Plant virus nanoparticles for vaccine applications

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Plant-based Nano-vaccines

Abstract

In the rapidly evolving field of nanotechnology, plant virus nanoparticles (pVNPs) are emerging as powerful tools in diverse applications ranging from biomedicine to materials science. The proteinaceous structure of plant viruses allows the capsid structure to be modified by genetic engineering and/or chemical conjugation with nanoscale precision. This means that pVNPs can be engineered to display peptides and proteins on their external surface, including immunodominant peptides derived from pathogens allowing pVNPs to be used for active immunization. In this context, pVNPs are safer than VNPs derived from mammalian viruses because there is no risk of infection or reversion to pathogenicity. Furthermore, pVNPs can be produced rapidly and inexpensively in natural host plants or heterologous production platforms.
In this review, we discuss the use of pVNPs for the delivery of peptide antigens to the host immune in pre-clinical studies with the final aim of promoting systemic immunity against the corresponding pathogens. Furthermore, we described the versatility of plant viruses, with innate immunostimulatory properties, in providing a huge natural resource of carriers that can be used to develop the next generation of sustainable vaccines.

Keywords:
Molecular farming, CVP, eVLP, VLP, vaccine, peptide production

1. **Introduction**

Many different platforms have been developed in the emerging field of nanotechnology, ranging from synthetic nanomaterials to naturally occurring bio-nanomaterials, the latter including protein cages and viral nanoparticles (VNPs).

VNPs are nanomaterials based on viruses [1]. From a materials science perspective, VNPs are interesting because they form self-assembling architectures that can easily be produced in milligram quantities in the laboratory. These naturally occurring nanostructures have many unique properties, including a quality-control system that guarantees that all the particles are monodisperse and nearly identical in shape and size. These properties are difficult to achieve when producing synthetic nanomaterials. Furthermore, VNPs-based nanomaterials are the result of the assembly of millions of identical proteins, resulting in a highly defined and precise three dimensional structures, with superior features as a scaffold than synthetic particles [2]. VNPs also tend to be symmetrical, polyvalent, soluble in water and stable in aqueous buffers, which are attractive features for biological applications [1].

Virus capsids occur naturally in many different sizes and shapes, including icosahedrons [3], tubes [4] and filaments[5]. Therefore, VNPs can be designed with similarly diverse forms. However, one common property shared by all capsids is their exceptional robustness, reflecting their natural function to enclose and protect viral nucleic acids, thus increasing the resistance of VNPs to extreme pH and temperatures. But because they are entirely composed of protein, VNPs are also biodegradable and biocompatible. Furthermore, VNPs are suitable for scaled-up production and industrial manufacturing, given their inexpensive production; in addition, in some cases ,VNPs can be self-replicating, and this aspect can decrease even more their production expenses [2]. Together, these features make VNPs one of the most advanced and versatile nanomaterials produced by nature.

Although viral nanotechnology is a novel and emerging field, recombinant virus-based materials have been used for nearly 50 years as vaccines and gene delivery vectors [6,7]. Several clinical vaccines are based on VNPs, including the Human papillomavirus (HPV) vaccines Gardasil produced by Merck and Cervarix produced by GSK [8]. Several gene therapies based on Adenovirus, Adeno-associated virus and Lentivirus are undergoing clinical trials [9]. For the past 20 years, chemists, materials scientists and engineers have developed a range of methodologies that can be applied to fine-tune and engineer VNPs for specific applications.

VNPs based on plant viruses (pVNPs) are particularly versatile because they pose no risk of infection in mammals. In contrast, VNPs based on mammalian viruses have the potential to revert to an infectious form. Experiments in mice have also shown that pVNPs have negligible toxicity. The administration of up to 100 mg (10^{16} particles) per kg body weight has been demonstrated without adverse effects, Cowpea Clorotic Mottle Virus particles have been used for *in vivo* imaging in a pre-clinical study, demonstrating their ability to spread in mice's organisms without leaving trace after 24 hours [10]. Biomaterials derived from plant viruses can be delivered intravenously and do not induce hemolysis or coagulation, and they are rapidly cleared from non-target organs and tissues in a matter of hours [11].

The applications of pVNPs can be divided into four key fields: 1) vaccine development; 2) engineering targeted imaging and/or therapeutic reagents; 3) data storage; and 4) the development of films and arrays for applications ranging from electronics to tissue engineering.

This review focuses on the use of pVNPs for vaccine development, describing the main platforms developed thus far and some examples of active immunization. However, the use of pVNP carriers for the delivery of drugs and imaging reagents is also a rapidly developing field, particularly for the diagnosis and treatment of cancer [1]. A groundbreaking example, in this framework, is the fusion to the viral capsid of doxorubicin, a chemotherapeutic agent. The resulting pVNP displays both immunotherapeutic and chemotherapeutic effects, resulting effective in the treatment of mice melanoma [12]. The reader interested in this area should consult the extensive work of Steinmetz and colleagues [12–15].

2. The Development of pVNPs-based platforms for displaying Subunit Vaccines

Unlike attenuated or killed pathogens, subunit vaccines comprise only a small antigenic component of a target pathogen so there is no risk of reversion to pathogenicity. Subunit vaccine design requires the selection of an immuno-dominant peptide that is able to induce a strong immune response which at least partially protects against the pathogen from which the peptide was derived. Subunit vaccines can be produced in many different expression systems, but one drawback is that multiple doses are required combined with an efficient but nontoxic adjuvant to confer an acceptable level protection. Poor immunogenicity has often limited the application of subunit vaccines [16]. This probably reflects the incomplete folding and/or poor presentation of the antigen subunits to the immune system. To enhance the immunogenicity of subunit vaccines, they can be combined with stronger adjuvants [17] or displayed as a multivalent array on a larger carrier molecule, such as a pVNP.

The structure of many plant viruses has been solved at high resolution thanks to a combination of DNA sequence analysis and techniques such as X-ray crystallography and cryo-electron microscopy (Table 1). Despite the large size of pVNPs, the availability of intricate structural models means that regioselective modification is usually achievable and predictable. Viruses can therefore function as nanoscale scaffolds allowing the multivalent attachment of functional ligands at defined positions.

Because pVNPs are genetically encoded biomaterials they can be modified by genetic engineering. This offers a clear advantage over any synthetic material because chemical modifications are not 100% efficient. The genetic insertion of heterologous coding sequences at defined positions within the genes encoding virus coat proteins allows peptides with diverse biological functions to be displayed on VNPs, usually on the external surface [18–20] but also occasionally on the surface of an internal cavity or lumen [21].

Bioconjugation can be used instead of (or in combination with) genetic engineering to display not only peptides but also entire complex proteins, organic molecules and polymers such as nucleic acids and polyethylene glycol (PEG) on the VNP surface [22–24]. Bioconjugation protocols require the presence of amino acids with reactive side chains such as lysine, cysteine or aspartate/glutamate, which are accessible to *N*-hydroxysuccinimidyl (NHS) chemistry, Michael addition to maleimides, and carbodiimide activation, respectively. This allows VNPs to be functionalized with antibodies, oligonucleotides, peptides, proteins, carbohydrates, fluorescent reagents and drugs. The bioconjugation with PEG, for example, has the aim to modulate the strong immune response given from the pVNPs, in order to improve their bioavailability, pharmacokinetics and to selectively target tissues [22,25]. Some viruses are also amenable to physical modifications. For example, Tobacco mosaic virus (TMV) can be transformed from its native rod-shape to a spherical form by increasing the temperature during particle assembly [26]. It is know that the shape affects the behavior of the particles: for example, in tumor homing Potato Virus X and Cowpea Mosaic Virus showed different profiles in bioaccumulation, with the former

showing a major penetration potential than the latter [27]. However, in both conformations, the highly repeated structure appear to function as a Pathogen Associated Molecular Pattern, which allow the design for new immunostimolation systems for the treatment of cancer [15].

3. The Production of VLPs and CVPs

The broad class of nanostructures known as pVNPs can be divided into two subclasses, namely virus-like particles (VLPs) and chimeric virus particles (CVPs). VLPs do not carry any viral nucleic acid and are therefore incapable of autonomous replication. They are produced by expressing the modified coat protein of the virus in heterologous cells, resulting in the production of coat protein molecules that self-assemble in to a VLP [28]. In contrast, CVPs function in much the same way as the native virus (i.e. they remain replication-competent) but they carry additional genetic material allowing them to display heterologous proteins or peptides [28]. VLPs rely on the ability of certain viruses to self-assemble without any priming from the viral genetic material whereas CVPs exploit both the virus coat protein and the genetic material. Therefore, VLPs can be produced in various heterologous expression systems (including bacteria) but the titers depend on the efficiency of expression, whereas CVPs must be produced in their natural plant hosts or a compatible species but the titers are often very high due to the intrinsic replicative ability of the virus.

Whichever strategy is chosen, it is important to ensure that the pVNPs assemble correctly even when the coat protein contains additional peptide sequences and that the peptides displayed on the surface maintain their biological/immunological activity. This is critical when CVPs are used because the production method involves replication in a host plant, and correct particle assembly is required for infection and systemic spreading. One of the major drawbacks of the CVP approach is the tendency for surface peptides to cause steric hindrance, thus inhibiting particle assembly [29]. The use of CVPs therefore has four inherent limitations. First, the characteristics of the foreign peptide can sometimes affect the infectivity of the virus [30,31]. Second, the ability of the chimeric virus to spread systemically in the whole plants can be inhibited by selectivity between the recombinant virus RNA and the chimeric coat protein [32], and virus–host interactions [31,33]. Third, in most epitope presentation systems, chimeric coat proteins do not benefit the virus so deletions that remove the insert are favored by natural selection [30], causing the CVP to revert to wild-type after several cycles of infection [31]. Fourth, special structural features of the chimeric coat protein can affect key domains required for assembly, preventing it from assembling into virus particles [34]. One way to overcome the inhibitory effects of inserted peptide sequences is to generate VNPs that combine some wild-type coat proteins and some fusion proteins, spreading the displayed peptides further apart. This overcoat strategy has been particularly successful for Potexviruses and is discussed in more detail below.

