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Plant Production of Vaccine Against HPV: A New Perspectives

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1. Introduction

Infection by human papilloma virus (HPV) attracted attention in connection with cervical cancer in humans (zur Hausen, 1996). HPV type 16 alone accounts for approximately 50% of all cases of cervical cancer. The virus icosahedral capsid is composed of the L1 major and the L2 minor proteins. L1 alone has the capacity to self-assemble into virus-like particles (VLPs) without participation of L2 or other proteins. Because of similar immunogenicity compared to infectious virions, VLPs can be produced and used as a safe prophylactic vaccine against viral transmission of cervical cancer. During recent years two highly efficient VLP-based HPV vaccines (e.g. Gardasil, Merck MSD and Cervarix, GlaxoSmithKline) become available. For commercial production of vaccines and recombinant therapeutics, plants are often considered as a cost-effective alternative with several benefits. Firstly, production in plants can be easily scaled up in the case of acute demand for production and secondly, produced proteins are unlikely to be contaminated by human or animal pathogens, toxins and oncogenic sequences. Moreover, plants provide a convenient environment for protein expression and storage including the possibility of direct administration as edible vaccine if expressed in the appropriate plant tissue. In this article, we report recent promising advances in the production of prophylactic and therapeutic vaccines against HPV by expression of the relevant antigens in plants, and discuss future prospects for the use of such vaccines.

2. HPV vaccines

2.1 Structure of HPV capsid and neutralizing epitopes on its surface

Papilloma viruses (PVs) are small tissue specific double-stranded DNA tumor viruses, classified in the taxonomic family of *Papillomaviridae*. The Human Papilloma Viruses (HPVs) are phylogenetically closely related with similar biological properties among each other and with animal papillomaviruses that are host-specific to other vertebrates including amphibians, reptiles, birds and a variety of land and sea mammals. Animal PVs have been studied either as disease carrier and transmitters in animals or as models of human PV infection (Brandsma et al.; 1994; Campo 1997). Due to etiological connection with the high-rate mortality cervical cancer, the main attention is concentrated on the genital high-risk HPV types 16, 18, 33 and 58 as the leading cause of cancer (Munoz et al., 2003). The low-risk types 6 and 11 are associated with benign epithelial papillomas or warts that occur in 5–12% of normal women (Heim et al,

1995), however, HPV6 and 11 are the most commonly diagnosed to coinfect and comorbid in immunosuppressed individuals with malignant HPVs (Jay & Moscicki, 2000).

Papillomavirus infection induces type-specific immune response, directed mainly against the major capsid protein L1, rather than L2 minor protein, which also participates in the formation of the shell of native HPV particles. The viral capsid is primarily composed of 360 copies of L1 protein organized into 72 L1-pentamers (capsomeres) and associated with 12 or more copies of the minor L2 protein. When expressed in various recombinant system, L1 readily self-assembles, even in the absence of L2, into noninfectious virus-like particles (VLPs). VLPs are also organized into 72 capsomeres of L1 protein (Fig. 1C) and are immunologically indistinguishable from the native virions (Fig. 1B). The 504-residue of the L1 protein chain contains 12 β -strands, 6 loops, and 5 α -helices that form “jelly roll” β -sandwich (Fig. 1D).

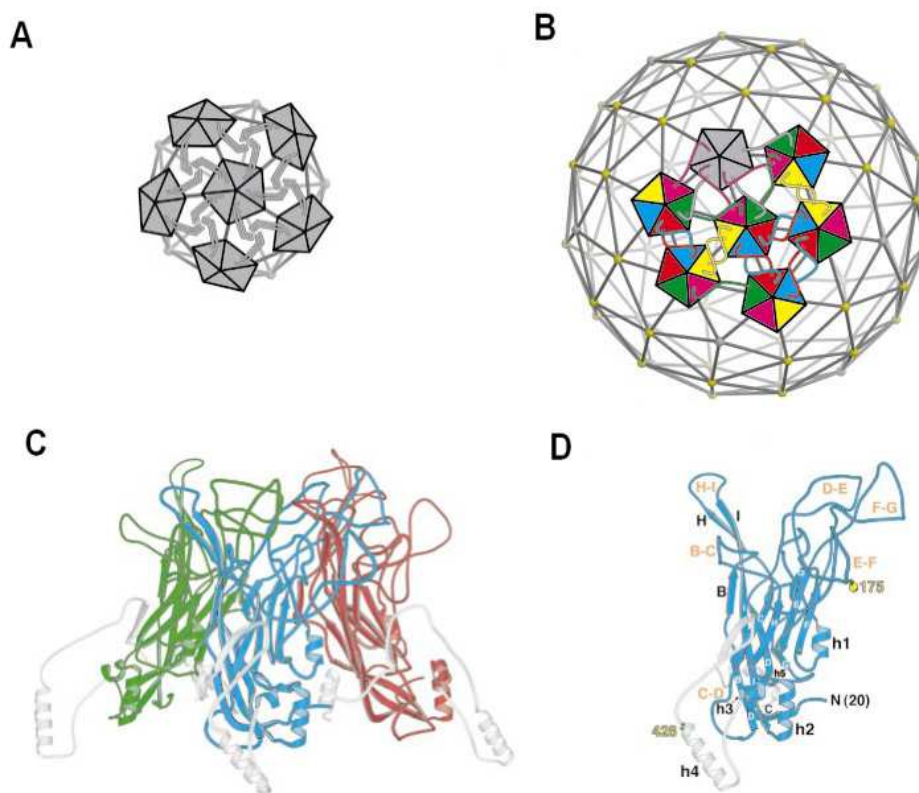


Fig. 1. Structure of the HPV capsid. (A) Small ($T=1$) VLPs derived from HPV-L1; (B) Full-size ($T=7$) Papillomavirus particles; (C) HPV16 capsomere (L1-pentamer) in the conformation found in small VLPs (Chen et al., 2000). Three subunits are shown in green, blue and red. The C'-terminal arms are in gray, to indicate that these portions of the subunit rearrange when L1-pentamers assemble into virions or into full-sized capsids; (D) The 3D structure of human papillomavirus 16 L1 monomer (HPV16L1). Secondary structural elements are labeled, with letters B–J for β -strands and h1–h5 for the 5 α -helices. Loops between strands are labeled B–C, C–D, etc. The first and the last residues are marked N (20) and C (474), respectively. The two cysteines that participate in the interpentamer disulphide bonding within the virion or in the virion-sized particles are shown in yellow, together with their residue numbers, 175 and 428. (Modis et al., 2002)

Some residues in the L1 protein, such as Asp202, Cys175, and Cys428 of HPV16 L1, are very important for VLP formation (Slupetzky et al., 2001), however some residues at the C'-

terminus can be truncated and replaced with heterologous epitopes or short polypeptides up to 60 amino acids without disrupting the assembly of VLPs (Paintsil et al., 1996; Müller et al., 1997; Paz De la Rosa et al., 2009). These chimeric VLPs (cVLPs) can induce strong immune responses against not only the inserted epitopes or polypeptides, but also the VLP shell (Freyschmidt et al., 2004; Varsani et al., 2003a; Xu et al., 2006). Experiments *in vitro* showed that a short N'-terminal segment of the L1 polypeptide chain acts as a switch between T=7 (72 L1-pentamer) and T=1 (12 L1-pentamer) VLP assembly (Fig. 1B) (Chen et al., 2000).

Structural analysis has revealed that BC, EF, FG, HI and DE hyper variable loops of L1 (Fig. 1D) are exposed on the outer surface of the L1-pentamer and form a broad pocket, which participate in receptor interaction. The rim of the pentamer pocket is extremely variable contrary to its floor. With a few exceptions, all HPV-neutralizing monoclonal antibodies analyzed so far are type-specific and recognize conformational epitopes within these surface-exposed hyper variable loops (Pastrana et al., 2004; Fleury et al., 2006). HPV16 and HPV11 VLPs epitopes recognized by neutralizing mAbs are shown on Fig. 2 (Rodén et al., 1997a; Ludmerer et al., 1997).

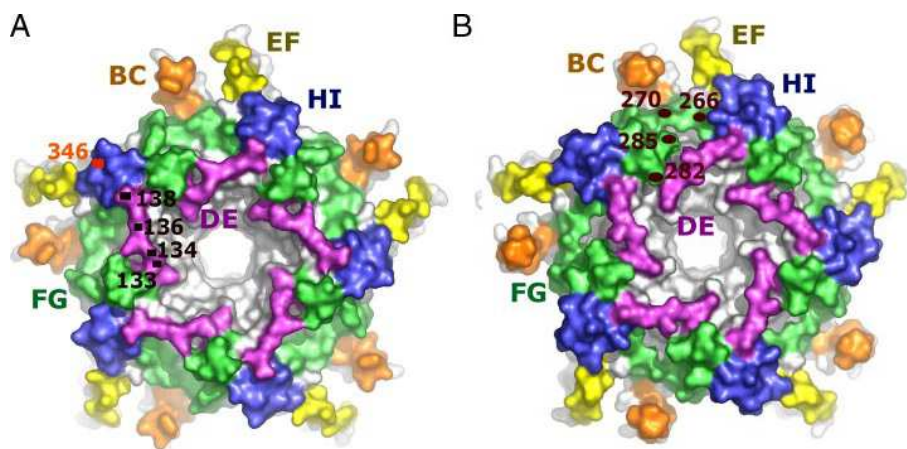


Fig. 2. Surface of the HPV 11 and 16 L1 pentamers. The surface loops are colored differently on the surface: BC (orange), DE (violet), EF (yellow), FG (green), and HI (slate). (A) HPV11; (B) HPV16. Neutralizing epitopes are schematically indicated on pentamers. Black squares, H11.F1 and H11.G5; orange square, H11.H3; red ellipse, H16.V5 and H16.E70. (Bishop et al., 2007)

2.2 HPV vaccines of the first generation. The need for second-generation vaccines

The first applied strategy for HPV prophylactic vaccination aimed on induction of neutralizing antibodies against L1 capsid proteins. Currently two vaccines composed of HPV-L1 self assembled into VLPs have been developed and are commercially available, a Glaxo-Smith Kline bivalent vaccine Cervarix and a Merck, Sharp and Dohme quadrivalent vaccine Gardasil (also marketed as Gardasil or Silgard). Bivalent vaccine Cervarix protects primarily against HPV 16 and 18 that are produced separately using a recombinant Baculovirus expression system. Purified VLPs of each HPV type are formulated with the AS04 adjuvant system composed of aluminium hydroxide and 3-O-desacyl-4-monophosphoryl lipid A (MPL). Cervarix is stored as a sterile turbid liquid suspension for intramuscular injection at 2-8°C with a proposed shelf life 3 years. Gardasil is combination of *Saccharomyces cerevisiae* produced HPV 16, 18, 6 and 11 VLPs and has amorphous

aluminum hydroxy-phosphate sulfate (AAHS) as an adjuvant. Administrations of vaccine to HPV naive women have demonstrated almost complete protection against infection by the targeted HPV types. Number of medical trials confirmed the safety of currently used vaccines, their efficiency to induce immune response was equal or even higher than that observed during a natural infection and to maintain protection for 5 and 7.3 years in the case of Gardasil and Cervarix, respectively. (McCormack & Joura, 2010; Schwarz, 2009). The induction of HPV neutralizing antibody reaches maximum titers at month 7 after the first vaccination, i.e. 1 month after administration of the last, third dose. Then the titer decline until month 24 and remain stable thereafter (Dillner et al., 2007). Interestingly, the immune response to the Gardasil, the tetravalent vaccine, inversely correlates with the age. The induction of neutralizing antibody is higher in males and females aged 10 to 15 than in those of an age group 16 to 23 year (Villa et al., 2005). For sexually active women in the general population the efficiency of vaccination is expected to be much lower. Moreover, total period of protection afforded by vaccination is not yet known (Wright et al., 2006).

Along with the questions who should be vaccinated and at what age the vaccination is the most effective (Villa, 2011), issues related to vaccine formulation, production and administration have to be also adequately resolved (Schiller & Nardelli-Haeffliger, 2006). Firstly, multispecific VLPs based vaccines are expensive to manufacture, since they are produced in eukaryotic cell culture and extensively purified. Both commercially available vaccines require 3 intramuscular injections over a 6 months course to achieve prophylaxis, and the direct vaccine cost excluding administration and medical visits is about USD 375 per recipient in the United States (Armstrong, 2010), reflecting also costly cold chain handling, distribution and storage of a vaccine. More to it, the protection with current vaccines is predominately type specific, and so they are not expected to protect against the almost 30% of cervical cancers that are HPV16 and 18 independent. Last, but not least, therapeutic activity against external genital lesions has not been reported (Villa, 2011).

Suitable vaccine formulations ensuring VLPs stability in liquid were established for both Gardasil (Shank-Retzlaff et al., 2006) and Cervarix (Le Tallec et al., 2009) vaccines. However during production fibrous aggregates of VLPs were occasionally observed. This is why marketed solutions of concentrated VLPs are protected against aggregation by high concentrations of salt. Many other factors including excipients maintaining pH, storage stability, temperature and time effectively influence VLPs. For example at high pH and low salt concentration, VLPs disassemble into capsomeres with weaker immune response than VLPs (Thönes et al., 2008) and denatured L1 protein does not induce any virus neutralizing antibody response (Shinje et al., 1991). Stabilization of protein antigens against aggregation and degradation in solution is important for antigen purification as well as vaccine formulation. Lyophilized VLPs might be an alternative to aqueous droplets for mucosal delivery in a powder formulation of a vaccine (Schiller & Nardelli-Haeffliger, 2006; Gerber et al., 2001). Papillomavirus virions are resistant to desiccation and retain their native conformation after freeze-drying (Roden et al., 1997b; Šmídková et al., 2010). However, both commercial vaccines contain an alum adjuvant, which during freeze-drying extensively coagulate into gel-like consistency, the state suspected to inhibit the release of antigen upon rehydration (Maa et al., 2006). The loss of efficient immune induction has been reported after freezing or freeze-drying of VLPs in aqueous solutions (Shinje et al., 1991). The use of non-ionic stabilizers, such as methylcellulose (Corbett et al., 2010) or polysorbate PS80 (Shi et al., 2005) helps to avoid this effect. Another solution could be the use of another, physically and chemically more suitable adjuvant(s), but this strategy would further add to the cost of vaccine

and principally could raise safety concerns. This is why convenient dry formulation, meaning at least longer shelf life at higher temperatures for marketing purposes is currently missing. The examples of the alternative adjuvants to alum are discussed in Section 3.

