

# Chapter 9

## Virus-Like Particles, a Versatile Subunit Vaccine Platform

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### 9.1 Introduction

Novel vaccine development requires a balance between eliciting a potent immune response while limiting the unintentional induction of hypersensitivity and off-target effects. Virus-like particles (VLPs) are a form of subunit vaccine consisting of self-assembling shells derived from virus capsid proteins. Due to the absence of viral genomic material, VLPs are rendered non-replicative and non-infectious, enhancing their safety profile. In comparison to other subunit vaccines, the resemblance of VLPs to their corresponding native virus provides enhanced immunogenicity and specificity. VLP capsid proteins retain their natural structural conformation, harbouring undamaged antigenic motifs in a more immunologically relevant state than an inactivated virus vaccine. VLPs can also resemble a live attenuated virus without replicative or infectious capacity due to structural similarity and utilisation of similar processing pathways. In general, VLPs are considered significantly safer than many other virally derived vaccines by avoiding potential hazards such as attenuated virus reversion or incomplete inactivation.

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The first VLP identified and studied was isolated from patients infected with Hepatitis B virus (HBV) in 1968 (Bayer et al. 1968). Due to the ability of some viral capsid proteins to spontaneously form stable particles, many viruses produce VLPs as a natural by-product of their infection cycle. Engerix (GlaxoSmithKline) was the first human VLP-based vaccine, licensed in 1989 for vaccination against HBV. Since then a number of VLP vaccines have been approved for clinical use, including Epaxal (Crucell) for Hepatitis A virus (HAV), Recombivax (Merck), Hepavax (Crucell), and many others for HBV, Gardasil (Merck), and Cervarix (GlaxoSmithKline) for human papillomavirus (HPV), and Inflflexal V (Crucell) for Influenza. Bolstered by the success of these vaccines many new VLPs are being developed, with a selection of examples summarised in Table 9.1. In addition, VLPs have also been produced from various non-human mammalian viruses, primarily for vaccination of livestock. Examples include porcine circovirus (Kim et al. 2002), bovine rotavirus (Rodriguez-Limas et al. 2011), chicken anaemia virus (Noteborn et al. 1998; Koch et al. 1995), SARS coronavirus (Liu et al. 2011), Nipah virus (Walpita et al. 2011), and swine vesicular stomatitis virus (Ko et al. 2005).

## 9.2 VLP Structural Conformation

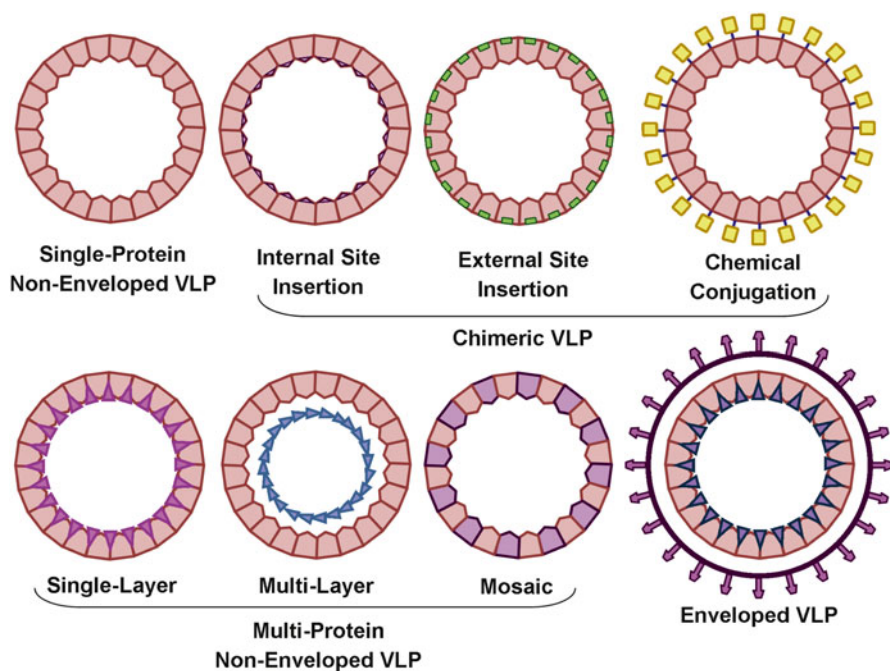
Spontaneous polymerisation of a range of viral capsid proteins can yield VLPs with authentic geometric symmetry, usually icosahedral, spherical or rod-like in shape, depending on the source virus. VLPs can be generally categorised into groups based on their structural complexity, including single-protein non-enveloped (e.g. VLPs derived from caliciviruses (Jiang et al. 1992), papillomaviruses (Kirnbauer et al. 1992), and parvoviruses (Lopez de Turiso et al. 1992)), multi-protein non-enveloped (e.g. VLPs derived from infectious bursal disease virus (Kibenge et al. 1999), poliovirus (Brautigam et al. 1993), and reoviruses (French et al. 1990; French and Roy 1990)) and enveloped VLPs (e.g. VLPs derived from Hantaan virus (Betenbaugh et al. 1995), hepatitis C virus (Baumert et al. 1998), influenza A (Latham and Galarza 2001), and retroviruses (Yamshchikov et al. 1995)) as illustrated in Fig. 9.1. While single-protein VLPs have a relatively simple structure, multi-protein VLPs can contain unique structural features such as several distinct capsid layers. For example, expression of various combinations of the VP2, VP4, VP6, and VP7 capsid proteins of rotavirus can produce stable VLPs with double or even triple capsid layers (Crawford et al. 1994; Sabara et al. 1991).

Multi-protein VLPs can also be produced from variant copies of the same protein derived from different viral strains. These mosaic VLPs efficiently confer protection against several strains of the same virus (Buonamassa et al. 2002). An alternative means of increasing VLP versatility is through the incorporation of antigens from heterologous sources. Chimeric VLPs contain antigenic material from a target source supported by a stable VLP framework. These antigens can be inserted as peptides into the VLP capsid protein or substructural secondary VLP proteins, or covalently coupled to the surface of VLP. Chimeric VLPs have an extensive range of potential applications, and will be discussed later in this chapter. Enveloped

**Table 9.1** Examples of VLPs licensed or in clinical trials

Vaccine target	Company/Institution	Administration (adjuvant)	Expression system	VLP platform	Antigen	References
Respiratory syncytial virus	Novavax	IM (aluminum phosphate)	Insect (SF-9 cells)	RSV	RSV-F protein	Glenn et al. (2013)
Norwalk virus	LigoCyte Pharmaceuticals	IN (±MPL, chitosan, mannitol, sucrose)	Insect (SF-9 cells)	NV	NV capsid protein	El-Kamary et al. (2010)
Hepatitis B (Engerix-B)	GSK	IM (aluminum hydroxide)	Yeast ( <i>S. cerevisiae</i> )	HBV	HBV surface antigen	Keating and Noble (2003)
Human papilloma virus (Gardasil)	Merck	IM (aluminum hydroxyphosphate sulphate)	Yeast ( <i>S. cerevisiae</i> )	HPV	HPV6/11/16/18L1	Romanowski (2011)
Human papilloma virus (Cervarix)	GSK	IM (aluminum hydroxide & MPL)	Insect (Hi Five cells)	HPV	HPV16/18 L1	Romanowski (2011)
Alzheimer's disease (CAD106)	Cytos Biotechnology/Novartis	SC, IM (unspecified)	Bacteria ( <i>E. coli</i> )	Qβ (carrier)	Ab 1-6	Lemere and Masliah (2010)
Human parvovirus B19 (VAL-VP705)	NHI/Meridian Life Science	IM (MF59)	Insect (SF-9 cells)	B19	B19 VP1, VP2	Bernstein et al. (2011)
Human immunodeficiency virus	British Biotech Pharmaceuticals/NAID	SC/IM, IM (aluminum hydroxide) Oral or rectal (none)	Yeast ( <i>S. cerevisiae</i> )	Ty p1 (carrier)	HIV-1 Gag p17/p24	Peters et al. (1997)
Rabies	Thomas Jefferson University	Oral (none)	Plant (Tg spinach)	Alfalfa mosaic virus (carrier)	Rabies GP/NP	Yusibov et al. (2002)
Influenza	Novavax	IM (none)	Insect (SF-9 cells)	Influenza virus	A/California/04/09 (H1N1) HA, NA	Lopez-Macias et al. (2011)
Influenza	Medicago	IM (none)	Plant (transient <i>N. benthamiana</i> )	Influenza virus	A/California/04/09 (H1N1) HA, NA	Medicago
Breast Cancer	Pevion Biotech	IM (none)	Cell-free	Influenza virosome (carrier)	Her2/neu	Wiedermann et al. (2010)
Hepatitis A (Eqaxal)	Cruceil	IM (none)	Cell-free	Influenza virosome (carrier)	Inactivated Hepatitis A vaccine	Bovier (2008)

Adapted from Kushnir et al. (2012). *Abbreviations:* GP glycoprotein, HA haemagglutinin, HBV, hepatitis B virus, HPV human papillomavirus, IM intramuscular, IN intra-nasal, NA neuraminidase, NP nucleocapsid protein, NV Norwalk virus, RSV respiratory syncytial virus, SC sub-cutaneous



**Fig. 9.1** VLP Structure. VLPs can be categorised based on characteristic structural features such as capsid protein composition, encapsulation inside a lipid bilayer envelope, and incorporation of antigens by recombinant insertion or chemical conjugation. Additional combinations other than those illustrated also exist, such as multi-protein chimeric VLPs and enveloped mosaic or chimeric VLPs

VLPs consist of either a single-protein or multi-protein VLPs encapsulated in a lipid bilayer captured from the cell membrane. Co-expression of haemagglutinin (HA), neuraminidase (NA), matrix protein M1, and ion channel protein M2 from influenza virus produces enveloped VLPs with the same size and morphology as native influenza virions, including the characteristic surface spikes HA and NA (Latham and Galarza 2001). The lipid bilayer of enveloped VLPs can also support the incorporation of transmembrane anchored proteins from multiple viral strains (enveloped mosaic VLPs) or even heterologous pathogens (enveloped chimeric VLPs) (Buonaguro et al. 2001; Halsey et al. 2008; Visciano et al. 2011). VLP structural complexity appears to have few limitations, with intriguing novel constructs still frequently theorised and investigated.

