV-2 receptor-binding domain elicit strong virus neutralization activity. *Nat. Commun.* 12: 324.
110. Dalvie, N. C., L. H. Tostanoski, S. A. Rodriguez-Aponte, K. Kaur, S. Bajoria, O. S. Kumru, A. J. Martinot, A. Chandrashekar, K. McMahan, N. B. Mercado, J. Yu, A. Chang, V. M. Giffin, F. Nampanya, S. Patel, L. Bowman, C. A. Naranjo, D. Yun, Z. Flinchbaugh, L. Pessaint, R. Brown, J. Velasco, E. Teow, A. Cook, H. Andersen, M. G. Lewis, D. L. Camp, J. M. Silverman, H. Kleanthous, S. B. Joshi, D. B. Volkin, S. Biswas, J. C. Love, and D. H. Barouch (2021) A modular protein subunit vaccine candidate produced in yeast confers protection against SARS-CoV-2 in non-human primates. *bioRxiv* https://doi.org/10.1101/2021.07.13.452251
111. Royal, J. M., C. A. Simpson, A. A. McCormick, A. Phillips, S. Hume, J. Morton, J. Shepherd, Y. Oh, K. Swope, J. L. DeBeauchamp, R. J. Webby, R. W. Cross, V. Borisevich, T. W. Geisbert, J. K. Demarco, B. Bratcher, H. Haydon, and G. P. Pogue (2021) Development of a SARS-CoV-2 vaccine candidate using plant-based manufacturing and a tobacco mosaic virus-like nano-particle. *Vaccines (Basel)* 9: 1347.
112. Guo, C., Y. Peng, L. Lin, X. Pan, M. Fang, Y. Zhao, K. Bao, R. Li, J. Han, J. Chen, T. Z. Song, X. L. Feng, Y. Zhou, G. Zhao, L. Zhang, Y. Zheng, P. Zhu, H. Hang, L. Zhang, Z. Hua, H. Deng, and B. Hou (2021) A pathogen-like antigen-based vaccine confers immune protection against SARS-CoV-2 in non-human primates. *Cell Rep. Med.* 2: 100448.
113. van Oosten, L., J. J. Altenburg, C. Fougeroux, C. Geertsema, F. van den End, W. A. C. Evers, A. H. Westphal, S. Lindhoud, W. van den Berg, D. C. Swarts, L. Deurhof, A. Suhrbier, T. T. Le, S. Torres Morales, S. K. Myeni, M. Kikkert, A. F. Sander, W. A. de Jongh, R. Dagil, M. A. Nielsen, A. Salanti, M. Søgaard, T. M. P. Keijzer, D. Weijers, M. H. M. Eppink, R. H. Wijffels, M. M. van Oers, D. E. Martens, and G. P. Pijlman (2021) Two-component nanoparticle vaccine displaying glycosylated spike S1 domain induces neutralizing antibody response against SARS-CoV-2 variants. *mBio* 12: e0181321.
114. Chang, X., A. Zeltins, M. O. Mohsen, Z. Gharailoo, L. Zha, X. Liu, S. Walton, M. Vogel, and M. F. Bachmann (2021) A novel double mosaic virus-like particle-based vaccine against SARS-CoV-2 incorporates both receptor binding motif (RBM) and fusion domain. *Vaccines (Basel)* 9: 1287.
115. Chevillard, C., A. Amen, S. Besson, D. Hannani, I. Bally, V. Dettling, E. Gout, C. J. Moreau, M. Buisson, S. Gallet, D. Fenel,

E. Vassal-Stermann, G. Schoehn, P. Poignard, M. C. Dagher, and P. Fender (2022) Elicitation of potent SARS-CoV-2 neutralizing antibody responses through immunization with a versatile adenovirus-inspired multimerization platform. *Mol. Ther.* 30: 1913-1925.
116. Ortega-Rivera, O. A., M. D. Shin, A. Chen, V. Beiss, M. A. Moreno-Gonzalez, M. A. Lopez-Ramirez, M. Reynoso, H. Wang, B. L. Hurst, J. Wang, J. K. Pokorski, and N. F. Steinmetz (2021) Trivalent subunit vaccine candidates for COVID-19 and their delivery devices. *J. Am. Chem. Soc.* 143: 14748-14765.