Several alternative methods of vaccine needle delivery have been developed. The tattoo delivery of DNA has been found as a cost-effective method that may be used in laboratory conditions when more rapid and more robust immune responses are required (Pokorná et al., 2009). The second method, a novel dry-coated densely packed Macroflux® microprojections array skin patch, was established as an alternate delivery system to intramuscular injection for delivering an alum adjuvanted vaccine Gardasil (Shi et al., 2005).

Besides the effort to improve the formulation, storing properties and methods of vaccine delivery, the first generation of HPV prophylactic vaccines based on IM delivery of HPV antigens of the recombinant VLPs reached its limits with two vaccines produced in baculovirus and yeast cell culture on the market. In parallel with development of these vaccines there were successful attempts to produce recombinant VLPs also in various plant expression systems (see Section 2.4). Plant expression of HPV vaccines pursues several objectives as the cost efficiency, production of uncontaminated safe product, scale up and potential edible vaccine format. Currently HPV VLPs are readily produced in several plant systems with newly developed technologies for industrial large-scale transient production that can successfully compete with current production of L1 based VLPs vaccines. Nevertheless the cost of clinical trials, of approval and implementation of new technologies is so inhibitory high that there will be hardly any HPV vaccine of the first generation produced in plants that will reach the market. More realistic expectation is that developed plant technologies will compete with established procedures for production of new, improved generation of vaccines, when their advantages will surplus currently marketed vaccines and expected market success will justify the cost of clinical trials and production.

2.3 Vaccines of the second generation

2.3.1 L2 based vaccines

The limitation of current vaccines is that neutralizing antibodies induced by immune response to L1 based VLPs are type-restricted (Wakabayashi et al., 2002). Addition of other HPV types VLPs to the existing vaccines would be viable approach only in the case of a small increase in the overall cost of the vaccination scheme. In contrast to L1, Pastrana et al. (2005) showed in *in vitro* assays that antigen determinant present on the N'-end of L2 coat protein can induce broad range of cross neutralizing antibodies in mouse and rabbit sera. These results raise the possibility that a monovalent vaccine could protect against a broad range of genital HPV types. Unfortunately, neutralizing antibody titers against the papillomavirus type from which the L2 vaccine was derived were generally higher than the titers against heterologous types and lower than those induced by L1 VLPs (Pastrana et al., 2005; Roden et al., 2000). This can be avoided by construction of concatenated multiple L2 fusion proteins derived from known cross-protective epitopes of several divergent HPV types. These fusion proteins, consisting of L2 epitopes of 3-22 HPV types, were able to induce high neutralizing antibody titers against all heterologous HPVs tested at a level comparable to that induced by L1 VLPs. In addition, L2 polypeptides have the advantage that they could be produced in *E. coli*, and therefore manufacturing would be easier and cheaper in comparison to production of VLPs. The most promising approach of the non-VLPs second generation of HPV vaccines includes L1 capsomeres and L2 protein (Stanley, 2010).

2.3.2 Therapeutical vaccines

The therapeutic vaccines reduce or eradicate existing disease or infections by targeting cells expressing tumor-associated or tumor-specific antigens on their surface (Ma et al., 2010). There are many different types of therapeutic vaccine candidates based on viral gene peptides and proteins (Xie et al., 2011; Kenter et al., 2008; Melief et al., 2007; Fiander et al., 2006), DNA (Alvarez-Salas et al.; 2008, Sheets et al., 2003) and various viral and bacterial vectors (Brandsma et al, 2009, Davison et al, 2003). They all aim to induce specific cell-mediated immunity and in most cases the targets are the E6 and E7 proteins. Whereas L1 and L2 are expressed only in terminally differentiated keratinocytes, E6 and E7 are constitutively expressed at all layers of epithelium-infected cells (Fig. 3).

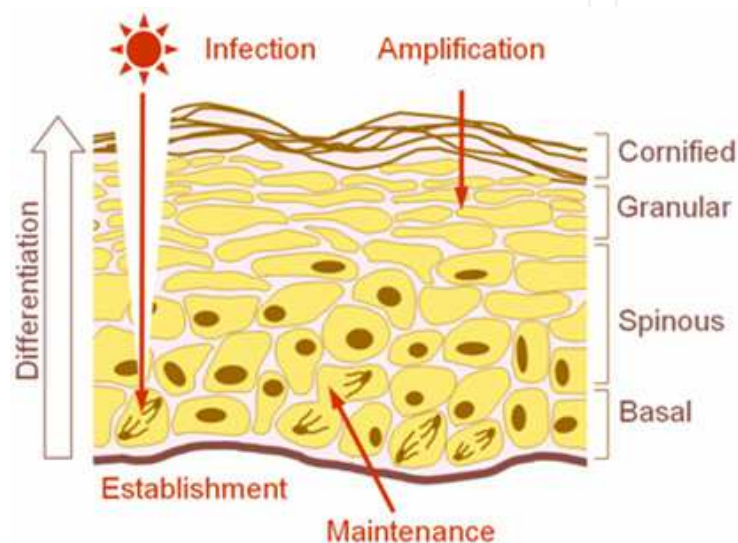


Fig. 3. Cervical stratified squamous epithelial cell architecture and the expression of HPV proteins after infection. Daughter cells of epithelial stem cells divide along the basement membrane and then mature vertically through the epithelium without further division. After introduction of HPV into stem cells in the basal layer of the epithelium, expression of viral non-structural proteins E1-E7 occurs. Under the regulation of these proteins, the dividing-cell population expands vertically and epithelial cell differentiation is delayed and is less complete. Viral proteins are expressed sequentially with differentiation as shown, and mature virions are produced only in the most superficial layers of the epithelium. Intraepithelial antigen-presenting cells (APCs) are depleted in the HPV-infected epithelium. (<http://www.ircm.qc.ca/LARECHERCHE/axes/Biologie/Virologie/Pages/Projets.aspx>)

E6 and E7 bind p53 and pRB human tumor suppressor genes (Duensing et al., 2000). These oncoproteins are involved in the malignant transformation of HPV-infected cells and are thought to be required for continued tumor growth. They are the primary targets of therapeutic vaccines, most of which have been designed to treat later stages of the disease. The E1 and E2 proteins are necessary for HPV replication within the cell before the virus is integrated into the host DNA (Doorbar et al., 1991). Because E1 and E2 are expressed already early in the progress of at HPV infection and at higher levels than E6 and E7, they may be the best targets for a therapeutic vaccine designed to treat early stages of the disease, such as low-grade dysplasia (Carvajal et al., 2007). Many candidate vaccines with therapeutic potential are currently tested in ongoing trials; however, there is low expectation

that any of the current therapeutic vaccines will have a substantial public health impact in the near future (Ma et al., 2010).

2.4 Vaccine production in plants

2.4.1 Posttranslational modification of the therapeutic proteins in plants

Therapeutic recombinant proteins are produced in many hosts from prokaryotes to human cells. When the protein of interest is of eukaryotic origin, one of the production objectives besides yield, solubility and stability is a posttranslational modifications (PTM) required for structural integrity and biological activity of the protein. Microbial expression systems are generally used for expression of simple proteins, because, PTM, including signal peptide cleavage, propeptide processing, protein folding, disulfide bond formation and glycosylation, might not be achieved in prokaryotes. Contrary to prokaryotes, plants are capable of PTM as other higher eukaryotes for safe and low cost production biologically active proteins (Dieryck et al., 1997; Ma et al., 1995). The correct folding and assembly of plant-produced antibody molecules, which requires interactions with several chaperones and with processing and glycosylation enzymes, illustrates that most co- and posttranslational events are similar in plants and mammals (Table 1).

Protein glycosylation is assumed as the most important PTM with significant effects on protein folding, conformation, distribution, stability and activity. In plant cells, as in other eukaryotic cells, N-glycosylation starts during co-translation in ER, when an oligosaccharide precursor is added to Asn residues that is constituent of the N-glycosylation-specific sequences Asn-X-Ser/Thr. The differences in the maturation of plant and mammalian N-glycans appear during the late processing in Golgi apparatus, when core alpha(1,6)-linked fucose residues and terminal sialic acid are attached in mammals, whereas beta(1,2)-xylose and core alpha(1,3)-fucose residues in plants (Fig.4).

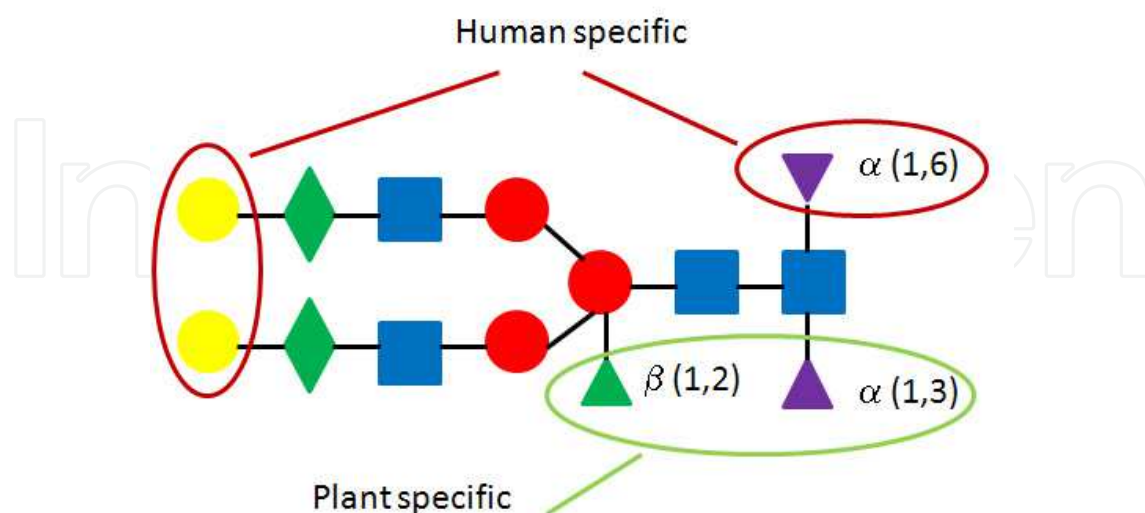


Fig. 4. N-glycan structures in human and plant. N-glycosylation patterns processed in plant cells differ from those of mammal

| Posttranslational modification | Location of the reaction in animal cell | Identification in plants and location |
|--|---|---|
| Glycosylation | | |
| O-glycosylation | Secretory pathway, nucleus and cytosol | Yes. Sugar addition on Hyp, Ser, Thr |
| N-glycosylation | Secretory pathway | Yes. Minor differences in modification |
| Glycation | | Yes |
| Proteoglycan | Secretory pathway | Yes |
| Attachment of fatty acids | | |
| S-acylation | Cytosol | Yes |
| N-myristoylation | Cytosol | Yes |
| Prenylation | Cytosol | Yes |
| Glypiation | Cytosol | Yes |
| Cholesterol link | Cytosol | Not identified. |
| Attachment of ions | | |
| Phosphorylation | Cytosol, secretory pathway | Yes. No mannose-6-phosphate in plant N-glycans |
| Sulfatation | Secretory pathway | Not identified in secretory pathway |
| Gamma-carboxylation | ER | Not identified. |
| Proteolysis | | |
| Cleavage of signal peptide | ER | Yes. ER |
| Cleavage of propeptide | ER/Golgi | Yes. ER, Golgi, vacuole |
| Other posttranslational modifications | | |
| Hydroxylation | ER | Yes. ER, Golgi |
| Cross-linking modifications | ER | Yes. ER, cytosol |
| Acetylation | Secretory pathway and cytosol | Yes. Function not yet understood in plants |
| Oligomerization | ER | Yes. ER |
| Selenoprotein | Cytosol | Not identified in higher plants, described in <i>C. reinhardtii</i> |
| Deamidation | Intra- and extracellular | Yes |
| Oxidation | Intra- and extracellular | Yes |

Table 1. Protein modifications in plant and animal cells. (Gomord & Faye, 2004, modified)

Plant specific residues were described to be constituents of the glycol-epitopes of some plant allergens, showing IgE binding and causing mediator release by human basophils (van Ree et al., 2000). Moreover, the injection of a plant glycoproteins or plant-made antibodies containing plant-specific N-glycans was found to elicit production of antibodies specific for beta(1,2)-xylose and alpha(1,3)-fucose-containing glyco-epitopes in most laboratory mammals and non-allergic human blood donors. Their presence may induce a rapid immune clearance of plant-glycosylated therapeutics from the blood stream (Bardor et al., 2003). One strategy to prevent the addition of immunogenic glycans is to store therapeutic proteins carrying KDEL signal within ER (Ko et al., 2003). Second strategy is based on the inhibition of Golgi glycosyltransferases of plants. The moss *Physcomitrella patens* is the only known plant with high frequency of homologous recombination, thus allowing relatively easy knocking out of target genes. The knocking out of alpha(1,3)-fucosyltransferase and beta(1,2)-xylosyltransferase genes in the moss *Physcomitrella patens* prevents the production of plant-specific glyco-epitopes without affecting the secretion of the protein (Koprivova et al., 2004). Third attractive strategy to “humanize” plant N-glycans is expressing of mammalian glycosyltransferases in plants. Like N-glycosylation, O-glycosylation is important for protein function. Surprisingly, little attention is paid so far to O-glycosylation status of plant produced therapeutic proteins.