### 9.3 Production of VLPs







VLPs are a natural by-product produced during the infection cycle of certain viruses (Bayer et al. 1968). The same characteristics that benefit efficient virus reproduction, such as spontaneously polymerising capsid proteins, also promote the

formation of VLPs; however, the isolation of VLPs produced from virally infected cells is not an efficient means of purification. An expansive range of protein expression systems have been developed for a variety of applications, and can be effectively commandeered for the production and purification of high quality VLPs. Recombinant expression of viral capsid proteins through tailored expression systems can also enable the production of VLPs from viruses not routinely cultured in laboratories. Common VLP expression systems include bacteria, yeast, insect cell lines, mammalian cell lines, plants, and cell-free cultures. Each expression system has its benefits and pitfalls as outlined in Table 9.2 (Rebeaud and Bachmann 2012). While most VLPs can be produced in multiple expression systems, the quaternary structural conformation of the capsid proteins produced can vary due to differences in post-translational modifications such as phosphorylation and glycosylation. This can have significant effects on the immunogenicity of VLPs, as these modifications are often essential for eliciting the desired immune response.

*Escherichia coli* (*E. coli*) has long been a primary laboratory workhorse bacterium, facilitating the expression and purification of recombinant proteins through plasmid transformation or bacteriophage vector delivery. Expression in *E. coli* is often preferred when producing small proteins with limited post-translational modifications; however, larger proteins with post-translational modifications require a more complex expression system (e.g. Chinese hamster ovary (CHO) mammalian cell line). The presence of endotoxins during downstream purification also presents a significant challenge for vaccine development from a bacterial expression system. Each VLP is unique, with optimal expression identified through trial and error by comparing the translated products of multiple expression systems. For example, Rabbit Haemorrhagic Disease Virus (RHDV) VLPs can be optimally produced by expressing the RHDV VP60 capsid protein in *Spodoptera frugiperda* (SF) cells using a recombinant baculovirus vector (Young et al. 2004, 2006; Peacey et al. 2007). Icosahedral T=3 VLPs with a diameter of around 40 nm spontaneously form when VP60 is expressed in SF21 cells. Each VLP contains 180 copies of the VP60 capsid protein, representing a relatively simple single-protein non-enveloped VLP. These VLPs structurally resemble native RHDV virions, as illustrated in Fig. 9.2 (Katpally et al. 2010; Wang et al. 2013).

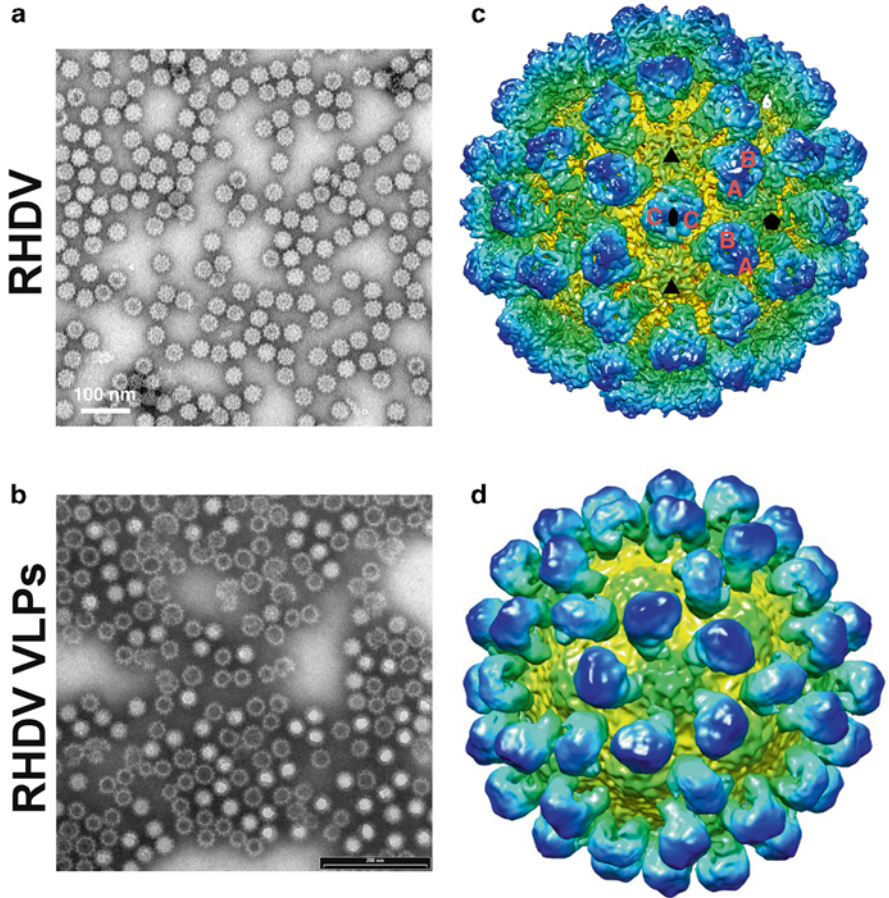
Production of VLPs in transgenic plants (e.g. tobacco, potato, tomato) is a relatively new concept with interesting applications. Expression of recombinant proteins in plants is achieved through transgene insertion into the nuclear or plastid genome, or using plant viral vectors. While plant cells do not have a mammalian-like post-translational modification system, plant-specific glycosylation can have an immunostimulatory effect. Some examples of VLPs produced in a transgenic plant system include Norwalk virus (Tacket 2007; Tacket et al. 2000), HIV-1 (Scotti et al. 2009), and influenza virus VLPs (Medicago). Another recently developed expression platform is the cell-free system. This usually consists of extracts from *E. coli* or yeast cells, and was developed primarily to enable the production of viral capsid proteins which have toxic intermediate protein forms. Development of VLPs containing unnatural amino acids (UAAs) has also been achieved using the cell-free system. The non-replenishing nature of a cell-free system renders this method highly demanding with some scalability limitations. The influenza vaccine

**Table 9.2** VLP expression systems

	Advantages	Limitations
<b>Bacteria</b> (e.g. <i>Escherichia coli</i> ) 	<ul style="list-style-type: none"> <li>• Rapid cell growth</li> <li>• Highest yield</li> <li>• Low production cost</li> <li>• Scalable</li> </ul>	<ul style="list-style-type: none"> <li>• No post-translational modification</li> <li>• Limited applications for mammalian VLPs</li> <li>• May form inclusion bodies</li> <li>• Requires removal of endotoxins</li> </ul>
<b>Yeast</b> (e.g. <i>Saccharomyces cerevisiae</i> ) 	<ul style="list-style-type: none"> <li>• Rapid cell growth</li> <li>• High yield</li> <li>• Low production cost</li> <li>• Scalable</li> <li>• Already has some regulatory approval</li> </ul>	<ul style="list-style-type: none"> <li>• Limited post-translational modification</li> <li>• May form inclusion bodies</li> </ul>
<b>Insect cells/Baculovirus</b> (e.g. <i>Spodoptera frugiperda</i> ) 	<ul style="list-style-type: none"> <li>• Average cell growth</li> <li>• High yield</li> <li>• Scalable</li> <li>• Complex post-translational modification</li> <li>• Formation of multi-protein VLP</li> </ul>	<ul style="list-style-type: none"> <li>• Requires removal of baculovirus proteins</li> <li>• May form inclusion bodies</li> </ul>
<b>Plant cells</b> (e.g. <i>Nicotiana</i> sp.) 	<ul style="list-style-type: none"> <li>• Rapid production</li> <li>• Low production cost</li> <li>• Scalable</li> </ul>	<ul style="list-style-type: none"> <li>• Limited post-translational modification</li> <li>• Relatively new system</li> </ul>
<b>Mammalian cells</b> (e.g. Chinese hamster ovary cells) 	<ul style="list-style-type: none"> <li>• Scalable</li> <li>• Complex post-translational modification</li> <li>• Formation of multi-protein VLP</li> <li>• VLP</li> </ul>	<ul style="list-style-type: none"> <li>• Slow growth</li> <li>• Low yield</li> <li>• Demanding culture conditions</li> <li>• High production cost</li> <li>• Potential infectious contamination</li> </ul>
<b>Cell free</b> 	<ul style="list-style-type: none"> <li>• Almost exclusive production of target protein</li> <li>• Limited cellular contaminants</li> <li>• Enables production of VLPs containing non-natural amino acids or toxic protein intermediates</li> </ul>	<ul style="list-style-type: none"> <li>• Very high production cost</li> <li>• Limited scalability</li> <li>• Relatively new system, not well characterised</li> </ul>

Adapted from Rebeaud and Bachmann (2012)

