HPV vaccine manufacture

Field of the Invention

The present invention relates to the field of human vaccines. More particularly, the present invention relates to a HPV vaccine manufacturing process, and to resulting HPV vaccine compositions.

Background

Papillomaviruses are small, highly species specific, DNA tumor viruses. Human papillomaviruses are DNA viruses that infect basal epithelial (skin or mucosal) cells. Over 100 individual human papillomaviruses (HPV) types have been described. HPVs are generally specific either for the squamous epithelium of the skin or mucosal surfaces and usually cause benign tumors (warts) that persist for several months or years.

Persistent infection with an oncogenic human papillomavirus (HPV) type causes cervical cancer, the second most common cause of cancer deaths among women worldwide. There is international consensus that "high-risk" HPV types, including types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73 and 82 can lead to cervical cancer and are associated with other mucosal anogenital and head and neck cancers (IARC monograph 2018 https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100B-11.pdf, Martinez-Portilla et al., 2019[1]; Arbyn M et al., 2014[2]).

Infections with other types, termed "low-risk," including types 6, 11, can cause benign or low-grade cervical tissue changes and genital warts (condyloma acuminata), which include growths on the cervix, vagina, vulva and anus in women and the penis, scrotum or anus in men. They also cause epithelial growths over the vocal cords of children and adults (juvenile respiratory papillomatosis or recurrent respiratory papillomatosis) that require surgical intervention (Bosch et al., 2013[3]).

Several prophylactic HPV vaccines are licensed so far. They all use virus-like particles (VLPs) comprised of recombinant L1 capsid proteins of individual HPV types.

The CERVARIX HPV vaccine contains HPV-16 and -18 VLPs produced in a Trichoplusia ni insect cell substrate using a baculovirus expression vector system and formulated with the immunostimulant 3-O-desacyl-4'-monophosphoryl lipid A (3D MPL, also known as MPL) and aluminum hydroxide salt.

GARDASIL-9 (a nine-valent HPV vaccine) is the evolution of GARDASIL (a four-valent HPV vaccine), and contains HPV-6, -11, -16, -18, -31, -33, -45, -52, and -58 VLPs produced in the yeast *Saccharomyces cerevisiae* and formulated with amorphous aluminum hydroxyphosphate sulphate salt. GARDASIL-9 contains VLPs from non-oncogenic types HPV-6 and -11, which are implicated in 75–90% of genital warts, and from oncogenic types HPV-16, -18, -31, -33, -45, -52 and -58 implicated in cervical, vulvar, vaginal, anal, oropharyngeal and other head and neck cancers.

As reviewed by Fox et al. 2017 [4] in "Current Status of Toll-Like Receptor 4 Ligand Vaccine Adjuvants", TLR4 ligands demonstrate excellent adjuvant properties and TLR4-based adjuvant formulations have been included in several clinical trials; in particular 3D-MPL, GLA, SLA, RC-529 that can be adsorbed onto aluminum, and others such as OM-174 (E. coli derived triacetylated disaccharide diphosphoryl compound), PET lipid A (hexa-acylated monosaccharide monophosphoryl compound), ONO-4007 (monosaccharide triacyl structure with two of the chains terminating in benzene rings and a sulphate head group). Additionally, dLOS is another TLR4 ligand. It is prepared by alkaline hydrolysis of LPS which lacks O-antigen and is expressed by *Escherichia coli* rough strain. Furthermore, upon combination with aluminum, dLOS and alum are capable of synergizing their adjuvant effects to HPV L1 VLPs (Eun et. al., 2014 [6]).

WO00/23105 discloses a process for the preparation of an adjuvanted HPV vaccines which consists of admixing HPV L1 VLPs and TLR4 ligands that were each pre-adsorbed onto a metallic salt.

There is a need for improved methods of preparing adjuvanted HPV vaccines.

Summary of the invention

In one aspect, the present invention provides a method for the preparation of a HPV vaccine composition comprising the steps of (i) adsorbing one or more HPV antigen(s) on a metallic salt and, (ii) adding a non adsorbed glycolipid based TLR4 ligand to the mixture obtained in (i).

In another aspect, the invention provides a HPV vaccine composition obtained by the method according to the invention.

In one aspect, there is provided a HPV vaccine composition as described herein for use in therapy.

In one aspect, there is provided a HPV vaccine composition as described herein for preventing or treating a HPV infection or associated disease in a subject.

In one aspect, there is provided the use of a HPV vaccine composition as described herein, in the manufacture of a medicament for the prevention or treatment of HPV infection in a subject.

In one aspect, there is provided a method of preventing or treating an infection or disease comprising the administration of an effective amount of a HPV vaccine composition as described herein to a patient in need thereof.

In one aspect, there is provided a method of preventing or treating an infection or disease caused by HPV comprising the administration of an effective amount of a HPV vaccine composition as described herein to a patient in need thereof.

Brief description of the drawings

FIG. 1: Picture of 9V DP after one day (A) and 7 days (B). From left to right: in-line AlOOH (Ix); in-line no Al added; in-line $AlPO_4$; in-line AlOOH (Croda); in-line AlOOH (Ix) with aggregated MPL; in-line $AlPO_4$ with aggregated MPL; in-line AlOOH (Croda) with aggregated MPL; 9V DP with AMB MPL $AlPO_4$; 9V DP with AMB MPL AlOOH; 9V DP with AMB aggregated MPL $AlPO_4$.

FIG. 2: Graph representing the evolution of the average median particle size measured by SLS over 4 time points (after formulation, one week, two weeks and six weeks) for the 9V DPs (without aggregated MPL).

FIG. 3: Distribution of the particle sizes of the different 9V DP formulations (with or w/o aggregated MPL) measured by SLS one day after their formulation. The average of 5 measurements per sample after formulation is shown.

FIG. 4: Distribution of the particle sizes of CERVARIX, GARDASIL-9 and the 9V DP (without MPL) measured by SLS. The average of 5 measurements per sample is shown.

FIG. 5: Distribution of the particle sizes of the different 9V DP formulations measured by SLS. The average of 5 measurements per sample is shown.

FIG. 6: Relative potency computed with MPL ref 10µg/mL depending on MPL concentration for each sample.

FIG. 7: Bioassay measuring the MPL activity of the different 9V DP formulations, measured by quantifying the TNF-α response. Relative potency computed with MPL ref 10µg/mL depending on MPL

concentration for each sample grouped by MPL concentration.

FIG. 8: Bioassay measuring the MPL activity of the different 9V DP formulations, measured by quantifying the TNF-α response. The results have been normalized to the value of the MPL bulk control that has been included in the measurements, resulting in the Relative Potency values.

List of sequences

SEQ ID NO: 1 – full length HPV 6 L1 amino acid sequence (GENBANK accession number: AAC80442.1)

SEQ ID NO: 2 – truncated HPV 6 L1 amino acid sequence

SEQ ID NO: 3 – full length HPV 11 L1 amino acid sequence (UNIPROT accession number: P04012.1)

SEQ ID NO: 4 – truncated HPV 11 L1 amino acid sequence

SEQ ID NO: 5 – full length HPV 16 L1 amino acid sequence (GENBANK accession number: AGC65525.1)

SEQ ID NO: 6 – truncated HPV 16 L1 amino acid sequence

SEQ ID NO: 7 – full length HPV 18 L1 amino acid sequence (GENBANK accession number: AAQ92369.1)

SEQ ID NO: 8 – truncated HPV 18 L1 amino acid sequence

SEQ ID NO: 9 – full length HPV 31 L1 amino acid sequence (GENBANK accession number: AAA46956.1)

SEQ ID NO: 10 – truncated HPV 31 L1 amino acid sequence

SEQ ID NO: 11 – full length HPV 33 L1 amino acid sequence (GENBANK accession number: ACV84009.1)

SEQ ID NO: 12 – truncated HPV 33 L1 amino acid sequence

SEQ ID NO: 13 – full length HPV 45 L1 amino acid sequence (GENBANK accession number: AAY86494.1)

SEQ ID NO: 14 – truncated HPV 45 L1 amino acid sequence

SEQ ID NO: 15 – full length HPV 52 L1 amino acid sequence (GENBANK accession number: ACM66882.1)

SEQ ID NO: 16 – truncated HPV 52 L1 amino acid sequence

SEQ ID NO: 17 – full length HPV 58 L1 amino acid sequence (GENBANK accession number: ACM66884)

SEQ ID NO: 18 – truncated HPV 58 L1 amino acid sequence

SEQ ID NO: 19 – HPV 6 L1 amino acid sequence

SEQ ID NO: 20 – HPV 11 L1 amino acid sequence

SEQ ID NO: 21 – HPV 16 L1 amino acid sequence

SEQ ID NO: 22 – HPV 18 L1 amino acid sequence

SEQ ID NO: 23 – HPV 31 L1 amino acid sequence

SEQ ID NO: 24 – HPV 33 L1 amino acid sequence

SEQ ID NO: 25 – HPV 45 L1 amino acid sequence

SEQ ID NO: 26 – HPV 52 L1 amino acid sequence

SEQ ID NO: 27 – HPV 58 L1 amino acid sequence

SEQ ID NO: 28 – HPV 16 L1 amino acid sequence

SEQ ID NO: 29 – HPV 18 L1 amino acid sequence

SEQ ID NO: 30 – full length HPV 26 L1 amino acid sequence (UNIPROT kB accession number: P36735.1)

SEQ ID NO: 31 – full length HPV 35 L1 amino acid sequence (GENBANK accession number: QJD38251.1)

SEQ ID NO: 32 – full length HPV 39 L1 amino acid sequence (GENBANK accession number: CAD1814503.1)

SEQ ID NO: 33 – full length HPV 51 L1 amino acid sequence (GENBANK accession number: ARQ82736.1)

SEQ ID NO: 34 – full length HPV 53 L1 amino acid sequence (GENBANK accession number: ACX32368.1)

SEQ ID NO: 35 – full length HPV 56 L1 amino acid sequence (GENBANK accession number: CAD1814189.1)

SEQ ID NO: 36 – full length HPV 59 L1 amino acid sequence (GENBANK accession number: AGU90696.1)

SEQ ID NO: 37 – full length HPV 66 L1 amino acid sequence (GENBANK accession number: ALT54954.1)

SEQ ID NO: 38 – full length HPV 67 L1 amino acid sequence (GENBANK accession number: CAD1814012.1)

SEQ ID NO: 39 – full length HPV 68 L1 amino acid sequence (GENBANK accession number: ACX32384.1)

SEQ ID NO: 40 – full length HPV 70 L1 amino acid sequence (GENBANK accession number: AGU90878.1)

SEQ ID NO: 41 – full length HPV 73 L1 amino acid sequence (GENBANK accession number: CAA63887.1)

SEQ ID NO: 42 – full length HPV 82 L1 amino acid sequence (GENBANK accession number: BAA90742.1)

Detailed description of the invention

The present invention is based on a counterintuitive finding by the inventors that a method for the preparation of an adjuvanted HPV vaccine where HPV antigens pre-adsorbed onto a metallic salt are combined with a TLR4 ligand which has not been pre-adsorbed onto a metallic salt ("free TLR4 ligand" or "non adsorbed TLR4 ligand"), are advantageous. Indeed, the inventors have surprisingly found that this method leads to enhanced TLR4 ligand biological activity as compared to the method disclosed in WO 00/23105 where the TLR4 ligand is pre-adsorbed onto a metallic salt prior to being combined with the HPV antigens. This improved formulation method is referred to herein as "in-line" formulation/production. This "in-line" method goes against the teaching of WO 00/23105 according to which the TLR4 ligand needs to be pre-adsorbed on a metallic salt prior to being combined with the pre-adsorbed HPV antigens, in order to avoid difficulties during quality control (QC) for the assessment of the proper adsorption of the TLR4 ligand and of the antigen.

In one **aspect**, the present invention provides a method for the preparation of a HPV vaccine composition comprising the steps of (i) adsorbing one or more HPV antigen(s) on a metallic salt and, (ii) adding a non adsorbed glycolipid based TLR4 ligand to the mixture obtained in (i).

As used herein, an "**in-line**" process or method is a process or method where antigens, in particular HPV antigens, are combined with a TLR4 ligand which has not been pre-adsorbed onto a metallic salt. The antigens may be pre-adsorbed onto a metallic salt.

As used herein, a "**salt**" is a chemical compound consisting of an ionic assembly of cations and anions, and a "**metallic salt**" is a salt comprising a metallic ion. Preferred metallic salt are aluminum salts comprising Al^{3+}.

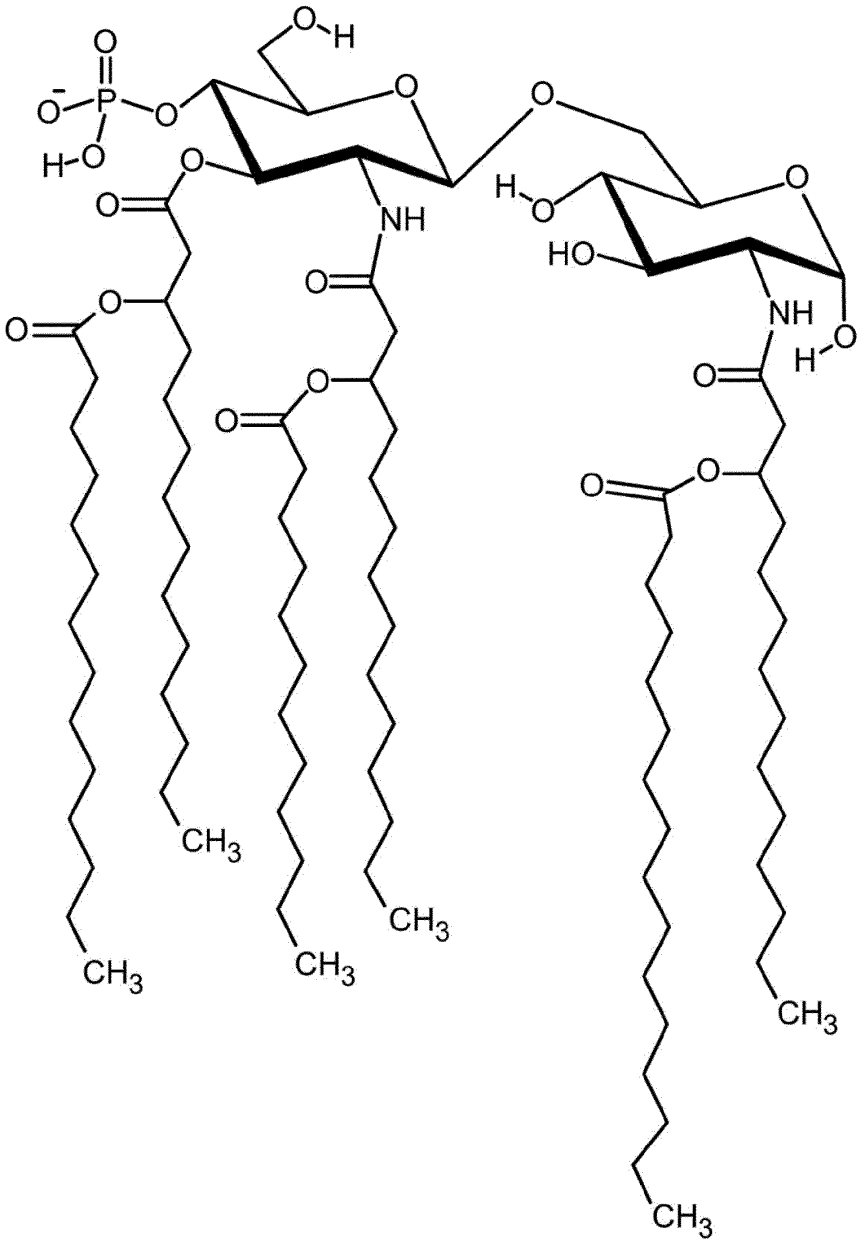
As used herein, a "**non adsorbed**" (or "free" or "not pre-adsorbed") glycolipid based TLR4 ligand is a glycolipid based TLR4 ligand which has not been pre-adsorbed on a metallic salt.

As used herein, a "**TLR4 ligand**" is a molecule capable of binding to TLR4 (Toll Like Receptor 4). TLR4 is a transmembrane protein, member of the toll-like receptor family, which belongs to the pattern recognition receptor (PRR) family. It is most well-known for recognizing lipopolysaccharide (LPS), a component present in many Gram-negative bacteria and some Gram-positive bacteria. Upon activation, TLR4 triggers the production of mature IL18. IL18 then drives the production of INF-γ by innate cells including natural killer cells (NK) and neutrophils, as well as memory CD8 T cells, that in turn promote TH1 immunity. Nontoxic TLR4 ligands can thus be used as adjuvants.