Plants offer an attractive system for the production of pVNP-based vaccines owning to their ability to produce large quantities of the particles at low cost, the proper assembly of the particles *in planta*, and the low-risk of introducing adventitious human pathogens during production [35]. In this framework, the Alfalfa Mosaic Virus has been exploited as an antigen presenting system for the rabies antigen and used as a candidate to boost the vaccination[36].

Plants do not require expensive fermentation facilities for biomass generation or the construction of duplicate facilities for scaled-up production. Hence, plant biomass generation and upstream processing capacity can be operated and scaled up in a flexible, cost-efficient manner that cannot be easily matched by current fermentation-based technologies [37].

A further advantage of plant-based production systems is that edible plants can be engineered to produce pVNPs, which can then be administered orally with minimal processing [38]. This novel

and cost-effective approach can be used to establish gut mucosal immunity by oral delivery. Plants can also be used to produce VLPs representing human viral pathogens for active immunization strategies. Examples include the use of plants to produce VLPs based on HPV and influenza virus at very high titers: chimeric Cucumber Mosaic Virus bearing an Hepatitis C antigen, for example has been proven to be a good candidate as an oral vaccine in rabbits [39].This specific use of plants to produce VLP-based vaccines is beyond the scope of this review but it has been extensively described elsewhere [40,41].

4. Platforms for the production of pVNPs

Many different plant viruses have been considered as pVNP platforms, differing in terms of the host species, the format (VLP or CVP) and the number of peptides that can be displayed per particle (Table 1). Only a few species have been studied in detailed proof-of-concept experiments and five of the most prevalent are discussed below.

Table 1: Features of pVNPs for peptide display.

pVNP	***Structural Resolution***	***Target peptide insertion point***	***Number of peptides / pVNP***	***Host***	***CVP***	***VLP***	***References***
TBSV	2.9 Å	C-terminus	180	*Nicotiana benthamiana* *Insect cell/baculovirus*	X	X	[42–44]
CPMV	2.9 Å	βB- βC loop of the Small CP βC′-βC″ loop of the small coat protein	60	*Nicotiana benthamiana* *Pisum sativum* *Spinacea oleracea* *Vicia sativa* *Vigna unguiculata* *Vigna radiata*	X	X	[31,45,46]
CCMV	3.2 Å	C-terminus N-terminus In *P. pastoris* into an external loop	180 (Particles are formed by 90 homodimers)	*Pichia pastoris* *Escherichia coli*	X	X	[47–50]
PLRV	-	N-terminus	-	*Insect cells and baculovirus*	-	X	[51,52]
TMV	-	N-terminus C-terminus Surface loop aa 59-65	> 2100	*Nicotiana benthamiana* *Nicotiana excelsiana*	X	-	[53,54]
JGMV	-	N-terminus C-terminus	-	*Escherichia coli*	-	X	[55]
PVX	-	N- terminus	1300	*Nicotiana benthamiana*	-		[28,56,57]
PhMV	3.8 Å	Chemical conjugation to lysine or cysteine	Lys: 160-180 Cys: 40- 60	*Escherichia coli*	-	X	[58,59]
TuMV	-	N-terminus	Up to 2000 copies for the CVPs. For the VLPs it depends on the length of the formed particles	*Brassica juncea* *Nicotiana benthamiana* *Escherichia coli*	X	X	[60]

PapMV	2.7 Å	12 aa – 187 aa – C-terminus N-terminus N-terminnus 12 aa before F13	For the VLPs it depends on the length of the formed particles	*Escherichia coli*	-	X	[61–66]
AltMV	13 Å	C-terminus	-	*Nicotiana benthamiana Portulaca grandiflora*	X	X	[67–69]
BaMV	5.6 Å	35 aa from the N-terminus	-	*Nicotiana benthamiana Chenopodium quinoa*	X	-	[70,71]
AlMV	-	N-terminus	-	*Nicotiana tabacum Nicotiana benthamiana*	X	-	[72–75]
CMV	3.2 Å	βH-βI loop	180	*Nicotiana benthamiana Nicotiana tabacum cv Xhanti*	X	X	[23,76–78]
CdMV	-	N-terminus C-terminus	-	*Escherichia coli*	-	X	[79]
AMCV	-	C-terminus	180	*Nicotiana benthamiana*	X	-	[80]
PPV	-	N-terminus	-	*Nicotiana clevandii*	X	-	[81]
PVY	-	N-terminus	2000	*Escherichia coli*	-	X	[82]
CymRSV	-	-	280	*Nicotiana benthamiana*	-	X	[83]

TBSV: Tomato Bushy Stunt Virus; **CPMV**: Cowpea Mosaic Virus; **CCMV**: Cowpea Clorotic Mottle Virus; **PLRV**: Potato Leafroll Virus; **TMV**: Tobacco Mosaic Virus; **JGMV**: Johnson Grass Mosaic Virus; **PVX**: Potato Virus X; **PhMV**: Physalis Mosaic Virus; **TuMV**: Turnip Mosaic Virus; **PapMV**: Papaya Mosaic Virus; **AltMV**: Alternanthera Mosaic Virus; **BaMV**: Bamboo Mosaic Virus; **AlMV**: Alfalfa Mosaic Virus; **CMV**: Cucumber Mosaic Virus; **CdMV**: Cardamom Mosaic Virus; **AMCV**: artichoke Mottled Crinkle Virus; **PPV**: Plum Pox Virus; **PVY**: Potato Virus Y; **CymRSV**: Cymbidium Ringspot Virus.

4.1 Cowpea mosaic virus

Cowpea mosaic virus (CPMV) has been studied extensively for its biomedical applications in the fields of cancer, cardiovascular medicine and vaccination [84,85]. CPMV has a bipartite RNA genome and a 28-nm icosahedral capsid comprising 60 copies each of the large (L) and small (s) coat proteins [86]. CPMV surface chemistry (inside and out) is well understood [87], allowing an exquisite level of functionalization. Additionally, the large-scale production of pVNPs based on CPMV can be achieved in its natural host (cowpea, *Vigna unguiculata*) and the model host tobacco species *Nicotiana benthamiana*, which is highly scalable and economic.

4.2 Potato virus X

Potato virus X (PVX) is the type member of the Potexvirus genus. It has a positive-sense RNA genome and forms filamentous and flexible rod-like particles measuring 515 nm in length and 13 nm in diameter, comprising 1270 identical 25-kDa coat protein subunits. The monopartite genome has a 5' cap and 3' poly(A) tail and it contains five major open reading frames (ORFs) encoding proteins for viral replication, movement and assembly. ORF5 encodes the coat protein, which is also required for cell-to-cell and long-distance movement [88]. The N-terminal part of the coat protein is exposed on the surface so most peptides displayed by PVX-based pVNPs have been

introduced by N-terminal fusion to the coat protein [89]. PVX naturally replicates in many solanaceous species and *N. benthamiana* plants are particularly susceptible to mechanical inoculation, producing milligram quantities of particles from 1 g of infected leaf material. Infectious cDNA clones of PVX genomic RNA are available and genetic modification protocols have been established for the production of CVPs. However, PVX coat protein subunits have not yet been shown to assemble into filamentous VLPs in the absence of RNA either *in vivo* or *in vitro*. Whereas genetic modification of PVX is well established, chemical bioconjugation methods are inefficient (21–86% depending on the conjugation strategy and the size of the target molecule) [25, 26]. PVX has been investigated for various applications ranging from vaccination to in vitro and in vivo diagnosis [12, 27, 28]. PVX virion assembly is sensitive to steric hindrance, so the characteristics of displayed peptides and their impact on the final structure have been studied in detail, revealing that peptide sizes, pI and the presence of specific amino acids play crucial roles [30,93–95]. In order to overcome size-limitation problems, an alternative 'overcoat' strategy was developed to improve the stability of chimeric particles based on the use of the Foot and mouth disease virus (FMDV) 2A co-translational dissociation sequence, allowing the simultaneous production of both wild-type coat proteins and fusion proteins that can assemble into stable particles without steric hindrance at the most effective wild-type: fusion protein ratio [96].

4.3 Tobacco mosaic virus

The TMV coat protein gene provides various possibilities for the insertion of heterologous sequences. The N-terminus has been used successfully [20] but the C-terminus is a much more common fusion site [97–99]. The latter can be achieved by fusion to threonine-158 at the extreme C-terminus or insertion into the surface loop between serine-154 and glycine-155 [32]. Both strategies produce viable and stable chimeric particles. It is unclear whether the four C-terminal amino acids are required for the efficient systemic movement of recombinant viruses and the assembly of chimeric particles. In infected tobacco plants, the viral coat protein may account for up to 7% of total host cell protein and can be easily purified from plant tissue in the form of VNPs. Several heterologous proteins and short peptides have been expressed in TMV as coat protein fusions without affecting viral replication and assembly [101–105].

4.4 Bamboo mosaic virus

Bamboo mosaic virus (BaMV) is related to PVX and has a similar capsid and genome [102–105]. However, it has a broader host range, infecting both monocotyledonous and dicotyledonous plants [101]. BaMV is not a pathogen in most crops and therefore may be ecologically safer for field use than other pVNPs [101]. A novel transgenic cell-suspension culture system has recently been developed, which continually produces self- replicating BaMV CVPs as well as coat proteins that self-assemble into VLPs displaying target epitopes [106]. The size of the epitope presented by BaMV-based CVPs is limited to 37 amino acids [70], and similar size constraints have been reported for other viruses [97,107–109]. As for PVX, the FMDV 2A co-translational dissociation sequence may therefore provide a solution to this challenge [93].