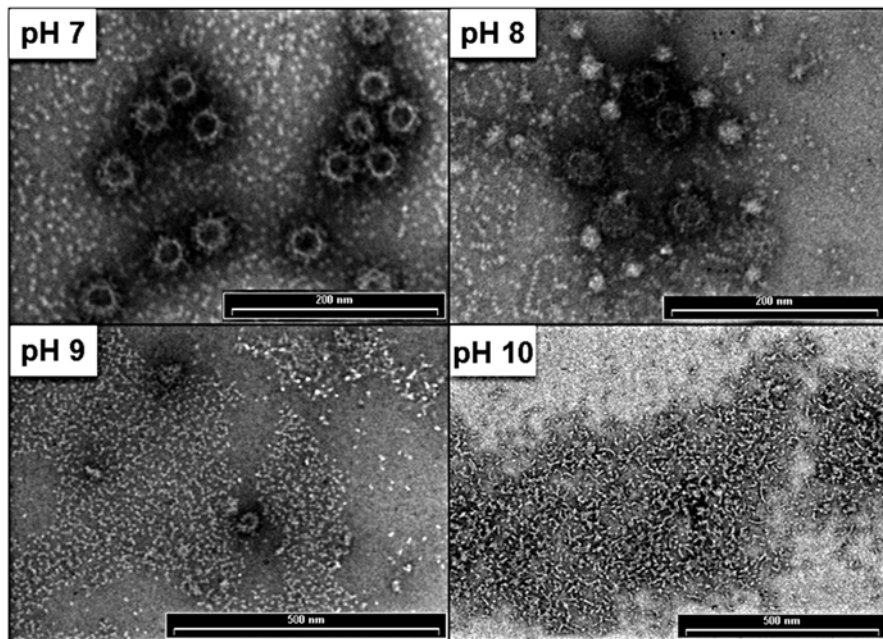




**Fig. 9.2** Comparison of RHDV and RHDV VLP structure. RHDV VLPs expressed in insect cells visibly share structural characteristics with the native virus as viewed by transmission electron microscopy (**a**, **b**) and 3D modelling from cryo-electron microscopy and crystallography (**c**, **d**). (**a**, **c**) Adapted from Wang et al. (2013). (**d**) Generously supplied by Thomas J. Smith (Katpally et al. 2010)

Inflexal V (Herzog et al. 2009), and the hepatitis A vaccine Epaxal (Bovier 2008) (Crucell) are two commercialised VLP vaccines that consist of virosomes produced in a cell-free expression system.

Following production, VLPs must be isolated from the expression system and purified to sufficient quality for downstream applications. VLPs are usually isolated through a combination of cell lysis, removal of cellular debris, VLP concentration, and selective purification. Some mammalian and insect cell lines secrete VLPs into the supernatant, negating the necessity for cell lysis (Vicente et al. 2011). Resilient cells (e.g. bacteria, plant cells) may require more robust mechanical manipulation such as ultrasonication, compression, abrasion, repeated freeze/thawing, or enzymatic



**Fig. 9.3** RHDV VLP stability at an alkaline pH. RHDV VLPs are visibly perturbed under transmission electron microscopy in an elevated solvent pH, resulting in irreversible particle disassembly

degradation for VLP release (Cull and McHenry 1990; Salazar and Asenjo 2007). VLPs can be purified by differential centrifugation; however, this can pose limitations on scalability. GMP production of vaccine-grade purified VLPs for commercial applications often involves industrial-scale protein purification methods such as size-exclusion, ion exchange, or affinity chromatography columns (Vicente et al. 2011). Optimal solvent conditions must also be identified to maintain VLP stability. Solution pH is critical, as some VLPs irreversibly denature and disassemble beyond a specific pH range. For example, RHDV VLPs deteriorate in an alkaline environment, with complete VLP disruption above pH 9 (Fig. 9.3). VLP solubility is another important consideration as some VLPs may aggregate at higher concentrations, forming an insoluble precipitate.

#### 9.4 VLPs as an Antigen Scaffold

VLPs are an incredibly versatile vaccination tool. In addition to harbouring multiple copies of immunologically recognisable antigens from their source virus, VLPs can also be used as a nanoparticulate delivery vector for heterogenous antigenic molecules. Each VLP can be considered a polymerised protein subunit vaccine,



supporting modifications such as recombinant peptide insertion and chemical conjugation of peptides, proteins, lysate, carbohydrates, and lipoproteins to form chimeric VLPs. Some VLPs can support insertion of short peptide sequences at specific sites in their structural capsid proteins without impairing VLP formation. For example, RHDV VP60 is known to retain its ability to spontaneously form VLPs despite recombinant insertion of peptides at the N-terminus, C-terminus, or at amino acid residue 306 (Crisci et al. 2009); however, these sites have restrictions on inserted peptide length and residue sequence. The N-terminus of RHDV VP60 can support an insertion of <33 amino acids (Peacey et al. 2007), while other viral proteins such as polyomavirus VP1 can support insertions ranging from 9 to 120 amino acids (Eriksson et al. 2011; Lasickiene et al. 2012; Mazeike et al. 2012; Middelberg et al. 2011). Inclusion of secondary capsid proteins, such as polyomavirus VP2, with a limited contribution to VLP stability, can support large insertions including truncated proteins (Tegerstedt et al. 2005). HBV core antigen (HBcAg) has been one of the most popular VLP forming viral capsid proteins for recombinant insertion of immunogenic epitopes. Notable recombinant HBc vaccines include Malariavax, which targets *Plasmodium falciparum* (*P. falciparum*); the protozoan responsible for malaria (Gregson et al. 2008), and a pan-influenza A vaccine against the influenza matrix protein 2 (M2e) ectodomain universally conserved between strains (Fiers et al. 2009). The phase I clinical trial of the recombinant influenza A M2e vaccine ACAM-FLU-A reported a 90 % seroconversion rate amongst participants after two vaccinations, with no severe adverse side-effects observed (Fiers et al. 2009).

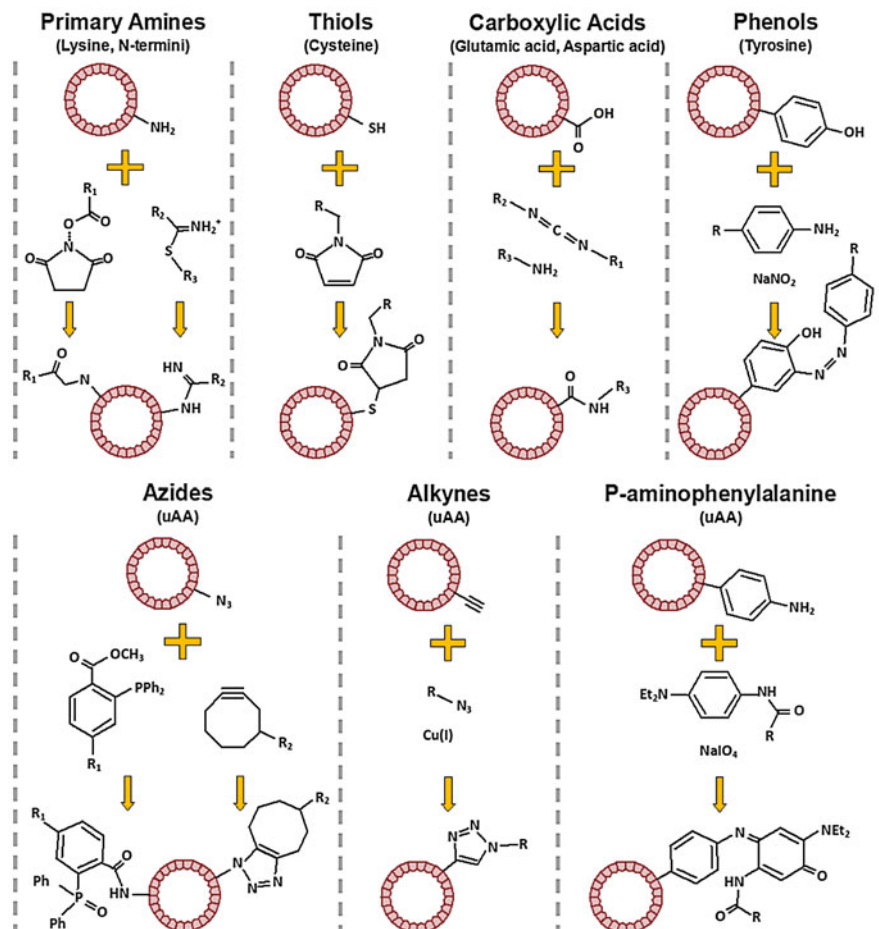
Recombinant non-mammalian VLPs can also be used to deliver immunogenic peptides derived from mammalian pathogens. Various plant viruses have demonstrated promise as recombinant antigen scaffolds, including alfalfa mosaic virus (AIMV) VLP containing epitopes from HIV-1 or rabies virus (Yusibov et al. 2002), cowpea mosaic virus (CPMV) VLP containing epitopes from *Bacillus anthracis* (Phelps et al. 2007) or HPV16 (Matic et al. 2011) and tobacco mosaic virus (TMV) VLP containing epitopes for *P. falciparum* (Turpen et al. 1995). The potential applications of chimeric VLPs extend far beyond the incorporation of epitopes from mammalian pathogens. VLPs containing auto-antigens naturally present in humans have been harnessed for a variety of novel roles, such as targeting angiotensin II to combat hypertension (Ambuhl et al. 2007; Maurer and Bachmann 2010), targeting A $\beta$  protein to treat Alzheimer's disease (Zamora et al. 2006; Wiessner et al. 2011; Chackerian et al. 2006), or interrupting cytokine signalling pathways (Spohn et al. 2008; Link and Bachmann 2010). VLPs that stimulate an immune response against nicotine have even been investigated for their potential to break smoking addiction (Maurer et al. 2005; Cornuz et al. 2008). The therapeutic application of VLPs can also be used to develop novel vaccines for cancer. For example, recombinant insertion of a truncated form of Her2 protein into polyomavirus VP1/VP2 VLP confers protection against Her2 positive mammary carcinoma (Tegerstedt et al. 2005).