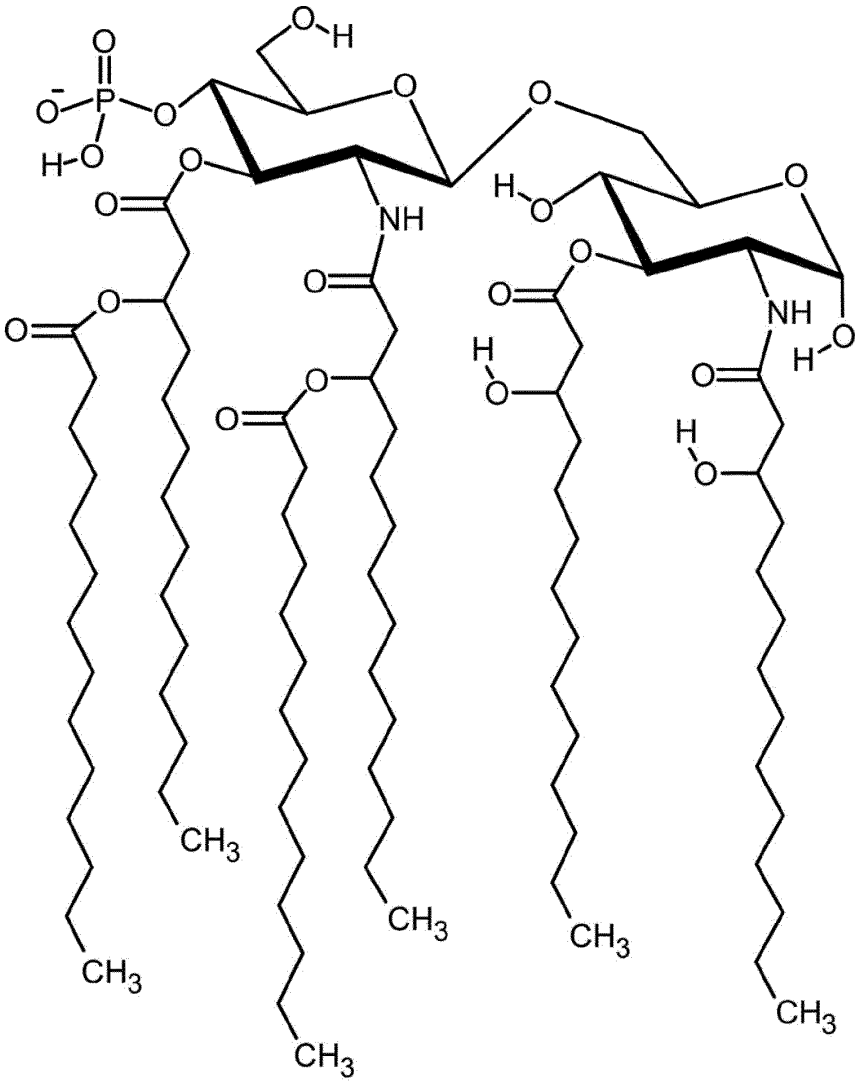
As used herein, a "**glycolipid based**" TLR4 ligand is a non toxic TLR4 ligand based on an oligosaccharide structure covalently linked to one or more lipid chains. Such a TLR4 ligand is suitable for use as an adjuvant in a vaccine composition. Glycolipid based TLR4 ligand can be derived from bacterial LPS, Lipid-A or chemically synthesized. Suitable glycolipid based TLR4 ligands include

- disaccharide glycolipids, such as MPL (or 3D-MPL), GLA, SLA and OM-174,
- monosaccharide glycolipids such as CCL-34, RC-529, PET Lipid A and ONO-4007, CRX601, and
- lipooligosaccharides such as dLOS,

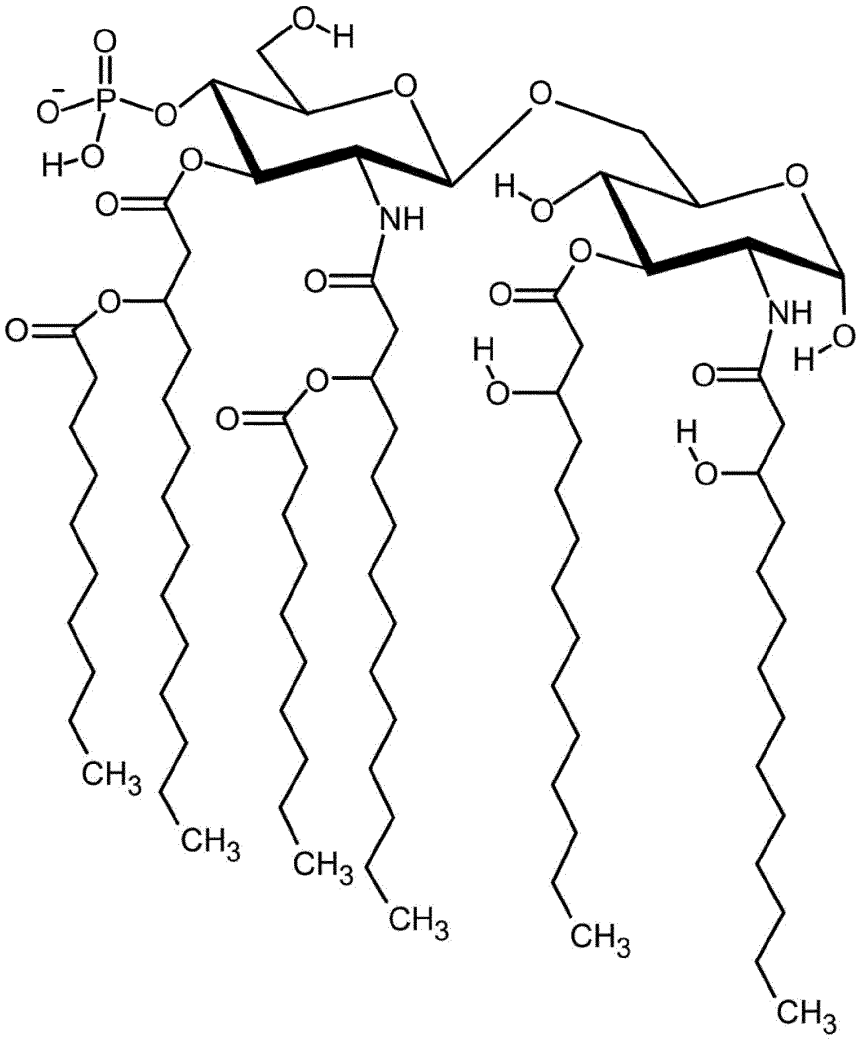
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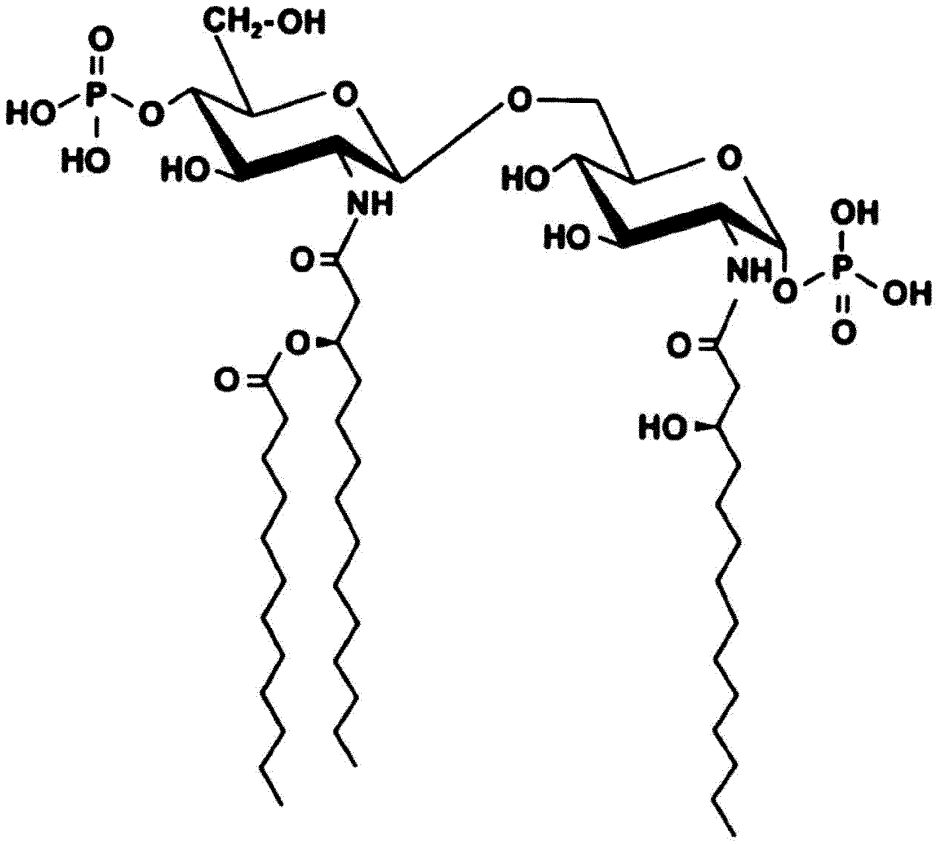
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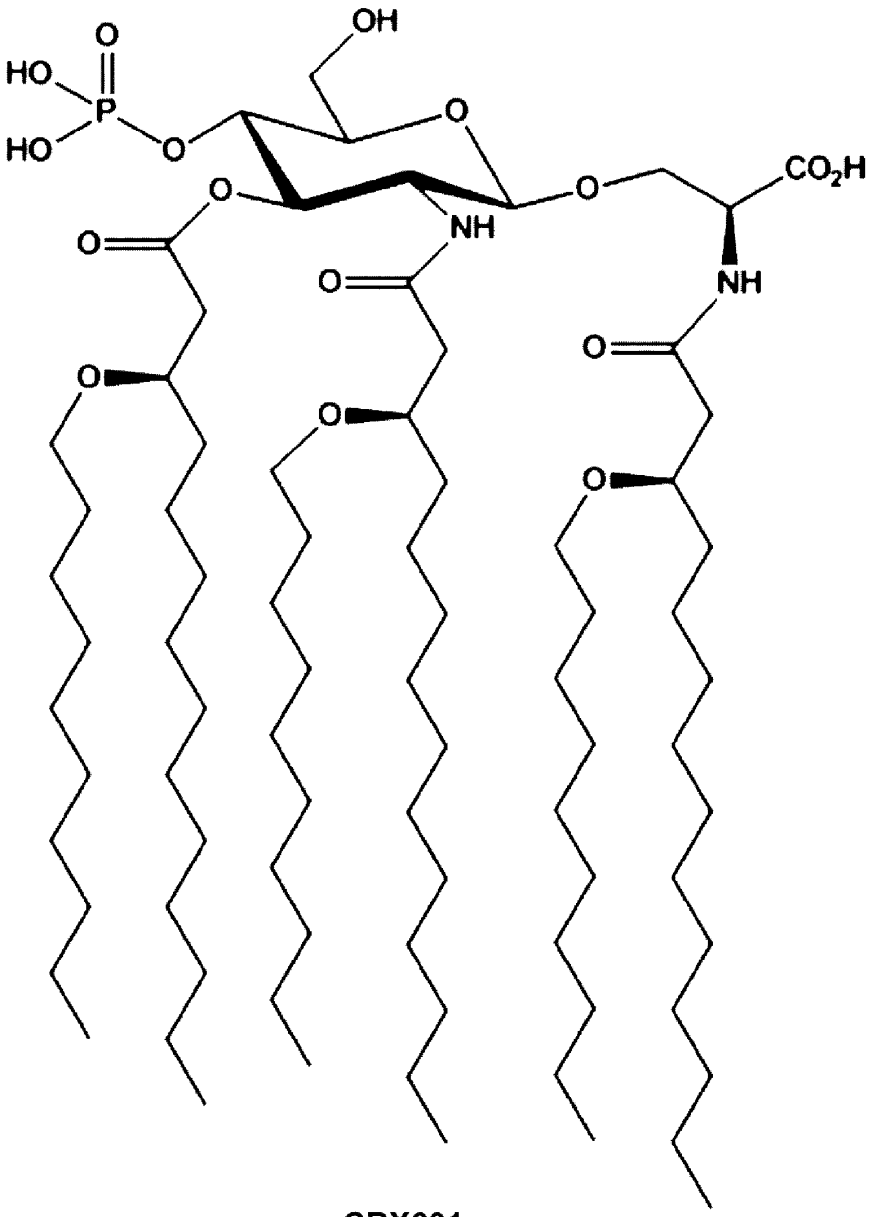
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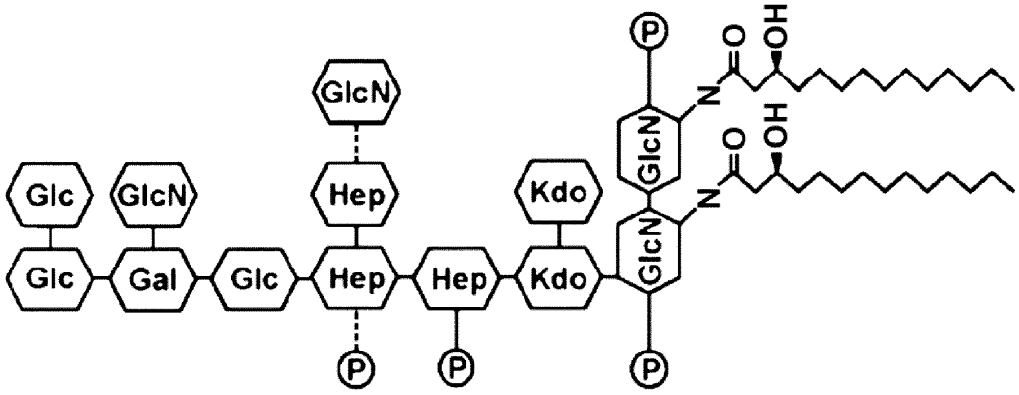
SLA



OM-174.



CRX601



dLOS

MPL (3-Deacylated monophoshoryl lipid A) is commercialized by GlaxoSmithKline Biologicals, and is referred to herein as MPL or 3D-MPL. See, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094. 3D-MPL primarily promotes CD4+ T cell responses with an IFN-γ (Th1) phenotype. 3D-MPL can be produced according to the methods disclosed in GB2220211 A. Chemically, it is a mixture of 3-deacylated monophosphoryl lipid A with 3, 4, 5 or 6 acylated chains. In the compositions of the present invention small particle 3D-MPL can be used. Small particle 3D-MPL has a particle size such that it can be sterile-filtered through a 0.22µm filter. Such preparations are described in WO94/21292.

GLA (glucopyranosyl lipid adjuvant) and **SLA** (second-generation lipid adjuvant) are two synthetic analogs of detoxified MPL. The structures of GLA and SLA are based on molecules in MPL except that in GLA the hexa-acyl chain is at the 3 position, whereas MPL is 3-deacylated. SLA is a version of GLA where the length of some acyl chains is different. (Reed et al., 2016[5]).

OM-174 is a purified water soluble dephosphorylated and triacetylated lipid A derived from E. coli. This compound is currently being developed as an adjuvant for therapeutic vaccination, and mainly for cancer applications (D'Agostini et al. 2005 [9]).

CCL-34 is a synthetic glycolipid that induces the activation of macrophages and the maturation of dendritic cells in a TLR4-dependent manner (Chou et al., 2020 [7]).

RC-529 is a chemically obtained MPL mimic, which combined with aluminum oxyhydroxide formed the adjuvant component of a hepatitis B virus vaccine licensed in Argentina (Fox et al. 2017 [4], Shimoyama and Fukase, 2020 [8]);

PET Lipid A is a synthetic hexa-acylated monosaccharide monophosphorylated TLR4 ligand. The combination of a liposomal formulation of this compound and a cancer antigen was tolerated in phase I clinical trial (Fox et al. 2017 [4], Hamdy et al. 2007 [10], Kawther et al.[13]).

ONO-4007 is a synthetic lipid A derivative featuring a monosaccharide triacyl structure, with two of the chains terminating in benzene rings and the head group phosphate replaced by a sulfate. A phase I immunotherapy trial was reported with this compound in 2002 (Kuramitsu et al. 1997 [11]).

CRX601 is an alkyl glucosaminide phosphate (AGP) described in WO98/50399 and US patent No. 6,303,347 (processes for preparation of AGPs are also described).

dLOS (deacylated lipooligosaccharide, also referred to as "CIA05") consists of a core oligosaccharide lacking the terminal glucose residue, a glucosamine disaccharide with two phosphate groups, and two N-linked acyl groups. dLOS induces Th1, Th2, and Th17-type immune responses in a dose dependent manner (Eun et al. 2014 [6], Seo Ri Wui et al.[14]).

In one embodiment, the glycolipid based TLR4 ligand is selected from disaccharide glycolipids, monosaccharide glycolipids and lipooligosaccharides. In a preferred embodiment, the glycolipid-based TLR4 ligand is selected from MPL, GLA, SLA, OM-174, CCL-34, RC-529, PET-Lipid A, ONO-4007, CRX601 and dLOS. In a preferred embodiment, the glycolipid based TLR4 ligand is MPL.

In one embodiment, the one or more HPV antigen(s) are selected from HPV late proteins L1 and L2, chimeric L1 proteins, chimeric L1/L2 proteins, and immunogenic fragments thereof. A "**late protein**" (L) is a viral protein that is formed after the virus replication. It usually surrounds the genetic material of a virus as a capsid. L1 proteins are the major proteins of the HPV capsid, and L2 proteins are the minor proteins of the HPV capsid. A chimeric L1 protein is a protein comprising parts of an L1 protein from a first HPV type and parts of an L1 protein from a second HPV type wherein the first and second HPV types are different. A chimeric L1/L2 protein is a protein comprising parts of an L1 HPV protein and parts of an L2 HPV protein, for example an L1 HPV protein expressing one or more conserved L2 epitopes.

Exemplary HPV L1 sequences are shown in table 1.