117. Chen, G. L., E. E. Coates, S. H. Plummer, C. A. Carter, N. Berkowitz, M. Conan-Cibotti, J. H. Cox, A. Beck, M. O'Callahan, C. Andrews, I. J. Gordon, B. Larkin, R. Lampley, F. Kaltovich, J. Gall, K. Carlton, J. Mendy, D. Haney, J. May, A. Bray, R. T. Bailer, K. A. Dowd, B. Brockett, D. Gordon, R. A. Koup, R. Schwartz, J. R. Mascola, B. S. Graham, T. C. Pierson, Y. Donastorg, N. Rosario, J. W. Pape, B. Hoen, A. Cabié, C. Diaz, J. E. Ledgerwood, and VRC 704 Study Team (2020) Effect of a Chikungunya virus-like particle vaccine on safety and tolerability outcomes: a randomized clinical trial. *JAMA* 323: 1369-1377.
118. Aslan Koşar, P., H. Tuncer, A. Cihangir Uğuz, J. Espino Palma, H. Darıcı, İ. Onaran, B. Çiğ, A. Koşar, and A. B. Rodriguez Moratinos (2015) The efficiency of poly(ADP-Ribose) polymerase (PARP) cleavage on detection of apoptosis in an experimental model of testicular torsion. *Int. J. Exp. Pathol.* 96: 294-300.
119. Loganathan, R., K. R. Selvaduray, K. Nesaretnam, and A. K. Radhakrishnan (2013) Tocotrienols promote apoptosis in human breast cancer cells by inducing poly(ADP-ribose) polymerase cleavage and inhibiting nuclear factor kappa-B activity. *Cell Prolif.* 46: 203-213.
120. Ku, C. R., H. J. Lee, S. K. Kim, E. Y. Lee, M. K. Lee, and E. J. Lee (2012) Resveratrol prevents streptozotocin-induced diabetes by inhibiting the apoptosis of pancreatic β-cell and the cleavage of poly (ADP-ribose) polymerase. *Endocr. J.* 59: 103-109.
121. P Park, E. J., J. W. Shin, Y. S. Seo, D. W. Kim, S. Y. Hong, W. I. Park, and B. M. Kang (2011) Gonadotropin-releasing hormone-agonist induces apoptosis of human granulosa-luteal cells via caspase-8, -9 and -3, and poly-(ADP-ribose)-polymerase cleavage. *Biosci. Trends* 5: 120-128.
122. Pal, H. C., I. Sehar, S. Bhushan, B. D. Gupta, and A. K. Saxena (2010) Activation of caspases and poly (ADP-ribose) polymerase cleavage to induce apoptosis in leukemia HL-60 cells by *Inula racemosa*. *Toxicol. In Vitro* 24: 1599-1609.
123. Mahfouz, R. Z., R. K. Sharma, K. Poenicke, R. Jha, U. Paasch, S. Grunewald, and A. Agarwal (2009) Evaluation of poly(ADP-ribose) polymerase cleavage (cPARP) in ejaculated human sperm fractions after induction of apoptosis. *Fertil. Steril.* 91: 2210-2220.
124. Zhang, P., H. Li, D. Chen, J. Ni, Y. Kang, and S. Wang (2007) Oleanolic acid induces apoptosis in human leukemia cells through caspase activation and poly(ADP-ribose) polymerase cleavage. *Acta Biochim. Biophys. Sin. (Shanghai)* 39: 803-809.
125. Rodríguez-Hernández, A., G. Brea-Calvo, D. J. Fernández-Ayala, M. Cordero, P. Navas, and J. A. Sánchez-Alcázar (2006) Nuclear caspase-3 and caspase-7 activation, and poly(ADP-ribose) polymerase cleavage are early events in camptothecin-induced apoptosis. *Apoptosis* 11: 131-139.
126. Yinjun, L., J. Jie, X. Weilai, and T. Xiangming (2004) Homoharringtonine mediates myeloid cell apoptosis via upregulation of pro-apoptotic bax and inducing caspase-3-mediated cleavage of poly(ADP-ribose) polymerase (PARP). *Am. J. Hematol.* 76: 199-204.