2.4.2 L1 based prophylactic vaccines and the yields of L1 produced in different plant systems

L1 when expressed in plants readily assemble into VLPs indistinguishable in size from baculovirus expressed VLPs in insect cells (Fig. 5). Sucrose sedimentation analysis also showed that there is a large amount of not, or only partially assembled molecules, presumably capsomeres (fractions 18–24), as well as other larger aggregates (fractions 1–8) when compared to the insect cell-produced protein (Maclean et al., 2007). L1VLPs can be produced either in transgenic plants stably transformed with an expression cassette or transiently using one of several available plant-virus derived expression systems. Initially published yields of L1 expression was low, in a range of 1% of the total soluble protein, which is far lower than industrial demand of more than 5% (Rybicky, 2010). For example, Warzecha et al. (2003) obtained approximately 20 ng HPV-11 L1 per g in transgenic potato tubers; Varsani et al., 2003b, 2006 obtained 4 ng HPV-16 L1 per g of leaf tissue in transgenic tobacco and approximately 40 ng per g of *Nicotiana benthamiana* leaves transiently transformed with a tobacco mosaic virus vector and Kohl et al. (2006) and Liu et al. (2005) achieved approximate yield of L1 at the range 0.05% TSP in transgenic tobacco.

Nevertheless, during passed years, necessary steps to improve L1 gene expression were recognized and applied. Firstly it was removal of the carboxy-terminal nuclear localization signal sequence (NLS) of L1 that has been shown to enhance expression in transgenic plants. Moreover, the results indicated that full-length L1 is localized essentially entirely within the nucleus (Fig. 6A), whereas cells that express truncated form of L1 in a diffuse pattern within entire cell (Fig. 6B.) (Warzecha et al., 2003). Transient expression of full-length L1 protein in cytoplasm of tobacco leaf cells after agroinfection was described by Šmídková et al. (2010) (Fig. 6C).

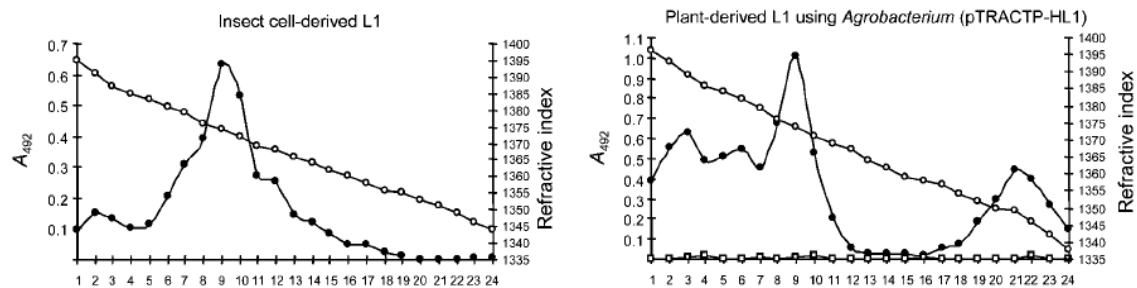


Fig. 5. Sedimentation analysis of transiently expressed HPV-16 L1 protein isolated from *Nicotiana benthamiana* plants (left panel) and insect cells (right panel) in sucrose gradient. The concentration of L1 in fractions was estimated by capture ELISA (closed circles); open squares – ELISA analysis of control, no expressing plants; open circles and right axis - refractive index. Fraction 1 corresponds to the bottom of the centrifuge tube (Maclean et al., 2007).

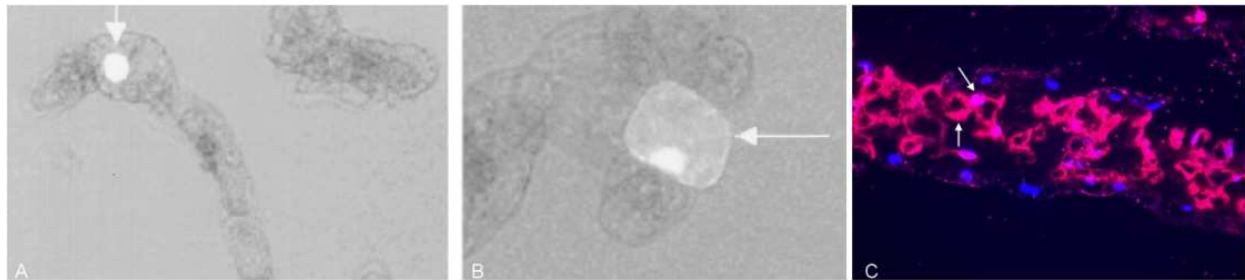


Fig. 6. Expression of HPV L1 proteins in tobacco cells. Transgenic tobacco expressing either full-length (A) or truncated (B) L1 coding sequence fused in frame at the carboxy terminus of GFP. Fluorescence appears as bright areas (Warzecha et al., 2003) (C) Localization of L1 in cryosections of *Nicotiana benthamiana* leaves agroinfected with plant TRV viral vector expressing optimized *L1h* gene (pTVL1h). The protein was detected by immunofluorescence microscopy at 400X magnification. Nuclei were counterstained with DAPI (Šmídková et al., 2010).

Biemelt et al. (2003) after failure to express L1 in transgenic potatoes, changed amino-acid codon usage of *L1* gene to that of potato (*L1p*) and of canonic human cells (*L1h*). Despite the presence of codons rarely used by plant cells and for plant genes atypical high GC content, expression of *L1h* led to high accumulation of L1 protein in transgenic plants, even higher than expression of *L1p*. The effect of increased GC content on expression efficiency of *L1* in plants has been confirmed by several groups (Maclean et al., 2007; Šmídková et al., 2010), nevertheless the published yields of L1 differ significantly one from each other, depending on the plant expression system used. For example the change of tobacco cultivar used for transgenic expression from *Nicotiana tabacum* cv. Xanthi to cv. SR1 allowed a 100-fold increase in expression of the native *L1* from viral isolate (Rybicky, 2010). The transient expression of the same *L1* using TMV plant viral expression vector resulted in further, one order of magnitude, increase of L1 over the expression in transgenic tobacco (Varsani et al., 2006). A strategy of optimization procedure for L1 transient expression described recently Šmídková et al. (2010). The results are summarized on Fig. 7, depicting A) The course of L1 expression from original virus isolate sequence (41% GC) and *L1p* sequences optimized for

Solanace plants expression (39% GC) and from canonic human cell optimized sequence *L1h* (61% GC); B) In three plants: tomato and *Nicotiana benthamiana* and *Nicotiana tabacum* tobaccos; C) In two plant viral expression vectors: PVX and TRV and by D) Two transformation methods: agroinfection (method in which a virus infects a host as a part of T-DNA of Ti plasmid carried by *Agrobacterium tumefaciens*) and *Agrobacterium* mediated transfer of expression cassette into cells after infiltration of leaves. The optimization of *L1h* transient expressed from pTRV vector after *Agrobacterium* infiltration of tomato host plants yielded 45 mg of VLPs per kilo of fresh leaves, the yield that is close to industrial acceptable level.

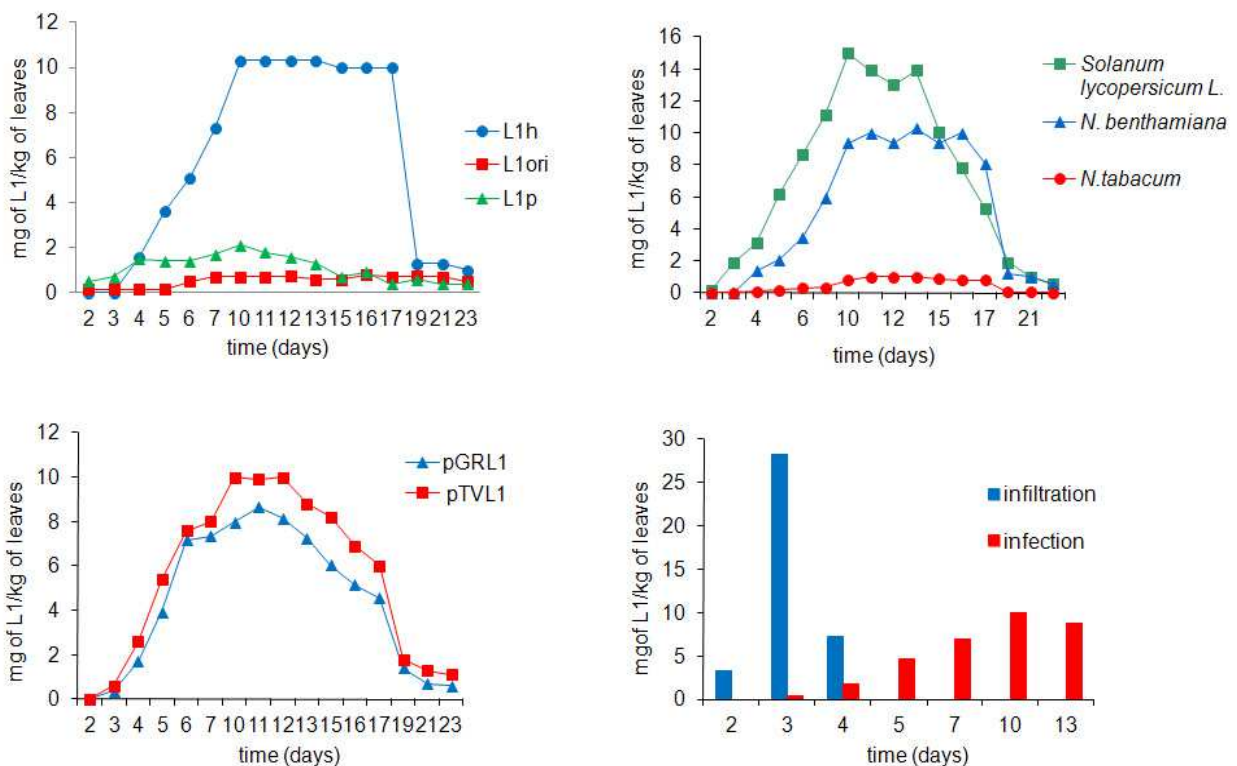


Fig. 7. Time course of the L1 transient expression. (A) Expression of original native HPV *L1* gene sequence *L1ori* and sequences optimized for expression either in plants *L1p* or in mammalian cells *L1h* from plant TRV-based vector pTV00; (B) Expression of *L1h* from pTV00 vector in leaves of *Nicotiana benthamiana*, *Nicotiana tabacum* and *Solanum lycopersicum* L. (tomato); (C) Comparison of the expression of *L1h* gene from plant tobamo virus (TRV) based expression vector pTV00 and from potato virus X (PVX) based vector pGR106; (D) The yield of L1protein reached by viral agroinfection or by *Agrobacterium* mediated transfer of expression cassette into leaf cells of *Nicotiana benthamiana* (Šmídková et al., 2010).

Nevertheless the highest yield (3 g/ kg fresh leaves; 24% TSP) of plant-produced L1 ever was achieved by Fernández-San Millán et al. (2008) when expressing unmodified *L1* sequence of primary HPV16 virus isolate from expression cassette stably integrated in tobacco chloroplasts genome. Expression in plant chloroplasts is an emerging system when compared to nuclear transformation. Plastid genome engineering offers many advantages over nuclear genome, including targeted recombination based integration, high levels of transgene expression due to high copy number, absence of epigenetic effects, transgene

containment via maternal inheritance and multi-gene expression in a single transformation event (Chebolu and Daniell, 2009). Recently the expressions of transgenes in plastids as high as 70% and 72% of total leaf protein was reported by Oey et al. (2009) and Ruhlman et al. (2010), respectively.

Results of L1 expression in various plant systems up to date are summarized in Table 2.

| Antigen | Production system and yield | Efficacy data |
|--|---|--|
| HPV-16 L1 | <i>Agrobacterium</i> -transformed <i>Nicotiana tabacum</i> cv. Xanthi plants Assembled in VLPs 4 µg/kg ww | Weakly immunogenic in rabbit |
| HPV-11 L1 | Transgenic potato tubers Assembled in VLPs 20 µg/kg | Weakly immunogenic in orally vaccinated mice |
| HPV-16 L1 | Transgenic potato tubers Assembled in VLPs 12 mg/kg | Weakly immunogenic in orally vaccinated mice |
| HPV-16 L1 | Transgenic tobacco plants Assembled in VLPs 20 mg/kg ww | Highly immunogenic in mice injected with purified product |
| HPV-16 L1 | Protein expressed in <i>Nicotiana benthamiana</i> by TMV-derived vector 40 µg/kg wet leaves | ND |
| HPV-11 L1 | Transgenic <i>N. tabacum</i> 2 mg/kg ww Transgenic <i>Arabidopsis thaliana</i> 12 mg/kg ww <i>N. benthamiana</i> via rTMV 10 mg/kg ww | ND |
| HPV-16 L1 | Agroinfiltrated <i>N. benthamiana</i> , human codon usage-optimized gene; protein targeted to chloroplasts, assembled in VLPs 500 mg/kg ww | Antibodies elicited in mice by injection of crudely purified extracts neutralized HPV-16 pseudovirion transfection of HEK293TT cells |
| HPV-16 L1- Rubisco/ ATPase peptide fusion | Protein produced in chloroplasts of transplantomic tobacco plants from native or chloroplast-optimized genes 60 mg/kg ww | ND |
| HPV-16 L1 | Protein produced from unmodified genes in chloroplasts of transplantomic tobacco plants 3 g/kg ww | Mice injected intraperitoneally with partially purified VLPs with Freund's or aluminium hydroxide adjuvants produced neutralizing antibodies |

Table 2. Plant-derived HPV antigens for the development of prophylactic vaccines. HPV: Human papillomavirus; ND: No data; PVA: Potato virus A; PVX: Potato virus X; rTMV: Recombinant tobacco mosaic virus; TMV: Tobacco mosaic virus; VLP: Virus-like particle; ww: Wet weight. (Giorgi et al., 2010)

2.4.3 The structure and stability of plant derived L1

The assembly of VLPs in plants after transient (Fig. 8 A, C) or stable (Fig. 8D) L1 expression was confirmed by electron microscopy of leaf crude extracts.