Table 1 *– Exemplary HPV L1 sequences*

Protein	SEQ ID NO	Sequence
HPV6 L1	1	MWRPSDSTVYVPPPNPVSKVVATDAYVTRTNIFYHASSSRLLAVGHPYF SIKRANKTVVPKVSGYQYRVFKVVLPDPNKFALPDSSLFDPTTQRLVWA CTGLEVGRGQPLGVGVSGHPFLNKYDDVENSGSGGNPGQDNRVNVGMDY KQTQLCMVGCAPPLGEHWGKGKQCTNTPVQAGDCPPLELITSVIQDGDM VDTGFGAMNFADLQTNKSDVPIDICGTTCKYPDYLQMAADPYGDRLFFF LRKEQMFARHFFNRAGEVGEPVPDTLIIKGSGNRTSVGSSIYVNTPSGS LVSSEAQLFNKPYWLQKAQGHNNGICWGNQLFVTVVDTTRSTNMTLCAS VTTSSTYTNSDYKEYMRHVEEYDLQFIFQLCSITLSAEVMAYIHTMNPS VLEDWNFGLSPPPNGTLEDTYRYVQSQAITCQKPTPEKQKPDPYKNLSF WEVNLKEKFSSELDQYPLGRKFLLQSGYRGRSSIRTGVKRPAVSKASAA PKRKRAKTKR
	2	MDSTVYVPPPNPVSKVVATDAYVTRTNIFYHASSSRLLAVGHPYFSIKR ANKTVVPKVSGYQYRVFKVVLPDPNKFALPDSSLFDPTTQRLVWACTGL EVGRGQPLGVGVSGHPFLNKYDDVENSGSGGNPGQDNRVNVGMDYKQTQ LCMAGCAPPLGEHWGKGKQCTNTPVQAGDCPPLELITSVIQDGDMVDTG FGAMNFADLQTNKSDVPIDICGTTCKYPDYLQMAADPYGDRLFFFLRKE QMFARHFFNRAGEVGEPVPDTLIIKGSGNRTSVGSSIYVNTPSGSLVSS EAQLFNKPYWLQKAQGHNNGICWGNQLFVTVVDTTRSTNMTLCASVTTS STYTNSDYKEYMRHVEEYDLQFIFQLCSITLSAEVVAYIHTMNPSVLED WNFGLSPPPNGTLEDTYRYVQSQAITCQKPTPEKQKPDPYKNLSFWEVN LKEKFSSELDQYPLGRKFLLQSGYRGRSSIRTGVKRPAVSKASAAPKRK RAKTKR
	19	MWRPSDSTVYVPPPNPVSKVVATDAYVTRTNIFYHASSSRLLAVGHPYF SIKRANKTVVPKVSGYQYRVFKVVLPDPNKFALPDSSLFDPTTQRLVWA CTGLEVGRGQPLGVGVSGHPFLNKYDDVENSGSGGNPGQDNRVNVGMDY KQTQLCMAGCAPPLGEHWGKGKQCTNTPVQAGDCPPLELITSVIQDGDM VDTGFGAMNFADLQTNRSDVPIDICGTTCKYPDYLQMAADPYGDRLFFF LRKEQMFARHFFNRAGEVGEPVPDTLIIKGSGNRTSVGSSIYVNTPSGS LVSSEAQLFNKPYWLQKAQGHNNGICWGNQLFVTVVDTTRSTNMTLCAS VTTSSTYTNSDYKEYMRHVEEYDLQFIFQLCSITLSAEVMAYIHTMNPS VLEDWNFGLSPPPNGTLEDTYRYVQSQAITCQKPTPEKQKPDPYKNLSF WEVNLKEKFSSELDQYPLGRKFLLQSGYRGRSSIRTGVKRPAVSKASAA PKRKRAKTKR
HPV11 L1	3	MWRPSDSTVYVPPPNPVSKVVATDAYVKRTNIFYHASSSRLLAVGHPYY SIKKVNKTVVPKVSGYQYRVFKVVLPDPNKFALPDSSLFDPTTQRLVWA CTGLEVGRGQPLGVGVSGHPLLNKYDDVENSGGYGGNPGQDNRVNVGMD YKQTQLCMVGCAPPLGEHWGKGTQCSNTSVQNGDCPPLELITSVIQDGD MVDTGFGAMNFADLQTNKSDVPLDICGTVCKYPDYLQMAADPYGDRLFF YLRKEQMFARHFFNRAGTVGEPVPDDLLVKGGNNRSSVASSIYVHTPSG SLVSSEAQLFNKPYWLQKAQGHNNGICWGNHLFVTVVDTTRSTNMTLCA SVSKSATYTNSDYKEYMRHVEEFDLQFIFQLCSITLSAEVMAYIHTMNP SVLEDWNFGLSPPPNGTLEDTYRYVQSQAITCQKPTPEKEKQDPYKDMS FWEVNLKEKFSSELDQFPLGRKFLLQSGYRGRTSARTGIKRPAVSKPST APKRKRTKTKK
	4	MSDSTVYVPPPNPVSKVVATDAYVKRTNIFYHASSSRLLAVGHPYYSIK KVNKTVVPKVSGYQYRVFKVVLPDPNKFALPDSSLFDPTTQRLVWACTG LEVGRGQPLGVGVSGHPLLNKYDDVENSGGYGGNPGQDNRVNVGMDYKQ

Protein	SEQ ID NO	Sequence
		TQLCMVGCAPPLGEHWGKGTQCSNTSVQNGDCPPLELITSVIQDGDMVD TGFGAMNFADLQTNKSDVPLDICGTVCKYPDYLQMAADPYGDRLFFYLR KEQMFARHFFNRAGTVGEPVPDDLLVKGGNNRSSVASSIYVHTPSGSLV SSEAQLFNKPYWLQKAQGHNNGICWGNHLFVTVVDTTRSTNMTLCASVS KSATYTNSDYKEYMRHVEEFDLQFIFQLCSITLSAEVMAYIHTMNPSVL EDWNFGLSPPPNGTLEDTYRYVQSQAITCQKPTPEKEKQDPYKDMSFWE VNLKEKFSSELDQFPLGRKFLLQSGYRGRTSARTGIKRPAVSKPSTAPK RKRTKTKK
	20	MWRPMSDSTVYVPPPNPVSKVVATDAYVKRTNIFYHASSSRLLAVGHPY YSIKKVNKTVVPKVSGYQYRVFKVVLPDPNKFALPDSSLFDPTTQRLVW ACTGLEVGRGQPLGVGVSGHPLLNKYDDVENSGGYGGNPGQDNRVNVGM DYKQTQLCMVGCAPPLGEHWGKGTQCSNTSVQNGDCPPLELITSVIQDG DMVDTGFGAMNFADLQTNKSDVPLDICGTVCKYPDYLQMAADPYGDRLF FYLRKEQMFARHFFNRAGTVGEPVPDDLLVKGGNNRSSVASSIYVHTPS GSLVSSEAQLFNKPYWLQKAQGHNNGICWGNHLFVTVVDTTRSTNMTLC ASVSKSATYTNSDYKEYMRHVEEFDLQFIFQLCSITLSAEVMAYIHTMN PSVLEDWNFGLSPPPNGTLEDTYRYVQSQAITCQKPTPEKEKQDPYKDM SFWEVNLKEKFSSELDQFPLGRKFLLQSGYRGRTSARTGIKRPAVSKPS TAPKRKRTKTKK
HPV16 L1	5	MEVTFIYILVITCYENDVNVYHIFFQMSLWLPSEATVYLPPVPVSKVVS TDEYVARTNIYYHAGTSRLLAVGHPYFPIKKPNNNKILVPKVSGLQYRV FRIHLPDPNKFGFPDTSFYNPDTQRLVWACVGVEVGRGQPLGVGISGHP LLNKLDDTENASAYAANAGVDNRECISMDYKQTQLCLIGCKPPIGEHWG KGSPCTNVAVNPGDCPPLELINTVIQDGDMVDTGFGAMDFTTLQANKSE VPLDICTSICKYPDYIKMVSEPYGDSLFFYLRREQMFVRHLFNRAGAVG ENVPDDLYIKGSGSTANLASSNYFPTPSGSMVTSDAQIFNKPYWLQRAQ GHNNGICWGNQLFVTVVDTTRSTNMSLCAAISTSETTYKNTNFKEYLRH GEEYDLQFIFQLCKITLTADVMTYIHSMNSTILEDWNFGLQPPPGGTLE DTYRFVTSQAIACQKHTPPAPKEDPLKKYTFWEVNLKEKFSADLDQFPL GRKFLLQAGLKAKPKFTLGKRKATPTTSSTSTTAKRKKRKL
	6	MLPSEATVYLPPVPVSKVVSTDEYVARTNIYYHAGTSRLLAVGHPYFPI KKPNNNKILVPKVSGLQYRVFRIHLPDPNKFGFPDTSFYNPDTQRLVWA CVGVEVGRGQPLGVGISGHPLLNKLDDTENASAYAANAGVDNRECISMD YKQTQLCLIGCKPPIGEHWGKGSPCTNVAVNPGDCPPLELINTVIQDGD MVDTGFGAMDFTTLQANKSEVPLDICTSICKYPDYIKMVSEPYGDSLFF YLRREQMFVRHLFNRAGAVGDNVPDDLYIKGSGSTANLASSNYFPTPSG SMVTSDAQIFNKPYWLQRAQGHNNGICWGNQLFVTVVDTTRSTNMSLCA AISTSETTYKNTNFKEYLRHGEEYDLQFIFQLCKITLTADIMTYIHSMN STILEDWNFGLQPPPGGTLEDTYRFVTSQAIACQKHTPPAPKEDPLKKY TFWEVNLKEKFSADLDQFPLGRKFLLQAGLEAKPKFTLGKRKATPTTSS TSTTAKRKKRKL
	21	MSLWLPSEATVYLPPVPVSKVVSTDEYVARTNIYYHAGTSRLLAVGHPY FPIKKPNNNKILVPKVSGLQYRVFRIHLPDPNKFGFPDTSFYNPDTQRL VWACVGVEVGRGQPLGVGISGHPLLNKLDDTENASAYAANAGVDNRECI SMDYKQTQLCLIGCKPPIGEHWGKGSPCTNVAVNPGDCPPLELINTVIQ DGDMVDTGFGAMDFTTLQANKSEVPLDICTSICKYPDYIKMVSEPYGDS LFFYLRREQMFVRHLFNRAGAVGENVPDDLYIKGSGSTANLASSNYFPT PSGSMVTSDAQIFNKPYWLQRAQGHNNGICWGNQLFVTVVDTTRSTNMS LCAAISTSETTYKNTNFKEYLRHGEEYDLQFIFQLCKITLTADVMTYIH

Protein	SEQ ID NO	Sequence
		SMNSTILEDWNFGLQPPPGGTLEDTYRFVTSQAIACQKHTPPAPKEDPL KKYTFWEVNLKEKFSADLDQFPLGRKFLLQAGLKAKPKFTLGKRKATPT TSSTSTTAKRKKRKL
	28	MSLWLPSEATVYLPPVPVSKVVSTDEYVARTNIYYHAGTSRLLAVGHPY FPIKKPNNNKILVPKVSGLQYRVFRIHLPDPNKFGFPDTSFYNPDTQRL VWACVGVEVGRGQPLGVGISGHPLLNKLDDTENASAYAANAGVDNRECI SMDYKQTQLCLIGCKPPIGEHWGKGSPCTNVAVNPGDCPPLELINTVIQ DGDMVDTGFGAMDFTTLQANKSEVPLDICTSICKYPDYIKMVSEPYGDS LFFYLRREQMFVRHLFNRAGAVGENVPDDLYIKGSGSTANLASSNYFPT PSGSMVTSDAQIFNKPYWLQRAQGHNNGICWGNQLFVTVVDTTRSTNMS LCAAISTSETTYKNTNFKEYLRHGEEYDLQFIFQLCKITLTADVMTYIH SMNSTILEDWNFGLQPPPGGTLEDTYRFVTSQAIACQKHTPPAPKEDPL KKYTFWEVNLKEKFSADLDQFPLGRKFLLQ
HPV18 L1	7	MALWRPSDNTVYLPPPSVARVVNTDDYVTRTSIFYHAGSSRLLTVGNPY FRVPAGGGNKQDIPKVSAYQYRVFRVQLPDPNKFGLPDTSIYNPETQRL VWACAGVEIGRGQPLGVGLSGHPFYNKLDDTESSHAATSNVSEDVRDNV SVDYKQTQLCILGCAPAIGEHWAKGTACKSRPLSQGDCPPLELKNTVLE DGDMVDTGYGAMDFSTLQDTKCEVPLDICQSICKYPDYLQMSADPYGDS MFFCLRREQLFARHFWNRAGTMGDTVPQSLYIKGTGMRASPGSCVYSPS PSGSIVTSDSQLFNKPYWLHKAQGHNNGVCWHNQLFVTVVDTTRSTNLT ICASTQSPVPGQYDATKFKQYSRHVEEYDLQFIFQLCTITLTADVMSYI HSMNSSILEDWNFGVPPPPTTSLVDTYRFVQSVAITCQKDAAPAENKDP YDKLKFWNV
	8	MRPSDNTVYLPPPSVARVVNTDDYVTRTSIFYHAGSSRLLTVGNPYFRV PAGGGNKQDIPKVSAYQYRVFRVQLPDPNKFGLPDTSIYNPETQRLVWA CAGVEIGRGQPLGVGLSGHPFYNKLDDTESSHAATSNVSEDVRDNVSVD YKQTQLCILGCAPAIGEHWAKGTACKSRPLSQGDCPPLELKNTVLEDGD MVDTGYGAMDFSTLQDTKCEVPLDICQSICKYPDYLQMSADPYGDSMFF CLRREQLFARHFWNRAGTMGDTVPQSLYIKGTGMRASPGSCVYSPSPSG SIVTSDSQLFNKPYWLHKAQGHNNGVCWHNQLFVTVVDTTRSTNLTICA STQSPVPGQYDATKFKQYSRHVEEYDLQFIFQLCTITLTADVMSYIHSM NSSILEDWNFGVPPPPTTSLVDTYRFVQSVAIACQKDAAPAENKDPYDK LKFWNVDLKEKFSLDLDQYPLGRKFLVQAGLRRKPTIGPRKRSAPSATT ASKPAKRVRVRARK
	22	MALWRPSDNTVYLPPPSVARVVNTDDYVTRTSIFYHAGSSRLLTVGNPY FRVPAGGGNKQDIPKVSAYQYRVFRVQLPDPNKFGLPDNSIYNPETQRL VWACAGVEIGRGQPLGVGLSGHPFYNKLDDTESSHAATSNVSEDVRDNV SVDYKQTQLCILGCAPAIGEHWAKGTACKSRPLSQGDCPPLELKNTVLE DGDMVDTGYGAMDFSTLQDTKCEVPLDICQSICKYPDYLQMSADPYGDS MFFCLRREQLFARHFWNRAGTMGDTVPQSLYIKGTGMRASPGSCVYSPS PSGSIVTSDSQLFNKPYWLHKAQGHNNGICWHNQLFVTVVDTTRSTNLT ICASTQSPVPGQYDATKFKQYSRHVEEYDLQFIFQLCTITLTADVMSYI HSMNSSILEDWNFGVPPPPTTSLVDTYRFVQSVAITCQKDAAPAENKDP YDKLKFWNVDLKEKFSLDLDQYPLGRKFLVQAGLRRKPTIGPRKRSAPS ATTSSKPAKRVRVRARK
	29	MALWRPSDNTVYLPPPSVARVVNTDDYVTRTSIFYHAGSSRLLTVGNPY FRVPAGGGNKQDIPKVSAYQYRVFRVQLPDPNKFGLPDNSIYNPETQRL VWACVGVEIGRGQPLGVGLSGHPFYNKLDDTESSHAATSNVSEDVRDNV SVDYKQTQLCILGCAPAIGEHWAKGTACKSRPLSQGDCPPLELKNTVLE

Protein	SEQ ID NO	Sequence
		DGDMVDTGYGAMDFSTLQDTKCEVPLDICQSICKYPDYLQMSADPYGDS MFFCLRREQLFARHFWNRAGTMGDTVPPSLYIKGTGMRASPGSCVYSPS PSGSIVTSDSQLFNKPYWLHKAQGHNNGVCWHNQLFVTVVDTTRSTNLT ICASTQSPVPGQYDATKFKQYSRHVEEYDLQFIFQLCTITLTADVMSYI HSMNSSILEDWNFGVPPPPTTSLVDTYRFVQSVAITCQKDAAPAENKDP YDKLKFWNVDLKEKFSLDLDQYPLGRKFLVQ
HPV31 L1	9	MSLWRPSEATVYLPPVPVSKVVSTDEYVTRTNIYYHAGSARLLTVGHPY YSIPKSDNPKKIVVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPETQR LVWACVGLEVGRGQPLGVGISGHPLLNKFDDTENSNRYAGGPGTDNREC ISMDYKQTQLCLLGCKPPIGEHWGKGSPCSNNAITPGDCPPLELKNSVI QDGDMVDTGFGAMDFTALQDTKSNVPLDICNSICKYPDYLKMVAEPYGD TLFFYLRREQMFVRHFFNRSGTVGESVPTDLYIKGSGSTATLANSTYFP TPSGSMVTSDAQIFNKPYWMQRAQGHNNGICWGNQLFVTVVDTTRSTNM SVCAAIANSDTTFKSSNFKEYLRHGEEFDLQFIFQLCKITLSADIMTYI HSMNPAILEDWNFGLTTPPSGSLEDTYRFVTSQAITCQKTAPQKPKEDP FKDYVFWEVNLKEKFSADLDQFPLGRKFLLQAGYRARPKFKAGKRSAPS ASTTTPAKRKKTKK
	10	MSLWRPSEATVYLPPVPVSKVVSTDEYVTRTNIYYHAGSARLLTVGHPY YSIPKSDNPKKIVVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPETQR LVWACVGLEVGRGQPLGVGISGHPLLNKFDDTENSNRYAGGPGTDNREC ISMDYKQTQLCLLGCKPPIGEHWGKGSPCSNNAITPGDCPPLELKNSVI QDGDMVDTGFGAMDFTALQDTKSNVPLDICNSICKYPDYLKMVAEPYGD TLFFYLRREQMFVRHFFNRSGTVGESVPTDLYIKGSGSTATLANSTYFP TPSGSMVTSDAQIFNKPYWMQRAQGHNNGICWGNQLFVTVVDTTRSTNM SVCAAIANSDTTFKSSNFKEYLRHGEEFDLQFIFQLCKITLSADIMTYI HSMNPAILEDWNFGLTTPPSGSLEDTYRFVTSQAITCQKTAPQKPKEDP FKDYVFWEVNLKEKFSADLDQFPLGRKFLLQAGYRARPKFKAGKRSAPS ASTTTPAKRKKTKK
	23	MSLWRPSEATVYLPPVPVSKVVSTDEYVTRTNIYYHAGSARLLTVGHPY YSIPKSDNPKKIVVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPETQR LVWACVGLEVGRGQPLGVGISGHPLLNKFDDTENSNRYAGGPGTDNREC ISMDYKQTQLCLLGCKPPIGEHWGKGSPCSNNAITPGDCPPLELKNSVI QDGDMVDTGFGAMDFTALQDTKSNVPLDICNSICKYPDYLKMVAEPYGD TLFFYLRREQMFVRHFFNRSGTVGESVPTDLYIKGSGSTATLANSTYFP TPSGSMVTSDAQIFNKPYWMQRAQGHNNGICWGNQLFVTVVDTTRSTNM SVCAAIANSDTTFKSSNFKEYLRHGEEFDLQFIFQLCKITLSADIMTYI HSMNPAILEDWNFGLTTPPSGSLEDTYRFVTSQAITCQKSAPQKPKEDP FKDYVFWEVNLKEKFSADLDQFPLGRKFLLQAGYRARPKFKAGKRSAPS ASTTTPAKRKKTKK
HPV33 L1	11	MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLAVGHPY FSIKNPTNAKKLLVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPDTQR LVWACVGLEIGRGQPLGVGISGHPLLNKFDDTETGNKYPGQPGADNREC LSMDYKQTQLCLLGCKPPTGEHWGKGVACTNAAPANDCPPLELINTIIE DGDMVDTGFGCMDFKTLQANKSDVPIDICGSTCKYPDYLKMTSEPYGDS LFFFLRREQMFVRHFFNRAGTLGEAVPDDLYIKGSGTTASIQSSAFFPT PSGSMVTSESQLFNKPYWLQRAQGHNNGICWGNQVFVTVVDTTRSTNMT LCTQVTSDSTYKNENFKEYIRHVEEYDLQFVFQLCKVTLTAEVMTYIHA MNPDILEDWQFGLTPPPSASLQDTYRFVTSQAITCQKTVPPKEKEDPLG