The selected site of recombinant insertion in chimeric VLP provides an inherent limitation on vaccine applications. Recognition of native unprocessed antigen is an essential component of B cell activation in the humoral immune system leading to antibody production. Internal site insertion prevents such interactions, and restricts

these sites to insertion of antigenic epitopes that utilise intracellular processing pathways. External site insertion is required for effective B cell activation and depending on the selected VLP this method of chimeric VLP design can have significantly increased complexity. While internal site insertions are typically cloned onto an internal terminus of the VLP capsid protein, external site insertion requires in-depth understanding of the capsid protein quaternary structure and the interactions between each protein subunit to identify the optimal site for insertion. For example, the RHDV VP60 capsid protein is arranged with its N-terminus facing internally and C-terminus facing externally; however, the quaternary structural conformation has the C-terminus folded backwards into the central protein bulk. Amino acid 306 of RHDV VP60 capsid protein was instead identified as a potential external insertion site for exposure of immunogenic peptides tailored for B cell recognition (Crisci et al. 2009).

An alternative means of avoiding the limitations imposed by recombinant peptide insertion is to utilise VLPs as a particulate scaffold for chemical conjugation of antigenic molecules such as peptides, proteins, lysate, carbohydrates, and lipoproteins. Advancement in coupling chemistry continues to expand the list of viable conjugation candidates, primarily limited by their effects on the resulting particles size, solubility, and processing. Conjugation uses a chemical linker as a bridge, commonly conjugating proteins by acylation of amino groups, alkylation of sulfhydryl groups, or the activation of carboxylic acid residues. Some of the most commonly used protein coupling chemistries are illustrated in Fig. 9.4 (Smith et al. 2013). Azide-alkyne click chemistry (Patel and Swartz 2011) and biotin-streptavidin complexes (Chackerian et al. 2008) are also used in VLP conjugation. Heterobifunctional crosslinkers have two reactive groups enabling efficient protein crosslinking; however, this has the unfortunate side-reaction of non-specific conjugation, which can result in protein aggregation in a complex solution such as tumour lysate. Bioorthogonal crosslinkers are a specific alternative that enables selective conjugation, limiting side-reactions by targeting chemical motifs absent in biological systems (e.g. phosphines).

Chemical conjugation of immunogenic peptides is a relatively simple alternative to recombinant insertion, using commercial protein crosslinkers such as Sulfo-SMCC (Thermo Fisher Scientific Inc., Rockford, IL, USA). Sulfo-SMCC is a heterobifunctional crosslinker which permits a stepwise conjugation process targeting the amino groups on the VLP surface (NHS-ester) and the sulfhydryl group (maleimide group) from a cysteine residue introduced at the N-terminus of a target epitope (Peacey et al. 2008). Direct comparison between recombinant insertion and chemical conjugation of an H2k<sup>b</sup>-restricted peptide from lymphocytic choriomeningitis virus (LCMV) gp33 in chimeric RHDV VLP indicated that the recombinant form induced a superior in vivo cytotoxic response (Li et al. 2013). Remarkably both the coupled and recombinant forms induced 20 % and 50 % tumour-free survival, respectively, following a single vaccination without adjuvant in mice grafted with Lewis' lung carcinoma tumours expressing gp33. The addition of a 4 week vaccination boost with VLP and adjuvant increased tumour-free survival to 70 % in mice vaccinated with the recombinant gp33 RHDV VLP (Li et al. 2013).



**Fig. 9.4** Chemical conjugation onto the surface of VLPs. Various molecules can be coupled onto the surface of VLPs through chemical conjugation, targeting primary amines (e.g. lysine, N-termini), thiols (e.g. cysteine), carboxylic acids (e.g. glutamic acid, aspartic acid), and phenols (e.g. tyrosine). Unnatural amino acids (UAAs) incorporated into the structure of VLPs can also be used as potential chemical conjugation targets by introducing reactive azide or alkyne groups. Adapted from Smith et al (2013)

Conjugation of large proteins or cell lysate onto the surface of VLPs enables the induction of an immune response without identification of specific immunogenic peptides. Tumour lysates derived from the MART-1 expressing melanoma cell line Mel888 conjugated onto RHDV VLP provides more efficient lysate delivery to dendritic cell antigen processing compartments and enhances MART-1-specific CD8<sup>+</sup> T cell stimulation compared to lysate alone (Win et al. 2012). The use of tumour lysate in VLP vaccines has potential applications for personalised anti-cancer immunotherapy.

## 9.5 Intraparticulate Encapsulation

Although VLPs are devoid of genomic material from their source virus, they are not necessarily always empty. Many viral structural proteins have an inherent ability to bind specific nucleic acids, facilitating packaging of the viral genome during the infection cycle. This property is retained in some VLPs and can be appropriated for encapsulation of negatively charged molecules such as oligonucleotides and chemical polymers. The primary limitations on intraparticulate encapsulation are the VLPs internal cavity volume and the availability of positively charged amino acids (Zeltins 2013). VLPs with the ability to encapsulate exogenous DNA have been explored as a possible means of gene delivery. VLPs produced from the VP1 capsid protein of the human polyomavirus John Cunningham (JC) virus are a promising candidate, with the ability to harbour DNA plasmids up to 14 kbp in length (Fang et al. 2012). Delivery of pEGFP-C1 plasmid into human epithelial kidney 293 (HEK293) cells and subsequent EGFP production was achieved by loading the plasmid into JC virus VLPs through osmotic shock, which disrupts the VLP structure and increases permeability (Ou et al. 1999). More recently in vivo plasmid loading during expression of VP1 in *E. coli* was found to result in superior plasmid loading over osmotic shock or VLP reassembly, validated with JC virus VLPs encapsulating pEGFP-N3 plasmid (Chen et al. 2010). This loading method was subsequently used with pUMVC1-tk plasmid containing the herpes simplex virus thymidine kinase (tk) suicide gene. JC virus VLPs containing pUMVC1-tk were found to selectively target human colon carcinoma (COLO-320 HSR) cells grafted in nude mice, significantly reducing tumour volume following administration of ganciclovir (Chen et al. 2010).

Chimeric VLPs have also been used to encapsulate DNA plasmids, enabling successful gene delivery upon cellular uptake. Recombinant insertion of the DNA binding site from HPV type 16 (HPV-16) L1 (VP60 $\Delta$ -L1BS) or L2 (VP60 $\Delta$ -L2BS) capsid protein onto the N-terminus of RHDV VP60 produces chimeric VLPs with the capacity to encapsulate pCMV- $\beta$  in vitro through VLP reassembly.  $\beta$ -Galactosidase expression was identified in a range of cell lines (Cos-7, R17, HuH-7, and CaCo<sub>2</sub>) treated with VP60 $\Delta$ -L1BS containing pCMV- $\beta$  plasmid (El Mehdaoui et al. 2000). It is possible that any VLP that supports internal site insertion may be capable of DNA plasmid encapsulation and delivery by incorporation of an appropriate DNA binding site. VLPs have even been used to induce the expression of their own structural capsid protein, enhancing immunogenicity by mimicking viral replication in vivo (Pichlmair et al. 2010). A variety of molecules other than nucleic acids have also been successfully encapsulated into VLPs, including enzymes into Q $\beta$  VLPs (Fiedler et al. 2010), polymerase into rotavirus VLPs (Boudreaux et al. 2013), and fluorophores into cucumber mosaic virus VLPs (Lu et al. 2012). Recombinant insertion of a heterodimeric coiled-coil amino acid motif at the N-terminus of a capsid protein from cowpea chlorotic mottle virus (CCMV) produced CCMV VLPs capable of encapsulating up to 15 EGFP proteins (Minten et al. 2009). Encapsulating exogenous proteins inside VLPs is an interesting concept with multiple potential therapeutic applications.

## 9.6 VLP Vaccine Immunogenicity

The immune response to VLP vaccines is primarily determined by particle size, capsid structure, and innate immunity activation. Particles larger than 200 nm require transportation to the lymph nodes by peripheral antigen presenting cells (APCs) at the vaccination site (e.g. macrophages, dendritic cells) (Manolova et al. 2008). VLPs are usually below 200 nm, facilitating free circulation and drainage directly to lymph nodes in addition to peripheral APC transportation. Once in the lymph node, free VLPs are internalised by resident APCs. The repetitive structure of VLPs promotes efficient uptake by APCs through mechanisms such as phagocytosis and macropinocytosis (Win et al. 2011; Xiang et al. 2006; Scheerlinck and Greenwood 2008). Due to VLPs consisting of native viral capsid proteins, they often contain receptor binding motifs that also enable receptor-mediated endocytosis. An example of this is the binding of haemagglutinin in influenza VLPs to the sialylose receptor on the cell surface (Pan et al. 2010).

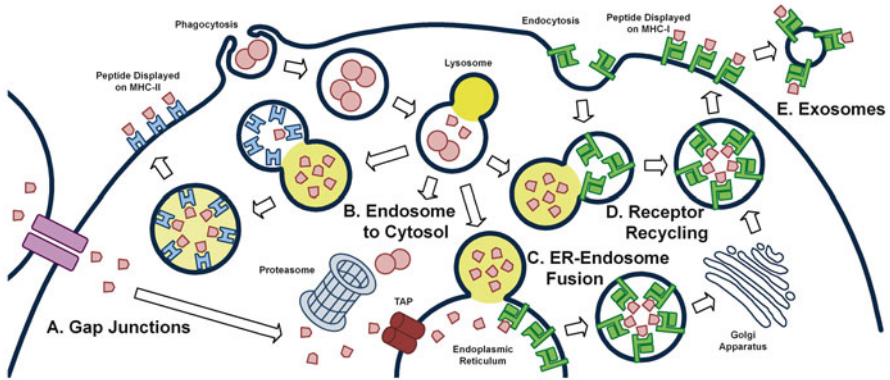
The processing of VLPs by APCs can lead to the stimulation of both the humoral and cell-mediated arms of the immune system. Historically vaccines have been designed to target the initiation of humoral immunity, generating vaccine-specific antibodies. While this type of immune response is essential for the clearance of extracellular pathogens, establishing protection against intracellular conditions such as viral infections and cancer often requires the generation of cell-mediated immunity. The ability of VLPs to stimulate both arms of the immune system is advantageous, as the combination of immune responses often work synergistically to ensure disease clearance and the initiation of effective immunological memory. The immune response to VLPs is summarised in Fig. 9.5.