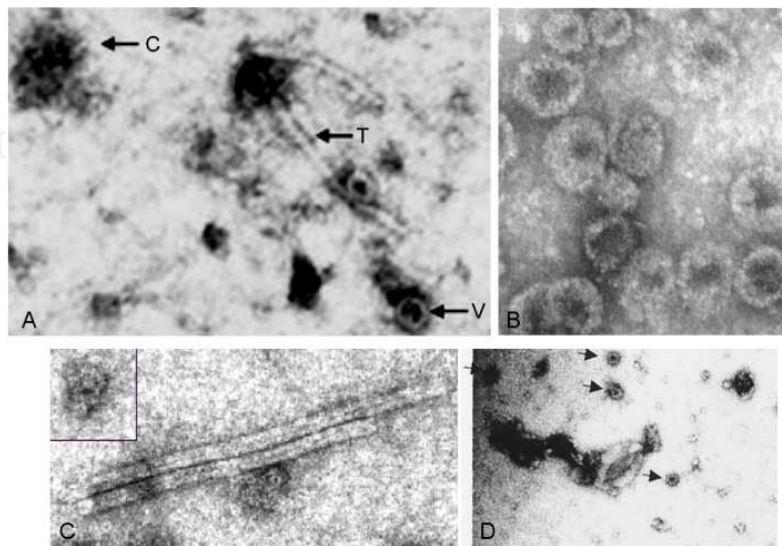


Fig. 8. Electron microscope images of uranyl acetate negatively stained HPV VLPs expressed in various systems: (A) Extracts prepared from freeze-dried leaves of *Nicotiana benthamiana* transiently expressing L1. V - HPV16L1 55-nm VLPs particles, C - HPV16 L1 capsomeres, T - flexible rods of plant TRV virus (Šmídková et al., 2010); (B) CsCl-purified baculovirus expressed VLPs in insect cells; (C) Crude extracts from top leaves of *Nicotiana benthamiana* expressing HPV VLPs from plant TMV virus. Two rods of TMV are shown together with VLPs (Varsani et al., 2006); (D) CsCl-purified VLPs from transgenic potato plants. VLPs have band density 1.32g/ml (Biemelt et al., 2003).

The structure of VLPs is not stable upon freezing and thawing, but plant expressed VLPs retain their structure during freeze-drying in both, the plant extracts and the plant tissue Fig. 8 and 9 (Maclean et al, 2007).

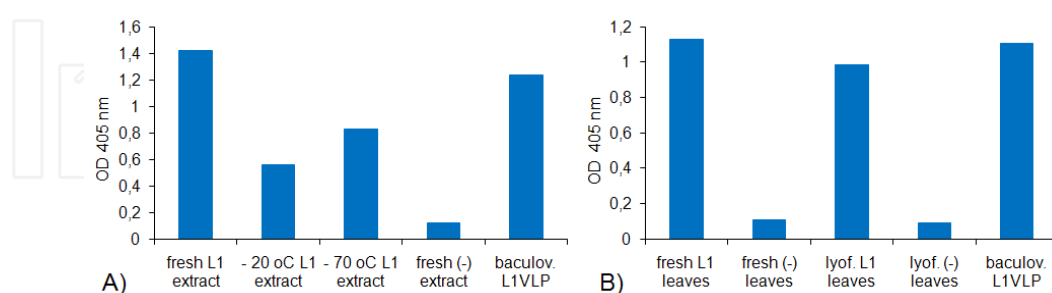


Fig. 9. Stability of L1 VLPs transiently expressed from pTV00 vector in *Nicotiana benthamiana* leaves: (A) after freezing and thawing extract from fresh leaves. Approximately 50% of VLPs loses 3D structure when extract is stored at -20°C or -70°C , respectively; (B) Upon freeze-drying of leaves and extraction cycle. More than 90 % of L1 retains conformation in extracts from freeze-dried leaves (*third column*) in comparison to extract prepared from fresh leaves (*first column*). Controls are extracts from leaves prepared the same way, but from plants inoculated with an empty pTV00 vector.

2.4.4 Immunogenicity of plant derived L1

The first report on production of HPV VLPs in plants and testing of their immunogenicity appeared in literature in 2003. Varsani et al. (2003b) was the first to express full-length native *HPV-16 L1* gene in transgenic *Nicotiana tabacum* cv. Xanthi. This plant-produced protein assembled into recognizable VLPs and was immunogenic, when injected into rabbits with Freund's incomplete adjuvant. Since then, several groups has observed induction of specific antibodies after subcutaneous injection of either purified plant-derived HPV16 L1 VLPs (Biemelt et al., 2003) or of the crude extract from the tobacco leaves expressing HPV16 L1 protein (Fig. 11A) (Maclean et al., 2007; Šmídková et al., 2010). Despite the fact that in plant extracts majority of expressed L1 protein was present as capsomeres besides VLPs Fig. 8A, the extracts were highly immunogenic without any additional adjuvant (Maclean et al., 2007, Šmídková et al., 2010). The antibodies induced by immunization with plant extract depicted on Fig. 8A preferentially recognized fully assembled L1 VLPs (Fig. 12A) and neutralized in vitro HPV16 virions (Fig. 12B). Plant expressed L1 in crude extract also induces CTL (Fig. 11B).

These findings suggests that principal antigenic determinant is either entire VLP or 3D structure specific for fully assembled VLPs and these antigens are present in crude extracts from plants transiently expressing L1 in enough quantity to elicit immune response equal or higher than purified VLPs from insect cells. Moreover immunization with plant L1 crude extracts induce cellular responses characteristic for active vaccine (Šmídková et al., 2010).

Mucosal delivery has several advantages over needle administration. Immune response is best achieved by direct application of a vaccine to mucosal surfaces and in addition mucosal application of a vaccine can also induce humoral, cell-mediated and systemic immune responses (Rigano & Walmsley, 2005). HPV VLPs are immunogenic when administered orally and stable in the environment of the gastrointestinal tract. Rose et al (1999) and Gerber et al. (2001) reported that as little as 1-10 micrograms are sufficient to induce high titers of L1 specific antibodies after oral application when administered with LT or CpG DNA adjuvants. Besides VLPs also capsomeres (L1-pentamers) and T=1 particles (12 L1-pentamers) depicted on Fig. 10 were investigated for oral immunogenicity in mice. Mutated L1 gene (L1_2xCysM) with two cysteines replaced by serines was used to generate capsomeres and T=1 particles. Compared to capsomeres, VLPs induced higher titers of neutralizing and IgA secreted antibodies, while cytotoxic T cell responses was comparable. The induction of secreted IgA antibodies was observed after oral but not after subcutaneous immunization (Thönes & Müller, 2007).

The concept of using tissue of plants expressing vaccine antigens as an edible vaccines attracted already a lot of attention and is still of special interest. A number of clinical studies demonstrated the induction of specific antibodies after oral immunization using crude plant material containing, for example, hepatitis B or Norwalk virus antigens (Lal et al., 2007). Likewise, oral immunization using crude potato tubers expressing L1 protein can induce specific antibody (Warzecha et al., 2003, Biemelt et al., 2003). Moreover, HPV L1-E6/E7 based chimeric VLPs have been successfully expressed in tomato fruits, which were able to elicit humoral and cytotoxic T-cell activity in mice (Paz De la Rosa et al., 2009).

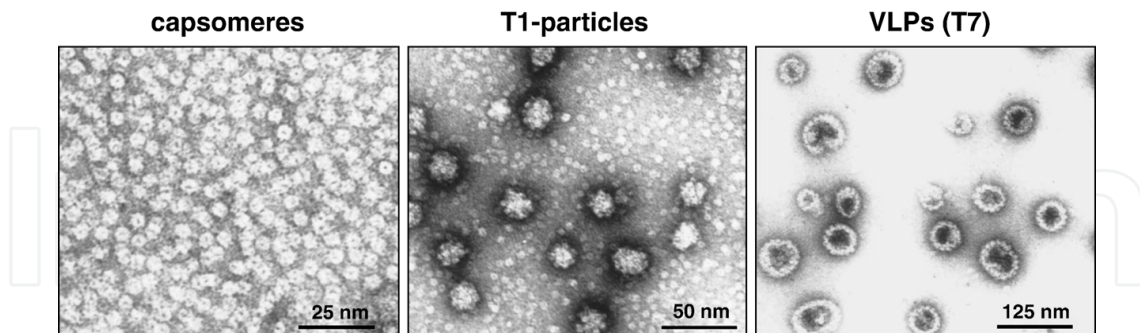


Fig. 10. Analysis of different L1 assembly forms by electron microscopy. Capsomeres (L1-pentamers), T1-particles (12 L1-pentamers) and VLPs (T7 particles of 71 L1-pentamers) purified from infected insect cells expressing the wild-type HPV 16 L1 gene (L1wt) or mutated L1 (L1_2xCysM) were analyzed by electron microscopy after uranyl acetate negative staining (Thönes & Müller, 2007).

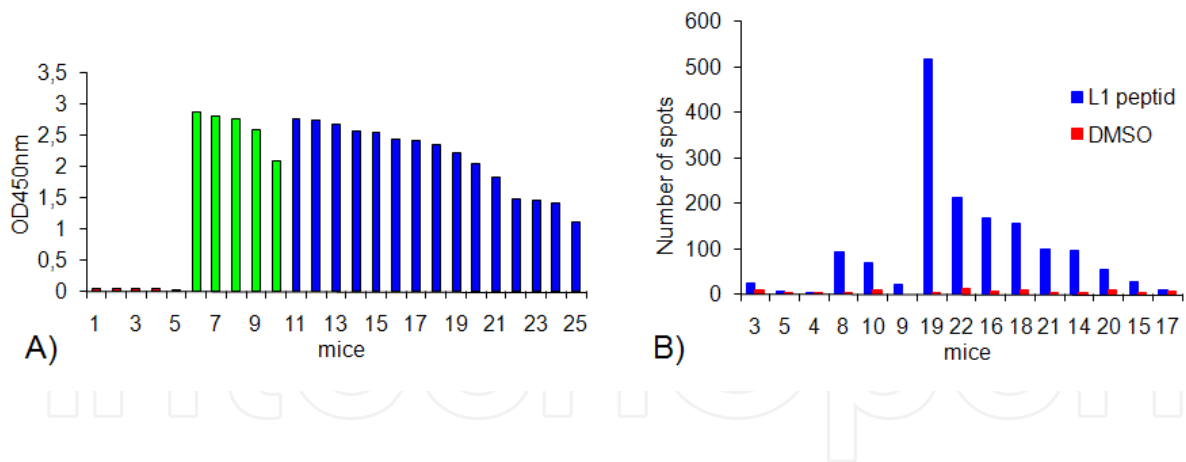


Fig. 11. Antibody (A) and cytotoxic T-lymphocytes (B) induction by L1-VLPs. Response elicited by control plant extracts (mice 1 - 5), control plant extract from *Nicotiana benthamiana* containing purified VLPs from insect cells (mice 6 - 10) and crude plant extracts from leaves expressing L1 (mice 11 - 25) in C57BL/6 mice. Collected sera from individual mice were tested by ELISA for induction of specific antibody (y-axis in OD 450 units). CTLs induction was measured by ELISPOT analysis of splenocytes recovered from scarified animals. The number of CTLs spots was recorded by an ELISPOT reader and expressed as a mean per 10^6 splenocytes

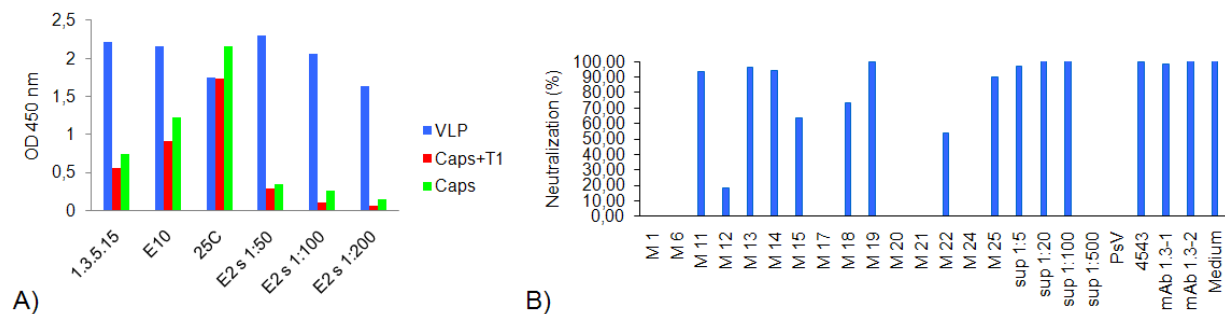


Fig. 12. (A) The specificity of mAb E2 toward various L1 assembly structures. VLPs, T1-particles (12 L1-pentamers) and capsomeres produced in insect cells were absorbed on the microtiter plates and analyzed by ELISA. Interactions of hybridoma E2 supernatant is compared to antibodies obtained after immunization with insect cell-derived VLPs (1.3.5.15, E10 and 25C). (B) Neutralization assay. Sera of mice and of E2 hybridoma supernatant were tested for neutralization of infection of 293T cells by HPV pseudovirions (Psv). Percent of neutralization obtained by incubation with mice sera diluted 1:50 and that of E2 hybridoma supernatant diluted 1:5, 1:20, 1:100, 1:500. Neutralization activity of mice sera was compared to a high titer rabbit polyclonal anti-L1 antiserum 4543 (100 %) and to the mAb's 1.3.1 and 1.3.2 as additional positive controls.