Protein	SEQ ID NO	Sequence
		KYTFWEVDLKEKFSADLDQFPLGRKFLLQAGLKAKPKLKRAAPTSTRTS SAKRKKVKK
	12	MTVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLAVGHPYFSIKNPTN AKKLLVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPDTQRLVWACVGL EIGRGQPLGVGISGHPLLNKFDDTETGNKYPGQPGADNRECLSMDYKQT QLCLLGCKPPTGEHWGKGVACTNAAPANDCPPLELINTIIEDGDMVDTG FGCMDFKTLQANKSDVPIDICGSTCKYPDYLKMTSEPYGDSLFFFLRRE QMFVRHFFNRAGTLGEAVPDDLYIKGSGTTASIQSSAFFPTPSGSMVTS ESQLFNKPYWLQRAQGHNNGICWGNQVFVTVVDTTRSTNMTLCTQVTSD STYKNENFKEYIRHVEEYDLQFVFQLCKVTLTAEVMTYIHAMNPDILED WQFGLTPPPSASLQDTYRFVTSQAITCQKTVPPKEKEDPLGKYTFWEVD LKEKFSADLDQFPLGRKFLLQAGLKAKPKLKRAAPTSTRTSSAKRKKVK K
	24	MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLAVGHPY FSIKNPTNAKKLLVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPDTQR LVWACVGLEIGRGQPLGVGISGHPLLNKFDDTETGNKYPGQPGADNREC LSMDYKQTQLCLLGCKPPTGEHWGKGVACTNAAPANDCPPLELINTIIE DGDMVDTGFGCMDFKTLQANKSDVPIDICGSTCKYPDYLKMTSEPYGDS LFFFLRREQMFVRHFFNRAGTLGEAVPDDLYIKGSGTTASIQSSAFFPT PSGSMVTSESQLFNKPYWLQRAQGHNNGICWGNQVFVTVVDTTRSTNMT LCTQVTSDSTYKNENFKEYIRHVEEYDLQFVFQLCKVTLTAEVMTYIHA MNPDILEDWQFGLTPPPSASLQDTYRFVTSQAITCQKTVPPKEKEDPLG KYTFWEVDLKEKFSADLDQFPLGRKFLLQAGLKAKPKLKRAAPTSTRTS SAKRKKVKK
HPV45 L1	13	MALWRPSDSTVYLPPPSVARVVSTDDYVSRTSIFYHAGSSRLLTVGNPY FRVVPNGAGNKQAVPKVSAYQYRVFRVALPDPNKFGLPDSTIYNPETQR LVWACVGMEIGRGQPLGIGLSGHPFYNKLDDTESAHAATAVITQDVRDN VSVDYKQTQLCILGCVPAIGEHWAKGTLCKPAQLQPGDCPPLELKNTII EDGDMVDTGYGAMDFSTLQDTKCEVPLDICQSICKYPDYLQMSADPYGD SMFFCLRREQLFARHFWNRAGVMGDTVPTDLYIKGTSANMRETPGSCVY SPSPSGSIITSDSQLFNKPYWLHKAQGHNNGICWHNQLFVTVVDTTRST NLTLCASTQNPVPSTYDPTKFKQYSRHVEEYDLQFIFQLCTITLTAEVM SYIHSMNSSILENWNFGVPPPPTTSLVDTYRFVQSVAVTCQKDTTPPEK QDPYDKLKFWTVDLKEKFSSDLDQYPLGRKFLVQAGLRRRPTIGPRKRP AASTSTASTASRPAKRVRIRSKK
	14	MALWRPSDSTVYLPPPSVARVVSTDDYVSRTSIFYHAGSSRLLTVGNPY FRVVPNGAGNKQAVPKVSAYQYRVFRVALPDPNKFGLPDSTIYNPETQR LVWACVGMEIGRGQPLGIGLSGHPFYNKLDDTESAHAATAVITQDVRDN VSVDYKQTQLCILGCVPAIGEHWAKGTLCKPAQLQPGDCPPLELKNTII EDGDMVDTGYGAMDFSTLQDTKCEVPLDICQSICKYPDYLQMSADPYGD SMFFCLRREQLFARHFWNRAGVMGDTVPTDLYIKGTSANMRETPGSCVY SPSPSGSIITSDSQLFNKPYWLHKAQGHNNGICWHNQLFVTVVDTTRST NLTLCASTQNPVPSTYDPTKFKQYSRHVEEYDLQFIFQLCTITLTAEVM SYIHSMNSSILENWNFGVPPPPTTSLVDTYRFVQSVAVTCQKDTTPPEK QDPYDKLKFWTVDLKEKFSSDLDQYPLGRKFLVQAGLRRRPTIGPRKRP AASTSTASTASRPAKRVRIRSKK
	25	MALWRPSDSTVYLPPPSVARVVNTDDYVSRTSIFYHAGSSRLLTVGNPY FRVVPSGAGNKQAVPKVSAYQYRVFRVALPDPNKFGLPDSTIYNPETQR LVWACVGMEIGRGQPLGIGLSGHPFYNKLDDTESAHAATAVITQDVRDN

Protein	SEQ ID NO	Sequence
		VSVDYKQTQLCILGCVPAIGEHWAKGTLCKPAQLQPGDCPPLELKNTII EDGDMVDTGYGAMDFSTLQDTKCEVPLDICQSICKYPDYLQMSADPYGD SMFFCLRREQLFARHFWNRAGVMGDTVPTDLYIKGTSANMRETPGSCVY SPSPSGSITTSDSQLFNKPYWLHKAQGHNNGICWHNQLFVTVVDTTRST NLTLCASTQNPVPNTYDPTKFKHYSRHVEEYDLQFIFQLCTITLTAEVM SYIHSMNSSILENWNFGVPPPPTTSLVDTYRFVQSVAVTCQKDTTPPEK QDPYDKLKFWTVDLKEKFSSDLDQYPLGRKFLVQAGLRRRPTIGPRKRP AASTSTASRPAKRVRIRSKK
HPV52 L1	15	MVQILFYILVIFYYVAGVNVFHIFLQMSVWRPSEATVYLPPVPVSKVVS TDEYVSRTSIYYYAGSSRLLTVGHPYFSIKNTSSGNGKKVLVPKVSGLQ YRVFRIKLPDPNKFGFPDTSFYNPETQRLVWACTGLEIGRGQPLGVGIS GHPLLNKFDDTETSNKYAGKPGIDNRECLSMDYKQTQLCILGCKPPIGE HWGKGTPCNNNSGNPGDCPPLQLINSVIQDGDMVDTGFGCMDFNTLQAS KSDVPIDICSSVCKYPDYLQMASEPYGDSLFFFLRREQMFVRHFFNRAG TLGDPVPGDLYIQGSNSGNTATVQSSAFFPTPSGSMVTSESQLFNKPYW LQRAQGHNNGICWGNQLFVTVVDTTRSTNMTLCAEVKKESTYKNENFKE YLRHGEEFDLQFIFQLCKITLTADVMTYIHKMDATILEDWQFGLTPPPS ASLEDTYRFVTSTAITCQKNTPPKGKEDPLKDYMFWEVDLKEKFSADLD QFPLGRKFLLQAGLQARPKLKRPASSAPRTSTKKKKVKR
	16	MPVPVSKVVSTDEYVSRTSIYYYAGSSRLLTVGHPYFSIKNTSSGNGKK VLVPKVSGLQYRVFRIKLPDPNKFGFPDTSFYNPETQRLVWACTGLEIG RGQPLGVGISGHPLLNKFDDTETSNKYAGKPGIDNRECLSMDYKQTQLC ILGCKPPIGEHWGKGTPCNNNSGNPGDCPPLQLINSVIQDGDMVDTGFG CMDFNTLQASKSDVPIDICSSVCKYPDYLQMASEPYGDSLFFFLRREQM FVRHFFNRAGTLGDPVPGDLYIQGSNSGNTATVQSSAFFPTPSGSMVTS ESQLFNKPYWLQRAQGHNNGICWGNQLFVTVVDTTRSTNMTLCAEVKKE STYKNENFKEYLRHGEEFDLQFIFQLCKITLTADVMTYIHKMDATILED WQFGLTPPPSASLEDTYRFVTSTAITCQKNTPPKGKEDPLKDYMFWEVD LKEKFSADLDQFPLGRKFLLQAGLQARPKLKRPASSAPRTSTKKKKVKR
	26	MSVWRPSEATVYLPPVPGSKVVSTDEYVSRTSIYYYAGSSRLLTVGHPY FSIKNTSSGNGKKVLVPKVSGLQYRVFRIKLPDPNKFGFPDTSFYNPET QRLVWACTGLEIGRGQPLGVGISGHPLLNKFDDTETSNKYAGKPGIDNR ECLSMDYKQTQLCILGCKPPIGEHWGKGTPCNNNSGNPGDCPPLQLINS VIQDGDMVDTGFGCMDFNTLQASKSDVPIDICSSVCKYPDYLQMASEPY GDSLFFFLRREQMFVRHFFNRAGTLGDPVPGDLYIQGSNSGNTATVQSS AFFPTPSGSMVTSESQLFNKPYWLQRAQGHNNGICWGNQLFVTVVDTTR STNMTLCAEVKKESTYKNENFKEYLRHGEEFDLQFIFQLCKITLTADVM TYIHKMDATILEDWQFGLTPPPSASLEDTYRFVTSTAITCQKNTPPKGK EDPLKDYMFWEVDLKEKFSADLDQFPLGRKFLLQAGLQARPKLKRPASS APRTSTKKKKVKR
HPV58 L1	17	MVLILCCTLAILFCVADVNVFHIFLQMSVWRPSEATVYLPPVPVSKVVS TDEYVSRTSIYYYAGSSRLLAVGNPYFSIKSPNNNKKVLVPKVSGLQYR VFRVRLPDPNKFGFPDTSFYNPDTQRLVWACVGLEIGRGQPLGVGVSGH PYLNKFDDTETSNRYPAQPGSDNRECLSMDYKQTQLCLIGCKPPTGEHW GKGVACNNNAAATDCPPLELFNSIIEDGDMVDTGFGCMDFGTLQANKSD VPIDICNSTCKYPDYLKMASEPYGDSLFFFLRREQMFVRHFFNRAGKLG EAVPDDLYIKGSGNTAVIQSSAFFPTPSGSIVTSESQLFNKPYWLQRAQ GHNNGICWGNQLFVTVVDTTRSTNMTLCTEVTKEGTYKNDNFKEYVRHV EEYDLQFVFQLCKITLTAEIMTYIHTMDSNILEDWQFGLTPPPSASLQD

Protein	SEQ ID NO	Sequence
		TYRFVTSQAITCQKTAPPKEKEDPLNKYTFWEVNLKEKFSADLDQFPLG RKFLLQSGLKAKPRLKRSAPTTRAPSTKRKKVKK
	18	MTVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLAVGNPYFSIKSPNN NKKVLVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPDTQRLVWACVGL EIGRGQPLGVGVSGHPYLNKFDDTETSNRYPAQPGSDNRECLSMDYKQT QLCLIGCKPPTGEHWGKGVACNNNAAATDCPPLELFNSIIEDGDMVDTG FGCMDFGTLQANKSDVPIDICNSTCKYPDYLKMASEPYGDSLFFFLRRE QMFVRHFFNRAGKLGEAVPDDLYIKGSGNTAVIQSSAFFPTPSGSIVTS ESQLFNKPYWLQRAQGHNNGICWGNQLFVTVVDTTRSTNMTLCTEVTKE GTYKNDNFKEYVRHVEEYDLQFVFQLCKITLTAEIMTYIHTMDSNILED WQFGLTPPPSASLQDTYRFVTSQAITCQKTAPPKEKEDPLNKYTFWEVN LKEKFSADLDQFPLGRKFLLQSGLKAKPRLKRSAPTTRAPSTKRKKVKK
	27	MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLAVGNPY FSIKSPNNNKKVLVPKVSGLQYRVFRVRLPDPNKFGFPDTSFYNPDTQR LVWACVGLEIGRGQPLGVGVSGHPYFNKFDDTETSNRYPAQPGSDNREC LSMDYKQTQLCLIGCKPPTGEHWGKGVACNNNAAATDCPPLELFNSIIE DGDMVDTGFGCMDFGTLQANKSDVPIDICNSTCKYPDYLKMASEPYGDS LFFFLRREQMFVRHFFNRAGKLGEAVPDDLYIKGSGNTAVIQSSAFFPT PSGSMVTSESQLFNKPYWLQRAQGHNNGICWGNQLFVTVVDTTRSTNMT LCTEVTKEGTYKNDNFKEYVRHVEEYDLQFVFQLCKITLTAEIMTYIHT MDSNILEDWQFGLTPPPSASLQDTYRFVTSQAITCQKTAPPKEKEDPLN KYTFWEVNLKEKFSADLDQFPLGRKFLLQSGLKAKPRLKRSAPTTRAPS TKRKKVKK
HPV L1	30	MALWRTSDSKVYLPPTPVSRVVNTDEYVTRTGIYYYAGSSRLLTLGHPYFSIPK TGQKAEIPKVSAYQYRVFRVHLPDPNKFGLPDPQLYNPDTERLVWACVGVEVGR GQPLGIGLSGHPLFNKLDDTENSHLATVNADTDNRDNVSVDNKQTQLCIIGCTP PLGEHWGIGTICKNTQTQRGDCPPLELISSIIEDGDMIDTGFGAMDFTALQATK SDVPIDISQSTCKYPDYLKMSADTYGNSMFFFLRREQLFARHFYNKAGAVGDAI PTTLYIKGAESGREPPTSSIYSATPSGSMVTSDAQLFNKPYWLQRAQGHNNGIC WGNQLFVTCVDTTRSTNLTISTLSAASASTPFKPSDYKQFIRHGEEYELQFIFQ LCKITLTTDVMAYIHLMNASILEDWNFGLTLPPTASLEDAYRFIKNSATTCQRN APPVPKEDPFQKFKFWDVDLKEKFSIDLDQFPLGRKFMLQAGIQRRPKLGTKRP LSSTSSSTKRKKRKLTK
HPV35 L1	31	MSLWRSNEATVYLPPVSVSKVVSTDEYVTRTNIYYHAGSSRLLAVGHPYYAIKK QDSNKIAVPKVSGLQYRVFRVKLPDPNKFGFPDTSFYDPASQRLVWACTGVEVG RGQPLGVGISGHPLLNKLDDTENSNKYVGNSGTDNRECISMDYKQTQLCLIGCR PPIGEHWGKGTPCNANQVKAGECPPLELLNTVLQDGDMVDTGFGAMDFTTLQAN KSDVPLDICSSICKYPDYLKMVSEPYGDMLFFYLRREQMFVRHLFNRAGTVGET VPADLYIKGTTGTLPSTSYFPTPSGSMVTSDAQIFNKPYWLQRAQGHNNGICWS NQLFVTVVDTTRSTNMSVCSAVSTSDSTYKNDNFKEYLRHGEEYDLQFIFQLCK ITLTADVMTYIHSMNPSILEDWNFGLTPPPSGTLEDTYRYVTSQAVTCQKPSAP KPKDDPLKNYTFWEVDLKEKFSADLDQFPLGRKFLLQAGLKARPNFRLGKRAAP ASTSKKSSTKRRKVKS
HPV39 L1	32	MAMWRSSDSMVYLPPPSVAKVVNTDDYVTRTGIYYYAGSSRLLTVGHPYFKVGM NGGRKQDIPKVSAYQYRVFRVTLPDPNKFSIPDASLYNPETQRLVWACVGVEVG RGQPLGVGISGHPLYNRQDDTENSPFSSTTNKDSRDNVSVDYKQTQLCIIGCVP AIGEHWGKGKACKPNNVSTGDCPPLELVNTPIEDGDMIDTGYGAMDFGALQETK SEVPLDICQSICKYPDYLQMSADVYGDSMFFCLRREQLFARHFWNRGGMVGDAI PAQLYIKGTDIRANPGSSVYCPSPSGSMVTSDSQLFNKPYWLHKAQGHNNGICW HNQLFLTVVDTTRSTNFTLSTSIESSIPSTYDPSKFKEYTRHVEEYDLQFIFQL CTVTLTTDVMSYIHTMNSSILDNWNFAVAPPPSASLVDTYRYLQSAAITCQKDA