4.5 Papaya mosaic virus

Papaya mosaic virus (PapMV) is another Potexvirus, with a similar genome structure to PVX and BaMV. It has a filamentous capsid, 500 nm in length and 14 nm in diameter. PapMV coat proteins have been expressed in *E. coli* and showed the capacity of self-assemble into VLPs. The coat protein has been separated from the genomic RNA by the degradation of purified virus particles using acetic acid, thus facilitating *in vitro* assembly. The extracted coat protein formed a variety of aggregates that included a disk-like helical structure, similar in architecture to the native virus

particle but comprising only 18–20 subunits. Furthermore, the addition of genomic RNA to the isolated disks triggered the *in vitro* assembly of long rod-shaped particles similar to the wild-type virus [67,110].

PapMV nanoparticles can be used as an adjuvant to improve the performance of vaccines [111] and cancer immunotherapy [112], or as a vaccine platform to trigger an immune response against specific peptide antigens [113]. In order to prevent long peptides interfering with the self-assembly of VLPs [49], a novel system was developed by coupling peptides to already self-assembled nanoparticles using the bacterial transpeptidase sortase A [62,100].

5. Development of pVNP Vaccines Displaying Antigenic Peptides

Many plant viruses have been used to display heterologous peptides for the development of vaccine candidates intended for active immunization. Some of these candidates have been tested *in vitro* and others have progressed to pre-clinical studies. The achievements of these investigations and the properties of the pVNPs and displayed peptides are summarized in Table 2. Interestingly, 10 of 36 studies demonstrated that the ability of pVNPs to induce an immunological response against the target peptide conferred protection against the corresponding pathogen in subsequent challenge experiments. In some studies, pVNPs were even able to confer partial cross-protection against related pathogens [98].

Figure **1**.

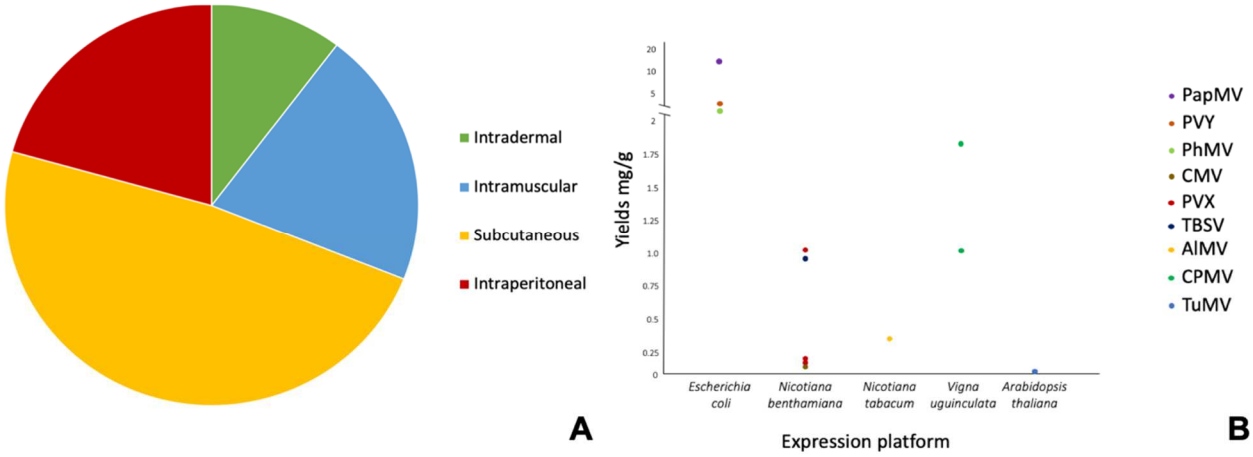

Figure 1. The production of pVNPs for pre-clinical development. A: The yields of pVNPs achieved in different production platforms. B: Relative frequency of different administration routes for pVNPs in pre-clinical studies.

In most cases the administration route was parenteral with a high proportion of subcutaneous injections (Figure 1A). The pVNPs were always administered in two or three doses. The average yield of the pVNPs was strongly dependent on which virus was used and which target peptide was displayed, resulting in a broad distribution (Figure 1B). The yields of pVNPs are also dependent the position of the heterologous peptide on the viral surface, a property that also affects the immunological properties of the pVNP [114].

Table 2: Survey of pVNP *in vitro* tests and pre-clinical studies.

pVNP	*Target Peptide*	*Host*	*Pre-clinical and in vitro studies*	*References*

TuMV	Human vascular endothelial growth factor receptor 3 (VEGFR-3)	*Arabidopsis thaliana*	Immunogenicity in mice	[60]
TMV	M2e epitope from influenza	*Nicotiana benthamiana*	Immunogenicity in vitro	[115]
TMV	Two peptides of CRPV	*Nicotiana tabacum, Nicotiana benthamiana*	Immunogenicity and challenging in rabbits	[98]
TMV	Peptide F20 (V141–P160 of FMDV VP1)	*Nicotiana tabacum cv. Samsun*	Immunogenicity in guinea pigs and swine and challenging	[107]
TMV	Peptide 9-14mer (TDAYNQKLSERRAN) of outer membrane (OM) protein F of Pseudomonas aeruginosa	*Nicotiana tabacum cv. Xanthi*	Immunogenicity and challenging in mice	[99]
TMV-U1	M2e epitope from influenza	*Nicotiana benthamiana*	Immunogenicity in BALB/c mice, virus challenging protection	[116]
BaMV	FMDV-VP1	*Nicotiana benthamiana, Chenopodium quinoa*	Immunogenicity in male and swine and challenging with the virus	[70]
BaMV	vvIBDV	*Nicotiana benthamiana, Chenopodium quinoa*	Immunogenicity, challenging with the virus and immunohistochemistry in chickens	[117]
PVX	HIV ELDKWA epitope from glycoprotein (gp) 41	*Nicotiana benthamiana*	immunogenicity in mice	[118]
PVX	HPV-16 L2 minor capsid protein (amino acids 108-120)	*Nicotiana benthamiana*	Immunogenicity in mice	[119]
PVX	H-2Db-restricted epitope ASNENMETM of influenza A virus nucleoprotein (NP)	*Nicotiana benthamiana*	Immunogenicity in C57BL/6J female mice	[28]
PVX	D2 peptide from *S. aureus* bronectin-binding protein (FnBP)	*Nicotiana benthamiana*	Immunogenicity in mice and rats	[120]
CPMV	D2 peptide from *S. aureus* bronectin-binding protein (FnBP)	*Nicotiana benthamiana*	Immunogenicity in mice and rats	[120]
CPMV	14 amino acid NIm-1A epitope from human rhinovirus 14 (HRV-14)	*Vigna unguiculata*	Immunogenicity	[114]
CPMV	Linear epitope from the VP2 capsid protein of mink enteritis virus (MEV)	*Vigna unguiculata*	Immunization of minks and challenging	[121]
CPMV	Peptide derived from the VP2 capsid protein of canine parvovirus (CPV)	*Vigna unguiculata*	Immunogenicity in dogs and challenging with the virus	[122]
CPMV	aa731- 752 of the gp41 of HIV-l strain IIIB	*Vigna unguiculata*	immunogenicity in mice C57/BL6 (H-2b), BALB/c (H-2d) mice)	[123]
AMCV	HIV-1 2F5 epitope (2F5e)	*Nicotiana benthamiana*	ELISA	[80]
AIMV	Chimeric peptide representing rabies G (amino acids 253–275) and N (amino acids 404–418) proteins	*Nicotiana tabacum cv. Samsun*	Immunogenicity and challenging with the virus and Immunogenicity in humans of experimental plant virus-based rabies vaccine consumed in food	[72]
AIMV	V3 loop of the HIV-1 MN isolate (MNV3),	*Nicotiana benthamiana*	Immunogenicity in mice	[73]
AlMV	21-mer peptide representing amino acids 170-190 of the respiratory syncytial virus (RSV) G protein	*Nicotiana tabacum cv. Samsun*	immunogenicity in vivo in non-human primates and in vitro in human dendritic cells	[74]
AlMV	Small loop 15 amino acid epitope from domain 4 of the Bacillus anthracis protective antigen (PA-D4s).	*Nicotiana tabacum cv. Samsun*	Immunogenicity in Balb/c mice	[75]
PapMV	Hepatitis C virus (HCV) E2 epitope	*Escherichia coli*	Immunogenicity in C3H/HeJ and Balb/c mice and in humans	[63]
PapMV	M2e influenza epitope	*Escherichia coli*	Immunogenicity in BALB7c mice and challenging with the virus	[124]

PapMV	M2e influenza epitope	*Escherichia coli*	Immunogenicity in Balb/c mice	[64]
PapMV	HA11 peptide influenza peptide	*Escherichia coli*	Immunogenicity in Balb/c mice	[61]
TBSV	16 amino acid epitopes of ricin toxin A chain	Insect cells and *baculovirus*	Immunogenicity in Balb/c mice	[125]
TBSV	13-amino-acid peptide derived from the V3 loop of human immunodeficiency virus (HIV-1) glyco- protein 120 (gp120)	*Nicotiana benthamiana*	Immunogenicity in mice	[126]
JGMV	27 amino acid peptide A of the Japanese encephalitis virus (JEV)	*Escherichia coli*	Immunogenicity in FVB/J mice and challenge with the virus	[55]
PhMV	FMDV infection-related B-cell epitopes of NSPs 3A, 3B and 3D	*Escherichia coli*	Immunogenicity in cattle, buffalo, sheep, pig samples	[127]
CMV	17 amino acid (aa) neutralizing epitope of NDV's fusion (F) protein (aa 65– 81), 8 aa neutralizing epitope of the NDV hem-agglutinin-neuraminidase (HN) protein (aa 346–353), and the same epitopes fused together in a tandem F–HN construct.	*Nicotiana benthamiana*	Immunogenicity in chickens	[77]
CMV	Peptide of HCV envelope protein E2 (R9 mimotope).	*Nicotiana tabacum cv. Xanthi*	Immunogenicity in rabbits, in vitro studies in humans	[76]
PVY	PreS1 or rubredoxin	*Escherichia coli*	Immunogenicity in Balb/c mice	[82]
CdMV	Kennedy peptide and the 2F5 and 4E10 epitopes of gp41 ofHIV.	*Escherichia coli*	Immunoreactivity with HIV patient sera	[79]
PPV	VP2 capsid protein of canine parvovirus (CPV)	*Nicotiana clevelandii*	Immunogenicity in mice and rabbits	[81]