Following internalisation VLPs are degraded and processed through the exogenous antigen processing pathway with immunogenic peptides loaded onto MHC class II (MHC-II) molecules for surface presentation. Processed antigens are recognised by CD4<sup>+</sup> T helper cells, resulting in activation and release of immunostimulatory cytokines, such as Interleukin (IL) 4, IL-5, which are essential for B cell activation or IL-2 and IFN $\gamma$ , which are essential for induction of cell-mediated immunity. For successful activation of the humoral immune system, B cells must also interact with native antigen through B cell receptors (BCRs). The repetitive nature of VLPs promotes crosslinking of BCRs, enhancing activation and promoting memory-cell formation. This stimulation is notably superior to soluble peptides or non-repetitive proteins (Jegerlehner et al. 2007; Bachmann and Jennings 2010). Antibodies produced in response to VLPs can neutralise native virions to prevent active infection, and memory-cell formation promotes long-term immunity. Notable examples of licensed VLP vaccines that successfully induce high titres of long-lasting neutralising antibodies include the Hepatitis B vaccine Enderix (Keating and Noble 2003) and the HPV vaccines Cervarix and Gardasil (Romanowski 2011).

Some VLPs are also cross-presented by APCs, primarily CD8<sup>+</sup> dendritic cells, facilitating loading of exogenous antigen onto MHC class I (MHC-I). With additional stimulation from co-stimulatory molecules (e.g. CD40, CD80/86), MHC-I







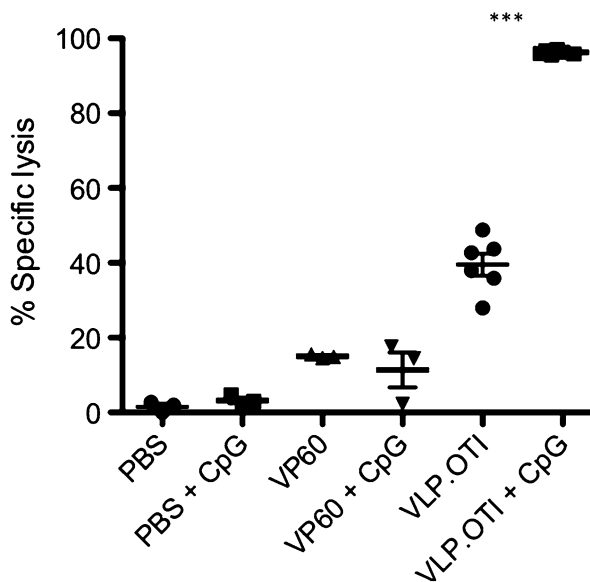
**Fig. 9.6** The immune response to VLPs. VLPs have the capacity to stimulate both the cell-mediated and humoral immune system. The desired immune response can be promoted through adjuvant selection

levels of antibody detected in the mucosae compared to unconjugated cholera toxin B (Kang et al. 2003).

Depending on the protein expression system used, some VLPs can contain exogenous immunogenic molecules such as bacterial RNA which promote APC activation through stimulation of pattern recognition receptors such as toll-like receptors (TLRs) (Rebeaud and Bachmann 2012). This can have an adjuvanting effect due to activation of the innate immune system; however, we have found that successful vaccination can be achieved in the absence of these adjuvanting molecules (Li et al. 2013). RHDV VLPs produced in insect cell cultures do not stimulate innate cells cultured *in vitro*, but effectively activate the cell-mediated and humoral immune systems *in vivo*. Despite the success of VLP vaccination alone, the inclusion of an adjuvant can still be beneficial in driving specific immune pathway activation and leading to further enhancement of immune stimulation.

A number of adjuvants have been used in humans; these include mineral salts (e.g. aluminum hydroxide, aluminum phosphate, and aluminum hydroxy phosphates), emulsions (e.g. MF59, AS01, and AS02), and microbial derivatives (monophosphoryl lipid A and CpG) (Rappuoli et al. 2011). Traditionally adjuvants were used to enhance vaccine immunogenicity by stimulating the humoral immune system to induce antibody production and isotype-switching. This was thought to be facilitated by formation of a slow-release antigen depot. Modern adjuvants are designed to directly target immune activation and to tailor vaccination to drive a specific immune response, enabling selective stimulation of the cell-mediated immune system. TLR agonists such as unmethylated CpG DNA can induce a potent cell-mediated response by stimulating upregulated expression of co-stimulatory molecules in innate immune cells. We have found that the addition of CpG to recombinant RHDV VLP containing the SIINFEKL peptide derived from the model antigen OVA (VLP.OTI) led to an enhancement in the generation of SIINFEKL-specific cytotoxic T cells (Fig. 9.7) (Scullion 2012). This association was also confirmed using the Lewis' lung carcinoma model with recombinant gp33 RHDV VLP (Li et al. 2013).

**Fig. 9.7** The effect of CpG adjuvant on in vivo cytotoxicity. Co-administration of RHDV VLP containing the SIINFEKL peptide (VLP.OTI) with CpG significantly increased % specific lysis of target cells loaded with SIINFEKL peptide (Scullion 2012)



Co-delivery of vaccine adjuvants and antigens to cells through physical association or linkage leads to enhanced immunogenicity and a reduction in adjuvant-related side effects. Viruses often bind molecules that facilitate entry into host cells. RHDV VLPs retain the ability of its native virus to bind carbohydrate moieties, providing a useful mechanism for modification and vaccine enhancement (Ruvoen-Clouet et al. 2000; McKee et al. 2012).  $\alpha$ -Galactosylceramide is a glycolipid adjuvant which was found to directly associate with RHDV VLP, forming a composite particle.  $\alpha$ -Galactosylceramide activates natural killer-like T cells, leading to the enhancement of both the innate and acquired immune responses (Bendelac et al. 2007). Prophylactic vaccination with recombinant gp33, RHDV VLP and  $\alpha$ -galactosylceramide led to the generation of gp33-specific T cells and enhanced protection against subcutaneous tumour challenge. The adjuvanting effect of  $\alpha$ -galactosylceramide was increased >10-fold when delivered as a composite particle compared to the co-delivery of unassociated  $\alpha$ -galactosylceramide and recombinant gp33.RHDV VLP (McKee et al. 2012).

## 9.7 Conclusion

The primary requirements of a successful vaccine include safety, reliability, and efficacy. Along with other subunit vaccines VLPs have an impeccable safety record, with some vaccines already approved for routine use. The absence of the viral genome renders VLPs incapable of infection or replication, preventing inadvertent reversion as has occurred with attenuated viruses. Extensive purification of VLPs

produced using protein expression systems helps to limit contamination with substances which might induce allergies or undesirable side effects. Each new VLP is extensively characterised during development, containing immunogenic epitopes in a more natural state than inactivated virus vaccines. The particulate nature of VLPs also provides some protection from degradation. VLPs are uniform in structure, providing consistency between preparations and a reliable vaccine. VLPs are readily processed by the immune system and can stimulate cytotoxic and humoral immune responses. VLPs can provide protection against their source virus, or they can harbour exogenous antigens. This unprecedented versatility in vaccine design enables the utilisation of VLP vaccines in a wide range of applications including prophylactic viral vaccines, therapeutic cancer vaccines, and even gene delivery. VLP vaccines have only begun to unveil their true potential as a versatile subunit vaccine platform.

## References

- Ambuhl PM, Tissot AC, Fulurija A, Maurer P, Nussberger J, Sabat R, Nief V, Schellekens C, Sladko K, Roubicek K, Pfister T, Rettenbacher M, Volk HD, Wagner F, Muller P, Jennings GT, Bachmann MF (2007) A vaccine for hypertension based on virus-like particles: preclinical efficacy and phase I safety and immunogenicity. *J Hypertens* 25(1):63–72. doi:10.1097/HJH.0b013e32800ff5d6
- Bachmann MF, Jennings GT (2010) Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nat Rev Immunol* 10(11):787–796. doi:10.1038/nri2868
- Baumert TF, Ito S, Wong DT, Liang TJ (1998) Hepatitis C virus structural proteins assemble into viruslike particles in insect cells. *J Virol* 72(5):3827–3836
- Bayer ME, Blumberg BS, Werner B (1968) Particles associated with Australia antigen in the sera of patients with leukaemia, Down's syndrome and hepatitis. *Nature* 218(5146):1057–1059
- Bendelac A, Savage PB, Teyton L (2007) The biology of NKT cells. *Annu Rev Immunol* 25:297–336. doi:10.1146/annurev.immunol.25.022106.141711
- Bernstein DI, El Sahly HM, Keitel WA, Wolff M, Simone G, Segawa C, Wong S, Shelly D, Young NS, Dempsey W (2011) Safety and immunogenicity of a candidate parvovirus B19 vaccine. *Vaccine* 29(43):7357–7363. doi:10.1016/j.vaccine.2011.07.080
- Betenbaugh M, Yu M, Kuehl K, White J, Pennock D, Spik K, Schmaljohn C (1995) Nucleocapsid- and virus-like particles assemble in cells infected with recombinant baculoviruses or vaccinia viruses expressing the M and the S segments of Hantaan virus. *Virus Res* 38(2–3):111–124
- Boudreaux C, Vile D, Gilmore B, Tanner J, Kelly D, McDonald S (2013) Rotavirus core shell subdomains involved in polymerase encapsidation into virus-like particles. *J Gen Virol* 94(Pt 8):1818–1826. doi:10.1099/vir.0.052951-0
- Bovier PA (2008) Recent advances with a virosomal hepatitis A vaccine. *Expert Opin Biol Ther* 8(8):1177–1185. doi:10.1517/14712598.8.8.1177
- Brautigam S, Snezhkov E, Bishop DH (1993) Formation of poliovirus-like particles by recombinant baculoviruses expressing the individual VP0, VP3, and VP1 proteins by comparison to particles derived from the expressed poliovirus polyprotein. *Virology* 192(2):512–524. doi:10.1006/viro.1993.1067
- Buonaguro L, Buonaguro FM, Tornesello ML, Mantas D, Beth-Giraldo E, Wagner R, Michelson S, Prevost MC, Wolf H, Giraldo G (2001) High efficient production of Pr55(gag) virus-like particles expressing multiple HIV-1 epitopes, including a gp120 protein derived from an Ugandan HIV-1 isolate of subtype A. *Antiviral Res* 49(1):35–47