Protein	SEQ ID NO	Sequence
		PAPEKKDPYDGLKFWNVDLREKFSLELDQFPLGRKFLLQARVRRRPTIGPRKRP AASTSSSSATKHKRKRVSK
HPV51 L1	33	MALWRTNDSKVYLPPAPVSRIVNTEEYITRTGIYYYAGSSRLITLGHPYFPIPK TSTRAAIPKVSAFQYRVFRVQLPDPNKFGLPDPNLYNPDTDRLVWGCVGVEVGR GQPLGVGLSGHPLFNKYDDTENSRIANGNAQQDVRDNTSVDNKQTQLCIIGCAP PIGEHWGIGTTCKNTPVPPGDCPPLELVSSVIQDGDMIDTGFGAMDFAALQATK SDVPLDISQSVCKYPDYLKMSADTYGNSMFFHLRREQIFARHYYNKLGSVGEDI PNDYYIKGSGNGRDPIESYIYSATPSGSMITSDSQIFNKPYWLHRAQGHNNGIC WNNQLFITCVDTTRSTNLTISTATAAVSPTFTPSNFKQYIRHGEEYELQFIFQL CKITLTTEVMAYLHTMDPTILEQWNFGLTLPPSASLEDAYRFVRNAATSCQKDT PPQAKPDPLAKYKFWDVDLKERFSLDLDQFALGRKFLLQVGVQRKPRPGLKRPA SSASSSSSSSAKRKRVKK
HPV53 L1	34	MAVWRPSDSKVYLPPTPVSKVITTDAYVKRTTIFYHAGSSRLLTVGHPYYPISK SGKTDIPKVSAFQYRVFRVRLPDPNKFGLPDTNIFNPDQERLVWACVGLEIGRG QPLGVGVSGHPLFNRLDDTESSSIAIQDTAPDSRDNISVDPKQTQLCIIGCAPA IGEHWTKGTACRSTPTTAGDCPPLELINSPIEDGDMVDTGFGALNFKALQESKS DVPLDIVQSTCKYPDYLKMSADAYGDSMWFYLRREQLFTRHFFNRSGVIGEEIP NDLYIKGSNDRDPPPSSVYVATPSGSMITSEAQLFNKPYWLQRAQGHNNGICWN NQLFVTVVDTTRNTNMTLSATTQSMSTYNSKQIKQYVRHAEEYELQFVFQLCKI SLSAEVMAYLHTMNSTLLEDWNIGLSPPVATSLEDKYRYVKSAAITCQKDQPPP EKQDPLSKYKFWEVNLQNSFSADLDQFPLGRKFLMQVGVRTEPPVSSKKRSAPT TSTSAPSSKRKRK
HPV56 L1	35	MATWRPSENKVYLPPTPVSKVVATDSYVKRTSIFYHAGSSRLLAVGHPYYSVTK DNTKTNIPKVSAYQYRVFRVRLPDPNKFGLPDTNIYNPDQERLVWACVGLEVGR GQPLGAGLSGHPLFNRLDDTESSNLANNNVIEDSRDNISVDGKQTQLCIVGCTP AMGEHWTKGAVCKSTQVTTGDCPPLALINTPIEDGDMIDTGFGAMDFKVLQESK AEVPLDIVQSTCKYPDYLKMSADAYGDSMWFYLRREQLFARHYFNRAGKVGETI PAELYLKGSNGREPPPSSVYVATPSGSMITSEAQLFNKPYWLQRAQGHNNGICW GNQLFVTVVDTTRSTNMTISTATEQLSKYDARKINQYLRHVEEYELQFVFQLCK ITLSAEVMAYLHNMNANLLEDWNIGLSPPVATSLEDKYRYVRSTAITCQREQPP TEKQDPLAKYKFWDVNLQDSFSTDLDQFPLGRKFLMQLGTRSKPAVATSKKRSA PTSTSTPAKRKRR
HPV59 L1	36	MALWRSSDNKVYLPPPSVAKVVSTDDYVTRTSIFYHAGSSRLLTVGHPYFKVPK GGNGRQDVPKVSAYQYRVFRVNLPDPNKFGLPDNTVYDPNSQRLVWACVGVEIG RGQPLGVGLSGHPFYNKLDDTENSHVASAVDNKDTRDNVSVDYKQTQLCIIGCV PAIGEHWTKGTACKPNTVVQGDCPPLELINTPIEDGDMVDTGYGAMDFKLLQDT KSEVPLDICQSICKYPDYLQMSADAYGDSMFFCLRREQVFARHFWNRSGTMGDQ IPESLYIKGTDIRATPGSYLYSPSPSGSVVTSDSQLFNKPYWLHKAQGLNNGIC WHNQLFLTVVDTTRSTNLSVCASTTSSIPNVYTPTSFKEYARHVEEFDLQFIFQ LCKITLTTEVMSYIHNMNTTILEDWNFGVTPPPTASLVDTYRFVQSAAVTCQKD TAPPVKQDPYDKLKFWTVDLKERFSADLDQFPLGRKFLLQLGARPKPTIGPRKR AAPAPTSTPSPKRVKRRKSSRK
HPV66 L1	37	MAMWRPSDNKVYLPPTPVSKVVATDTYVKRTSIFYHAGSSRLLAVGHPYYSVSK SGTKTNIPKVSAYQYRVFRVRLPDPNKFGLPDPSFYNPDQERLVWACVGLEVGR GQPLGAGLSGHPLFNRLDDTEVSNLAGNNVIEDSRDNISVDCKQTQLCIVGCAP ALGEHWTKGAVCKSTPGNTGDCPPLALVNTPIEDGDMVDTGFGAMDFKLLQESK AEVPLDIVQSTCKYPDYLKMSADAYGDSMWFYLRREQLFARHYFNRAGNVGEAI PTDLYWKGGNGRDPPPSSVYVATPSGSMITSEAQLFNKPYWLQRAQGHNNGICW GNQVFVTVVDTTRSTNMTINAAKSTLTKYDAREINQYLRHVEEYELQFVFQLCK ITLTAEVMAYLHNMNNTLLDDWNIGLSPPVATSLEDKYRYIKSTAITCQREQPP AEKQDPLAKYKFWEVNLQDSFSADLDQFPLGRKFLMQLGPRPPPRPKASVSASKR RAAPTSSSSSPAKRKKR
HPV67 L1	38	MSVWRPSEATVYLPPVPVSKVVSTDEYVSRTSIYYYAGSSRLLAVGHPYFSIPN PSNTKKVLVPKVSGLQYRVFRVHLPDPNKFGFPDTSFYNPDTQRLVWACVGIEI

Protein	SEQ ID NO	Sequence
		GRGQPLGVGISGHPLLNKFDDTETNNKYPSQPGTDNRECLSMDAKQTQLCIIGC KPPTGEHWGKGTPCSGNSTGPGACPPLELMNTVIEDGDMIDTGFGCMDFKSLQA NKSDVPLDICTSICKYPDYLGMASEAYGDSLFFFLRREQMFVRHLFNRAGKLGE DVPTDLYFKGSANTSALQTSAFFPTPSGSMVSSESQLFNKPYWLQRAQGHNNGI CWGNQIFVTVVDTTRSTNMTLCSEEKSEATYKNENFKEYLRHVEEYDLQFIFQL CKISLTANVMQYIHTMNPDILEDWQFGLTPPPSGNLQDTYRFVTSQAITCQKTA TPTAKEDPLKKYSFWEINLKEKFSADLDQFPLGRKFLLQAGFTAKPKLKRSSPS SSSSSSAKRKKVKR
HPV68 L1	39	MALWRSSDNMVYLPPPSVAKVVNTDDYVTRTGIYYYAGTSRLLTVGHPYFKVPM SGGRKQDIPKVSAYQYRVFRVSLPDPNKFSLPESTLYNPDTQRLVWACVGVEIG RGQPLGVGLSGHPLYNRLDDTENSPFSSNKNPKDSRDNVSVDYKQTQLCIIGCV PAIGEHWAKGKSCKPSNVQPGDCPPLELVNTPIQDGDMIDTGYGAMDFSTLQET KSEVPLDICQSVCKYPDYLQMSADVYGDSMFFCLRRDQLFARHFWNRGGMVGDT IPTELYIKGTDIRDSPSSYVYAPSPSGSMVSSDSQLFNKPNWLHKAQGHNNGIC WHNQLFLTVVDTTRSTNFTLSTTTESAVPNIYDPNKFKEYVRHVEEYDLQFIFQ LCTITLSTDVMSYIHTMNPAILDDWNFGVAPPPSASLVDTYRYLQSAAITCQKD APAPTKKDPYDGLNFWNVNLKEKFSSELDQFPLGRKFLLQAGVRRRPTIGPRKR PATAPTASTSKHKRKRVSK
HPV70 L1	40	MALWRSSDNTVYLPPPSVAKVVNTDDYVTRTGIYYYAGSSRLLTVGHPYFKVPV NGGRKQEIPKVSAYQYRVFRVSLPDPNKFGLPDPSLYNPDTQRLVWACIGVEIG RGQPLGVGVSGHPLYNRLDDTENSHFSSAVNTQDSRDNVSVDYKQTQLCIIGCV PAMGEHWAKGKACKSTTVQQGDCPPLELVNTAIEDGDMIDTGYGAMDFRTLQET KSEVPLDICQSVCKYPDYLQMSADVYGDSMFFCLRKEQLFARHFWNRGGMVGDT IPSELYIKGTDIRDRPGTHVYSPSPSGSMVSSDSQLFNKPYWLHKAQGHNNGIC WHNQLFITVVDTTRSTNFTLSACTETAIPAVYSPTKFKEYTRHVEEYDLQFIFQ LCTITLTADVMAYIHTMNPAILDNWNIGVTPPPSASLVDTYRYLQSAAIACQKD APAPEKKDPYDDLKFWNVDLKEKFSTELDQFPLGRKFLLQVGARRRPTIGPRKR PASAKSSSSASKHKRKRVSK
HPV73 L1	41	MWRPTDAKVYLPPVSVSKVVSTDEYVTRTNIYYYAGSTRLLAVGHPYFPIKDSQ KRKTIVPKVSGLQYRVFRLRLPDPNKFGFPDASFYNPDKERLVWACSGVEVGRG QPLGIGTSGNPFMNKLDDTENAPKYIAGQNTDGRECMSVDYKQTQLCILGCRPP LGEHWGPGTPCTSQTVNTGDCPPLELKNTPIQDGDMIDVGFGAMDFKALQANKS DVPIDISNTTCKYPDYLGMAADPYGDSMWFYLRREQMFVRHLFNRAGDTGDKIP DDLMIKGTGNTATPSSCVFYPTPSGSMVSSDAQLFNKPYWLQKAQGQNNGICWH NQLFLTVVDTTRSTNFSVCVGTQASSSTTTYANSNFKEYLRHAEEFDLQFVFQL CKISLTTEVMTYIHSMNSTILEEWNFGLTPPPSGTLEETYRYVTSQAISCQRPQ PPKETEDPYAKLSFWDVDLKEKFSAELDQFPLGRKFLLQLGMRARPKLQASKRS ASATTSATPKKKRAKRI
HPV82 L1	42	MALWRTNDSKVYLPPAPVSRIVNTEEYITRTGIYYYAGSSRLITLGHPYFSIPK TNTRAEIPKVSAFQYRVFRVQLPDPNKFGLPDPNLFNPDTDRLVWGCVGVEVGR GQPLGVGLSGHPLFNKYDDTENSRFANGNDQQDVRDNISVDNKQTQLCIIGCAP PIGEHWATGTTCKNVPVPQGDCPPLELVSTVIEDGDMVDTGFGAMDFANLQATK SDVPLDIAQSVCKYPDYLKMSADTYGNSMFFHLRREQIFARHYYNKAGVVGDAI PDKAYIKGTGAGRDPISSYIYSATPSGSMITSDSQIFNKPYWLHRAQGHNNGIC WNNQLFITCVDTTKSTNLTISTAVTPSVAQTFTPANFKQYIRHGEEYELQFIFQ LCKITLTTEIMAYLHTMDSTILEQWNFGLTLPPSASLEDAYRFVKNAATSCHKD SPPQAKEDPLAKYKFWNVDLKERFSLDLDQFALGRKFLLQIGAQRKPRPGLKRP APSSSASSSAKRKRVKK

In a preferred embodiment, the one or more HPV antigen(s) are HPV L1 proteins or immunogenic fragments thereof.

As used herein, an "**immunogenic fragment**" refers to a fragment of a reference antigen containing one or more epitopes (e.g., linear, conformational or both) capable of stimulating a host's immune system to make a humoral and/or cellular antigen-specific immunological response (i.e. an immune response which specifically recognizes a naturally occurring polypeptide, e.g., a viral or bacterial protein). An "**epitope**" is that portion of an antigen that determines its immunological specificity. T- and B-cell epitopes can be identified empirically (e.g. using PEPSCAN or similar methods). In a preferred embodiment, the immunogenic fragment induces an immune response, suitably a humoral or Tcell response, which is similar to the immune response induced by the reference antigen. Suitably, the immunogenic fragment induces a humoral or T cell response in mice which is not more than 10-fold lower, more suitably not more than 5-fold lower, not more than 2-fold lower or not lower, than the immune response induced by the reference antigen.

As used herein, an "**immunogenic fragment of a HPV L1 protein**" refers to a fragment of a naturally-occurring HPV L1 protein of at least 50, 60, 100, 200, 300, 400, 450 or more amino acids, or a peptide having an amino acid sequence of at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 99.5% sequence identity to a naturally-occurring HPV L1 protein (or to a fragment of a naturally-occurring HPV L1 protein of at least about 50, 60, 100, 200, 300, 400, 450 or more amino acids). Thus, an immunogenic fragment of a HPV L1 protein may be a fragment of a naturally occurring HPV L1 protein, of at least 50 amino acids, and may comprise one or more amino acid substitutions, deletions or additions. Any of the HPV L1 protein immunogenic fragments may additionally comprise an initial methionine residue where required.

In one embodiment, the one or more HPV antigen(s) form VLPs. A "**virus like particle**" (VLP) is a self-assemblage of structural capsid proteins that mimics a virus but does not comprise the genetic material of a virus. A VLP is therefore not infectious. Suitably, the one or more HPV antigen(s) form VLPs comprising HPV L1, L2, chimeric L1 and/or chimeric L1/L2 proteins or immunogenic fragments thereof. In a preferred embodiment, the one or more HPV antigen(s) form VLPs consisting of HPV L1 proteins or immunogenic fragments thereof.

HPV VPLs and methods for the production of VLPs are well known in the art. VLPs typically are produced recombinantly from the HPV L1 protein of the virus and can also include the L2 protein. See for example WO9420137, US5985610, WO9611272, EP595935 for VLPs. Suitable expression systems for HPV VLPs, in particular L1 VLPs include without limitation, any prokaryotic and/or eukaryotic

system(s) including baculoviruses, adenoviruses, SV40, *E. coli,* CHO cells, vaccinia virus, insect viruses, yeast, bacteriophage virus or modified viruses, agrobacteria, DNA plasmids, vectors and the like. The host cell for expression of the L1 coding sequence is dependent on the expression system used. Examples of suitable host cells include, without limitation, bacteria (such as *E. coli*), microorganisms such as yeast (such as *Saccharomyces cerevisiae*), mammalian cells (eukaryotic) and insect cells. Methods for producing HPV VLPs in E. coli are disclosed in China patent No: ZL200610140613.0 and in Pan H, et al., 2017[15], in Gu, Y. et al., 2017[16], in Wang D., et al., 2017[17] and in Wei M. et al. 2018[18]. When using the baculovirus expression system, insect cells, such as Sf-9 or Sf-21 are preferred. HPV VLPs can also be produced in plants such as tobacco plants using recombinant *Agrobacterium* constructs (see eg. Naupu, P. N. et al, 2020[12]).

In one embodiment, the HPV L1 VLPS are produced in *E. coli*, yeast cells or in a baculovirus expression system. In a preferred embodiment, the HPV L1 VLPS are produced in *E. coli*.

In one embodiment, the one or more HPV antigen(s) comprise at least two, for example at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine HPV antigen.

In a preferred embodiment, the one or more HPV antigen(s) comprise at least nine HPV antigens.

In one embodiment, the one or more HPV antigen(s) are from HPV types selected from HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 67, 68, 70, 73 and 82.

In one embodiment, the one or more HPV antigen(s) are from HPV types selected from HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 52, 58 and 59.

In a preferred embodiment, the one or more HPV antigen(s) are from HPV types selected from HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58.

In one embodiment, the one or more HPV antigen(s) comprise HPV antigen(s) from HPV types 16 and 18.

In one embodiment, the one or more HPV antigen(s) comprise HPV antigen(s) from HPV types 6, 11, 16, and 18.

In a preferred embodiment, the one or more HPV antigen(s) comprise or consist of HPV antigen(s) from HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58.

In one embodiment, the one or more HPV antigen(s) comprise HPV antigen(s) from HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58, and optionally HPV antigen(s) from HPV types 35, 39 and/or 59.

In one embodiment, the one or more HPV antigen(s) comprise L1 VLPs from HPV types 16 and 18.

In one embodiment, the one or more HPV antigen(s) comprise L1 VLPs from HPV types 6, 11, 16, and 18.

In one embodiment, the one or more HPV antigen(s) comprise L1 VLPs from HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58 L1 VLPs, and optionally from HPV types 35, 39 and/or 59.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 6 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 1, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 1.

The sequence of the HPV 6 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 6 L1 protein. Suitably, the HPV 6 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 6 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 6 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 1 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 6 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 1 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In a preferred embodiment, the one or more HPV antigen(s) comprise a HPV 6 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 2 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a HPV 6 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 19 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 11 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 3, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 3.

The sequence of the HPV 11 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 11 L1 protein. Suitably, the HPV 11 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 11 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 11 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 3 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 11 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 3 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In a preferred embodiment, the one or more HPV antigen(s) comprise a HPV 11 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 4 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a HPV 11 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 20 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 16 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 5, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 5.

The sequence of the HPV 16 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 16 L1 protein. Suitably, the HPV 16 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 16 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 16 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 5 or to a variant thereof, for example 40 amino acids or less, 30

amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 16 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 5 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In a preferred embodiment, the one or more HPV antigen(s) comprise a HPV 16 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 6 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a HPV 16 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 21 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a HPV 16 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 28 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 18 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 7, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 7.