The dose of pVNP administered in each pre-clinical study strongly depended on the animal model, ranging from 0.5 to 400 µg/dose. In most cases, an adjuvant was also administered, with incomplete Freund's adjuvant and aluminum hydroxide the most widely used. The pre-clinical studies were mainly structured as a comparison between animals treated with pVNPs displaying the target peptide and a placebo group in which animals were treated with wild-type pVNPs lacking the displayed peptide. In a small number of cases, the experimental design also included a control group treated with the synthetic peptide. In these experiments, the quantity of peptide displayed by the pVNP was lower that the dose used for the synthetic peptide. Only one study included a control group in which the pVNPs were administered without an adjuvant [28]. This revealed that pVNPs administered with an adjuvant treatment achieved the greatest level of protection, and that pVNPs without adjuvant conferred the same protective effect as the synthetic peptide administered with the adjuvant. In other studies, the use of the synthetic peptide as a comparator was limited to indirect ELISA tests to detect the presence of specific antibodies [60,123]. Overall, these studies indicate that pVNPs can act as both as a carrier and an adjuvant, probably reflecting the particulate and repetitive nature of the virion [128].

The particulate nature of VLPs and CVPs also allows them to induce potent T-cell responses by interacting with antigen-presenting cells (APCs), especially dendritic cells (DCs) [129]. Cytotoxic T cells do not recognize native antigens, but are activated by processed peptides derived therefrom presented in association with MHC class I molecules [130]. The best way to induce T-cell activation by vaccination is to mimic the process of a natural infection, including the recognition, uptake, and processing of particulate antigens, and the presentation of processed peptides to cytotoxic T cells in order to activate them and trigger their proliferation [131]. DCs can efficiently carry out these processes by (i) taking up antigens in the cytosol and presenting processed peptide

antigens on MHC class I molecules or receptors by cross-presentation, and (ii) by presenting the peptides on MHC class II molecules as part of the classical antigen-processing pathway [131]. Thus, in addition to stimulating helper T cells, DCs can also stimulate the conversion of naive T cells into cytotoxic T lymphocytes (CTLs), which subsequently eliminate intracellular pathogens or cancer cells via the presentation of antigens on MHC class I molecules, effectively bridging innate and acquired immunity. DCs preferentially take up particulate antigens by phagocytosis or macropinocytosis if the particle diameter is 20–300 nm, the size range of most viruses [132]. Therefore, pVNPs are the ideal size for DC and macrophage uptake and antigen processing to initiate antigen cross-presentation. Due to the high density of epitopes on their surface, the uptake of a single pVNP feeds thousands of epitopes into the processing and presentation machinery of APCs, further enhancing their ability to stimulate CTLs.

The presence of pVNPs can also directly activate B cells, leading to high antibody titers and long-lasting B-cell memory, even in the absence of adjuvants. Like mammalian viruses, the quasi-crystalline surface of pVNPs with arrays of repetitive epitopes is a prime target for B-cell recognition [133] and can efficiently crosslink epitope-specific immunoglobulins on the cell surface. Particulate antigens with repetitive epitopes spaced at 5–10-nm intervals may be unique to microbial surfaces, and vertebrate B cells have therefore evolved to specifically recognize and respond vigorously to these types of antigens [134]. The oligomerization of immunoglobulins in this manner forms a strong activation signal that leads to B-cell proliferation and migration, the upregulation of MHC class II molecules, T helper cell activation, IgM and IgG production and secretion, and the generation of long-lasting memory B cells [133]. An advantage of CVPs over VLPs is that the viral RNA may trigger Toll-like receptor 7 on antigen-presenting cells, hence boosting the immune response like an adjuvant [135].

Another characteristic of pVNPs is that certain plant viruses (e.g. PapMV) are inherently highly immunogenic, triggering a strong humoral response against the coat protein. However, pre-existing antibodies recognizing virus coat do not appear to affect the ability of pVNPs to boost the humoral response toward heterologous antigens displayed on their surface [124].

In summary, pVNPs can be used to carry immunodominant peptides for active immunization strategies, and their intrinsic properties, such as the ability to display arrays of antigens on a scaffold with a repetitive structure, may help to promote their immunological efficacy.

6. Consent for Publication

All authors read and approved the final manuscript.

7. Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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9. References

[1] Wege, C.; Lomonossoff, G.P. *Virus-Derived Nanoparticles for Advanced Technologies: Methods and Protocols*; Methods in Molecular Biology; Springer Science+Business Media: New York, NY, **2018**.
[2] Koudelka, K.J.; Pitek, A.S.; Manchester, M.; Steinmetz, N.F. Virus-Based Nanoparticles as Versatile Nanomachines. *Annu. Rev. Virol.*, **2015**, *2*, 379–401.
[3] Sainsbury, F.; Cañizares, M.C.; Lomonossoff, G.P. *Cowpea Mosaic* Virus: The Plant Virus–Based Biotechnology Workhorse. *Annu. Rev. Phytopathol.*, **2010**, *48*, 437–455.
[4] Chu, S.; Brown, A.D.; Culver, J.N.; Ghodssi, R. Tobacco Mosaic Virus as a Versatile Platform for Molecular Assembly and Device Fabrication. *Biotechnol. J.*, **2018**, *13*, 1800147.
[5] Röder, J.; Dickmeis, C.; Commandeur, U. Small, Smaller, Nano: New Applications for Potato Virus X in Nanotechnology. *Front. Plant Sci.*, **2019**, *10*.
[6] Dane, D.S.; Cameron, C.H.; Briggs, M. VIRUS-LIKE PARTICLES IN SERUM OF PATIENTS WITH AUSTRALIA-ANTIGEN-ASSOCIATED HEPATITIS. *The Lancet*, **1970**, *295*, 695–698.
[7] Jagadish, M.N.; Ward, C.W.; Gough, K.H.; Tulloch, P.A.; Whittaker, L.A.; Shukla, D.D. Expression of Potyvirus Coat Protein in Escherichia Coli and Yeast and Its Assembly into Virus-like Particles. *J. Gen. Virol.*, **1991**, *72*, 1543–1550.
[8] Chackerian, B. Virus-like Particles: Flexible Platforms for Vaccine Development. *Expert Rev. Vaccines*, **2007**, *6*, 381–390.
[9] Kotterman, M.A.; Chalberg, T.W.; Schaffer, D.V. Viral Vectors for Gene Therapy: Translational and Clinical Outlook. *Annu. Rev. Biomed. Eng.*, **2015**, *17*, 63–89.
[10] Kaiser, C.R.; Flenniken, M.L.; Gillitzer, E.; Harmsen, A.L.; Harmsen, A.G.; Jutila, M.A.; Douglas, T.; Young, M.J. Biodistribution Studies of Protein Cage Nanoparticles Demonstrate Broad Tissue Distribution and Rapid Clearance in Vivo. *Int. J. Nanomedicine*, **2007**, *2*, 715–733.
[11] Bruckman, M.A.; Randolph, L.N.; VanMeter, A.; Hern, S.; Shoffstall, A.J.; Taurog, R.E.; Steinmetz, N.F. Biodistribution, Pharmacokinetics, and Blood Compatibility of Native and PEGylated Tobacco Mosaic Virus Nano-Rods and -Spheres in Mice. *Virology*, **2014**, *449*, 163–173.
[12] Le, D.H.T.; Lee, K.L.; Shukla, S.; Commandeur, U.; Steinmetz, N.F. Potato Virus X, a Filamentous Plant Viral Nanoparticle for Doxorubicin Delivery in Cancer Therapy. *Nanoscale*, **2017**, *9*, 2348–2357.
[13] Steinmetz, N.F.; Mertens, M.E.; Taurog, R.E.; Johnson, J.E.; Commandeur, U.; Fischer, R.; Manchester, M. Potato Virus X as a Novel Platform for Potential Biomedical Applications. *Nano Lett.*, **2010**, *10*, 305–312.
[14] Czapar, A.E.; Steinmetz, N.F. Plant Viruses and Bacteriophages for Drug Delivery in Medicine and Biotechnology. *Curr. Opin. Chem. Biol.*, **2017**, *38*, 108–116.
[15] Lizotte, P.H.; Wen, A.M.; Sheen, M.R.; Fields, J.; Rojanasopondist, P.; Steinmetz, N.F.; Fiering, S. In Situ Vaccination with Cowpea Mosaic Virus Nanoparticles Suppresses Metastatic Cancer. *Nat. Nanotechnol.*, **2016**, *11*, 295–303.
[16] Bobbala, S.; Hook, S. Is There an Optimal Formulation and Delivery Strategy for Subunit Vaccines? *Pharm. Res.*, **2016**, *33*, 2078–2097.
[17] Bastola, R.; Noh, G.; Keum, T.; Bashyal, S.; Seo, J.-E.; Choi, J.; Oh, Y.; Cho, Y.; Lee, S. Vaccine Adjuvants: Smart Components to Boost the Immune System. *Arch. Pharm. Res.*, **2017**, *40*, 1238–1248.
[18] Gleiter, S.; Lilie, H. Coupling of Antibodies via Protein Z on Modified Polyoma Virus-like Particles. *Protein Sci. Publ. Protein Soc.*, **2001**, *10*, 434–444.
[19] Hajitou, A.; Trepel, M.; Lilley, C.E.; Soghomonyan, S.; Alauddin, M.M.; Marini, F.C.; Restel, B.H.; Ozawa, M.G.; Moya, C.A.; Rangel, R.; Sun, Y.; Zaoui, K.; Schmidt, M.; von Kalle, C.; Weitzman, M.D.; Gelovani, J.G.; Pasqualini, R.; Arap, W. A Hybrid Vector for Ligand-Directed Tumor Targeting and Molecular Imaging. *Cell*, **2006**, *125*, 385–398.
[20] McCormick, C.J.; Maucourant, S.; Griffin, S.; Rowlands, D.J.; Harris, M. Tagging of NS5A Expressed from a Functional Hepatitis C Virus Replicon. *J. Gen. Virol.*, **2006**, *87*, 635–640.