2.4.5 Plant based therapeutic and second-generation vaccines

Capsomeres can be used as a potential cost-saving substitute of VLPs, as L1-pentameric capsomeres are considered thermo-stable, the advantageous feature for the use in developing countries where cold chain administration and delivery of vaccine is difficult to maintain (Stanley et al., 2008). A promising step towards a capsomeres-based vaccine was described by Yuan et al. (2001) when dogs were completely protected against canine oral papillomavirus (COPV) infection by capsomeres vaccination. Capsomeres have been also reported to induce neutralizing antibodies and L1-specific cytotoxic T-lymphocytes (CTLs) upon oral, intranasal and subcutaneous immunization (Dell et al., 2006; Thönes & Müller, 2007; Schadlich et al., 2009). To increase immunogenicity, L1_{2xCys} mutant version of HPV16 L1 protein was fused with LTB as an adjuvant and expressed in chloroplasts (Waheed et al, 2011a, 2011b).

Plant expressed HPV antigens to be used as therapeutic vaccine to date are summarized in Table 3:

| Antigen | Production system and yield | Efficacy data |
|--|---|---|
| HPV-16 E7 | <i>N. benthamiana</i> tobacco leaves infected with PVX-E7 3-4 µg/g fresh leaves | 40% of mice immunized with E7-containing crude leaf extract were protected from growth of cancer induced by E7-expressing C3 cells |
| HPV-16 E7 | <i>N. benthamiana</i> tobacco leaves infected with PVX-E7; protein targeted to secretory pathway 15-20 µg/g fresh leaves | 80% of mice immunized with E7-containing crude leaf extract were protected from growth of cancer induced by E7-expressing C3 cells |
| HPV-16 E7 | <i>N. benthamiana</i> tobacco leaves infected with PVX-E7; protein targeted to secretory pathway | Mice vaccinated orally with freeze-dried E7-containing leaf extract mixed with feed produced high titer of anti-E7 antibodies |
| HPV-16 E7 | <i>N. benthamiana</i> tobacco leaves infected with PVX-E7 | Dendritic cells pulsed with E7-containing leaf extract were able to prime naive lymphocytes to induce E7-specific CTLs |
| LicKM-E7GGG | <i>N. benthamiana</i> tobacco leaves infected with LicKM-E7GGG, using a launch vector expression system 400 µg purified protein per gram of fresh leaves | Purified protein injected into mice induced IgG and CTL response and protected them against challenge with E7-expressing tumor cells in both prophylactic and therapeutic vaccination regimen |
| 11-kDa Zera zein-derived peptide-E7 mut | <i>N. benthamiana</i> via agroinfiltration | Mice vaccinated with the protein were protected against tumor cells expressing E7 |
| PVX CP-HPV-16 E7GGG | DNA vaccine | DNA vaccine was able to protect vaccinated mice from the growth of tumors induced by E7-expressing TC-1 cells |
| PVX CP-HPV-16 E7 | Tobacco chloroplast | ND |
| SAP-KQ-E7GGG | a) DNA vaccine b) Expressed in <i>N. benthamiana</i> | a) DNA vaccine tested in therapeutic setting was able to block tumor growth in the 40% of challenged mice b) Not performed |
| HPV-16 L2-PVA CP-E7 epitope fused protein | Expressed by PVX in <i>Nicotiana tabacum</i> , <i>N. benthamiana</i> using <i>Agrobacterium tumefaciens</i> -mediated inoculation | ND |
| HPV-16 VLPs carrying L1 fused to a string of epitopes from E6 and E7 | Tomato seedling cotyledons HPV-16 VLPs carrying L1 fused to string of epitopes from E6 and E7 using <i>A. tumefaciens</i> -mediated inoculation | Mice injected with chimeric VLPs were able to develop neutralizing antibodies and specific CTLs |

Table 3. Plant-derived HPV antigens for the development of therapeutic vaccines. CP: Coat protein; CTL: Cytotoxic T lymphocyte; HPV: Human papillomavirus; ND: No data; PVA: Potato virus A; PVX: Potato virus X; SAP-KQ: Mutagenized type I ribosome inhibiting proteins from *Saponaria officinalis*; VLP: Virus-like particle. (Giorgi et al., 2010)

The important proof of the L2 plant-produced nonhuman papillomavirus vaccine efficiency was published in connection with the purified rTMV particles displaying cottontail rabbit papillomavirus (CRPV) or rabbit oral papillomavirus (ROPV) L2 protein peptides on their

surface (Palmer et al., 2006). This pseudovirion system was also used to express entire native L1 gene of CRPV. Intramuscular injection with of CRPV L1-containing concentrated plant extract derived from transgenic tobacco protected rabbits against CRPV challenge (Kohl et al., 2006).

Since VLPs based vaccines are not effective in the therapy of diseases, an important goal is development of anti-HPV vaccines with either therapeutic or both prophylactic and therapeutic properties. Few studies were performed with viral oncoproteins expressed in plants. HPV-16 E7 was transiently expressed using a viral vector based on potato virus X (PVX) in the *Nicotiana benthamiana*, *Nicotiana rustica*, *Nicotiana tabacum*, *Chenopodium quinoa* and the tomato *Solanum lycopersicum* L. cv. Micro-Tom. The highest expression of HPV-16 E7 3–4 µg/g of fresh leaves was achieved in *Nicotiana benthamiana* and the expressed E7 induced specific humoral and cell-mediated immune responses in mice (Franconi et al., 2002, 2006). The efficiency of chimeric constructs when E7 is fused to other proteins and expressed in plants was also reported. The expression of HPV-16 E7 fused with the PVX CP in tobacco chloroplasts has been recently reported. The expression of the fusion protein in this system was higher than E7 alone (Morgenfeld et al., 2009). Mutated E7, E7GGG, which lacks the retinoblastoma binding site, and thus the native transformation potential, was fused to the *Clostridium thermocellum* b-1,3-1,4-glucanase (LicKM) as a carrier molecule for expression in plants. The expression of fusion protein in *Nicotiana benthamiana* yielded 400 µg of purified protein per gram of leaf (Musyichuk et al., 2007). Injection of the purified LicKM-E7GGG fusion protein into mice induced both E7-specific IgG and cytotoxic T-cell responses, and protected mice against challenge with E7-expressing tumor cells (Massa et al., 2007). The successful expression of chimeric HPV-16 L1 protein fused to a string of three E7 and one E6 epitopes in transgenic tomatoes demonstrates that a combination prophylactic / therapeutic HPV vaccine could be produced in plants (Paz de la Rosa et al., 2009; Monroy-García et al., 2011). Unfortunately, while the produced VLPs stimulated both antibody and T-cell responses, yields were low in the range 0.05 – 0.1% of total soluble protein. Similarly, combined vaccine based on minor capsid protein L2 and an epitope of E7 oncoprotein was successfully expressed in plants, when an epitope of the L2 protein and an epitope of E7 oncoprotein were fused to the N'- and C'-end of PVA CP, respectively. The construct was cloned into a PVX-based vector and transiently expressed in plants using *Agrobacterium*-mediated inoculation (Čeřovská et al., 2008).

3. Vaccine formulation

3.1 Parenteral administration

3.1.1 Adjuvants

The goal of vaccination is to generate a strong immune response to the administered antigen. Papillomavirus VLPs themselves are good “inducers” of immune response and antigen determinants present on their surface are able to activate dendritic cells (DCs) for triggering T-cell activation (Bontkes et al., 2005; Yang et al., 2005). Nevertheless, for efficient clinical use additional adjuvants are needed not only to enhance the immune response, but also assuring achievement of appropriate type of protective immunity in each situation.

The aluminum (alum) salts or gel-based adjuvant formulations used e.g. in HPV Gardasil vaccine are currently approved in vaccines licensed for human use in the US. Nevertheless a

significant number of compounds tested for adjuvant effect are clearly more effective than alum, albeit usually accompanied with a higher toxicity as e.g. Freund's complete adjuvant. This is the main reason preventing their use as adjuvants in human vaccine formulations. As adjuvants were successfully tested low toxic mutants of the cholera toxin (CT) (Yamamoto et al., 1997) and *E.coli* heat-labile enterotoxin (LT) (Chong et al., 1998). The inactive B-subunits of these toxins proved to be a strong mucosal (oral, nasal, vaginal, etc.) adjuvants (e.g. Salmonella toxin B subunit is used in commercial Cervarix vaccine) for a wide variety of antigens in mice and other animal species so far tested, however their use in humans is limited (Chong et al., 1998). This is why there is a growing interest to develop new adjuvants eliciting high mucosal, humoral and cellular immune response accompanied by negligible or low toxicity.

| Antigen delivery systems | Immunopotentiators |
|--------------------------------------|---|
| Insoluble aluminum compounds | MPL and synthetic derivates |
| Calcium phosphate | MDP and derivatives |
| Liposomes | Oligonucleotides (CpG, etc.) |
| Virosomes™ | Double-stranded RNA (dsRNA) |
| ISCOMS® | Alternative pathogen-associated Molecular patterns (PAMPs) (<i>E. coli</i> heat Labile enterotoxin (LTB); flagellin) |
| Microparticles (e.g., PLG) | Saponins (Quils, QS-21) |
| Emulsions (e.g., MF59, Montanides) | Small-molecule immune potentiators (SMIPs) (e.g., resiquimod [R848]) |
| Virus-like particles & viral vectors | Cytokines & chemokines |

Table 4. Examples of adjuvant classes (O'Hagan & Rappuoli, 2004)

3.1.2 Adjuvant effect of plant extracts

Plant extracts are known to contain various compounds, which supposedly have immunostimulatory and immunosuppressive effects (Wagner & Proksh, 1985). Plant crude extracts and their components were tested for their adjuvant capacity. The extract from leaves of *Nicotiana bethamiana* co-administrated subcutaneously with HPV16E7 (Franconi et al., 2002) or HPV16L1 VLP's (Maclean et. al, 2007; Šmídková et al., 2010) enhanced specific humoral and cellular immune response in tested mice. Freund's adjuvant added to the plant extract did not increased noticeably humoral response elicited by HPV16L1 VLP's in subcutaneously immunized mice and results indicate that the addition of Freund's adjuvant to plant extract might be even deleterious (Maclean et. al, 2007). The study of Isfar et al. (2004) compares adjuvant effect of CT and of aqueous extract of *Solanum torvum* (STE). STE was shown to evoke an increase in IgA titer comparable to that of CT when co-administrated with ovalbumin intraperitoneally. No acute toxic effects were evident with the used dose range. Plant extract has been shown to induce DC maturation of dendritic cells. This effect was not caused by lipopolysaccharide (LPS) but rather by presence of heat-resistant products mimicking the effect of LPS in foliar extract (Di Bonito et al, 2009).

Probably the most studied plant compound with adjuvant effect is the saponin fractions isolated from *Quillaja saponaria* (Newman et al., 1992). The mechanism of saponin effect is complex and, apart from direct cellular stimulation, there is also evidence that saponins may

enhance oral immunization by protection of antigen from degradation by digestive enzymes and by increasing permeability of the intestine to macromolecules (Campbell, 1995).

All these findings are promising for development of needle-free administration route of immunization as an alternative to intramuscular vaccine application. For this purposes intranasal, intravaginal, transdermal, sublingual and intramuscular administration routes were tested for systemic immune responses against HPV16L1 using (Cho et al., 2010). The sublingual route provided the most effective mucosal secretory IgA (sIgA) and serum IgG responses, cholera toxin subunit B (CTB) showed the most promising adjuvant activity.

3.2 HPV L1 antigens as an edible vaccine?

The majority of currently licensed vaccines are administered parenterally, even though they have the disadvantages of patient reluctance to tolerate needle sticks and lack of mucosal immune induction (Velasquez et al 2010). Edible vaccine represents further approach to self-administrated nonparenteral vaccine that could solve the problem of high cost and need for appropriate storage of currently available preventive HPV vaccines.

Thönes & Müller (2007) investigated the oral immunogenicity of different assembly forms of HPV 16 L1: T7-VLPs, T1 particles and capsomeres produced from Baculovirus expression vector in insect cells and showed that all three assembly forms induce humoral and cellular immune responses after oral vaccination of mice. The anti-L1 antibodies were conformation-specific and showed neutralizing activity in a pseudovirion-based assay. They also investigated whether adjuvants have an effect on oral immunogenicity when co-administrated with different L1 forms. Besides saponins, which were significantly toxic if applied orally, co-administration of either CpG DNA or *Escherichia coli* heat-labile enterotoxin LT(R192G) had no apparent enhancing effect on the production of anti-L1 antibodies. Compared to capsomeres, VLPs induced stronger humoral immune responses while the CTL responses were induced at comparable levels.

To establish an edible HPV16 vaccine Sasagawa et al., (2005) constructed a recombinant HPV16 L1-expressing *Schizosaccharomyces pombe* yeast strain to be administrated as freeze-dried yeast powder orally as an edible vaccine, with or without the mucosal adjuvant heat-labile toxin LT (R192G), to mice. After the third immunization, none of the mice that received the edible HPV16 vaccine showed specific antibody responses, whereas all of the positive controls that were administered intranasally with 5 µg of HPV16-virus-like particles (VLP) had serum IgG, and genital IgA and IgG that reacted with HPV16-VLP in enzyme-linked immunosorbent assays (ELISAs).

HPV L1 antigens that proof to be highly immunogenic when administrated parenterally induce only mild or none response when administrated orally. In light of these experiments it seems unlikely that current design of L1 based HPV vaccines will reach the market as an edible replacement of existing vaccines. More research is needed to establish vaccine concentration and formulation to boost its effect. It is also obvious that the highly phrased concept of edible vaccine administrated as plants or fruits for direct consumption in the less developed countries is rather romantic dream than reality and have to be corrected. It is now clear that if there will be an edible vaccine, it will have complex formulation that will be strictly controlled.