- Buonamassa DT, Greer CE, Capo S, Yen TS, Galeotti CL, Bensi G (2002) Yeast coexpression of human papillomavirus types 6 and 16 capsid proteins. *Virology* 293(2):335–344. doi:[10.1006/viro.2001.1289](https://doi.org/10.1006/viro.2001.1289)
- Chackerian B, Rangel M, Hunter Z, Peabody DS (2006) Virus and virus-like particle-based immunogens for Alzheimer's disease induce antibody responses against amyloid-beta without concomitant T cell responses. *Vaccine* 24(37–39):6321–6331. doi:[10.1016/j.vaccine.2006.05.059](https://doi.org/10.1016/j.vaccine.2006.05.059)
- Chackerian B, Durfee MR, Schiller JT (2008) Virus-like display of a neo-self antigen reverses B cell anergy in a B cell receptor transgenic mouse model. *J Immunol* 180(9):5816–5825
- Chen LS, Wang M, Ou WC, Fung CY, Chen PL, Chang CF, Huang WS, Wang JY, Lin PY, Chang D (2010) Efficient gene transfer using the human JC virus-like particle that inhibits human colon adenocarcinoma growth in a nude mouse model. *Gene Ther* 17(8):1033–1041. doi:[10.1038/gt.2010.50](https://doi.org/10.1038/gt.2010.50)
- Cornuz J, Zwahlen S, Jungi WF, Osterwalder J, Klingler K, van Melle G, Bangala Y, Guessous I, Muller P, Willers J, Maurer P, Bachmann MF, Cerny T (2008) A vaccine against nicotine for smoking cessation: a randomized controlled trial. *PLoS One* 3(6):e2547. doi:[10.1371/journal.pone.0002547](https://doi.org/10.1371/journal.pone.0002547)
- Crawford SE, Labbe M, Cohen J, Burroughs MH, Zhou YJ, Estes MK (1994) Characterization of virus-like particles produced by the expression of rotavirus capsid proteins in insect cells. *J Virol* 68(9):5945–5952
- Crisci E, Almanza H, Mena I, Cordoba L, Gomez-Casado E, Caston JR, Fraile L, Barcena J, Montoya M (2009) Chimeric calicivirus-like particles elicit protective anti-viral cytotoxic responses without adjuvant. *Virology* 387(2):303–312. doi:[10.1016/j.virol.2009.02.045](https://doi.org/10.1016/j.virol.2009.02.045)
- Cull M, McHenry CS (1990) Preparation of extracts from prokaryotes. *Methods Enzymol* 182:147–153
- El Mehdaoui S, Touze A, Laurent S, Sizaret PY, Rasschaert D, Coursaget P (2000) Gene transfer using recombinant rabbit hemorrhagic disease virus capsids with genetically modified DNA encapsidation capacity by addition of packaging sequences from the L1 or L2 protein of human papillomavirus type 16. *J Virol* 74(22):10332–10340
- El-Kamary SS, Pasetti MF, Mendelman PM, Frey SE, Bernstein DI, Treanor JJ, Ferreira J, Chen WH, Sublett R, Richardson C, Bargatzke RF, Szein MB, Tacket CO (2010) Adjuvanted intranasal Norwalk virus-like particle vaccine elicits antibodies and antibody-secreting cells that express homing receptors for mucosal and peripheral lymphoid tissues. *J Infect Dis* 202(11):1649–1658. doi:[10.1086/657087](https://doi.org/10.1086/657087)
- Eriksson M, Andreasson K, Weidmann J, Lundberg K, Tegerstedt K, Dalianis T, Ramqvist T (2011) Murine polyomavirus virus-like particles carrying full-length human PSA protect BALB/c mice from outgrowth of a PSA expressing tumor. *PLoS One* 6(8):e23828. doi:[10.1371/journal.pone.0023828](https://doi.org/10.1371/journal.pone.0023828)
- Fang CY, Lin PY, Ou WC, Chen PL, Shen CH, Chang D, Wang M (2012) Analysis of the size of DNA packaged by the human JC virus-like particle. *J Virol Methods* 182(1–2):87–92. doi:[10.1016/j.jviromet.2012.03.017](https://doi.org/10.1016/j.jviromet.2012.03.017)
- Fiedler JD, Brown SD, Lau JL, Finn MG (2010) RNA-directed packaging of enzymes within virus-like particles. *Angew Chem Int Ed Engl* 49(50):9648–9651. doi:[10.1002/anie.201005243](https://doi.org/10.1002/anie.201005243)
- Fiers W, De Filette M, El Bakkouri K, Schepens B, Roose K, Schotsaert M, Birkett A, Saelens X (2009) M2e-based universal influenza A vaccine. *Vaccine* 27(45):6280–6283. doi:[10.1016/j.vaccine.2009.07.007](https://doi.org/10.1016/j.vaccine.2009.07.007)
- French TJ, Roy P (1990) Synthesis of bluetongue virus (BTV) corelike particles by a recombinant baculovirus expressing the two major structural core proteins of BTV. *J Virol* 64(4):1530–1536
- French TJ, Marshall JJ, Roy P (1990) Assembly of double-shelled, viruslike particles of bluetongue virus by the simultaneous expression of four structural proteins. *J Virol* 64(12):5695–5700
- Glenn GM, Smith G, Fries L, Raghunandan R, Lu H, Zhou B, Thomas DN, Hickman SP, Kpamegan E, Boddapati S, Piedra PA (2013) Safety and immunogenicity of a Sf9 insect cell-derived respiratory syncytial virus fusion protein nanoparticle vaccine. *Vaccine* 31(3):524–532. doi:[10.1016/j.vaccine.2012.11.009](https://doi.org/10.1016/j.vaccine.2012.11.009)

- Gregson AL, Oliveira G, Othoro C, Calvo-Calle JM, Thorton GB, Nardin E, Edelman R (2008) Phase I trial of an alhydrogel adjuvanted hepatitis B core virus-like particle containing epitopes of *Plasmodium falciparum* circumsporozoite protein. *PLoS One* 3(2):e1556. doi:[10.1371/journal.pone.0001556](https://doi.org/10.1371/journal.pone.0001556)
- Groothuis TA, Neeffjes J (2005) The many roads to cross-presentation. *J Exp Med* 202(10):1313–1318. doi:[10.1084/jem.20051379](https://doi.org/10.1084/jem.20051379)
- Halsey RJ, Tanzer FL, Meyers A, Pillay S, Lynch A, Shephard E, Williamson AL, Rybicki EP (2008) Chimaeric HIV-1 subtype C Gag molecules with large in-frame C-terminal polypeptide fusions form virus-like particles. *Virus Res* 133(2):259–268. doi:[10.1016/j.virusres.2008.01.012](https://doi.org/10.1016/j.virusres.2008.01.012)
- Herzog C, Hartmann K, Kunzi V, Kursteiner O, Mischler R, Lazar H, Gluck R (2009) Eleven years of Inflexal V-a virosomal adjuvanted influenza vaccine. *Vaccine* 27(33):4381–4387. doi:[10.1016/j.vaccine.2009.05.029](https://doi.org/10.1016/j.vaccine.2009.05.029)
- Jegerlehner A, Maurer P, Bessa J, Hinton HJ, Kopf M, Bachmann MF (2007) TLR9 signaling in B cells determines class switch recombination to IgG2a. *J Immunol* 178(4):2415–2420
- Jiang X, Wang M, Graham DY, Estes MK (1992) Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. *J Virol* 66(11):6527–6532
- Kang SM, Yao Q, Guo L, Compans RW (2003) Mucosal immunization with virus-like particles of simian immunodeficiency virus conjugated with cholera toxin subunit B. *J Virol* 77(18):9823–9830
- Katpally U, Voss NR, Cavazza T, Taube S, Rubin JR, Young VL, Stuckey J, Ward VK, Virgin HW 4th, Wobus CE, Smith TJ (2010) High-resolution cryo-electron microscopy structures of murine norovirus 1 and rabbit hemorrhagic disease virus reveal marked flexibility in the receptor binding domains. *J Virol* 84(11):5836–5841. doi:[10.1128/JVI.00314-10](https://doi.org/10.1128/JVI.00314-10)
- Keating GM, Noble S (2003) Recombinant hepatitis B vaccine (Engerix-B): a review of its immunogenicity and protective efficacy against hepatitis B. *Drugs* 63(10):1021–1051
- Kibenge FS, Qian B, Nagy E, Cleghorn JR, Wadowska D (1999) Formation of virus-like particles when the polyprotein gene (segment A) of infectious bursal disease virus is expressed in insect cells. *Can J Vet Res* 63(1):49–55
- Kim Y, Kim J, Kang K, Lyoo YS (2002) Characterization of the recombinant proteins of porcine circovirus type2 field isolate expressed in the baculovirus system. *J Vet Sci* 3(1):19–23
- Kimbauer R, Booy F, Cheng N, Lowy DR, Schiller JT (1992) Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. *Proc Natl Acad Sci U S A* 89(24):12180–12184
- Ko YJ, Choi KS, Nah JJ, Paton DJ, Oem JK, Wilsden G, Kang SY, Jo NI, Lee JH, Kim JH, Lee HW, Park JM (2005) Noninfectious virus-like particle antigen for detection of swine vesicular disease virus antibodies in pigs by enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol* 12(8):922–929. doi:[10.1128/CDLI.12.8.922-929.2005](https://doi.org/10.1128/CDLI.12.8.922-929.2005)
- Koch G, van Roozelaar DJ, Verschueren CA, van der Eb AJ, Noteborn MH (1995) Immunogenic and protective properties of chicken anaemia virus proteins expressed by baculovirus. *Vaccine* 13(8):763–770
- Kushnir N, Streatfield SJ, Yusibov V (2012) Virus-like particles as a highly efficient vaccine platform: diversity of targets and production systems and advances in clinical development. *Vaccine* 31(1):58–83. doi:[10.1016/j.vaccine.2012.10.083](https://doi.org/10.1016/j.vaccine.2012.10.083)
- Lasickiene R, Gedvilaite A, Norkiene M, Simanaviciene V, Sezaite I, Dekaminaviciute D, Shikova E, Zvirbliene A (2012) The use of recombinant pseudotype virus-like particles harbouring inserted target antigen to generate antibodies against cellular marker p16INK4A. *ScientificWorldJournal* 2012:263737. doi:[10.1100/2012/263737](https://doi.org/10.1100/2012/263737)
- Latham T, Galarza JM (2001) Formation of wild-type and chimeric influenza virus-like particles following simultaneous expression of only four structural proteins. *J Virol* 75(13):6154–6165. doi:[10.1128/JVI.75.13.6154-6165.2001](https://doi.org/10.1128/JVI.75.13.6154-6165.2001)
- Lemere CA, Masliah E (2010) Can Alzheimer disease be prevented by amyloid-beta immunotherapy? *Nat Rev Neurol* 6(2):108–119. doi:[10.1038/nrneuro.2009.219](https://doi.org/10.1038/nrneuro.2009.219)