[21] Beterams, G.; Böttcher, B.; Nassal, M. Packaging of up to 240 Subunits of a 17 KDa Nuclease into the Interior of Recombinant Hepatitis B Virus Capsids. *FEBS Lett.*, **2000**, *481*, 169–176.
[22] Raja, K.S.; Wang, Q.; Gonzalez, M.J.; Manchester, M.; Johnson, J.E.; Finn, M.G. Hybrid Virus−Polymer Materials. 1. Synthesis and Properties of PEG-Decorated Cowpea Mosaic Virus. 5.
[23] Smith, M.L.; Lindbo, J.A.; Dillard-Telm, S.; Brosio, P.M.; Lasnik, A.B.; McCormick, A.A.; Nguyen, L.V.; Palmer, K.E. Modified Tobacco Mosaic Virus Particles as Scaffolds for Display of Protein Antigens for Vaccine Applications. *Virology*, **2006**, *348*, 475–488.
[24] Meunier, S.; Strable, E.; Finn, M.G. Crosslinking of and Coupling to Viral Capsid Proteins by Tyrosine Oxidation. *Chem. Biol.*, **2004**, *11*, 319–326.
[25] Dogic, Z.; Fraden, S. Phase Behavior of Rod-like Virus and Polymer Mixture. *ArXivcond-Mat0010269*, **2000**.
[26] Atabekov, J.; Nikitin, N.; Arkhipenko, M.; Chirkov, S.; Karpova, O. Thermal Transition of Native Tobacco Mosaic Virus and RNA-Free Viral Proteins into Spherical Nanoparticles. *J. Gen. Virol.*, **2011**, *92*, 453–456.
[27] Shukla, S.; Ablack, A.L.; Wen, A.M.; Lee, K.L.; Lewis, J.D.; Steinmetz, N.F. Increased Tumor Homing and Tissue Penetration of the Filamentous Plant Viral Nanoparticle *Potato Virus X*. *Mol. Pharm.*, **2013**, *10*, 33–42.
[28] Lico, C.; Mancini, C.; Italiani, P.; Betti, C.; Boraschi, D.; Benvenuto, E.; Baschieri, S. Plant-Produced Potato Virus X Chimeric Particles Displaying an Influenza Virus-Derived Peptide Activate Specific CD8+ T Cells in Mice. *Vaccine*, **2009**, *27*, 5069–5076.
[29] Scholthof, H.B.; Scholthof, K.-B.G.; Jackson, A.O. PLANT VIRUS GENE VECTORS FOR TRANSIENT EXPRESSION OF FOREIGN PROTEINS IN PLANTS. *Annu. Rev. Phytopathol.*, **1996**, *34*, 299–323.
[30] Lico, C.; Capuano, F.; Renzone, G.; Donini, M.; Marusic, C.; Scaloni, A.; Benvenuto, E.; Baschieri, S. Peptide Display on Potato Virus X: Molecular Features of the Coat Protein-Fused Peptide Affecting Cell-to-Cell and Phloem Movement of Chimeric Virus Particles. *J. Gen. Virol.*, **2006**, *87*, 3103–3112.
[31] Porta, C.; Spall, V.E.; Findlay, K.C.; Gergerich, R.C.; Farrance, C.E.; Lomonossoff, G.P. Cowpea Mosaic Virus-Based Chimaeras. Effects of Inserted Peptides on the Phenotype, Host Range, and Transmissibility of the Modified Viruses. *Virology*, **2003**, *310*, 50–63.
[32] Schneemann, A. The Structural and Functional Role of RNA in Icosahedral Virus Assembly. *Annu. Rev. Microbiol.*, **2006**, *60*, 51–67.
[33] Ahlquist, P.; Schwartz, M.; Chen, J.; Kushner, D.; Hao, L.; Dye, B.T. Viral and Host Determinants of RNA Virus Vector Replication and Expression. *Vaccine*, **2005**, *23*, 1784–1787.
[34] Cañizares, M.C.; Lomonossoff, G.P.; Nicholson, L. Development of Cowpea Mosaic Virus-Based Vectors for the Production of Vaccines in Plants. *Expert Rev. Vaccines*, **2005**, *4*, 687–697.
[35] Chen, Q.; Lai, H. Plant-Derived Virus-like Particles as Vaccines. *Hum. Vaccines Immunother.*, **2013**, *9*, 26–49.
[36] Yusibov, V. Expression in Plants and Immunogenicity of Plant Virus-Based Experimental Rabies Vaccine. *Vaccine*, **2002**, *20*, 3155–3164.
[37] Merlin, M.; Gecchele, E.; Capaldi, S.; Pezzotti, M.; Avesani, L. Comparative Evaluation of Recombinant Protein Production in Different Biofactories: The Green Perspective. *BioMed Res. Int.*, **2014**, *2014*, 136419.
[38] Merlin, M.; Pezzotti, M.; Avesani, L. Edible Plants for Oral Delivery of Biopharmaceuticals. *Br. J. Clin. Pharmacol.*, **2017**, *83*, 71–81.
[39] Nuzzaci, M.; Vitti, A.; Condelli, V.; Lanorte, M.T.; Tortorella, C.; Boscia, D.; Piazzolla, P.; Piazzolla, G. In Vitro Stability of Cucumber Mosaic Virus Nanoparticles Carrying a Hepatitis C Virus-Derived Epitope under Simulated Gastrointestinal Conditions and in Vivo Efficacy of an Edible Vaccine. *J. Virol. Methods*, **2010**, *165*, 211–215.
[40] Noris, E. Self-Assembling Plant-Derived Vaccines Against Papillomaviruses. *Methods Mol.*

Biol. Clifton NJ, **2018**, *1776*, 85–95.
[41] Scotti, N.; Rybicki, E.P. Virus-like Particles Produced in Plants as Potential Vaccines. *Expert Rev. Vaccines*, **2013**, *12*, 211–224.
[42] Harrison, S.C.; Olson, A.J.; Schutt, C.E.; Winkler, F.K.; Bricogne, G. Tomato Bushy Stunt Virus at 2.9 A Resolution. *Nature*, **1978**, *276*, 368–373.
[43] Olson, A.J.; Bricogne, G.; Harrison, S.C. Structure of Tomato Busy Stunt Virus IV. The Virus Particle at 2.9 A Resolution. *J. Mol. Biol.*, **1983**, *171*, 61–93.
[44] Grasso, S.; Lico, C.; Imperatori, F.; Santi, L. A Plant Derived Multifunctional Tool for Nanobiotechnology Based on Tomato Bushy Stunt Virus. *Transgenic Res.*, **2013**, *22*, 519–535.
[45] Lin, T.; Clark, A.J.; Chen, Z.; Shanks, M.; Dai, J.-B.; Li, Y.; Schmidt, T.; Oxelfelt, P.; Lomonossoff, G.P.; Johnson, J.E. Structural Fingerprinting: Subgrouping of Comoviruses by Structural Studies of Red Clover Mottle Virus to 2.4-Å Resolution and Comparisons with Other Comoviruses. *J. Virol.*, **2000**, *74*, 493–504.
[46] Saunders, K.; Sainsbury, F.; Lomonossoff, G.P. Efficient Generation of Cowpea Mosaic Virus Empty Virus-like Particles by the Proteolytic Processing of Precursors in Insect Cells and Plants. *Virology*, **2009**, *393*, 329–337.
[47] Hassani-Mehraban, A.; Creutzburg, S.; van Heereveld, L.; Kormelink, R. Feasibility of Cowpea Chlorotic Mottle Virus-like Particles as Scaffold for Epitope Presentations. *BMC Biotechnol.*, **2015**, *15*, 80.
[48] Speir, J.A.; Munshi, S.; Wang, G.; Baker, T.S.; Johnson, J.E. Structures of the Native and Swollen Forms of Cowpea Chlorotic Mottle Virus Determined by X-Ray Crystallography and Cryo-Electron Microscopy. *Struct. Lond. Engl. 1993*, **1995**, *3*, 63–78.
[49] Burgess, J.; Motoyoshi, F.; Fleming, E.N. Structural Changes Accompanying Infection of Tobacco Protoplasts with Two Spherical Viruses. *Planta*, **1974**, *117*, 133–144.
[50] Wu, Y.; Li, J.; Yang, H.; Seoung, J.; Lim, H.-D.; Kim, G.-J.; Shin, H.-J. Targeted Cowpea Chlorotic Mottle Virus-Based Nanoparticles with Tumor-Homing Peptide F3 for Photothermal Therapy. *Biotechnol. Bioprocess Eng.*, **2017**, *22*, 700–708.
[51] Terradot, L.; Souchet, M.; Tran, V.; Giblot Ducray-Bourdin, D. Analysis of a Three-Dimensional Structure of Potato Leafroll Virus Coat Protein Obtained by Homology Modeling. *Virology*, **2001**, *286*, 72–82.
[52] Lamb, J.W.; Duncan, G.H.; Reavy, B.; Gildow, F.E.; Mayo, M.A.; Hay, R.T. Assembly of Virus-like Particles in Insect Cells Infected with a Baculovirus Containing a Modified Coat Protein Gene of Potato Leafroll Luteovirus. *J. Gen. Virol.*, **1996**, *77*, 1349–1358.
[53] Watson, J.D. The Structure of Tobacco Mosaic Virus. I. X-Ray Evidence of a Helical Arrangement of Sub-Units around the Longitudinal Axis. *Biochim. Biophys. Acta*, **1954**, *13*, 10–19.
[54] Namba, K.; Stubbs, G. Structure of Tobacco Mosaic Virus at 3.6 A Resolution: Implications for Assembly. *Science*, **1986**, *231*, 1401–1406.
[55] Saini, M.; Vrati, S. A Japanese Encephalitis Virus Peptide Present on Johnson Grass Mosaic Virus-like Particles Induces Virus-Neutralizing Antibodies and Protects Mice against Lethal Challenge. *J. Virol.*, **2003**, *77*, 3487–3494.
[56] Parker, L.; Kendall, A.; Stubbs, G. Surface Features of Potato Virus X from Fiber Diffraction. *Virology*, **2002**, *300*, 291–295.
[57] Lico, C.; Benvenuto, E.; Baschieri, S. The Two-Faced Potato Virus X: From Plant Pathogen to Smart Nanoparticle. *Front. Plant Sci.*, **2015**, *6*, 1009.
[58] Masarapu, H.; Patel, B.K.; Chariou, P.L.; Hu, H.; Gulati, N.M.; Carpenter, B.L.; Ghiladi, R.A.; Shukla, S.; Steinmetz, N.F. Physalis Mottle Virus-Like Particles as Nanocarriers for Imaging Reagents and Drugs. *Biomacromolecules*, **2017**, *18*, 4141–4153.
[59] Krishna, S.S.; Sastri, M.; Savithri, H.S.; Murthy, M.R. Structural Studies on the Empty Capsids of Physalis Mottle Virus. *J. Mol. Biol.*, **2001**, *307*, 1035–1047.
[60] Sánchez, F.; Sáez, M.; Lunello, P.; Ponz, F. Plant Viral Elongated Nanoparticles Modified for Log-Increases of Foreign Peptide Immunogenicity and Specific Antibody Detection. *J.*