The sequence of the HPV 18 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 18 L1 protein. Suitably, the HPV 18 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 18 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 18 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 7 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 18 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 7 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In a preferred embodiment, the one or more HPV antigen(s) comprise a HPV 18 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 8 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a HPV 18 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 22, or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a HPV 18 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 29, or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 31 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 9, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 9.

The sequence of the HPV 31 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 31 L1 protein. Suitably, the HPV 31 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 31 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 31 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 9 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 31 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 9 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In a preferred embodiment, the one or more HPV antigen(s) comprise a HPV 31 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 10 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a HPV 31 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 23 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 33 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 11, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 11.

The sequence of the HPV 33 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 33 L1 protein. Suitably, the HPV 33 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the

corresponding naturally occurring HPV 33 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 33 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 11 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 33 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 11 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In a preferred embodiment, the one or more HPV antigen(s) comprise a HPV 33 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 12 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a HPV 33 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 24 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 45 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 13, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 13.

The sequence of the HPV 45 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 45 L1 protein. Suitably, the HPV 45 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 45 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 45 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 13 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 45 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 13 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In a preferred embodiment, the one or more HPV antigen(s) comprise a HPV 45 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 14 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a HPV 45 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 25 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 52 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 15, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 15.

The sequence of the HPV 52 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 52 L1 protein. Suitably, the HPV 52 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 52 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 52 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 15 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 52 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 15 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In a preferred embodiment, the one or more HPV antigen(s) comprise a HPV 52 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 16 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a HPV 52 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 26 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 58 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 17, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 17.

The sequence of the HPV 58 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 58 L1 protein. Suitably, the HPV 58 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the

corresponding naturally occurring HPV 58 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 58 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 17 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 58 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 17 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In a preferred embodiment, the one or more HPV antigen(s) comprise a HPV 58 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 18 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a HPV 58 L1 protein comprising or consisting of the amino acid sequence SEQ ID NO: 27 or a variant thereof.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 35 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 31, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 31.

The sequence of the HPV 35 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 35 L1 protein. Suitably, the HPV 35 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 35 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 35 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 31 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 35 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 31 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 39 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 32, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 32.

The sequence of the HPV 39 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 39 L1 protein. Suitably, the HPV 39 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 39 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 39 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 32 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 39 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 32 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 59 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 36, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 36.

The sequence of the HPV 59 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 59 L1 protein. Suitably, the HPV 59 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 59 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 59 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 36 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 59 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 36 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 26 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 30, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 30.

The sequence of the HPV 26 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 26 L1 protein. Suitably, the HPV 26 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 26 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 26 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 30 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 26 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 30 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 39 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 32, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 32.

The sequence of the HPV 39 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 39 L1 protein. Suitably, the HPV 39 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 39 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 39 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 32 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 39 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 32 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 51 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 33, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 33.

The sequence of the HPV 51 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 51 L1 protein. Suitably, the HPV 51 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 51 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 51 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 33 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 51 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 33 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 53 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 34, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 34.

The sequence of the HPV 53 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 53 L1 protein. Suitably, the HPV 53 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 53 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 53 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 34 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 53 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 34 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 56 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 35, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 35.

The sequence of the HPV 56 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 56 L1 protein. Suitably, the HPV 56 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 56 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 56 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 35 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 56 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 35 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 66 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 37, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 37.

The sequence of the HPV 66 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 66 L1 protein. Suitably, the HPV 66 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 66 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 66 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 37 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 66 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 37 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 67 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 38, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 38.

The sequence of the HPV 67 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 67 L1 protein. Suitably, the HPV 67 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 67 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 67 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 38 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 67 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 38 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 68 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 39, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 39.

The sequence of the HPV 68 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 68 L1 protein. Suitably, the HPV 68 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 68 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 68 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 39 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 68 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 39 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 70 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 40, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 40.

The sequence of the HPV 70 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 70 L1 protein. Suitably, the HPV 70 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 70 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 70 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 40 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 70 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 40 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 73 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 41, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 41.

The sequence of the HPV 73 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 73 L1 protein. Suitably, the HPV 73 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 73 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 73 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 41 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 73 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 41 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

In one embodiment, the one or more HPV antigen(s) comprise a **HPV 82 L1** protein comprising or consisting of an amino-acid sequence which is at least 80% identical to SEQ ID NO: 42, suitably at least 90%, at least 95%, 99% or 100% identical to SEQ ID NO: 42.

The sequence of the HPV 82 L1 protein may be C-terminally or N-terminally truncated compared to the corresponding naturally occurring HPV 82 L1 protein. Suitably, the HPV 82 L1 protein comprises a C-terminal truncation or N-terminal truncation of 50 amino acids or less compared to the corresponding naturally occurring HPV 82 L1 protein, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 82 L1 protein comprises a C-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 42 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less. In one embodiment, the HPV 82 L1 protein comprises a N-terminal truncation of 50 amino acids or less compared to SEQ ID NO: 42 or to a variant thereof, for example 40 amino acids or less, 30 amino acids or less, 20 amino acids or less, 15 amino acids or less, 10 amino acids or less, 5 amino acids or less.

As used herein, a "**variant**" is a protein that differs in sequence from a reference antigen sequence but retains at least one essential property of the reference antigen. Changes in the sequence of protein variants may be limited or conservative, so that the sequences of the reference protein and the variant are closely similar overall and, in many regions, identical. A variant and reference antigen can differ in amino acid sequence by one or more substitutions, additions or deletions in any combination. A variant of an antigen can be naturally occurring such as an allelic variant, or can be a variant that is not known to occur naturally. Non-naturally occurring variants of nucleic acids and polypeptides may be made by mutagenesis techniques or by direct synthesis. In a preferred embodiment, the essential property retained by the variant is the ability to induce an immune response, suitably a humoral or Tcell response, which is similar to the immune response induced by the reference antigen. Suitably, the variant induces a humoral or Tcell response in mice which is not more than 10-fold lower, more suitably not more than 5-fold lower, not more than 2-fold lower or not lower, than the immune response induced by the reference antigen. Suitably, a HPV antigen variant has an amino acid sequence which is at least 60%, 65%, 70%, 75% 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 99,5% identical to the reference antigen sequence. Suitable HPV antigen variants include truncations, deletions, substitution, or insertion mutants. Suitable HPV L1 variants include

truncated or mutated L1 proteins, for example truncations removing a nuclear localization signal and/or DNA binding patterns, or mutations inactivating a nuclear localization signal and/or DNA binding patterns.

In one embodiment the one or more HPV antigens are pre-adsorbed onto an aluminum metallic salt, preferably selected from aluminum oxide hydroxide (AlOOH), aluminum phosphate ($AlPO_4$), aluminum hydroxyphosphate sulfate ($AlHO_9PS^{-3}$) and aluminum hydroxyphosphate ($AlHO_5P$). In a preferred embodiment, the HPV antigens comprise HPV L1 VLPs pre-adsorbed onto an aluminum metallic salt, preferably selected from AlOOH, $AlPO_4$ and $AlHO_9PS^{-3}$. In a more preferred embodiment, the HPV antigens comprise L1 VLPs from HPV types 6, 11, 16, 18, 31, 33, 45, 51 and 58, each independently pre-adsorbed onto an aluminum metallic salt, preferably selected from AlOOH, $AlPO_4$ and $AlHO_9PS^{-3}$. In a more preferred embodiment, the HPV L1 VLPs from HPV types 6, 11, 16, 18, 31, 33, 45, 51 and 58 are independently pre-adsorbed onto AlOOH. The metallic salt onto which the one or more HPV antigens are pre-adsorbed is not adsorbed onto a glycolipid based TLR4 ligand.

In one embodiment, the method further comprises the step of adding a metallic salt to the HPV vaccine composition. In one embodiment, the metallic salt is added concomitantly to the addition of the non adsorbed glycolipid based TLR4 ligand in step (ii). In another embodiment, the metallic salt is added prior to the addition of the non adsorbed glycolipid based TLR4 ligand in step (ii). In another embodiment, the metallic salt is added after to the addition of the non adsorbed glycolipid based TLR4 ligand in step (ii). In a preferred embodiment, the metallic salt is added prior to the addition of the non adsorbed glycolipid based TLR4 ligand in step (ii). Suitably, the added metallic salt is selected from AlOOH, $AlPO_4$ and $AlHO_9PS^{-3}$. In a preferred embodiment, the added metallic salt is AlOOH.

In one **aspect**, the invention provides a HPV vaccine composition obtained by the method according to the invention.

In one embodiment, the HPV vaccine composition comprises at least two, for example at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine HPV antigen. In a preferred embodiment, the HPV vaccine composition comprises at least nine HPV antigens.

In one embodiment, the HPV vaccine composition comprises HPV antigen(s) from HPV types selected from HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 67, 68, 70, 73 and 82.

In one embodiment, the HPV vaccine composition comprises HPV antigen(s) from HPV types selected from HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 52, 58 and 59.

In a preferred embodiment, the HPV vaccine composition comprises HPV antigen(s) from HPV types selected from HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58.

In one embodiment, the HPV vaccine composition comprises HPV antigen(s) from HPV types 16 and 18.

In one embodiment, the HPV vaccine composition comprises HPV antigen(s) from HPV types 6, 11, 16, and 18.

In one embodiment, the HPV vaccine composition comprises HPV antigen(s) from HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58.

In one embodiment, the HPV vaccine composition comprises HPV antigen(s) from HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58, and optionally HPV antigen(s) from HPV types 35, 39 and/or 59.

In one embodiment, the HPV vaccine composition comprises HPV L1 VLPs from HPV types 16 and 18.

In one embodiment, the HPV vaccine composition comprises HPV L1 VLPs from HPV types 6, 11, 16, and 18.

In one embodiment, the HPV vaccine composition comprises HPV L1 VLPs L1 VLPs from HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58 L1 VLPs, and optionally from HPV types 35, 39 and/or 59.

In one embodiment, the amount of the metallic ion which is part of the metallic salt in the HPV vaccine composition is from 100 to 500 μg/dose, suitably from 200 to 500 μg/dose, from 300 to 500 μg/dose, from 400 to 500 μg/dose or about 500 μg/dose. In a preferred embodiment, the amount of the metallic ion which is part of the metallic salt in the HPV vaccine composition is 500 μg/dose.

In one embodiment, the concentration of the metallic ion which is part of the metallic salt in the HPV vaccine composition is from 200 μg/ml to 1mg/ml, suitably from 400 μg/ml to 1mg/ml, from 600 μg/ml to 1mg/ml, from 800 μg/ml to 1mg/ml or about 1mg/ml. In a preferred embodiment, the concentration of the metallic ion which is part of the metallic salt in the HPV vaccine composition is 1mg/ml.

In a preferred embodiment, the metallic ion which is part of the metallic salt is Al^{3+} and the amount of Al^{3+} in the HPV vaccine composition is from 100 to 500 μg/dose, suitably from 200 to 500 μg/dose, from 300 to 500 μg/dose, from 400 to 500 μg/dose or about 500 μg/dose. In a preferred embodiment, the amount of Al^{3+} in the HPV vaccine composition is 500 μg/dose.

In a preferred embodiment, the metallic ion which is part of the metallic salt is Al^{3+} and the concentration of Al^{3+} in the HPV vaccine composition is from 200 μg/ml to 1mg/ml, suitably from 400 μg/ml to 1mg/ml, from 600 μg/ml to 1mg/ml, from 800 μg/ml to 1mg/ml or about 1mg/ml. In a preferred embodiment, the concentration of Al^{3+} in the HPV vaccine composition is 1mg/ml.

In one embodiment, the amount of glycolipid based TLR4 ligand in the HPV vaccine composition is from 1 to 200 μg/dose, preferably from 2 to 100 μg/dose, from 5 to 75 μg/dose, from 10 to 50 μg/dose or about 50 μg/dose. In a preferred embodiment, the amount of glycolipid based TLR4 ligand in the HPV vaccine composition is 50 μg/dose.

In one embodiment, the amount of glycolipid based TLR4 ligand in the HPV vaccine composition is from 2 to 400 μg/ml, preferably from 4 to 200 μg/ml, from 10 to 150 μg/ml, from 20 to 100 μg/ml or about 100 μg/ml. In a preferred embodiment, the amount of glycolipid based TLR4 ligand in the HPV vaccine composition is 100 μg/ml.

In a preferred embodiment, the glycolipid based TLR4 ligand is MPL, and the amount of MPL in the HPV vaccine composition is from 10 to 50 μg/dose, preferably from 20 to 50 μg/dose, from 30 to 50 μg/dose, from 40 to 50 μg/dose or about 50 μg/dose. In a preferred embodiment, the amount of MPL in the HPV vaccine composition is 50 μg/dose.

In a preferred embodiment, the glycolipid based TLR4 ligand is MPL, and the concentration of MPL in the HPV vaccine composition is from 20 to 100 μg/ml, preferably from 40 to 100 μg/ml, from 60 to 100 μg/ml, from 80 to 100 μg/ml or about 100 μg/ml. In a preferred embodiment, the concentration of MPL in the HPV vaccine composition is 100 μg/ml.

In one embodiment, the ratio of glycolipid based TLR4 ligand:metallic ion in the HPV vaccine composition is from 1:2 to 1: 20, preferably from 1:3 to 1:15, more preferably from 1:5 to 1:10, for example 1:5, 1:6, 1:7, 1:8, 1:9 or 1:10.

In a preferred embodiment, the metallic ion is Al^{3+}, the glycolipid based TLR4 ligand is MPL, and the ratio of MPL: Al^{3+} in the HPV vaccine composition is from 1:2 to 1:20, preferably from 1:3 to 1:15, more preferably from 1:5 to 1:10, for example 1:5, 1:6, 1:7, 1:8, 1:9 or 1:10.

Preferably, TLR4 ligand biological activity (or bioactivity) in the HPV vaccine composition of the invention is enhanced as compared to HPV vaccine composition obtained by a method where a TLR4 ligand is pre-adsorbed onto a metallic salt prior to being combined with the HPV antigens. Biological activity of a TLR4 ligand corresponds to its ability to induce the production of pro-inflammatory cytokines, such as TNF-α, and can be measured for example by using the assay described in example 1 (section entitled 'MPL Bioassay').

Preferably, the pH of the HPV vaccine composition is between 6.0 and 7.5, preferably between 6.5 and 7.2, for example about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.2 or about 7.2.

The amount of the one or more HPV antigens in the HPV vaccine composition can vary with the condition, sex, age and weight of the individual, the administration route of the vaccine. The amount can also be varied with the number of HPV antigens types. Suitably the delivery is of an amount of HPV antigens suitable to generate an immunologically protective response. Suitably each vaccine dose comprises between 5 and 100 µg of each HPV antigen.

Suitably, the HPV vaccine composition can comprise pharmaceutically acceptable carriers. Suitably, the HPV vaccine composition may be prepared for administration by being suspended or dissolved in a pharmaceutically acceptable carrier. A "**pharmaceutically acceptable carrier**" includes any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, sucrose, trehalose, lactose, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The HPV vaccine composition may also contain a pharmaceutically acceptable diluent, such as water, saline, glycerol, etc. Additionally, auxiliary

substances, such as wetting or emulsifying agents, pH buffering substances, surfactants, anti-oxidants, and the like, may be present. Sterile pyrogen-free, phosphate buffered physiologic saline is a typical carrier. The appropriate carrier may depend in large part upon the route of administration.

In one embodiment, the HPV vaccine composition comprises a surfactant, suitably a polysorbate (PS) such as PS20, PS60, PS80 or a poloxamer such as poloxamer 188. In a preferred embodiment, the surfactant comprises or consists of PS80. Suitably, the amount of the surfactant in the HPV vaccine composition is from 5 to 200 µg/dose, preferably from 10 to 100 µg/dose, more preferably from 20 to 50 µg/dose. In a preferred embodiment, the surfactant is PS80 and the amount of PS80 in the HPV vaccine composition is from 5 to 200 µg/dose, preferably from 10 to 100 µg/dose, more preferably from 20 to 50 µg/dose. Suitably, the concentration of the surfactant in the HPV vaccine composition is from 10 to 400 µg/mL, preferably from 20 to 200 µg/mL, more preferably from 40 to 100 µg/mL. In a preferred embodiment, the surfactant is PS80 and the concentration of PS80 in the HPV vaccine composition is from 10 to 400 µg/mL, preferably from 20 to 200 µg/mL, more preferably from 40 to 100 µg/mL.

In one embodiment, the HPV vaccine composition comprises a buffering agent. Suitably, the concentration of the buffering agent in the HPV vaccine composition is from 5 to 20 mM, preferably from 7,5 to 15 mM, for example 10 mM. In a preferred embodiment, the buffering agent comprises or consists of Na_2HPO_4/NaH_2PO_4. Suitably, the concentration of Na_2HPO_4/NaH_2PO_4 in the HPV vaccine composition is from 5 to 20 mM, preferably from 7,5 to 15 mM, for example 10 mM.

Suitably, the HPV vaccine composition is in liquid form, or in lyophilised form that is reconstituted into a liquid form prior to administration.

Suitably, the HPV vaccine composition is to be administered to a subject by any route as is known in the art, for example by intramuscular administration. Suitably, the subject is a vertebrate, such as a mammal, e.g. a human, a non-human primate, or a veterinary mammal (livestock or companion animals). In a preferred embodiment, the subject is a human.

The HPV vaccine composition according to the invention are useful to prevent or treat persistent HPV infection or subclinical HPV infection of the human anogenital epithelium, such as cervical epithelium, or HPV-related conditions such as CIN1 or LSIL, CIN2 and CIN3 lesions.

In one aspect, there is provided a HPV vaccine composition as described herein for use in therapy.

In one aspect, there is provided a HPV vaccine composition as described herein for preventing or treating a HPV infection or associated disease in a subject.

In one aspect, there is provided the use of a HPV vaccine composition as described herein, in the manufacture of a medicament for the prevention or treatment of HPV infection in a subject.

In one aspect, there is provided a method of preventing or treating an infection or disease comprising the administration of an effective amount of a HPV vaccine composition as described herein to a patient in need thereof.