A good nonparenteral alternative for vaccine delivery could be nasal immunization, which already proved to be effective in tests with animals. The obstacles imposed by the normal process of mucociliary clearance limiting residence time of applied antigens could be circumvented by presence of an inert *in situ* gelling polysaccharide (GelSite) extracted from Aloe vera for nasal delivery of NV VLP antigen (Hefferon, 2010). The nasal cavity is a promising site for vaccine delivery because it is easy to access, is highly vascularized, has a relatively large surface area, has low proteolytic activity, and is able to induce systemic immunity as well as both local and distal mucosal immunity via the Common Mucosal Immune System (CMIS)

4. Conclusions

The major reason for the vaccine production in plants is that the vaccine antigen production is safe and could be potentially cheap and both transient and transgenic productions are scalable. Biologically active proteins can be produced more easily in plants than in other eukaryotic systems; and that the use of food plants could eventually allow edible and/or oral vaccines to be produced cheaper. The recent reports indicate very high yields of human vaccine candidates to be obtained via plastid transformation or large scale transient expression what could enable to meet the expected requirement of antigen for oral route as is required parenterally for the same immune response. A recent review on human trials of plant-based oral vaccines summarizing human studies of oral transgenic plant derived vaccines against enterotoxigenic *E. coli* infection, norovirus and HBV adds weight to the growing body of evidence that plant-made oral vaccines to these viruses are not only feasible, but could be effective (Rybicki, 2010). Nevertheless there is still long way to go from improvement of antigen yields, to formulation of the vaccine including auxiliary factors improving efficacy and stability, to translation of the proposed vaccines into clinical trials and, not least, governmental and/or regulatory body approvals.

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6. References

- Alvarez-Salas, L.M. (2008). Amolimogene bepiplasmid, a DNA-based therapeutic encoding the E6 and E7 epitopes from HPV, for cervical and anal dysplasia. *Current opinion in molecular therapeutics*, Vol.10, No.6, (December 2008), pp. 622-6288, ISSN 1464-8431
- Armstrong, E. P. (2010). Prophylaxis of cervical cancer and related cervical disease: a review of the cost-effectiveness of vaccination against oncogenic HPV types. *Journal of managed care pharmacy*, Vol.16, No.3, (April 2010), pp. 217-230, ISSN 1083-4087
- Bardor, M.; Faveeuw, C.; Fitchette, A.C.; Gilbert, D.; Galas, L.; Trottein, F.; Faye, L. & Lerouge, P. (2003). Immunoreactivity in mammals of two typical plant glyco-epitopes, core alpha(1,3)-fucose and core xylose. *Glycobiology*, Vol.13, No.6, (June 2003), pp.427-434, ISSN 0959-6658

- Biemelt, S.; Sonnewald, U.; Gaimbacher, P.; Willmitzer, L. & Müller, M. (2003). Production of human papillomavirus type 16 virus-like particles in transgenic plants. *Journal of virology*, Vol.77, No. 17, (September 2003), pp.9211-9220, ISSN 0022-538X
- Bishop, B.; Dasgupta, J.; Klein, M.; Garcea, R.L.; Christensen, N.D.; Zhao, R. & Chen, X.S. (2007). Crystal structures of four types of human papillomavirus L1 capsid proteins: Understanding the specificity of neutralizing monoclonal antibodies. *The Journal of Biological Chemistry*, Vol.282, No.43, (October 2007), pp. 31803-31811, ISSN 0021-9258
- Bontkes, H.J.; Ruizendaal, J.J.; Kramer, D.; Meijer, C.J. & Hooijberg, E. (2005). Plasmacytoid dendritic cells are present in cervical carcinoma and become activated by human papillomavirus type 16 virus-like particles. *Gynecologic Oncology*, Vol.96, No.3, (March 2005), pp. 897-901, ISSN 0090-8258
- Brandsma, J. L. (1994). Animal models of human-papillomavirus-associated oncogenesis. *Intervirology*, Vol.37, No.3-4, (March 1994), pp. 189-200, ISSN 0300-5526
- Brandsma, J.L.; Shlyankevich, M.; Su, Y.; Zeltermann, D.; Rose, J.K. & Buonocore, L. (2010). Reversal of papilloma growth in rabbits therapeutically vaccinated against E6 with naked DNA and/or vesicular stomatitis virus vectors. *Vaccine*, Vol.29, No.52, (December 2010), pp. 8345-8351, ISSN 1873-2518
- Campbell, J.B. (1995). Saponins. In: *The theory and practical application of adjuvants*, Stewart-Tull, D.E.S. (Ed.), pp. 95-127, John Wiley & Sons, New York
- Campo, M.S. (1997). Vaccination against papillomavirus in cattle. *Clinics in dermatology*, Vol.15, No.2, (July 1997), pp. 275-83, ISSN 0738-081X
- Carvajal, A. A.; de la Garza, A.; Quiroz, B. J.; Zea, E. V.; Estrada, I. D.; Fuentez, E. M.; Contreras, M. L.; Andrade-Manzano, A.; Padilla, S. & Varela, A. R. (2007). MVA E2 recombinant vaccine in the treatment of human papillomavirus infection in men presenting intraurethral flat condyloma: a phase I/II study. *Biodrugs*, Vol. 21, No.1, (January 2007), pp. 47-59, ISSN 1173-8804
- Čeřovská, N.; Hoffmeisterová, H.; Pečenková, T.; Moravec, T.; Synková, H.; Plchová, H. & Velemínský J. (2008). Transient expression of HPV16 E7 peptide (aa 44-60) and HPV16 L2 peptide (aa 108-120) on chimeric potyvirus-like particles using potato virus X-based vector. *Protein Expression and Purification*, Vol.58, No.1, (August 2008), pp. 154-161, ISSN 1046-5928
- Chebolu, S. & Daniell, H. (2009). Chloroplast-derived vaccine antigens and biopharmaceuticals: expression, folding, assembly and functionality. *Current topics in microbiology and immunology*, Vol.332, (July 2009), 33-54, ISSN 0070-217X
- Chen, X. S.; Garcea, R. L.; Goldberg, I.; Casini, G. & Harrison, S. C. (2000). Structure of small virus-like particles assembled from the L1 protein of human papillomavirus 16. *Molecular Cell*, Vol. 5, No.3, (March 2000), pp. 557-567, ISSN 1097-2765
- Cho, H.J.; Kim, J.Y.; Lee Y., Kim, J.M.; Kim, Y.B.; Chun, T. & Oh, Y.K. (2010). Enhanced humoral and cellular immune responses after sublingual immunization against human papillomavirus 16 L1 protein with adjuvants. *Vaccine*, Vol. 28, No.14, (March 2010), pp. 2598-2606, ISSN 1873-2518
- Chong, C.; Friberg, M. & Clements, J.D. (1998). LT (R192G), a non-toxic mutant of the heat-labile enterotoxin of *Escherichia coli*, elicits enhanced humoral and cellular immune responses associated with protection against lethal oral challenge with *Salmonella* spp. *Vaccine*, Vol.16, No.7, (April 1998), pp.732-740, ISSN 0264-410X

- Davidson, E.J.; Boswell, C.M.; Sehr, P.; Pawlita, M.; Tomlinson, A.E.; McVey, R.J.; Dobson, J.; Roberts, J.S.; Hickling, J.; Kitchener, H.C. & Stern, P.L. (2003). Immunological and clinical responses in women with vulval intraepithelial neoplasia vaccinated with a vaccinia virus encoding human papillomavirus 16/18 oncoproteins. *Cancer research*, Vol.63, No.18, (September 2003), pp. 6032-6041, ISSN 0008-5472
- Dell, K.; Koesters, R.; Linnebacher, M.; Klein, C. & Gissmann, L. (2006). Intranasal immunization with human papillomavirus type 16 capsomeres in the presence of non-toxic cholera toxin-based adjuvants elicits increased vaginal immunoglobulin levels. *Vaccine*, Vol.24, No.13, (March 2006), pp. 2238-2247, ISSN 0264-410X
- Di Bonito, P.; Grasso, F.; Mangino, G.; Massa, S.; Illiano, E.; Franconi, R.; Fanales-Belasio, E.; Falchi, M.; Affabris, E. & Giorgi, C. (2009). Immunomodulatory activity of a plant extract containing human papillomavirus 16-E7 protein in human monocyte-derived dendritic cells. *International journal of immunopathology and pharmacology*, Vol.22, No. 4, (October-December 2009), pp. 967-978, ISSN 0394-6320
- Dieryck, W.; Pagnier, J.; Poyart, C.; Marden, M.C.; Gruber, V.; Bournat, P.; Baudino, S. & Merot, B. (1997). Human haemoglobin from transgenic tobacco. *Nature*, Vol.386, No.6620, (March 1997), pp. 29-30, ISSN 0028-0836
- Dillner, J.; Arbyn, M. & Dillner, L. (2007). Translational mini-review series on vaccines: Monitoring of human papillomavirus vaccination. *Clinical and experimental immunology*, Vol.148, No.2, (May 2007), pp. 199-207, ISSN 0009-9104
- Doorbar, J.; Ely, S.; Sterling, J.; McLean, C. & Crawford, L. (1991). Specific interaction between HPV-16 E1-E4 and cytokeratins results in collapse of the epithelial cell intermediate filament network. *Nature*, Vol. 352, No.6338, (August 1991), pp. 824-827, ISSN 0028-0836
- Duensing, S.; Lee, L. Y.; Duensing, A.; Basile, J.; Piboonniyom, S.; Gonzalez, S.; Crum, C.P. & Munger, K. (2000). The human papillomavirus type 16 E6 and E7 oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.97, No. 18, (August 2000), pp. 10002-10007, ISSN 0027-8424
- Fiander, A.N.; Tristram, A.J.; Davidson, E.J.; Tomlinson, A.E.; Man, S.; Baldwin, P.J.; Sterling, J.C. & Kitchener HC. (2006). Prime-boost vaccination strategy in women with high-grade, noncervical anogenital intraepithelial neoplasia: clinical results from a multicenter phase II trial. *International journal of gynecological cancer*, Vol.16, No.3, (May-June 2006), pp. 1075-1081, ISSN 1048-891X
- Fernández-San Millán, A.; Ortigosa, S.M.; Hervás-Stubbs, S.; Corral-Martínez, P.; Seguí-Simarro, J.M., Gaétan, J., Coursaget, P. & Veramendi, J. (2008). Human papillomavirus L1 protein expressed in tobacco chloroplasts self-assembles into virus-like particles that are highly immunogenic. *Plant biotechnology journal*, Vol.6, No.5, (Jun 2008), pp. 427-44, ISSN 1467-7652
- Fleury, M.J.; Touzé, A.; Alvarez, E.; Carpentier, G.; Clavel, C.; Vautherot, J.F. & Coursaget, P. (2006). Identification of type-specific and cross-reactive neutralizing conformational epitopes on the major capsid protein of human papillomavirus type 31. *Archive of virology*, Vol.151, No.8, (August 2006), pp. 1511-1523, ISSN 0304-8608
- Franconi, R.; Di Bonito, P.; Dibello, F.; Accardi, L.; Müller, A.; Cirilli, A.; Simeone, P.; Donà, G.; Venuti, A. & Giorgi, C. (2002). Plant-derived human papillomavirus 16 E7

- oncprotein induces immune response and specific tumor protection. *Cancer Research*, Vol.62, No.13, (July 2002), pp. 3654-3658, ISSN 0008-5472
- Franconi, R.; Massa, S.; Illiano, E.; Mullar, A.; Cirilli, A.; Accardi, L.; Di Bonito, P.; Giorgi, C. & Venuti, A. (2006). Exploiting the plant secretory pathway to improve the anticancer activity of a plant-derived HPV16 E7 vaccine. *International journal of immunopathology and pharmacology*, Vol. 19, No.1, (January-March 2006), pp. 187-197, ISSN 0394-6320
- Freyschmidt, E.J.; Alonso, A.; Hartmann, G. & Gissmann, L. (2004). Activation of dendritic cells and induction of T cell responses by HPV 16 L1/E7 chimeric virus-like particles are enhanced by CpG ODN or sorbitol. *Antiviral Therapy*, Vol.9, No.4, (August 2004), pp. 479-489, ISSN 1359-6535
- Gerber, S.; Lane, C.; Brown, D.M.; Lord, E.; DiLorenzo, M.; Clements, J.D.; Rybicki, E., Williamson, A.L. & Rose, R.C. (2001). Human papillomavirus virus-like particles are efficient oral immunogens when coadministered with Escherichia coli heat-labile enterotoxin mutant R192G or CpG DNA. *Journal of Virology*, Vol.75, No.10, (May 2001), pp. 4752-60, ISSN 0022-538X
- Giorgi, C.; Franconi, R. & Rybicki, E.P. (2010). Human papillomavirus vaccines in plants. *Expert Review of Vaccines*, Vol. 9, No.8, (August 2010), pp. 913-924, ISSN 1744-8395
- Gomorod, V. & Faye L. (2004). Posttranslational modification of therapeutic proteins in plants. *Current opinion in plant biology*, Vol.7, No.2, (April 2004), pp. 171-181, ISSN 1369-5266
- Hefferon, K.L. (2010). The mucosal immune response to plant-derived vaccines. *Pharmaceutical research*, Vol.27, No.10, (October 2010), pp. 2040-2042, ISSN 0724-8741
- Heim, K.; Christensen, N.D.; Hoepfl, R.; Wartusch, B.; Pinzger, G.; Zeimet, A.; Baumgartner, P.; Kreider, J.W. & Dapunt, O. (1995). Serum IgG, IgM, and IgA reactivity to human papillomavirus types 11 and 6 viruslike particles in different gynecologic patient groups. *The Journal of Infectious Diseases*, Vol.172, No.2, (August 1995), pp. 395-402, ISSN 0022-1899
- Corbett H.J.; Fernando, G.J.P.; Chen, X.; Frazer, I.H. & Kendall, M.A.F. (2010). Skin vaccination against cervical cancer associated human papillomavirus with a novel micro-projection array. *PLoS One*, Vol.5, No.10, (October 2010), pp. e13460, ISSN 1932-6203
- Israfi, D.A.; Lajis, N.H.; Somchit, M.N. & Sulaiman, M.R. (2004). Enhancement of ovalbumin-specific IgA responses via oral boosting with antigen coadministered with an aqueous *Solanum torvum* extract. *Life Science*, Vol.75, No.4, (January 2004), pp. 397-406, ISSN 0024-3205
- Jay, N. & Moscicki, A.B. (2000). Human papillomavirus infections in women with HIV disease: prevalence, risk, and management. *The AIDS Reader*, Vol.10, No.11, (November 2000), pp. 659-668, ISSN 1053-0894
- Kenter, G.G.; Welters, M.J.; Valentijn, A.R.; Lowik, M.J.; Berends-van der Meer, D.M.; Vloon, A.P.; Drijfhout, J.W.; Wafelman, A.R.; Oostendorp, J.; Fleuren, G.J.; Offringa, R.; van der Burg, S.H. & Melief, C.J. (2008). Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high-risk human papillomavirus 16 in end-stage cervical cancer patients shows low toxicity and robust