519 *Biotechnol.*, **2013**, *168*, 409–415.
520 [61] Rioux, G.; Babin, C.; Majeau, N.; Leclerc, D. Engineering of Papaya Mosaic Virus
521 (PapMV) Nanoparticles through Fusion of the HA11 Peptide to Several Putative Surface-Exposed
522 Sites. *PLoS ONE*, **2012**, *7*, e31925.
523 [62] Thérien, A.; Bédard, M.; Carignan, D.; Rioux, G.; Gauthier-Landry, L.; Laliberté-Gagné,
524 M.-È.; Bolduc, M.; Savard, P.; Leclerc, D. A Versatile Papaya Mosaic Virus (PapMV) Vaccine
525 Platform Based on Sortase-Mediated Antigen Coupling. *J. Nanobiotechnology*, **2017**, *15*.
526 [63] Denis, J.; Majeau, N.; Acosta-Ramirez, E.; Savard, C.; Bedard, M.-C.; Simard, S.; Lecours,
527 K.; Bolduc, M.; Pare, C.; Willems, B.; Shoukry, N.; Tessier, P.; Lacasse, P.; Lamarre, A.; Lapointe,
528 R.; Lopez Macias, C.; Leclerc, D. Immunogenicity of Papaya Mosaic Virus-like Particles Fused to a
529 Hepatitis C Virus Epitope: Evidence for the Critical Function of Multimerization. *Virology*, **2007**,
530 *363*, 59–68.
531 [64] Carignan, D.; Thérien, A.; Rioux, G.; Paquet, G.; Gagné, M.-È.L.; Bolduc, M.; Savard, P.;
532 Leclerc, D. Engineering of the PapMV Vaccine Platform with a Shortened M2e Peptide Leads to an
533 Effective One Dose Influenza Vaccine. *Vaccine*, **2015**, *33*, 7245–7253.
534 [65] Yang, S.; Wang, T.; Bohon, J.; Gagné, M.-È.L.; Bolduc, M.; Leclerc, D.; Li, H. Crystal
535 Structure of the Coat Protein of the Flexible Filamentous Papaya Mosaic Virus. *J. Mol. Biol.*, **2012**,
536 *422*, 263–273.
537 [66] Babin, C.; Majeau, N.; Leclerc, D. Engineering of Papaya Mosaic Virus (PapMV)
538 Nanoparticles with a CTL Epitope Derived from Influenza NP. *J. Nanobiotechnology*, **2013**, *11*, 10.
539 [67] Tremblay, M.-H.; Majeau, N.; Gagné, M.-E.L.; Lecours, K.; Morin, H.; Duvignaud, J.-B.;
540 Bolduc, M.; Chouinard, N.; Paré, C.; Gagné, S.; Leclerc, D. Effect of Mutations K97A and E128A
541 on RNA Binding and Self Assembly of Papaya Mosaic Potexvirus Coat Protein. *FEBS J.*, **2006**,
542 *273*, 14–25.
543 [68] Donchenko, E.K.; Pechnikova, E.V.; Mishyna, M.Yu.; Manukhova, T.I.; Sokolova, O.S.;
544 Nikitin, N.A.; Atabekov, J.G.; Karpova, O.V. Structure and Properties of Virions and Virus-like
545 Particles Derived from the Coat Protein of Alternanthera Mosaic Virus. *PLoS ONE*, **2017**, *12*.
546 [69] Tyulkina, L.G.; Skurat, E.V.; Frolova, O.Y.; Komarova, T.V.; Karger, E.M.; Atabekov, I.G.
547 New Viral Vector for Superproduction of Epitopes of Vaccine Proteins in Plants. *Acta Naturae*,
548 **2011**, *3*, 73–82.
549 [70] Yang, C.-D.; Liao, J.-T.; Lai, C.-Y.; Jong, M.-H.; Liang, C.-M.; Lin, Y.-L.; Lin, N.-S.; Hsu,
550 Y.-H.; Liang, S.-M. Induction of Protective Immunity in Swine by Recombinant Bamboo Mosaic
551 Virus Expressing Foot-and-Mouth Disease Virus Epitopes. *BMC Biotechnol.*, **2007**, *7*, 62.
552 [71] DiMaio, F.; Chen, C.-C.; Yu, X.; Frenz, B.; Hsu, Y.-H.; Lin, N.-S.; Egelman, E.H. The
553 Molecular Basis for Flexibility in the Flexible Filamentous Plant Viruses. *Nat. Struct. Mol. Biol.*,
554 **2015**, *22*, 642–644.
555 [72] Yusibov, V.; Hooper, D.C.; Spitsin, S.V.; Fleysh, N.; Kean, R.B.; Mikheeva, T.; Deka, D.;
556 Karasev, A.; Cox, S.; Randall, J.; Koprowski, H. Expression in Plants and Immunogenicity of Plant
557 Virus-Based Experimental Rabies Vaccine. *Vaccine*, **2002**, *20*, 3155–3164.
558 [73] Yusibov, V.; Modelska, A.; Steplewski, K.; Agadjanyan, M.; Weiner, D.; Hooper, D.C.;
559 Koprowski, H. Antigens Produced in Plants by Infection with Chimeric Plant Viruses Immunize
560 against Rabies Virus and HIV-1. *Proc. Natl. Acad. Sci.*, **1997**, *94*, 5784–5788.
561 [74] Yusibov, V.; Mett, V.; Mett, V.; Davidson, C.; Musiychuk, K.; Gilliam, S.; Farese, A.;
562 Macvittie, T.; Mann, D. Peptide-Based Candidate Vaccine against Respiratory Syncytial Virus.
563 *Vaccine*, **2005**, *23*, 2261–2265.
564 [75] Brodzik, R.; Bandurska, K.; Deka, D.; Golovkin, M.; Koprowski, H. Advances in Alfalfa
565 Mosaic Virus-Mediated Expression of Anthrax Antigen in Planta. *Biochem. Biophys. Res.*
566 *Commun.*, **2005**, *338*, 717–722.
567 [76] Piazzolla, G.; Nuzzaci, M.; Tortorella, C.; Panella, E.; Natilla, A.; Boscia, D.; De Stradis,
568 A.; Piazzolla, P.; Antonaci, S. Immunogenic Properties of a Chimeric Plant Virus Expressing a
569 Hepatitis C Virus (HCV)-Derived Epitope: New Prospects for an HCV Vaccine. *J. Clin. Immunol.*,