- Li K, Peers-Adams A, Win SJ, Scullion S, Wilson M, Young VL, Jennings P, Ward VK, Baird MA, Young SL (2013) Antigen incorporated in virus-like particles is delivered to specific dendritic cell subsets that induce an effective antitumor immune response in vivo. *J Immunother* 36(1):11–19. doi:[10.1097/CJI.0b013e3182787f5e](https://doi.org/10.1097/CJI.0b013e3182787f5e)
- Link A, Bachmann MF (2010) Immunodrugs: breaking B- but not T-cell tolerance with therapeutic anticytokine vaccines. *Immunotherapy* 2(4):561–574. doi:[10.2217/imt.10.30](https://doi.org/10.2217/imt.10.30)
- Liu YV, Massare MJ, Barnard DL, Kort T, Nathan M, Wang L, Smith G (2011) Chimeric severe acute respiratory syndrome coronavirus (SARS-CoV) S glycoprotein and influenza matrix 1 efficiently form virus-like particles (VLPs) that protect mice against challenge with SARS-CoV. *Vaccine* 29(38):6606–6613. doi:[10.1016/j.vaccine.2011.06.111](https://doi.org/10.1016/j.vaccine.2011.06.111)
- Lopez de Turiso JA, Cortes E, Martinez C, Ruiz de Ybanez R, Simarro I, Vela C, Casal I (1992) Recombinant vaccine for canine parvovirus in dogs. *J Virol* 66(5):2748–2753
- Lopez-Macias C, Ferat-Osorio E, Tenorio-Calvo A, Isibasi A, Talavera J, Arteaga-Ruiz O, Arriaga-Pizano L, Hickman SP, Allende M, Lenhard K, Pincus S, Connolly K, Raghunandan R, Smith G, Glenn G (2011) Safety and immunogenicity of a virus-like particle pandemic influenza A (H1N1) 2009 vaccine in a blinded, randomized, placebo-controlled trial of adults in Mexico. *Vaccine* 29(44):7826–7834. doi:[10.1016/j.vaccine.2011.07.099](https://doi.org/10.1016/j.vaccine.2011.07.099)
- Lu X, Thompson JR, Perry KL (2012) Encapsidation of DNA, a protein and a fluorophore into virus-like particles by the capsid protein of cucumber mosaic virus. *J Gen Virol* 93(Pt 5):1120–1126. doi:[10.1099/vir.0.040170-0](https://doi.org/10.1099/vir.0.040170-0)
- Manolova V, Flace A, Bauer M, Schwarz K, Saudan P, Bachmann MF (2008) Nanoparticles target distinct dendritic cell populations according to their size. *Eur J Immunol* 38(5):1404–1413. doi:[10.1002/eji.200737984](https://doi.org/10.1002/eji.200737984)
- Matic S, Rinaldi R, Masenga V, Noris E (2011) Efficient production of chimeric human papillomavirus 16L1 protein bearing the M2e influenza epitope in *Nicotiana benthamiana* plants. *BMC Biotechnol* 11:106. doi:[10.1186/1472-6750-11-106](https://doi.org/10.1186/1472-6750-11-106)
- Maurer P, Bachmann MF (2010) Immunization against angiotensins for the treatment of hypertension. *Clin Immunol* 134(1):89–95. doi:[10.1016/j.clim.2009.06.003](https://doi.org/10.1016/j.clim.2009.06.003)
- Maurer P, Jennings GT, Willers J, Rohner F, Lindman Y, Roubicek K, Renner WA, Muller P, Bachmann MF (2005) A therapeutic vaccine for nicotine dependence: preclinical efficacy, and phase I safety and immunogenicity. *Eur J Immunol* 35(7):2031–2040. doi:[10.1002/eji.200526285](https://doi.org/10.1002/eji.200526285)
- Mazeike E, Gedvilaite A, Blohm U (2012) Induction of insert-specific immune response in mice by hamster polyomavirus VP1 derived virus-like particles carrying LCMV GP33 CTL epitope. *Virus Res* 163(1):2–10. doi:[10.1016/j.virusres.2011.08.003](https://doi.org/10.1016/j.virusres.2011.08.003)
- McKee SJ, Young VL, Clow F, Hayman CM, Baird MA, Hermans IF, Young SL, Ward VK (2012) Virus-like particles and alpha-galactosylceramide form a self-adjuvanting composite particle that elicits anti-tumor responses. *J Control Release* 159(3):338–345. doi:[10.1016/j.jconrel.2012.02.015](https://doi.org/10.1016/j.jconrel.2012.02.015)
- Medicago I News Release 17 June, 2013. Medicago receives authorization from health Canada and commences phase ii clinical trial for an H5N1 vaccine
- Middelberg AP, Rivera-Hernandez T, Wibowo N, Lua LH, Fan Y, Magor G, Chang C, Chuan YP, Good MF, Batzloff MR (2011) A microbial platform for rapid and low-cost virus-like particle and capsomere vaccines. *Vaccine* 29(41):7154–7162. doi:[10.1016/j.vaccine.2011.05.075](https://doi.org/10.1016/j.vaccine.2011.05.075)
- Minten IJ, Hendriks LJ, Nolte RJ, Cornelissen JJ (2009) Controlled encapsulation of multiple proteins in virus capsids. *J Am Chem Soc* 131(49):17771–17773. doi:[10.1021/ja907843s](https://doi.org/10.1021/ja907843s)
- Noteborn MH, Verschueren CA, Koch G, Van der Eb AJ (1998) Simultaneous expression of recombinant baculovirus-encoded chicken anaemia virus (CAV) proteins VP1 and VP2 is required for formation of the CAV-specific neutralizing epitope. *J Gen Virol* 79(Pt 12):3073–3077
- Ou WC, Wang M, Fung CY, Tsai RT, Chao PC, Hseu TH, Chang D (1999) The major capsid protein, VP1, of human JC virus expressed in *Escherichia coli* is able to self-assemble into a capsid-like particle and deliver exogenous DNA into human kidney cells. *J Gen Virol* 80(Pt 1):39–46