In one aspect, there is provided a method of preventing or treating an infection or disease caused by HPV comprising the administration of an effective amount of a HPV vaccine composition as described herein to a patient in need thereof.

It will be readily understood that the immunogenic compositions of the invention are suited for use in regimens involving repeated delivery over time for therapeutic purposes.

Sequence Comparison

For the purposes of comparing two closely-related polynucleotide or polypeptide sequences, the "sequence identity" or "% identity" between a first sequence and a second sequence may be calculated using an alignment program, such as the Basic Local Alignment Search Tool (BLAST) using standard settings. The percentage identity is the number of identical residues divided by the length of the alignment, multiplied by 100. An alternative definition of identity is the number of identical residues divided by the number of aligned residues, multiplied by 100. Alternative methods include using a gapped method in which gaps in the alignment, for example deletions in one sequence relative to the other sequence, are considered. Polypeptide or polynucleotide sequences are said to be identical to other polypeptide or polynucleotide sequences, if they share 100% sequence identity over their entire length.

A "difference" between two sequences refers to an insertion, deletion or substitution, e.g., of a single amino acid residue in a position of one sequence, compared to the other sequence.

For the purposes of comparing a first, reference polypeptide sequence to a second, comparison polypeptide sequence, the number of additions, substitutions and/or deletions made to the first sequence to produce the second sequence may be ascertained. An addition is the addition of one

amino acid residue into the sequence of the first polypeptide (including addition at either terminus of the first polypeptide). A substitution is the substitution of one amino acid residue in the sequence of the first polypeptide with one different amino acid residue. A deletion is the deletion of one amino acid residue from the sequence of the first polypeptide (including deletion at either terminus of the first polypeptide).

Suitable substitutions in the sequences of the present invention may be conservative substitutions. A conservative substitution comprises the substitution of an amino acid with another amino acid having a physico-chemical property similar to the amino acid that is substituted (see, for example, Stryer *et al*, Biochemistry, 5th Edition 2002, pages 44-49). Preferably, the conservative substitution is a substitution selected from the group consisting of: (i) a substitution of a basic amino acid with another, different basic amino acid; (ii) a substitution of an acidic amino acid with another, different acidic amino acid; (iii) a substitution of an aromatic amino acid with another, different aromatic amino acid; (iv) a substitution of a non-polar, aliphatic amino acid with another, different non-polar, aliphatic amino acid; and (v) a substitution of a polar, uncharged amino acid with another, different polar, uncharged amino acid. A basic amino acid is preferably selected from the group consisting of arginine, histidine, and lysine. An acidic amino acid is preferably aspartate or glutamate. An aromatic amino acid is preferably selected from the group consisting of phenylalanine, tyrosine and tryptophane. A non-polar, aliphatic amino acid is preferably selected from the group consisting of alanine, valine, leucine, methionine and isoleucine. A polar, uncharged amino acid is preferably selected from the group consisting of serine, threonine, cysteine, proline, asparagine and glutamine. In contrast to a conservative amino acid substitution, a non-conservative amino acid substitution is the exchange of one amino acid with any amino acid that does not fall under the above-outlined conservative substitutions (i) through (v).

Terms

Unless otherwise explained in the context of this disclosure, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Definitions of common terms in molecular biology can be found in Benjamin Lewin, Genes V, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. The term "plurality" refers to two or more. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Additionally, numerical limitations given with respect to concentrations or levels of a substance, such as an antigen, are intended to be approximate. Thus, where a concentration is indicated to be at least (for example) 200 pg, it is intended that the concentration be understood to be at least approximately (or "about" or "~") 200 pg.

The term "comprises" means "includes." Thus, unless the context requires otherwise, the word "comprises," and variations such as "comprise" and "comprising" will be understood to imply the inclusion of a stated compound or composition (*e.g.*, nucleic acid, polypeptide, antigen) or step, or group of compounds or steps, but not to the exclusion of any other compounds, composition, steps, or groups thereof.

Amino acid sequences provided herein are designated by either single-letter or three-letter nomenclature, as is known in the art (see, e.g., Eur. J. Biochem. 138:9-37(1984)).

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below.

The present invention will now be further described by means of the following non-limiting examples.

EXAMPLES

Example 1: Materials and Methods

Materials

- **HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58 L1 VLPs** were obtained as disclosed in China patent No: ZL200610140613.0 and in Pan H, et al., 2017[15], in Gu, Y. et al., 2017[16], in Wang D., et al., 2017[17] and in Wei M. et al. 2018[18]. The amino acid sequences of the HPV type 6, 11, 16, 18, 31, 33, 45, 52 and 58 L1 proteins are shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16 and 18 respectively.
- **Aluminum salts:**

- **AlOOH (Croda),** provided at a concentration of around 10mg/mL;
- **AlOOH (Ix)** was prepared as follow: 1mol/L NaOH solution was slowly added to 0.33mol/L $AlCl_3$ solution with stirring at 68±2°C. The amount of NaOH was 89.2% of the weight of $AlCl_3$ solution, and the addition of NaOH solution was stopped when the pH reached up to 5.6±0.5. The mixture was then stirred for more than 2 hours at 68±2°C. Then, the solution was filtered with a 10±5 µm filter and distributed into 5L blue bottle. Finally the aluminum hydroxide was autoclaved at 121 °C for 30 minutes, and stored at 2~8°C.

- **MPL** was produced internally (GSK) as a powder as described in GB2220211 A. The powder was then microfluidized in water to form a suspension, the so called MPL liquid Bulk.
- **HPV VLP antigen AMBs (Adsorbed Mono Bulks):** each of the 9 AMBs were made by mixing an Ag working solution (V2/3) with an adjuvant working solution (V1/3) containing Alum, except for HPV-16 which was obtained by mixing Ag working solution (V2/5) with adjuvant working solution (V1/5) and was completed with water (V2/5).

The Ag working solution was prepared from a single serotype Ag bulk, diluted with NaCl, Polysorbate 80 (PS-80) and Na_2HPO_4/NaH_2PO_4 buffer (pH 6) to a concentration of 1350 µg/mL of Ag. The phosphate concentration was 25 mM. The Ag working solution was sterile filtered on two 0.22µm PALL Supor EKV filters with PES (Poly Ether Sulfone) membrane in series.

The adjuvant working solution was prepared from AlOOH adjuvant bulk. The Al^{3+} concentration was diluted with NaCl and WFI (water for injection) to prepare the adjuvant working solution at a target Al^{3+} concentration of 4200 µg/mL (i.e. 155.66 mM Al^{3+}). The size of the AlOOH particles in the adjuvant working solution was 21 to 34 nm, which allowed for a sterile filtration on two 0.22µm PALL Supor EKV filters with PES membrane in series. The mixing of the Ag and adjuvant working solutions resulted in a molar ratio of 16.67 mM PO_4 to 51.89 mM Al^{3+} (or 1/3.11) in the final AMB.

The AMBs had a target Ag concentration of 900 µg/mL for serotypes 6/11/18/31/33/45/52/58 and 540 µg/mL for HPV16. The target Al^{3+} concentration for the AMBs was 1400 µg/mL, except for HPV16 for which it is 840 µg/mL.

- **MPL AMB without phosphate addition:** water was added, and under agitation, aluminum was added until 514.3 µg/mL Al^{3+}. MPL (in liquid bulk or aggregated was added) at 321.4 µg/mL of final volume. Stirring was pursued for 30 minutes. The samples were then stored at 4°C. The MPL was aggregated by addition of NaCl 1500 mM to MPL liquid bulk to obtain a final concentration of 0.7 mg/mL MPL in 428 mM of NaCl.
- **MPL AMB with phosphate addition:** in water, MPL liquid bulk was added up to 321.4 µg/mL of final volume. Al^{3+} was then added under agitation up to 515.3 µg/mL, followed by a 100 mM solution of Na_2HPO_4/NaH_2PO_4 at pH 5.67 up to a 6.12 mM concentration. Stirring was pursued for 30 minutes. The samples were then stored at 4°C.
- **9V formulations using an MPL AMB:** water for injection (WFI) was added, as well as homogenized MPL AMB previously formed ad 100 µg/mL. The 9 antigen AMB were homogenized and added to the mixture under continuous agitation. Stirring was pursued for 15 minutes. The 9V drug product was stored at 4°C until further use.
- **In-line formulation of 9V drug product with added Alum:** the 9 antigen AMBs were homogenized in a separate flask. Alum was added ad 500 µg/dose in WFI, and the 9 antigen AMBs were added under continuous agitation, followed by MPL (liquid bulk or aggregated) ad 50 µg/dose. Stirring was pursued for 30 minutes. The obtained 9V drug product was stored at 4°C until further use.
- **In-line formulation of 9V drug product without added Alum:** the 9 antigen AMBs were homogenized and added to WFI under continuous agitation. MPL liquid bulk was then added ad 50 µg/dose and stirring was pursued for 30 minutes. The obtained 9V drug product was stored at 4°C until further use.

The antigen (Ag) concentrations for each of HPV types 6/11/16/18/31/33/45/52/58 in the 9V formulations were 60/80/120/40/40/40/40/40/40 µg/mL, respectively.

Methods

- Visual observation

At each timepoint (one day, 7 days and 14 days after formulation), the samples were visually inspected.

- pH measurements

In order to assess the effect of time on pH, the pH of each sample was taken just after formulation (t=0, timepoint 1), after 7days for MPL AMB and 6 days for DP (drug product) (timepoint 2) and after 14days for MPL AMB and 13 days for DP (timepoint 3).

- Size measurement

The particle size of the 9V drug product was measured by static light scattering (SLS) over time, from the day after the 9V formulation and 2 days after the formulation for MPL AMB up to 6 weeks.

Dynamic light scattering (DLS) was also tested on aggregated MPL solution, in-line 9V DP with AlOOH (Ix) and in-line 9V DP with AlOOH (Ix) with aggregated MPL.

Samples were taken after formulation and stored in Eppendorf overnight. They were resuspended by rotation for 1 minute at 30 rpm.

- Completeness of adsorption of HPV antigen

The purpose of this analysis was to evaluate if the HPV antigen (Ag) present under the form of a VLP was properly adsorbed on aluminum.

Centrifugation of the sample (16.000 g. 15 minutes) was performed in order to provide the alum and the adsorbed antigen as sediments. Subsequently the protein content in the supernatant (SN) was measured by µBCA. In µBCA the protein content was quantified in an indirect manner by measuring the colorimetric changes induced by the chelation of BCA (bicinchoninic acid) with reduced Cu^{+} ions in the presence of protein.

- MPL Bioassay

The biological activity of MPL was tested by assessing its ability to induce pro-inflammatory cytokine production (*i.e.* TNF-α) by the human monocytic cell line U937. In this assay, the cell line was differentiated into macrophage in presence of PMA and stimulated to express the TLR4 receptor which binds to MPL and cytokines secretion via TLR-4 pathway. In the presence of MPL this receptor initiates an intracellular cascade leading to the production of TNF-α. The TNF-α binds to beads coated with TNF-α specific Ab. A secondary Ab coated with a fluorophore recognizes the bound TNF-α. A FACS system is

used to count and characterize the TNF-α containing beads. This signal can be linked back to the TNF-α concentration.

The production of TNF-α was measured in the supernatants with CBA (Cytometric Bead Array) Flex kits (Becton Dickinson) leading to absolute values of cytokine production in pg/ml. The data generated were expressed as Relative Potency (RP) by performing ratio between each measure (replicate) of TNFα cytokine secretion after stimulation with MPL based formulations at 3 concentrations (1, 3 and 10 µg/mL) and the arithmetic mean value of the quadruplicates of the reference MPL lot.

- MPL adsorption assessment

The purpose of this analysis was to quantify the MPL content in the supernatant of the formulation after one day. The sample was first centrifuged at 16 000 g for 15 minutes to ensure that all aluminum is pelleted. The supernatant was then recovered and analyzed by RP-HPLC fluo. The detected MPL allowed determination of the completeness of MPL adsorption.

Example 2: List of tested formulations

The following formulations were tested in two sets of experiments.

Experiment A

Formulation	Sample name
9V DP in-line formulation	
In-line AlOOH (Ix) added	20HPC03001
In-line no Al added	20HPC03002
In-line $AlPO_4$ added	20HPC03003
In-line AlOOH (Croda) added	20HPC03004
9V DP in-line formulation with aggregated MPL	
In-line AlOOH (Ix) added + aggregated MPL	20HPC03005
In-line $AlPO_4$ added + aggregated MPL	20HPC03006
In-line AlOOH (Croda) added + aggregated MPL	20HPC03007
9V DP formulation with AMB MPL (not in-line)	
9V DP with AMB MPL $AlPO_4$	20HPC03008

9V DP with AMB MPL AlOOH (Croda)	20HPC03009
9V DP with AMB aggregated MPL $AlPO_4$	20HPC03010

DP: Drug product

Experiment B

Formulation	**Sample name**
9V DP with MPL adsorbed on AlOOH (Ix) with PO_4 at RT	20HPC01007 20HPC02007
9V DP with MPL adsorbed on $AlPO_4$ at RT	20HPC01037 20HPC02027
9V DP with MPL adsorbed on AlOOH (Ix) without PO_4 at RT	20HPC01017 20HPC02043
9V DP with MPL added 'in-line' AlOOH (Ix) added	20HPC010272 0HPC02051
9V DP without MPL (420µg Al^{3+}/dose)	20HPC02061
Cervarix-like control (100µg MPL on 500µg ALOOH/ dose)	20HPC02071
9V placebo	20HPC02082
Dilution buffer	20HPC02083

Example 3: Visual observation

Experiment A - No major change was observed between the various formulations at the two time points (**FIG. 1**). The aluminum layer was thinner after 7 days and no flocculation was observed.

Experiment B - All samples were visually inspected and were all found to be opalescent.

Example 4: pH measurements

Experiment A - Measured pH are summarized in **Table 2.** The pH of the samples did not evolve over time. In a range of 0.5 pH units, the pH was similar for each drug product formulation.

Table 2*: pH measured for every sample at each timepoint.* ***1*** *is t=0 (after formulation);* ***2*** *is 6 days;* ***3*** *is 13 days.*

Formulation	Sample name	pH at 1	pH at 2	pH at 3
9V DP In-line AlOOH (Ix) added	20HPC03001	6.86	6.91	6.87
9V DP In-line no Al added	20HPC03002	7.25	7.25	7.21
9V DP In-line $AlPO_4$ added	20HPC03003	6.86	6.94	6.97
9V DP In-line AlOOH (Croda) added	20HPC03004	7.16	7.19	7.16
9V DP In-line AlOOH (Ix) added + aggregated MPL	20HPC03005	6.85	6.86	6.89
9V DP In-line $AlPO_4$ added + aggregated MPL	20HPC03006	6.9	6.96	6.94
9V DP In-line AlOOH (Croda) added + aggregated MPL	20HPC03007	7.14	7.1	7.15
9V DP with MPL AMB $AlPO_4$	20HPC03008	7.08	7.07	7.1
9V DP with MPL AMB AlOOH (Croda)	20HPC03009	7.16	7.25	7.18
9V DP with aggregated MPL AMB $AlPO_4$	20HPC03010	7.06	7.1	7.08

Experiment B - The pH was measured three hours after the formulation of the 9V DP, the resulting data is presented in **table 3**. The long-term storage pH of the HPV AMBs was about 6.7 (data not shown). It is to be noted that the stability of the VLPs is ensured at this pH.

***Table 3**: pH measured for the 9V drug products 3 hours after formulation.*

Formulation	Sample name	pH
9V based on MPL AMB + AlOOH (Ix) + PO_4	20HPC02007	6.85
9V based on MPL AMB + $AlPO_4$	20HPC02027	6.94
9V based on MPL AMB + AlOOH (Ix)	20HPC02043	6.76
MPL in-line (AlOOH Ix)	20HPC02051	6.78
9V without MPL & 420µg Al/dose	20HPC02061	7.07

Example 5: Size measurements

Experiment A - The average median particle size of 5 consecutive measurements are given in **table 4** and in **FIG. 2**. Size distribution is shown for all 9V DPs (with or w/o aggregated MPL) in **FIG. 2** and **FIG. 3. FIG. 4** shows size distribution for CERVARIX, GARDASIL-9 and a 9V DP composed by the 9 serotypes in the matrix without addition of MPL. DLS analysis were also performed on in-line 9V DP with AlOOH (Ix) and in-line 9V DP with AlOOH (Ix) with aggregated MPL. No difference was observed between aggregated and non-aggregated MPL.

FIG. 2 highlights the decrease of the size of the particles over 4 time points (1 day after formulation, one week, two weeks and six weeks), and **FIG. 3** shows that all 9V DP formulations have a nearly identical monomodal peak with a median between 29 to 32µm depending on the formulation. **FIG. 4** shows that the 3 reference DP have a similar size which is smaller in comparison with the tested 9V DP formulations. The 9V DP without MPL was also found to have 2 distinct populations when the 2 commercial DP were mainly composed by one population.

***Table 4**: Static Light Scattering measurements performed on all samples. The median of the peak shown are the average of 5 consecutive measurements. The measurement was done with a new sample at 3 different timepoints. **1** is t=0 (after formulation); **2** is 6 days; **3** is 13 days; **4** is 6 weeks after formulation.*

Formulation	Sample name	Dx (50) at 1	Dx (50) at 2	Dx (50) at 3	Dx (50) at 4
9V DP In-line AlOOH (Ix) added	20HPC03001	31.1	26.5	23.3	7.74
9V DP In-line no Al added	20HPC03002	33.5	30.7	24.9	7.39
9V DP In-line $AlPO_4$ added	20HPC03003	33.4	29	25	8.27

9V DP In-line AlOOH (Croda) added	20HPC03004	31.4	28.5	24	8.7
9V DP In-line AlOOH (Ix) added + aggregated MPL	20HPC03005	29.8	25.8	24.9	18.3
9V DP In-line $AlPO_4$ added + aggregated MPL	20HPC03006	29.1	23.4	19.8	12.5
9V DP In-line AlOOH (Croda) added + aggregated MPL	20HPC03007	33.7	28.1	23.4	17.2
9V DP with MPL AMB $AlPO_4$	20HPC03008	34.1	29.3	24.9	9.83
9V DP with MPL AMB AlOOH (Croda)	20HPC03009	34.3	29.4	24.4	17.6
9V DP with aggregated MPL AMB $AlPO_4$	20HPC03010	31.9	23.9	19.6	14.7
CERVARIX	/	6.28	/	/	/
GARDASIL	/	9.13	/	/	/
9V DP provided by Innovax	/	13.1	/	/	/

Experiment B - SLS measurements of the different formulations of the 9V DPs provided similar results for all formulations in particle size and distribution, as illustrated in **table 5** and **FIG. 5**. The addition of MPL slightly increased the overall size of the particles as shown by the values in the last two columns of **table 5**. On the other hand, the 9V DP with MPL adsorbed on $AlPO_4$ showed a slight tail towards smaller particle sizes.