2005, *25*, 142–152.
[77] Natilla, A.; Nemchinov, L.G. Improvement of PVX/CMV CP Expression Tool for Display of Short Foreign Antigens. *Protein Expr. Purif.*, **2008**, *59*, 117–121.
[78] Natilla, A.; Hammond, R.W.; Nemchinov, L.G. Epitope Presentation System Based on Cucumber Mosaic Virus Coat Protein Expressed from a Potato Virus X-Based Vector. *Arch. Virol.*, **2006**, *151*, 1373–1386.
[79] Damodharan, S.; Gujar, R.; Pattabiraman, S.; Nesakumar, M.; Hanna, L.E.; Vadakkuppattu, R.D.; Usha, R. Expression and Immunological Characterization of Cardamom Mosaic Virus Coat Protein Displaying HIV Gp41 Epitopes: HIV Gp41 Epitopes on CdMV Coat Protein. *Microbiol. Immunol.*, **2013**, *57*, 374–385.
[80] Arcangeli, C.; Circelli, P.; Donini, M.; Aljabali, A.A.A.; Benvenuto, E.; Lomonossoff, G.P.; Marusic, C. Structure-Based Design and Experimental Engineering of a Plant Virus Nanoparticle for the Presentation of Immunogenic Epitopes and as a Drug Carrier. *J. Biomol. Struct. Dyn.*, **2014**, *32*, 630–647.
[81] Fernández-Fernández, M.R.; Martínez-Torrecuadrada, J.L.; Casal, J.I.; García, J.A. Development of an Antigen Presentation System Based on Plum Pox Potyvirus. *FEBS Lett.*, **1998**, *427*, 229–235.
[82] Kalnciema, I.; Skrastina, D.; Ose, V.; Pumpens, P.; Zeltins, A. Potato Virus Y-Like Particles as a New Carrier for the Presentation of Foreign Protein Stretches. *Mol. Biotechnol.*, **2012**, *52*, 129–139.
[83] Rubino, L.; Stradis, A.D.; Russo, M.; Martelli, G.P. GENERATION OF VIRUS-LIKE PARTICLES IN PLANTS EXPRESSING THE CAPSID PROTEIN OF CYMBIDIUM RINGSPOT VIRUS. *J. Plant Pathol.*, **2011**, *93*, 751–756.
[84] Wen, A.M.; Shukla, S.; Saxena, P.; Aljabali, A.A.A.; Yildiz, I.; Dey, S.; Mealy, J.E.; Yang, A.C.; Evans, D.J.; Lomonossoff, G.P.; Steinmetz, N.F. Interior Engineering of a Viral Nanoparticle and Its Tumor Homing Properties. *Biomacromolecules*, **2012**, *13*, 3990–4001.
[85] Ganguly, R.; Wen, A.M.; Myer, A.B.; Czech, T.; Sahu, S.; Steinmetz, N.F.; Raman, P. Anti-Atherogenic Effect of Trivalent Chromium-Loaded CPMV Nanoparticles in Human Aortic Smooth Muscle Cells under Hyperglycemic Conditions in Vitro. *Nanoscale*, **2016**, *8*, 6542–6554.
[86] Sainsbury, F.; Sack, M.; Stadlmann, J.; Quendler, H.; Fischer, R.; Lomonossoff, G.P. Rapid Transient Production in Plants by Replicating and Non-Replicating Vectors Yields High Quality Functional Anti-HIV Antibody. *PloS One*, **2010**, *5*, e13976.
[87] Meshcheriakova, Y.; Durrant, A.; Hesketh, E.L.; Ranson, N.A.; Lomonossoff, G.P. Combining High-Resolution Cryo-Electron Microscopy and Mutagenesis to Develop Cowpea Mosaic Virus for Bionanotechnology. *Biochem. Soc. Trans.*, **2017**, *45*, 1263–1269.
[88] Draghici, H.-K.; Pilot, R.; Thiel, H.; Varrelmann, M. Functional Mapping of PVX RNA-Dependent RNA-Replicase Using Pentapeptide Scanning Mutagenesis—Identification of Regions Essential for Replication and Subgenomic RNA Amplification. *Virus Res.*, **2009**, *143*, 114–124.
[89] Kendall, A.; Bian, W.; Maris, A.; Azzo, C.; Groom, J.; Williams, D.; Shi, J.; Stewart, P.L.; Wall, J.S.; Stubbs, G. A Common Structure for the Potexviruses. *Virology*, **2013**, *436*, 173–178.
[90] Venter, P.A.; Dirksen, A.; Thomas, D.; Manchester, M.; Dawson, P.E.; Schneemann, A. Multivalent Display of Proteins on Viral Nanoparticles Using Molecular Recognition and Chemical Ligation Strategies. *Biomacromolecules*, **2011**, *12*, 2293–2301.
[91] Wen, A.M.; Ryan, M.J.; Yang, A.C.; Breitenkamp, K.; Pokorski, J.K.; Steinmetz, N.F. Photodynamic Activity of Viral Nanoparticles Conjugated with C60. *Chem. Commun.*, **2012**, *48*, 9044.
[92] Tinazzi, E.; Merlin, M.; Bason, C.; Beri, R.; Zampieri, R.; Lico, C.; Bartoloni, E.; Puccetti, A.; Lunardi, C.; Pezzotti, M.; Avesani, L. Plant-Derived Chimeric Virus Particles for the Diagnosis of Primary Sjögren Syndrome. *Front. Plant Sci.*, **2015**, *6*.
[93] Cruz, S.S.; Chapman, S.; Roberts, A.G.; Roberts, I.M.; Prior, D.A.; Oparka, K.J. Assembly and Movement of a Plant Virus Carrying a Green Fluorescent Protein Overcoat. *Proc. Natl. Acad.*

621 *Sci.*, **1996**, *93*, 6286–6290.
622 [94] Betti, C.; Lico, C.; Maffi, D.; D'Angeli, S.; Altamura, M.M.; Benvenuto, E.; Faoro, F.;
623 Baschieri, S. Potato Virus X Movement in Nicotiana Benthamiana: New Details Revealed by
624 Chimeric Coat Protein Variants. *Mol. Plant Pathol.*, **2012**, *13*, 198–203.
625 [95] Uhde-Holzem, K.; Schlösser, V.; Viazov, S.; Fischer, R.; Commandeur, U. Immunogenic
626 Properties of Chimeric Potato Virus X Particles Displaying the Hepatitis C Virus Hypervariable
627 Region I Peptide R9. *J. Virol. Methods*, **2010**, *166*, 12–20.
628 [96] Marconi, G.; Albertini, E.; Barone, P.; De, F.; Lico, C.; Marusic, C.; Rutili, D.; Veronesi, F.;
629 Porceddu, A. In Planta Production of Two Peptides of the Classical Swine Fever Virus (CSFV) E2
630 Glycoprotein Fused to the Coat Protein of Potato Virus X. *BMC Biotechnol.*, **2006**, 9.
631 [97] Bendahmane, M.; Koo, M.; Karrer, E.; Beachy, R.N. Display of Epitopes on the Surface of
632 Tobacco Mosaic Virus: Impact of Charge and Isoelectric Point of the Epitope on Virus-Host
633 Interactions. *J. Mol. Biol.*, **1999**, *290*, 9–20.
634 [98] Palmer, K.E.; Benko, A.; Doucette, S.A.; Cameron, T.I.; Foster, T.; Hanley, K.M.;
635 McCormick, A.A.; McCulloch, M.; Pogue, G.P.; Smith, M.L.; Christensen, N.D. Protection of
636 Rabbits against Cutaneous Papillomavirus Infection Using Recombinant Tobacco Mosaic Virus
637 Containing L2 Capsid Epitopes. *Vaccine*, **2006**, *24*, 5516–5525.
638 [99] Staczek, J.; Bendahmane, M.; Gilleland, L.B.; Beachy, R.N.; Gilleland, H.E. Immunization
639 with a Chimeric Tobacco Mosaic Virus Containing an Epitope of Outer Membrane Protein F of
640 Pseudomonas Aeruginosa Provides Protection against Challenge with P. Aeruginosa. *Vaccine*,
641 **2000**, *18*, 2266–2274.
642 [100] Clancy, K.W.; Melvin, J.A.; McCafferty, D.G. Sortase Transpeptidases: Insights into
643 Mechanism, Substrate Specificity, and Inhibition. *Biopolymers*, **2010**, *94*, 385–396.
644 [101] Hamamoto, H.; Sugiyama, Y.; Nakagawa, N.; Hashida, E.; Matsunaga, Y.; Takemoto, S.;
645 Watanabe, Y.; Okada, Y. A New Tobacco Mosaic Virus Vector and Its Use for the Systemic
646 Production of Angiotensin-I-Converting Enzyme Inhibitor in Transgenic Tobacco and Tomato.
647 *Biotechnol. Nat. Publ. Co.*, **1993**, *11*, 930–932.
648 [102] Fitchen, J.; Beachy, R.N.; Hein, M.B. Plant Virus Expressing Hybrid Coat Protein with
649 Added Murine Epitope Elicits Autoantibody Response. *Vaccine*, **1995**, *13*, 1051–1057.
650 [103] Sugiyama, Y.; Hamamoto, H.; Takemoto, S.; Watanabe, Y.; Okada, Y. Systemic Production
651 of Foreign Peptides on the Particle Surface of Tobacco Mosaic Virus. *FEBS Lett.*, **1995**, *359*, 247–
652 250.
653 [104] Turpen, T.H.; Reinl, S.J.; Charoenvit, Y.; Hoffman, S.L.; Fallarme, V.; Grill, L.K. Malarial
654 Epitopes Expressed on the Surface of Recombinant Tobacco Mosaic Virus. *Biotechnol. Nat. Publ.*
655 *Co.*, **1995**, *13*, 53–57.
656 [105] Casper, S.J.; Holt, C.A. Expression of the Green Fluorescent Protein-Encoding Gene from a
657 Tobacco Mosaic Virus-Based Vector. *Gene*, **1996**, *173*, 69–73.
658 [106] Muthamilselvan, T.; Lee, C.-W.; Cho, Y.-H.; Wu, F.-C.; Hu, C.-C.; Liang, Y.-C.; Lin, N.-S.;
659 Hsu, Y.-H. A Transgenic Plant Cell-Suspension System for Expression of Epitopes on Chimeric
660 Bamboo Mosaic Virus Particles. *Plant Biotechnol. J.*, **2016**, *14*, 231–239.
661 [107] Jiang, L.; Li, Q.; Li, M.; Zhou, Z.; Wu, L.; Fan, J.; Zhang, Q.; Zhu, H.; Xu, Z. A Modified
662 TMV-Based Vector Facilitates the Expression of Longer Foreign Epitopes in Tobacco. *Vaccine*,
663 **2006**, *24*, 109–115.
664 [108] Uhde, K.; Fischer, R.; Commandeur, U. Expression of Multiple Foreign Epitopes Presented
665 as Synthetic Antigens on the Surface of Potato Virus X Particles. *Arch. Virol.*, **2005**, *150*, 327–340.
666 [109] Zhang, Y.; Li, J.; Pu, H.; Jin, J.; Zhang, X.; Chen, M.; Wang, B.; Han, C.; Yu, J.; Li, D.
667 Development of *Tobacco Necrosis Virus A* as a Vector for Efficient and Stable Expression of
668 FMDV VP1 Peptides. *Plant Biotechnol. J.*, **2010**, *8*, 506–523.
669 [110] Sit, T.L.; Leclerc, D.; AbouHaidar, M.G. The Minimal 5' Sequence for in Vitro Initiation of
670 Papaya Mosaic Potexvirus Assembly. *Virology*, **1994**, *199*, 238–242.
671 [111] Savard, C.; Guérin, A.; Drouin, K.; Bolduc, M.; Laliberté-Gagné, M.-E.; Dumas, M.-C.;