- Pan YS, Wei HJ, Chang CC, Lin CH, Wei TS, Wu SC, Chang DK (2010) Construction and characterization of insect cell-derived influenza VLP: cell binding, fusion, and EGFP incorporation. *J Biomed Biotechnol* 2010:506363. doi:[10.1155/2010/506363](https://doi.org/10.1155/2010/506363)
- Patel KG, Swartz JR (2011) Surface functionalization of virus-like particles by direct conjugation using azide-alkyne click chemistry. *Bioconjug Chem* 22(3):376–387. doi:[10.1021/bc100367u](https://doi.org/10.1021/bc100367u)
- Peacey M, Wilson S, Baird MA, Ward VK (2007) Versatile RHDV virus-like particles: incorporation of antigens by genetic modification and chemical conjugation. *Biotechnol Bioeng* 98(5):968–977. doi:[10.1002/bit.21518](https://doi.org/10.1002/bit.21518)
- Peacey M, Wilson S, Perret R, Ronchese F, Ward VK, Young V, Young SL, Baird MA (2008) Virus-like particles from rabbit hemorrhagic disease virus can induce an anti-tumor response. *Vaccine* 26(42):5334–5337. doi:[10.1016/j.vaccine.2008.07.074](https://doi.org/10.1016/j.vaccine.2008.07.074)
- Peters BS, Cheingsong-Popov R, Callow D, Foxall R, Patou G, Hodgkin K, Weber JN (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(3):231–235
- Phelps JP, Dang N, Rasochova L (2007) Inactivation and purification of cowpea mosaic virus-like particles displaying peptide antigens from *Bacillus anthracis*. *J Virol Methods* 141(2):146–153. doi:[10.1016/j.jviromet.2006.12.008](https://doi.org/10.1016/j.jviromet.2006.12.008)
- Pichlmair A, Habjan M, Unger H, Weber F (2010) Virus-like particles expressing the nucleocapsid gene as an efficient vaccine against Rift Valley fever virus. *Vector Borne Zoonotic Dis* 10(7):701–703. doi:[10.1089/vbz.2009.0248](https://doi.org/10.1089/vbz.2009.0248)
- Rappuoli R, Mandl CW, Black S, De Gregorio E (2011) Vaccines for the twenty-first century society. *Nat Rev Immunol* 11(12):865–872. doi:[10.1038/nri3085](https://doi.org/10.1038/nri3085)
- Rebeaud F, Bachmann M (2012) Virus-like particles as efficient delivery platform to induce a potent immune response. In: Baschieri S (ed) *Innovation in vaccinology: from design, through to delivery and testing*. Springer Science + Business Media, Dordrecht, pp 87–122
- Rodriguez-Limas WA, Tyo KE, Nielsen J, Ramirez OT, Palomares LA (2011) Molecular and process design for rotavirus-like particle production in *Saccharomyces cerevisiae*. *Microb Cell Fact* 10:33. doi:[10.1186/1475-2859-10-33](https://doi.org/10.1186/1475-2859-10-33)
- Romanowski B (2011) Long term protection against cervical infection with the human papillomavirus: review of currently available vaccines. *Hum Vaccin* 7(2):161–169
- Ruvoen-Clouet N, Ganiere JP, Andre-Fontaine G, Blanchard D, Le Pendu J (2000) Binding of rabbit hemorrhagic disease virus to antigens of the ABH histo-blood group family. *J Virol* 74(24):11950–11954
- Sabara M, Parker M, Aha P, Cosco C, Gibbons E, Parsons S, Babiuk LA (1991) Assembly of double-shelled rotaviruslike particles by simultaneous expression of recombinant VP6 and VP7 proteins. *J Virol* 65(12):6994–6997
- Salazar O, Asenjo JA (2007) Enzymatic lysis of microbial cells. *Biotechnol Lett* 29(7):985–994. doi:[10.1007/s10529-007-9345-2](https://doi.org/10.1007/s10529-007-9345-2)
- Scheerlinck JP, Greenwood DL (2008) Virus-sized vaccine delivery systems. *Drug Discov Today* 13(19–20):882–887. doi:[10.1016/j.drudis.2008.06.016](https://doi.org/10.1016/j.drudis.2008.06.016)
- Scotti N, Alagna F, Ferraiolo E, Formisano G, Sannino L, Buonaguro L, De Stradis A, Vitale A, Monti L, Grillo S, Buonaguro FM, Cardi T (2009) High-level expression of the HIV-1 Pr55gag polyprotein in transgenic tobacco chloroplasts. *Planta* 229(5):1109–1122. doi:[10.1007/s00425-009-0898-2](https://doi.org/10.1007/s00425-009-0898-2)
- Scullion S (2012) Investigating cytotoxic T cell responses to RHDV virus-like particles: a thesis submitted for the degree of Master of Science at the University of Otago, Dunedin, New Zealand. MSc, University of Otago
- Smith MT, Hawes AK, Bundy BC (2013) Reengineering viruses and virus-like particles through chemical functionalization strategies. *Curr Opin Biotechnol* 24(4):620–626. doi:[10.1016/j.copbio.2013.01.011](https://doi.org/10.1016/j.copbio.2013.01.011)
- Spohn G, Keller I, Beck M, Grest P, Jennings GT, Bachmann MF (2008) Active immunization with IL-1 displayed on virus-like particles protects from autoimmune arthritis. *Eur J Immunol* 38(3):877–887. doi:[10.1002/eji.200737989](https://doi.org/10.1002/eji.200737989)
- Tacket CO (2007) Plant-based vaccines against diarrheal diseases. *Trans Am Clin Climatol Assoc* 118:79–87

- Tacket CO, Mason HS, Losonsky G, Estes MK, Levine MM, Arntzen CJ (2000) Human immune responses to a novel norwalk virus vaccine delivered in transgenic potatoes. *J Infect Dis* 182(1):302–305. doi:[10.1086/315653](https://doi.org/10.1086/315653)
- Tegerstedt K, Lindencrona JA, Curcio C, Andreasson K, Tullus C, Forni G, Dalianis T, Kiessling R, Ramqvist T (2005) A single vaccination with polyomavirus VP1/VP2Her2 virus-like particles prevents outgrowth of HER-2/neu-expressing tumors. *Cancer Res* 65(13):5953–5957. doi:[10.1158/0008-5472.CAN-05-0335](https://doi.org/10.1158/0008-5472.CAN-05-0335)
- Turpen TH, Reinl SJ, Charoenvit Y, Hoffman SL, Fallarme V, Grill LK (1995) Malarial epitopes expressed on the surface of recombinant tobacco mosaic virus. *Biotechnology* 13(1):53–57
- Vicente T, Roldao A, Peixoto C, Carrondo MJ, Alves PM (2011) Large-scale production and purification of VLP-based vaccines. *J Invertebr Pathol* 107(Suppl):S42–S48. doi:[10.1016/j.jip.2011.05.004](https://doi.org/10.1016/j.jip.2011.05.004)
- Visciano ML, Diomede L, Tagliamonte M, Tornesello ML, Asti V, Bomsel M, Buonaguro FM, Lopalco L, Buonaguro L (2011) Generation of HIV-1 virus-like particles expressing different HIV-1 glycoproteins. *Vaccine* 29(31):4903–4912. doi:[10.1016/j.vaccine.2011.05.005](https://doi.org/10.1016/j.vaccine.2011.05.005)
- Walpita P, Barr J, Sherman M, Basler CF, Wang L (2011) Vaccine potential of Nipah virus-like particles. *PLoS One* 6(4):e18437. doi:[10.1371/journal.pone.0018437](https://doi.org/10.1371/journal.pone.0018437)
- Wang X, Xu F, Liu J, Gao B, Liu Y, Zhai Y, Ma J, Zhang K, Baker TS, Schulten K, Zheng D, Pang H, Sun F (2013) Atomic model of rabbit hemorrhagic disease virus by cryo-electron microscopy and crystallography. *PLoS Pathog* 9(1):e1003132. doi:[10.1371/journal.ppat.1003132](https://doi.org/10.1371/journal.ppat.1003132)
- Wiedermann U, Wiltshcke C, Jasinska J, Kundi M, Zurbriggen R, Garner-Spitzer E, Bartsch R, Steger G, Pehamberger H, Scheiner O, Zielinski CC (2010) A virosomal formulated Her-2/neu multi-peptide vaccine induces Her-2/neu-specific immune responses in patients with metastatic breast cancer: a phase I study. *Breast Cancer Res Treat* 119(3):673–683
- Wiessner C, Wiederhold KH, Tissot AC, Frey P, Danner S, Jacobson LH, Jennings GT, Luond R, Ortmann R, Reichwald J, Zurini M, Mir A, Bachmann MF, Staufenbiel M (2011) The second-generation active Abeta immunotherapy CAD106 reduces amyloid accumulation in APP transgenic mice while minimizing potential side effects. *J Neurosci* 31(25):9323–9331. doi:[10.1523/JNEUROSCI.0293-11.2011](https://doi.org/10.1523/JNEUROSCI.0293-11.2011)
- Win SJ, Ward VK, Dunbar PR, Young SL, Baird MA (2011) Cross-presentation of epitopes on virus-like particles via the MHC I receptor recycling pathway. *Immunol Cell Biol* 89(6):681–688. doi:[10.1038/icb.2010.161](https://doi.org/10.1038/icb.2010.161)
- Win SJ, McMillan DG, Errington-Mais F, Ward VK, Young SL, Baird MA, Melcher AA (2012) Enhancing the immunogenicity of tumour lysate-loaded dendritic cell vaccines by conjugation to virus-like particles. *Br J Cancer* 106(1):92–98. doi:[10.1038/bjc.2011.538](https://doi.org/10.1038/bjc.2011.538)
- Xiang SD, Scholzen A, Minigo G, David C, Apostolopoulos V, Mottram PL, Plebanski M (2006) Pathogen recognition and development of particulate vaccines: does size matter? *Methods* 40(1):1–9. doi:[10.1016/j.ymeth.2006.05.016](https://doi.org/10.1016/j.ymeth.2006.05.016)
- Yamshchikov GV, Ritter GD, Vey M, Compans RW (1995) Assembly of SIV virus-like particles containing envelope proteins using a baculovirus expression system. *Virology* 214(1):50–58. doi:[10.1006/viro.1995.9955](https://doi.org/10.1006/viro.1995.9955)
- Young SL, Buchan G, Slobbe LJ, Williman J, Wilson M, Ward V (2004) Novel vaccine formulations to enhance Type 1 immune responses. *Immunology* 1:555–563
- Young SL, Wilson M, Wilson S, Beagley KW, Ward V, Baird MA (2006) Transcutaneous vaccination with virus-like particles. *Vaccine* 24(26):5406–5412. doi:[10.1016/j.vaccine.2006.03.052](https://doi.org/10.1016/j.vaccine.2006.03.052)
- Yusibov V, Hooper DC, Spitsin SV, Fleysh N, Kean RB, Mikheeva T, Dekka D, Karasev A, Cox S, Randall J, Koprowski H (2002) Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine. *Vaccine* 20(25–26):3155–3164
- Zamora E, Handisurya A, Shafti-Keramat S, Borchelt D, Rudow G, Conant K, Cox C, Troncoso JC, Kirnbauer R (2006) Papillomavirus-like particles are an effective platform for amyloid-beta immunization in rabbits and transgenic mice. *J Immunol* 177(4):2662–2670
- Zeltins A (2013) Construction and characterization of virus-like particles: a review. *Mol Biotechnol* 53(1):92–107. doi:[10.1007/s12033-012-9598-4](https://doi.org/10.1007/s12033-012-9598-4)