***Table 5**: Particle size measurements of the different 9V DP formulations by SLS. The average result of 5 measurements are shown.*

		Percentiles on particle diameter (µm)		
Formulation	**Sample name**	**Dx (10%)**	**Dx (50%)**	**Dx (90%)**
9V based on MPL AMB + AlOOH (Ix) + PO_4	20HPC02007	14.2	33.2	65.3
9V based on MPL AMB + $AlPO_4$	20HPC02027	13.6	32.3	63.3
9V based on MPL AMB + AlOOH (Ix)	20HPC02043	14.2	34	68.7
MPL in-line (AlOOH Ix)	20HPC02051	14.7	36.5	75.5
9V without MPL & 420µg Al/dose	20HPC02061	13.8	31.1	59.9

Example 6: completeness of adsorption of HPV antigen

Experiment A - The quantity of total Ag which was not adsorbed on aluminum is disclosed in **table 6** which reveals that all formulations adsorbed nearly all HPV antigen.

***Table 6**: Amount of HPV antigen detected in the supernatant obtained by µBCA. This value was used to determine the completeness of adsorption in % showed in the third column. Limit of quantification is 6 µg/mL.*

Sample	**Sample name**	**Protein in SN (µg/mL)**	**Adsorption completeness (%)**
9V DP In-line AlOOH (Ix) added	20HPC03001	4.2	>99
9V DP In-line no Al added	20HPC03002	4.3	>99
9V DP In-line $AlPO_4$ added	20HPC03003	4.3	>99
9V DP In-line AlOOH (Croda) added	20HPC03004	4.9	>99
9V DP In-line AlOOH (Ix) added + aggregated MPL	20HPC03005	7.7	98.6

9V DP In-line $AlPO_4$ (Croda) added + aggregated MPL	20HPC03006	7.5	98.6
9V DP In-line AlOOH (Croda) added + aggregated MPL	20HPC03007	8.5	98.4
9V DP with MPL AMB $AlPO_4$	20HPC03008	3.7	>99
9V DP with MPL AMB AlOOH (Croda)	20HPC03009	3.3	>99
9V DP with aggregated MPL AMB $AlPO_4$	20HPC03010	7.5	98.6

Experiment B - The results are presented in **table 7.** As 540 µg of HPV protein was present per 1 mL of 9V drug product, only around 1% of protein was not adsorbed and found in the supernatant of the formulation. It was remarkable that for the formulation without MPL, the adsorption was the lowest with >2% of HPV antigen not adsorbed.

***Table 7**: Results of the µBCA assay, quantifying the amount of non adsorbed HPV antigen that was present in the supernatant of the different 9V drug products after centrifugation. *The LoQ is 6 µg/mL.*

Formulation	Sample name	Protein in SN (µg/mL)	Adsorption completeness (%)
9V based on MPL AMB +AlOOH (Ix) + PO_4	20HPC02007	7.2	98.7
9V based on MPLAMB +$AlPO_4$	20HPC02027	4.2*	>98.9
9V based on MPL AMB + AlOOH (Ix)	20HPC02043	6.6	98.7
MPL in-line (AlOOH Ix)	20HPC02051	7.1	98.7
9V without MPL & 420µg Al/dose	20HPC02061	11.4	97.9

Example 7: MPL activity assessment

Experiment A - The results are summarized in **table 8**. Those values are shown in a graph in **FIG. 6** and **FIG. 7**.

***Table 8**: Relative potency and its CV (coefficient of variation) computed with MPL ref 10µg/mL for each sample at each concentration. (1,3,10 µg of MPL/mL)*

		9V DP in-line							9V DP with MPL AMB			
		20HPC03001	20HPC03002	20HPC03004	20HPC03003	20HPC03006	20HPC03007	20HPC03005		20HPC03008	20HPC030010	
		AlOOH (Ix) added	No Al added	AlOOH (Croda) added	$AlPO_4$ added	Aggregated MPL with $AlPO_4$ added	Aggregated MPL with AlOOH (Croda) added	Aggregated MPL with AlOOH (Ix) added	Cervarix (not in-line)	9V with MPL AMB on $AlPO_4$	9V with aggregated MPL on AMB with $AlPO_4$	
N		4	4	4	4	4	4	4	4	4	4	
1 µg/mL	**Mean**	0.27	0.26	0.21	0.22	0.14	0.14	0.10	0.14	0.18	0.16	
	CV(%)	8.73	6.54	10.49	5.28	32.03	43.34	28.75	10.17	5.53	31.59	
3 µg/mL	**Mean**	0.42	0.38	0.30	0.29	0.19	0.18	0.16	0.24	0.27	0.17	
	CV(%)	10.21	5.63	9.16	9.69	26.51	30.80	24.01	13.54	5.36	24.17	
10 µg/mL	**Mean**	0.33	0.24	0.17	0.18	0.17	0.10	0.09	0.38	0.17	0.12	
	CV(%)	13.56	2.23	16.40	2.50	21.76	21.00	17.05	48.37	7.63	13.93	

FIG.s 6 and 7 show the relative potency (RP) of the various samples that were prepared. Formulations made with aggregated MPL had a lower RP than formulations with non aggregated MPL(liquid bulk). The in-line formulation with Al (Ix) had the highest RP followed by the in-line with no addition of aluminum suggesting a higher in vitro bioactivity. The other in-line formulations with non aggregated MPL showed comparable relative potency.

The formulation based on MPL AMB showed the lowest response compared to the in-line formulation.

Experiment B - A statistical analysis of the relative potencies for the 9V DP formulations is shown in **FIG. 8**. The 9V DP with the in-line formulation provided the best relative potencies at the three

concentrations in comparison to the 9V DP obtained with MPL AMB. The response was dependent on the concentration of MPL that was used (10 µg/mL vs 3 µg/mL vs 1 µg/mL), whatever the formulation.

Example 8: completeness of adsorption of MPL

Experiment A - All in-line formulations showed a complete adsorption of MPL as highlighted in **table 9**. The MPL AMB $AlPO_4$ adsorption was incomplete.

Table 9: *MPL content in supernatant quantified by RP-HPLC-FLUO. The completeness of adsorption of MPL on aluminium was determined with this value. The values indicate the concentration of MPL (µg/mL) present in the supernatant. <25µg/mL means that no peak was detected.*

Formulation	Sample name	MPL in SN (µg/mL)	Adsorption completeness (%)
9V DP In-line AlOOH (Ix) added	20HPC03001	<25	>75
9V DP In-line no Al added	20HPC03002	<25	>75
9V DP In-line $AlPO_4$ added	20HPC03003	<25	>75
9V DP In-line AlOOH (Croda) 9V DP	20HPC03004	<25	>75
9V DP with MPL AMB $AlPO_4$	20HPC03008	<25	>75
9V DP with MPL AMB AlOOH (Corda)	20HPC03009	<25	>75

Experiment B - As illustrated in **table 10**, no free MPL was detected in the supernatant of the 9V DPs using the RP-HPLC-FLR method whether they were obtained with an in-line formulation or with MPL AMB.

Table 10: *Completeness of adsorption of MPL on Alum in the 9V DP formulations, measured by the RP-HPLC-FLR (fluo) method (LoQ: 25 µg/mL). The values indicate the concentration of MPL (µg/mL) present in the supernatant.*

Formulation	Sample name	MPL in SN (µg/mL)	Adsorption completeness (%)
9V based on MPL AMB + AlOOH (Ix) + PO_4	20HPC02007	<25	>75
9V based on MPL AMB + $AlPO_4$	20HPC02027	<25	>75
9V based on MPL AMB + AlOOH (Ix)	20HPC02043	<25	>75
MPL in-line (AlOOH Ix)	20HPC02051	<25	>75
9V without MPL & 420µg Al/dose	20HPC02061	ND	ND

References

1. R.J. Martinez-Portilla, M. Rial-Crestelo, M.A. Mejia-Ugarte, J.L. Lopez-Velazquez, *Risk of cervical cancer and squamous intraepithelial lesions according to human papillomavirus high-risk serotypes detected by qualitative real-time in vitro PCR,* European journal of gynaecological oncology, XL, n. 1, 2019.
2. Arbyn, M., Tommasino, M., Depuydt, C., & Dillner, J. (2014). Are 20 human papillomavirus types causing cervical cancer?. *The Journal of pathology*, *234*(4), 431-435.
3. Bosch, F. X., Broker, T. R., Forman, D., Moscicki, A. B., Gillison, M. L., Doorbar, J., ... & Cuzick, J. (2013). Comprehensive control of human papillomavirus infections and related diseases. *Vaccine*, *31*, H1-H31.
4. C.B. Fox, D. Carter, R.M. Kramer, A.M. Beckman, S.G. Reed, *Current Status of Toll-Like Receptor 4 Ligand Vaccine Adjuvants*, 2017, Chapter 6, Immunopotentiators in Modern Vaccines.
5. S.G. Reed, F Hsu, D. Carter, M. T Orr, The Science of Vaccine Adjuvants: Advances in TLR4 Ligand Adjuvants, Current Opinion in Immunology, 2016, 41: 85-90
6. Ji Eun Han, Seo Ri Wui, Kwang Sung Kim, Yang Je Cho, Wan Je Cho, Na Gyong Lee, *Characterization of the Structure and Immunostimulatory Activity of a Vaccine Adjuvant, De-O-Acylated Lipooligosaccharide*, PLOS ONE, January 2014, Volume 9, Issue 1, e85838
7. Chou, Yi-Ju, et al., *Vaccine adjuvant activity of a TLR4-activating synthetic glycolipid by promoting autophagy*, Scientific Reports 10.1 (2020): 1-15.
8. Atsushi Shimoyama and Koichi Fukase, *Chemically Synthesized TLR4 Ligands, Their Immunological Functions, and Potential as Vaccine Adjuvant*, 2020, In: Rossetti C., Peri F. (eds) The Role of Toll-Like Receptor 4 in Infectious and Non Infectious Inflammation. Progress in Inflammation Research, vol 87. Springer, Cham.
9. Cartesio D'Agostini, Francesca Pica, Giuseppe Febbraro, Sandro Grelli, Carlo Chiavarolib, Enrico Garacia, *Antitumour effect of OM-174 and Cyclophosphamide on murine B16 melanoma in different experimental conditions*, International Immunopharmacology 5 (2005) 1205–1212.
10. Samar Hamdy, Azita Haddadi, Vishwa Somayaji, David Ruan, John Samuel, *Pharmaceutical analysis of synthetic lipid A-based vaccine adjuvants in poly (d,l-lactic-co-glycolic acid) nanoparticle formulations*, Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 914–923

11. Kuramitsu, Yasuhiro, Nishibe, Manabu, Ohiro, Youichi, et al. *A new synthetic lipid A analog, ONO-4007, stimulates the production of tumor necrosis factor-[alpha] in tumor tissues, resulting in the rejection of transplanted rat hepatoma cells*. Anticancer Drugs. 1997;**8**(**5**):500-508.
12. Naupu, P. N., van Zyl, A. R., Rybicki, E. P., & Hitzeroth, I. I. (2020). Immunogenicity of Plant-Produced Human Papillomavirus (HPV) Virus-Like Particles (VLPs). *Vaccines*, *8*(4), 740.
13. Kawther K. Ahmed, Sean M. Geary, and Aliasger K. Salem, *Development and Evaluation of Biodegradable Particles Co-loaded with Antigen and the Toll-like Receptor Agonist, Pentaerythritol Lipid A, as a Cancer Vaccine*, J Pharm Sci. 2016 March; 105(3): 1173–1179.
14. Seo Ri Wui, Hye Kyeong Kim, Ji Eun Han, Jong Min Kim, Yeon Hee Kim, Jeong Hoon Chun, Yang Je Cho, Na Gyong Lee, *A combination of the TLR4 agonist CIA05 and alum promotes the immune responses to Bacillus anthracis protective antigen in mice*, International Immunopharmacology 11 (2011) 1195–1204
15. Pan H#, Li Z#, Wang J, Song S, Wang D, Wei M, Gu Y, Zhang J, Li S*, Xia N*. (2017) Bacterially expressed human papillomavirus type 6 and 11 bivalent vaccine: Characterization, antigenicity and immunogenicity. Vaccine 35, 3222-3231.
16. Gu, Y.#, Wei, M.#, Wang, D., Li, Z., Xie, M., Pan, H., Wu, T., Zhang, J., Li, S*., and Xia, N*. (2017) Characterization of an Escherichia coli-derived human papillomavirus type 16 and 18 bivalent vaccine. Vaccine 35, 4637-4645.
17. Wang D#, Fan F#, Li Z, Liu X, Song S, Wei S, He M, Lin Y, Li Z, Wei M, Yu H, Gu Y, Li S*, Xia N, Stop codon mutagenesis for homogenous expression of human papillomavirus L1 protein in Escherichia coli[J]. Protein Expr Purif, 2017. 133: 110-120.
18. Wei M#, Wang D#, Li Z#, Song S, Kong X, Mo X, Yang Y, He M, Li Z, Huang B, Lin Z, Pan H, Zheng Q, Yu H, Gu Y, Zhang J, Li S*, Xia N*. N-terminal truncations on L1 proteins of human papillomaviruses promote their soluble expression in Escherichia coli and self-assembly in vitro. Emerging Microbes & Infections. 2018 Sep 26;7(1):160.

Claims

1. A method for the preparation of a HPV vaccine composition comprising the following steps:
   (i) adsorption of one or more HPV antigen(s) on a metallic salt, and
   (ii) addition of a non adsorbed glycolipid based TLR4 ligand to the mixture obtained in (i).

2. The method according to claim 1, wherein the one or more HPV antigen(s) are selected from HPV late proteins L1, L2, chimeric L1 and chimeric L1/L2 proteins, preferably HPV L1 proteins

3. The method according to claim 2, wherein the one or more HPV antigen(s) are selected from HPV L1 VLPs (virus like particles).

4. The method according to claim 3, wherein the HPV L1 VLPs are produced in *E. coli*, yeast cells or in a baculovirus expression system.

5. The method according to claim 4, wherein the HPV L1 VLPs are produced in *E. coli*.

6. The method according to any one of the preceding claims, wherein each of the one or more HPV antigen(s) are from a HPV type selected from HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 67, 68, 70, 73 and 82, more preferably from HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58.

7. The method according to any one of the preceding claims, wherein the one or more HPV antigen(s) comprise HPV antigen(s) from HPV types 16 and 18.

8. The method according to any one of the preceding claims, wherein the one or more HPV antigen(s) comprise HPV antigen(s) from HPV types 6, 11, 16, and 18.

9. The method according to any one of the preceding claims, wherein the one or more HPV antigen(s) comprise HPV antigen(s) from HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58.

10. The method according to claim 9, wherein the one or more HPV antigen(s) further comprise HPV antigen(s) from HPV types 35, 39 and/or 59.

11. The method according to any one of the preceding claims, wherein the one or more HPV antigen(s) comprise L1 VLPs from HPV types 16 and 18.

12. The method according to any one of the preceding claims, wherein the one or more HPV antigen(s) comprise L1 VLPs from HPV types 6, 11, 16, and 18.

13. The method according to any one of the preceding claims, wherein the one or more HPV antigen(s) comprise or consist of L1 VLPs from HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58.

14. The method according to claim 13, wherein the one or more HPV antigen(s) further comprise L1 VLPs from HPV types 35, 39 and/or 59 L1 VLPs.

15. The method according to any one of the preceding claims, wherein the metallic salt comprises aluminum.

16. The method according to claim 15, wherein the metallic salt is selected from AlOOH, $AlPO_4$, $AlHO_9PS^{-3}$ and $AlHO_5P$.

17. The method according to any one of the preceding claims, wherein the glycolipid based TLR4 agonist is selected from MPL, GLA, SLA, OM-174, CCL-34, RC-529, PET-Lipid A, ONO-4007 and dLOS, preferably MPL.

18. The method according to any one of the preceding claims further comprising the step of adding a metallic salt to the HPV vaccine composition.

19. The method of any one of the preceding claims, wherein
    - the one or more HPV antigen(s) comprise or consist of L1 VLPs from HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58,
    - the metallic salt comprises aluminum, and
    - the glycolipid based TLR4 agonist is MPL.

20. A HPV vaccine composition obtained by the method of any one of the preceding claims.

21. The HPV vaccine composition of claim 20 wherein the metallic ion which is part of the metallic salt is Al^{3+} and the amount of Al^{3+} in the HPV vaccine composition is from 100 to 500 µg/dose.

22. The HPV vaccine composition of claim 20 or 21 wherein the glycolipid based TLR4 ligand is MPL and the amount of MPL in the HPV vaccine composition is from 10 to 50 µg/dose.

23. The HPV vaccine composition of claim 20, 21 or 22, wherein the ratio of glycolipid based TLR4 ligand:metallic salt in the HPV vaccine composition is from 1:2 to 1: 20.

24. The HPV vaccine of any one of claims 20 to 23 for use in therapy.

25. The HPV vaccine of any one of claims 20 to 23 for preventing or treating a HPV infection or associated disease in a subject.

26. A method of preventing or treating an infection or disease caused by HPV comprising the administration of an effective amount of the HPV vaccine of any one of claims 20 to 23to a patient in need thereof.