- immunogenicity. *Clinical cancer research*, Vol.14, No.1, (January 2008), pp. 169-177, ISSN 1078-0432
- Ko, K.; Tekoah, Y.; Rudd, P.M.; Harvey, D.J.; Dwek, R.A.; Spitsin, S.; Hanlon, C.A.; Rupprecht, C.; Dietzschold, B.; Golovkin, M. & Koprowski, H. (2003). Function and glycosylation of plant-derived antiviral monoclonal antibody. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.100, No.13, (June 2003), p.p. 8013-8018, ISSN 0027-8424
- Kohl, T.; Hitzeroth, I.I.; Stewart, D.; Varsani, A.; Govan, V. A.; Christensen, N. D.; Williamson, A.L. & Rybicki E. P. (2006). Plant-produced cottontail rabbit papillomavirus L1 protein protects against tumor challenge: a proof-of-concept study. *Clinical and vaccine immunology*, Vol.13, No.8, (January 2006), pp. 845-853, ISSN 1556-6811
- Koprivova, A.; Stemmer, C.; Altmann, F.; Hoffmann, A.; Kopriva, S.; Gorr, G.; Reski, R. & Decker, E.L. (2004). Targeted knockouts of *Physcomitrella* lacking plant-specific immunogenic N-glycans. *Plant biotechnology journal*, Vol.2, No.6, (November 2004), p.p. 517-523, ISSN 1467-7644
- Le Tallec, D.; Doucet, D.; Elouahabi, A.; Harvengt, P.; Deschuyteneer, M. & Deschamps, M. (2009). Cervarix, the GSK HPV-16/HPV-18 AS04-adjuvanted cervical cancer vaccine, demonstrates stability upon long-term storage and under simulated cold chain break conditions. *Human vaccines*, Vol.5, No.7, (July 2009), pp. 467-474, ISSN 1554-8619
- Lal, P.; Ramachandran, V.G.; Goyal, R. & Sharma, R. (2007). Edible vaccines: current status and future. *Indian journal of medical microbiology*, Vol.25, No.2, (April 2007), pp. 93-102, ISSN 0255-0857
- Liu, H.L.; Li, W.S.; Lei, T.; Zheng, J.; Zhang, Z.; Yan, X.F.; Wang, Z.Z.; Wang, Y.L. & Si, L.S. (2005). Expression of human papillomavirus type 16 L1 protein in transgenic tobacco plants. *Acta Biochim. Biophys. Sin. (Shanghai)* 37, 153-158. *Acta biochimica et biophysica Sinica (Shanghai)*, Vol.37, No.3, (March 2005), pp. 153-158, ISSN 1672-9145
- Ludmerer, S.W.; Benincasa, D.; Mark, G.E. 3rd & Christensen, N.D. (1997). A neutralizing epitope of human papillomavirus type 11 is principally described by a continuous set of residues which overlap a distinct linear, surface-exposed epitope. *Journal of virology*, Vol.71, No.5, (May 1997), pp. 467-474, ISSN 0022-538X
- Ma, B.; Xu, Y.; Hung, C.F. & Wu T.C. (2010). HPV and Therapeutic Vaccines: Where are We in 2010? *Current cancer therapy reviews*, Vol.6, No.2, (May 2010), pp. 81-103, ISSN 1573-3947
- Ma, J.K.; Hiatt, A.; Hein, M.; Vine, N.D.; Wang, F.; Stabila, P.; van Dolleweerd, C.; Mostov, K. & Lehner, T. (1995). Generation and assembly of secretory antibodies in plants. *Science*, Vol.268, No.5211, (May 1995), p.p. 716-719, ISSN 0036-8075
- Maa, Y.F.; Shu, C.; Ameri, M.; Zuleger, C.; Che, J.; Osorio, J.E.; Payne, L.G. & Chen, D. (2003). Optimization of an alum-adsorbed vaccine powder formulation for epidermal powder immunization. *Pharmaceutical Research*, Vol.20, No.7, (July 2003), pp. 969-977, ISSN 0724-8741
- Maclean, J.; Koekemoer, M.; Olivier, A.J.; Stewart, D.; Hitzeroth I.I.; Rademacher, T.; Fischer, R.; Williamson, A.L. & Rybicki, E.P. (2007). Optimization of human papillomavirus type 16 (HPV-16) L1 expression in plants: comparison of the suitability of different

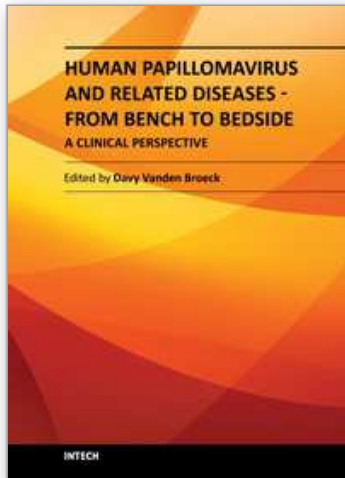
- HPV-16 L1 gene variants and different cell-compartment localization. *Journal of General Virology*, Vol.88, No.Pt 5, (May 2007), pp. 1460-1469, ISSN 0022-1317
- Massa, S.; Franconi, R.; Brandi, R.; Müller, A.; Mett, V.; Yusibov, V. & Venuti, A. (2007). Anti-cancer activity of plant-produced HPV16 E7 vaccine. *Vaccine*, Vol.25, No.16, (April 2007), pp. 3018-3021, ISSN 0264-410X
- McCormack, P.L. & Joura, E.A. (2010). Quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine (Gardasil®): a review of its use in the prevention of premalignant genital lesions, genital cancer and genital warts in women. *Drugs*, Vol.70, No.18, (December 2010), pp. 2449-2474, ISSN 0264-410X
- Melief, C.J.; Welters, M.J.; Lowik, M.J.; Vloon, A.P. & Kenter, G.G. (2007). Long peptide vaccine-induced migration of HPV16-specific type 1 and 2 T cells into the lesions of VIN III patients associated with complete clinical responses. *Cancer Immunity*, Vol.7, Suppl.1, (May 2007), pp. 20, ISSN 1424-9634
- Modis, Y.; Trus, B.L. & Harrison, S.C. (2002). Atomic model of the papillomavirus capsid. *The EMBO Journal*, Vol. 21, No.18, (September 2002), pp. 4754-4762, ISSN 0261-4189
- Monroy-García, A.; Gómez-Lim, M.A.; Weiss-Steider, B.; la Rosa, G. V & Hernández-Montes, J. (2011). A novel HPV 16 L1-based chimeric virus-like particle containing E6 and E7 seroreactive epitopes permits highly specific detection of antibodies in patients with CIN 1 and HPV-16 infection. *Virology Journal*, Vol. 8, (February 2002), pp. 59, ISSN 1743-422X
- Morgenfeld, M., Segretin, M.E. & Wirth, S. (2009). Potato virus X coat protein fusion to human papillomavirus 16 E7 oncoprotein enhance antigen stability and accumulation in tobacco chloroplast. *Molecular Biotechnology*, Vol.43, No.3, (July 2009), pp. 243-249, ISSN 1073-6085.
- Müller, M.; Zhou, J.; Reed, T.D.; Rittmüller, C.; Burger, A.; Gabelsberger, J.; Braspenning, J. & Gissmann, L. (1997). Chimeric papillomavirus-like particles. *Virology*, Vol.234, No.1, (July 1997), pp. 93-111, ISSN 0042-6822
- Munoz, N.; Bosch, F.X.; de Sanjose, S.; Herrero, R.; Castellsague, X.; Shah, K.V.; Snijders, P.J. & Meijer, C.J. (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. *The new england journal of medicine*, Vol.348, No.6, (February 2003) pp. 518-527, ISSN 0028-4793
- Musiychuk, K.; Stephenson, N.; Bi, H.; Farrance, C.E.; Orozovic, G.; Brodelius, M.; Brodelius, P.; Horsey, A.; Ugulava, N.; Shamloul, A.M.; Mett, V.; Rabindran, S.; Streatfield, S.J. & Yusibov V. (2007). A launch vector for the production of vaccine antigens in plants. *Influenza and Other Respiratory Viruses*, Vol.1, No.1, (January 2007) pp. 19-25, ISSN 1750-2640
- Newman, M.J.; Wu, J.; Gardner, B.H.; Munroe, K.J.; Leombruno, D.; Recchia, J.; Kensil, C.R. & Coughlin, R.T. (1992). Saponin adjuvant induction of ovalbumin-specific CD8+ cytotoxic T lymphocyte responses. *Journal of Immunology*, Vol.148, No.8, (April 1992), pp. 2357-2362, ISSN 0022-1767
- Oey, M.; Lohse, M.; Kreikemeyer, B. & Bock, R. (2009). Exhaustion of the chloroplast protein synthesis capacity by massive expression of a highly stable protein antibiotic. *The Plant Journal*, Vol.57, No.3, (February 2009), pp. 436-445., ISSN 1365-313X
- O'Hagan, D.T. & Rappuoli R. (2004). Novel approaches to vaccine delivery. *Pharmaceutical Research*, Vol.21, No.4, pp. 1519-1530, ISSN 0724-8741

- Paintsil, J.; Müller, M.; Picken, M.; Gissmann, L. & Zhou, J. (1996). Carboxyl terminus of bovine papillomavirus type-1 L1 protein is not required for capsid formation. *Virology*, Vol.223, No.1, (September 1996), pp. 238-244, ISSN 0042-6822
- Palmer, K.E.; Benko, A.; Doucette, S.A.; Cameron, T.I.; Foster, T.; Hanley, K.M., McCormick, A.A.; McCulloch, M.; Pogue G.P.; Smith, M.L. & Christensen, N.D. (2006). Protection of rabbits against cutaneous papillomavirus infection using recombinant tobacco mosaic virus containing L2 capsid epitopes. *Vaccine*, Vol.24, No. 26, (May 2006), pp. 5516-5525, ISSN 0264-410X
- Pastrana, D.V.; Buck, C.B.; Pang, Y.Y.; Thompson, C.D.; Castle, P.E.; FitzGerald, P.C.; Kruger Kjaer, S.; Lowy, D.R. & Schiller, J.T.(2004). Reactivity of human sera in a sensitive, high-throughput pseudovirus-based papillomavirus neutralization assay for HPV16 and HPV18. *Virology*, Vol.321, No.2, (April 2004), pp. 205-216, ISSN 0042-6822
- Pastrana, D.V.; Gambhira, R.; Buck, C.B.; Pang, Y.Y.; Thompson, C.D.; Culp, T.D.; Christensen, N.D.; Lowy, D.R.; Schiller, J.T. & Roden R.B. (2005). Cross-neutralization of cutaneous and mucosal Papillomavirus types with anti-sera to the amino terminus of L2. *Virology*, Vol.337, No.2, (July 2005), pp. 365-372, ISSN 0042-6822
- Paz De la Rosa, G.; Monroy-García, A.; Mora-García, M.L.; Reynaga Peña, C.G.; Hernández-Montes, J.; Weiss-Steider B. & Gómez Lim, M.A. (2009). An HPV 16 L1-based chimeric human papillomavirus-like particles containing a string of epitopes produced in plants is able to elicit humoral and cytotoxic T-cell activity in mice. *Virology Journal*, Vol. 6, (January 2009), pp. 2 ISSN 1743-422X
- Pokorná, D., Poláková, I., Kindlová, M., Dušková, M., Ludviková, V., Gabriel, P., Kutinová, L., Müller, M. & Šmahel M. (2009). Vaccination with human papillomavirus type 16-derived peptides using a tattoo device. *Vaccine*, Vol.27, No.27, (April 2009), pp. 3519-3529, ISSN ISSN 0264-410X
- Rigano, M.M. & Walmsley, A.M. (2005). Expression systems and developments in plant-made vaccines. *Immunology and cell biology*, Vol.83, No.3, pp. 271-277, ISSN 0818-9641
- Roden, R.B.; Armstrong, A.; Haderer, P.; Christensen, N.D.; Hubbert, N.L.; Lowy, D.R.; Schiller, J.T. & Kirnbauer, R. (1997a). Characterization of a human papillomavirus type 16 variant-dependent neutralizing epitope. *Journal of Virology*, Vol.71, No.8, (August 1997), pp. 6247-6252, ISSN 0022-538X
- Roden, R.B.; Lowy, D.R. & Schiller, J.T. (1997b). Papillomavirus is resistant to desiccation. *The Journal of infectious diseases*, Vol.176, No.4, (October 1997), pp. 1076-1079, ISSN 0022-1899
- Roden, R.B.; Yutzy, W.I.; Fallon, R.; Inglis, S; Lowy, D.R. & Schiller, J.T. (2000). Minor capsid protein of human genital papillomaviruses contains subdominant, cross-neutralizing epitope. *Virology*, Vol.270, No. 2, (May 2000), pp. 254 - 257, ISSN 0042-6822
- Rose, R. C.; Lane, C.; Wilson, S.; Suzich J. A., Rybicki, E.P. & A. L. Williamson A. L. (1999). Oral vaccination of mice with human papillomavirus virus-like particles induces systemic virus-neutralizing antibodies. *Vaccine*, Vol.17, No.17, (April 1999), pp. 2129- 2135, ISSN 0264-410X