Majeau, N.; Leclerc, D. Improvement of the Trivalent Inactivated Flu Vaccine Using PapMV Nanoparticles. *PloS One*, **2011**, *6*, e21522.
[112] Lebel, M.-È.; Chartrand, K.; Tarrab, E.; Savard, P.; Leclerc, D.; Lamarre, A. Potentiating Cancer Immunotherapy Using Papaya Mosaic Virus-Derived Nanoparticles. *Nano Lett.*, **2016**, *16*, 1826–1832.
[113] Leclerc, D. Plant Viral Epitope Display Systems for Vaccine Development. *Curr. Top. Microbiol. Immunol.*, **2014**, *375*, 47–59.
[114] Taylor, K.M.; Lin, T.; Porta, C.; Mosser, A.G.; Giesing, H.A.; Lomonossoff, G.P.; Johnson, J.E. Influence of Three-Dimensional Structure on the Immunogenicity of a Peptide Expressed on the Surface of a Plant Virus. *J. Mol. Recognit. JMR*, **2000**, *13*, 71–82.
[115] Petukhova, N.; Gasanova, T.; Ivanov, P.; Atabekov, J. High-Level Systemic Expression of Conserved Influenza Epitope in Plants on the Surface of Rod-Shaped Chimeric Particles. *Viruses*, **2014**, *6*, 1789–1800.
[116] Petukhova, N.V.; Gasanova, T.V.; Stepanova, L.A.; Rusova, O.A.; Potapchuk, M.V.; Korotkov, A.V.; Skurat, E.V.; Tsybalova, L.M.; Kiselev, O.I.; Ivanov, P.A.; Atabekov, J.G. Immunogenicity and Protective Efficacy of Candidate Universal Influenza A Nanovaccines Produced in Plants by Tobacco Mosaic Virus-Based Vectors. *Curr. Pharm. Des.*, **2013**, *19*, 5587–5600.
[117] Chen, T.-H.; Chen, T.-H.; Hu, C.-C.; Liao, J.-T.; Lee, C.-W.; Liao, J.-W.; Lin, M.-Y.; Liu, H.-J.; Wang, M.-Y.; Lin, N.-S.; Hsu, Y.-H. Induction of Protective Immunity in Chickens Immunized with Plant-Made Chimeric Bamboo Mosaic Virus Particles Expressing Very Virulent Infectious Bursal Disease Virus Antigen. *Virus Res.*, **2012**, *166*, 109–115.
[118] Marusic, C.; Rizza, P.; Lattanzi, L.; Mancini, C.; Spada, M.; Belardelli, F.; Benvenuto, E.; Capone, I. Chimeric Plant Virus Particles as Immunogens for Inducing Murine and Human Immune Responses against Human Immunodeficiency Virus Type 1. *J. Virol.*, **2001**, *75*, 8434–8439.
[119] Cerovska, N.; Hoffmeisterova, H.; Moravec, T.; Plchova, H.; Folwarczna, J.; Synkova, H.; Ryslava, H.; Ludvikova, V.; Smahel, M. Transient Expression of Human Papillomavirus Type 16 L2 Epitope Fused to N- and C-Terminus of Coat Protein of Potato Virus X in Plants. *J. Biosci.*, **2012**, *37*, 125–133.
[120] Brennan, F.R.; Jones, T.D.; Longstaff, M.; Chapman, S.; Bellaby, T.; Smith, H.; Xu, F.; Hamilton, W.D.O.; Flock, J.-I. Immunogenicity of Peptides Derived from a Fibronectin-Binding Protein of S. Aureus Expressed on Two Different Plant Viruses. *Vaccine*, **1999**, *17*, 1846–1857.
[121] Dalsgaard, K.; Uttenthal, A.; Jones, T.D.; Xu, F.; Merryweather, A.; Langeveld, J.P.M.; Boshuizen, R.S.; Porta, C.; Vela, C.; Casal, J.I.; Meloen, R.H.; Rodgers, P.B. Plant-Derived Vaccine Protects Target Aniinals against a Viral Disease. **1997**, *15*, 5.
[122] Langeveld, J.P.M.; Brennan, F.R.; Martínez-Torrecuadrada, J.L.; Jones, T.D.; Boshuizen, R.S.; Vela, C.; Casal, J.I.; Kamstrup, S.; Dalsgaard, K.; Meloen, R.H.; Bendig, M.M.; Hamilton, W.D.O. Inactivated Recombinant Plant Virus Protects Dogs from a Lethal Challenge with Canine Parvovirus. *Vaccine*, **2001**, *19*, 3661–3670.
[123] McLain, L.; Durrani, Z.; Wisniewski, L.A.; Porta, C.; Lomonossoff, G.P.; Dimmock, N.J. Stimulation of Neutralizing Antibodies to Human Immunodeficiency Virus Type 1 in Three Strains of Mice Immunized with a 22 Amino Acid Peptide of Gp41 Expressed on the Surface of a Plant Virus. *Vaccine*, **1996**, *14*, 799–810.
[124] Denis, J.; Acosta-Ramirez, E.; Zhao, Y.; Hamelin, M.-E.; Koukavica, I.; Baz, M.; Abed, Y.; Savard, C.; Pare, C.; Lopez Macias, C.; Boivin, G.; Leclerc, D. Development of a Universal Influenza A Vaccine Based on the M2e Peptide Fused to the Papaya Mosaic Virus (PapMV) Vaccine Platform. *Vaccine*, **2008**, *26*, 3395–3403.
[125] Kumar, S.; Ochoa, W.; Singh, P.; Hsu, C.; Schneemann, A.; Manchester, M.; Olson, M.; Reddy, V. Tomato Bushy Stunt Virus (TBSV), a Versatile Platform for Polyvalent Display of Antigenic Epitopes and Vaccine Design. *Virology*, **2009**, *388*, 185–190.
[126] Joelson, T.; Akerblom, L.; Oxelfelt, P.; Strandberg, B.; Tomenius, K.; Morris, T.J.

Presentation of a Foreign Peptide on the Surface of Tomato Bushy Stunt Virus. *J. Gen. Virol.*, **1997**, *78 (Pt 6)*, 1213–1217.
[127] Hema, M.; Nagendrakumar, S.B.; Yamini, R.; Chandran, D.; Rajendra, L.; Thiagarajan, D.; Parida, S.; Paton, D.J.; Srinivasan, V.A. Chimeric Tymovirus-like Particles Displaying Foot-and-Mouth Disease Virus Non-Structural Protein Epitopes and Its Use for Detection of FMDV-NSP Antibodies. *Vaccine*, **2007**, *25*, 4784–4794.
[128] Jegerlehner, A.; Storni, T.; Lipowsky, G.; Schmid, M.; Pumpens, P.; Bachmann, M.F. Regulation of IgG Antibody Responses by Epitope Density and CD21-Mediated Costimulation. *Eur. J. Immunol.*, **2002**, *32*, 3305–3314.
[129] Roldão, A.; Mellado, M.C.M.; Castilho, L.R.; Carrondo, M.J.; Alves, P.M. Virus-like Particles in Vaccine Development. *Expert Rev. Vaccines*, **2010**, *9*, 1149–1176.
[130] Townsend, A.; Bodmer, H. Antigen Recognition by Class I-Restricted T Lymphocytes. *Annu. Rev. Immunol.*, **1989**, *7*, 601–624.
[131] Bachmann, M.F.; Lutz, M.D.B.; Layton, G.T.; Harris, S.J.; Fehr, T.; Rescigno, M.; Ricciardi-Castagnoli, P. Dendritic Cells Process Exogenous Viral Proteins and Virus-like Particles for Class I Presentation to CD8+ Cytotoxic T Lymphocytes. *Eur. J. Immunol.*, **1996**, *26*, 2595–2600.
[132] Fifis, T.; Gamvrellis, A.; Crimeen-Irwin, B.; Pietersz, G.A.; Li, J.; Mottram, P.L.; McKenzie, I.F.C.; Plebanski, M. Size-Dependent Immunogenicity: Therapeutic and Protective Properties of Nano-Vaccines against Tumors. *J. Immunol.*, **2004**, *173*, 3148–3154.
[133] Bachmann, M.F.; Zinkernagel, R.M. NEUTRALIZING ANTIVIRAL B CELL RESPONSES. *Annu. Rev. Immunol.*, **1997**, *15*, 235–270.
[134] Bachmann, M.; Rohrer, U.; Kundig, T.; Burki, K.; Hengartner, H.; Zinkernagel, R. The Influence of Antigen Organization on B Cell Responsiveness. *Science*, **1993**, *262*, 1448–1451.
[135] Jobsri, J.; Allen, A.; Rajagopal, D.; Shipton, M.; Kanyuka, K.; Lomonossoff, G.P.; Ottensmeier, C.; Diebold, S.S.; Stevenson, F.K.; Savelyeva, N. Plant Virus Particles Carrying Tumour Antigen Activate TLR7 and Induce High Levels of Protective Antibody. *PLOS ONE*, **2015**, *10*, e0118096.