- Ruhlman, T.; Verma, D.; Samson, N. & Daniell, H. (2010) The role of heterologous chloroplast sequence elements in transgene integration and expression. *Plant Physiology*, Vol.152, No.4., (April 2010) ,pp. 2088–2104, ISSN 1532-2548
- Rybicki E.P. (2010). Plant-made vaccines for humans and animals. *Plant Biotechnology Journal*, Vol.8, No.5, (June 2010) ,pp. 620–637, ISSN 1467-7644
- Sasagawa, T.; Tani, M.; Basha, W.; Rose, R.C.; Tohda, H.; Giga-Hama, Y.; Azar, K.K.; Yasuda, H.; Sakai, A. & Inoue, M. (2005). A human papillomavirus type 16 vaccine by oral delivery of L1 protein. *Virus Research*, Vol.110, No.1-2, (June 2005), pp. 81–90, ISSN 0168-1702
- Schadlich, L.; Senger, T.; Gerlach, B.; Mucke, N.; Klein, C.; Bravo, I.G.; Müller, M. & Gissmann, L. (2009). Analysis of modified human papillomavirus type 16 L1 capsomeres: the ability to assemble into larger particles correlates with higher immunogenicity. *Journal of Virology*, Vol.83, No.15, (August 2009), pp. 7690–7705, ISSN 0022-538X
- Shank-Retzlaff, M.L.; Zhao, Q.; Anderson, C; Hamm, M.; High, K.; Nguyen, M.; Wang, F.; Wang, N.; Wang, B.; Wang, Y.; Washabaugh, M.; Sitrin, R. & Shi, L. (2006). Evaluation of the thermal stability of Gardasil. *Human Vaccines*, Vol.2, No.4, (July-August 2006), pp. 147–154, ISSN 1554-8600
- Sheets, E.E.; Urban, R.G.; Crum, C.P.; Hedley, M.L.; Politch, J.A.; Gold, M.A.; Muderspach, L.I.; Cole, G.A. & Crowley-Nowick, P.A. (2003). Immunotherapy of human cervical high-grade cervical intraepithelial neoplasia with microparticle-delivered human papillomavirus 16 E7 plasmid DNA. *American journal of obstetrics and gynecology*, Vol.188, No.4, (April 2003), pp. 916-926, ISSN 0002-9378
- Shi, L.; Sanyal, G.; Ni, A.; Luo, Z.; Doshna, S.; Wang, B.; Graham, T. L.; Wang, N. & Volkin, D. B. (2005). Stabilization of human papillomavirus virus-like particles by non-ionic surfactants. *Journal of pharmaceutical sciences*, Vol.94, No.7, (July 2005), pp. 1538-1551, ISSN 0022-3549
- Schiller, J.T. & Nardelli-Haeffliger, D. (2006). Chapter 17: Second generation HPV vaccines to prevent cervical cancer. *Vaccine*, Vol. 24, Suppl 3, (August 2006), pp. S3/147-153, ISSN 0264-410X
- Schwarz, T.F. (2009). Clinical update of the AS04-adjuvanted human papillomavirus-16/18 cervical cancer vaccine, Cervarix. *Advances in therapy*. Vol.26, No.11, (November 2009), pp.983-998, ISSN 0741-238X
- Shinje, G.; Neil, D.C.; John, W.K. & Jenson, A.B. (1991). Comparison of neutralization of BPV-1 infection of C127 cells and bovine fetal skin xenografts. *International journal of cancer*, Vol.49, No.2, (September 1991), pp. 285–289, ISSN 0020-7136
- Slupetzky, K.; Shafti-Keramat, S.; Lenz, P.; Brandt, S.; Grassauer, A.; Sara, M. & Kirnbauer, R. (2001). Chimeric papillomavirus-like particles expressing a foreign epitope on capsid surface loops. *The Journal of general virology*. 82, No.Pt 11, (November 2001), pp. 2799-2804, ISSN 0022-1317
- Šmídková, M.; Müller, M.; Thönes, N.; Puiko, K.; Angelisová, P.; Velemínský J. & Angelis K.J. (2010). Transient expression of human papillomavirus type 16 virus-like particles in tobacco and tomato using a tobacco rattle virus expression vector. *Biologia Plantarum*, Vol. 54, No.3, (September 2009), pp. 451-460, ISSN 0006-3134
- Stanley, M. (2010). Prospects for new human papillomavirus vaccines. *Current opinion in infectious diseases*, Vol.23, No.1, (February 2010), pp. 70-75, ISSN 0951-7375

- Thönes, N. & Müller, M. (2007). Oral immunization with different assembly forms of the HPV 16 major capsid protein L1 induces neutralizing antibodies and cytotoxic T-lymphocytes. *Virology*, Vol.369, No.2, (September 2007), pp. 375–388, ISSN 0042-6822
- Thönes, N.; Herreiner, A.; Schadlich, L.; Piuko, K. & Müller, M. (2008). A Direct Comparison of Human Papillomavirus Type 16 L1 Particles Reveals a Lower Immunogenicity of Capsomeres than Viruslike Particles with Respect to the Induced Antibody Response. *Journal of Virology*, Vol.82, No.11, (Jun 2008), pp. 5472–5485, ISSN 0022-538X
- van Ree, R.; Cabanes-Macheteau, M.; Akkerdaas, J.; Milazzo, J.P.; Loutelier-Bourhis, C.; Rayon, C.; Villalba, M.; Koppelman, S.; Aalberse, R.; Rodriguez, R.; Faye, L. & Lerouge P. (2000). Beta(1,2)-xylose and alpha(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. *The Journal of biological chemistry*, Vol.275, No.15, (April 2000), pp.114751-11458, ISSN 0021-9258
- Varsani, A.; Williamson, A.L.; deVilliers, D., Becker, I., Christensen, N.D. & Rybicki E.P. (2003a). Chimeric human papillomavirus type 16 (HPV-16) L1 particles presenting the common neutralizing epitope for the L2 minor capsid protein of HPV-6 and HPV-16. *Journal of Virology*, Vol.77, No.15, (August 2003), pp. 8386–8393, ISSN 0022-538X
- Varsani, A.; Williamson, A.L.; Rose, R.C.; Jaffer, M. & Rybicki, E.P. (2003b). Expression of Human papillomavirus type 16 major capsid protein in transgenic *Nicotiana tabacum* cv. Xanthi. *Archives of virology*, Vol.148, No., (9), (September 2003), pp. 1771–1786, ISSN 0304-8608
- Varsani, A.; Williamson, A.L.; Stewart, D. & Rybicki, E.P. (2006). Transient expression of Human papillomavirus type 16 L1 protein in *Nicotiana benthamiana* using an infectious tobamovirus vector. *Virus research*, Vol.120, No. 1-2, (March 2006), pp. 91–96, ISSN 0168-1702
- Velasquez, L.S.; Shira, S.; Berta, A.N.; Kilbourne, J.; Medi, B.M.; Tizard, I.; Ni, Y.; Arntzen, C.J. & Herbst-Kralovetz, M.M. (2011). Intranasal delivery of Norwalk virus-like particles formulated in an in situ gelling, dry powder vaccine. *Vaccine*, Vol.29, No. 32., (June 2011), pp. 5221– 5231, ISSN 0264-410X
- Villa, L.L.; Costa, R.L.; Petta, C.A.; Andrade, R.P.; Ault, K.A.; Giuliano, A.R.; Wheeler, C.M.; Koutsky, L.A.; Malm, C.; Lehtinen, M.; Skjeldestad, F.E.; Olsson, S.E.; Steinwall, M.; Brown, D.R.; Kurman, R.J.; Ronnett, B.M.; Stoler, M.H.; Ferenczy, A.; Harper, D.M.; Tamms, G.M.; Yu, J.; Lupinacci, L.; Railkar, R.; Taddeo, F. J.; Jansen, K. U.; Esser, M.T.; Sings, H.L.; Saah, A. J. & Barr, E. (2005). Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *The lancet oncology*, Vol.6, No.5, (May 2005), pp. 271-278, ISSN 1470-2045
- Villa, L. (2011). HPV prophylactic vaccination: The first years and what to expect from now. *Cancer Letters*, Vol.305, No.2, (Jun 2011), pp. 106–112, ISSN 0304-3835
- Wagner, H. & Proksh, A. (1985). Immunostimulatory drugs of fungi and higher plants. In: Wagner H, editor. *Economic and medicinal plant research*, pp. 113-153, Academic Press, New York

- Waheed, M.T.; Thönes, N.; Müller, M.; Hassan, S.W.; Razavi, M.; Lössl, E.; Kaul, H.P. & Lössl, A.G. (2011a). Transplastomic expression of a modified human papillomavirus L1 protein leading to the assembly of capsomeres in tobacco: a step towards cost-effective second-generation vaccines. *Transgenic research*, Vol.20, No.2, (Jun 2011), pp. 271–282, ISSN 0962-8819
- Waheed, M.T.; Thönes, N.; Müller, M.; Hassan, S.W.; Gottschamel, J.; Lössl, E., Kaul H.P. & Lössl A.G. (2011b). Plastid expression of a double-pentameric vaccine candidate containing human papillomavirus-16 L1 antigen fused with LTB as adjuvant: transplastomic plants show pleiotropic phenotypes. *Plant Biotechnology Journal*, Vol.9, No.6, (March 2011), pp. 651-660, ISSN 1467-7652
- Wakabayashi, M.T.; Da Silva, D.M.; Potkul, R.K. & Kast, W.M. (2002). Comparison of human papillomavirus type 16 L1 chimeric virus-like particles versus L1/L2 chimeric virus-like particles in tumor prevention. *Intervirology*, Vol.45, No.4-6, (April 2002), pp. 300–307, ISSN 0300-5526
- Warzecha, H.; Mason, H.S.; Lane, C.; Tryggvesson, A.; Rybicki, E.; Williamson, A.L.; Clements, J.D. & Rose, R.C. (2003). Oral immunogenicity of human papillomavirus-like particles expressed in potato. *Journal of Virology*, Vol.77, No. 16, (August 2003) pp. 8702-8711, ISSN 0022-538X
- Wright, T.C.; Bosch, F.X.; Franco, E.L.; Cuzick, J.; Schiller, J.T.; Garnett, G.P. & Meheus, A. (2006). Chapter 30: HPV vaccines and screening in the prevention of cervical cancer; conclusions from a 2006 workshop of international experts. *Vaccine*, Vol. 24, Suppl 3, (August 2006) pp. S3251-261, ISSN 0264-410X
- Xie Q.; Zhou Z.X.; Li Z.L. & Zeng Y. (2011). Transforming activity of a novel mutant of HPV16 E6E7 fusion gene. *Virologica Sinica*, Vol.26, No.3, (Jun 2011), pp. 206-213, ISSN 1995-820X
- Xu, Y.F.; Zhang, Y.Q.; Xu, X.M. & Song, G.X. (2006). Papillomavirus virus like particles as vehicles for the delivery of epitopes or genes. *Archives of virology*, Vol.151, No.11, (November 2006) pp. 2133-2148, ISSN 0304-8608
- Yamamoto, S.; Kiyono, H.; Yamamoto, M.; Imaoka, K.; Fujihashi, K.; Van Ginkel, F.W.; Noda, M.; Takeda, Y. & McGhee, J.R. (1997). A nontoxic mutant of cholera toxin elicits Th2-type responses for enhanced mucosal immunity. *Proceedings of the National Academy of Science USA*, Vol. 94, No.10, (May 1997), pp. 5267–5272, ISSN 0027-8424
- Yang, R.; Murillo, F.M.; Cui, H.; Blosser, R.; Uematsu, S.; Takeda, K.; Akira, S.; Viscidi, R.P. & Roden, R.B. (2004). Papillomavirus-like particles stimulate murine bone marrow-derived dendritic cells to produce alpha interferon and Th1 immune responses via MyD88. *Journal of Virology*, Vol.78, No.20, (October 2004), pp. 11152–11160, ISSN 0022-538X
- Yuan, H.; Estes, P.A.; Chen, Y.; Newsome, J.; Olcese, V.A.; Garcea, R.L. & Schlegel, R. (2001). Immunization with a pentameric L1 fusion protein protects against papillomavirus infection. *Journal of virology*, Vol.75, No.17, (September 2001), pp. 7848–7853, ISSN 0022-538X
- zur Hausen, H. (1996). Papillomavirus infections – a major cause of human cancers. *Biochimica et biophysica acta*, Vol.1288, No.2, (October 1996), pp. F55-F78, ISSN 0006-3002
- <http://www.ircm.qc.ca/LARECHERCHE/axes/Biologie/Virologie/Pages/Projets.aspx>



Human Papillomavirus and Related Diseases - From Bench to Bedside - A Clinical Perspective

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Cervical cancer is the second most prevalent cancer among women worldwide, and infection with Human Papilloma Virus (HPV) has been identified as the causal agent for this condition. The natural history of cervical cancer is characterized by slow disease progression, rendering the condition, in essence, preventable and even treatable when diagnosed in early stages. Pap smear and the recently introduced prophylactic vaccines are the most prominent prevention options, but despite the availability of these primary and secondary screening tools, the global burden of disease is unfortunately still very high. This book will focus on the clinical aspects of HPV and related disease, highlighting the latest developments in this field.

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