



(51) International Patent Classification:
A61K 39/12 (2006.01) *C07K 14/01* (2006.01)

(21) International Application Number:
PCT/EP2011/058963

(22) International Filing Date:
31 May 2011 (31.05.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10164764.2 2 June 2010 (02.06.2010) EP

(71) Applicant (for all designated States except US):
DEUTSCHES KREBSFORSCHUNGSZENTRUM
[DE/DE]; Im Neuenheimer Feld 280, 69120 Heidelberg
(DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SEITZ, Hannah**
[DE/DE]; Burgweg 9-11, 69117 Heidelberg (DE).
MÜLLER, Martin [DE/DE]; Siedlerweg 2, 69151
Neckargemünd (DE). **TOMMASINO, Massimo** [IT/FR];
940 route du Bas Privas, F-69390 Charly-Lyon (FR). **OT-**
TONELLO, Simone [IT/IT]; Via Palestro 1, I-43100
Parma (IT). **BOLCHI, Angelo** [IT/IT]; Spieriore 33,
I-43037 Lesignano de' Bagni (IT).

(74) Agent: **DICK, Alexander**; Herzog Fießer & Partner, Du-
denstr. 46, 68167 Mannheim (DE).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD,
ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI,
NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD,
SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG,
ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: PAPILLOMAVIRUS L2 C-TERMINAL PEPTIDES FOR VACCINATION

(57) Abstract: The current invention is concerned with papillomavirus vaccines. Specifically, it relates to a C-terminal peptide of a papillomavirus L2 protein or a fusion polypeptide comprising it for immunizing a subject against papillomavirus infection. The invention further contemplates the use of such a peptide or fusion polypeptide in treating or preventing a papillomavirus-related disease such as cancer. Further, a vaccine comprising the said peptide or fusion polypeptide is provided.



Papillomavirus L2 C-terminal peptides for vaccination

The current invention is concerned with papillomavirus vaccines. Specifically, it relates to a C-terminal peptide of a papillomavirus L2 protein or a fusion polypeptide comprising it for immunizing a subject against papillomavirus infection. The invention further contemplates the use of such a peptide or fusion polypeptide in treating or preventing a papillomavirus-related disease such a cancer. Further, a vaccine comprising the said peptide or fusion polypeptide is provided.

Papillomaviruses (PV) infect the skin and mucosa, especially of the anogenital and respiratory tracts of humans and other mammals and are thus divided into mucosal and skin types. Most PV types cause benign lesions like warts or papilloma.

Bovine PV (BPV) can cause cutaneous warts in cattle which are usually non-problematic and can regress spontaneously due to the immune response of the animal (as reviewed by Nasir, L., Campo, M.S., 2008. Bovine papillomaviruses: their role in the aetiology of cutaneous tumours of bovids and equids. *Veterinary dermatology* 19, 243-54.). They are, however, economically damaging to the owners of show cattle, since affected animals are not admitted to trade shows due to the high contagiousness of the infection. Even more economic damage is caused by genital warts, since they lead to a decrease or even the loss of reproductive functions both in male and in female cattle. Warts on teats may cause mastitis and interfere with suckling and milking. In addition to cutaneous warts, BPV-4 infections have been recognised to be a co-factor for the development of cancer of the gastrointestinal tract while BPV types 1 and 2 are associated with cancer of the urinary bladder (as reviewed by (Nasir, Campo, 2008)). Apart from infecting cattle, BPV-1 and -2 have been implicated in the development of persistent skin tumours in equids termed sarcoids (reviewed by Chambers, G., Ellsmore, V.A., O'Brien, P.M., Reid, S.W., Love, S., Campo, M.S., Nasir, L., 2003. Association of bovine papillomavirus with the equine sarcoid. *The Journal of general virology* 84, 1055-62), while BPV DNA has not been detected on the healthy skin of horses or in samples with non-sarcoid equine lesions (Carr, E.A., Theon, A.P., Madewell, B.R., Griffey, S.M., Hitchcock, M.E., 2001. Bovine papillomavirus DNA in neoplastic and nonneoplastic tissues obtained from horses with and without sarcoids in the western United States. *American journal of veterinary research* 62, 741-4; Nasir, L., McFarlane, S.T., Torrontegui, B.O., Reid, S.W., 1997. Screening for bovine papillomavirus in peripheral blood cells of donkeys with and without sarcoids. *Research in veterinary science* 63, 289-90; Otten, N., von Tscharnier, C., Lazary, S., Antczak, D.F., Gerber, H., 1993. DNA of bovine papillomavirus

type 1 and 2 in equine sarcoids: PCR detection and direct sequencing. Archives of virology 132, 121-31).

For human PV (HPV), association of some types with various forms of malignancies has, however, been established. Accordingly, mucosal HPV types are divided into low-risk types not associated with malignancies, high-risk types with an established association with cancer development, e.g. in cervical cancer, and putative high-risk types, where such an association is presumed.

Persistent infection with high-risk human papillomaviruses is a major risk factor for the development of cervical cancer and occurs in approximately 20% of infected women. Lesions resulting from persistent infection can progress to cervical intraepithelial grade I lesions (CIN1). 20% of these CIN1 lesions progress into CIN2 lesions, and some of these into CIN3 lesions and cervical cancer. The process of progression to CIN3 and cervical cancer is slow, often taking more than ten years after initial infection with the virus. It is also known that high-risk HPVs can cause vulvar, anal, vaginal, penile and oropharyngeal cancer, as well as vaginal intraepithelial neoplasia, anal intraepithelial neoplasia, vulvar intraepithelial neoplasia, and penile intraepithelial neoplasia.

The late PV proteins L1 and L2 are structural proteins building up the viral capsid. The major capsid protein L1 is currently used in prophylactic vaccinations against HPV infection in humans. The formulations CervarixTM and Gardasil[®] both contain virus-like particles (VLPs) consisting of L1 protein. Gardasil[®] includes VLPs of HPV types 6, 11, 16 and 18, which are expressed in and purified from *S.cerevisiae*. CervarixTM consists of only HPV types 16 and 18. The VLPs for CervarixTM are produced in insect cells infected with recombinant baculoviruses.

Introduction of L1-VLP-based vaccines is a significant step forward in the protection of humans from HPV-related disease, especially HPV-related cancer. There is, however, one major drawback to this vaccination, namely the fact that the L1 proteins are diverse in sequence among the various HPV types. Thus, vaccination using an L1 protein from a certain HPV type will usually not protect against infection with a different strain. This is also the reason why L1 proteins from two and four HPV types have been included in the current vaccines, respectively.

On the other hand, the L2 protein, which is the minor capsid protein of PV, was found to be highly conserved in some regions of its sequence. Accordingly, vaccination with N-terminal L2 peptides induced cross-protection against several other PV types in mice; however, peptides derived from L2 were found to be of low immunogenicity, making vaccination difficult to perform.

The technical problem underlying the present invention, thus, could be seen as the provision of means and methods for complying with the aforementioned needs. The said technical problem is solved by the embodiments characterized in the claims and herein below.

Accordingly, the present invention relates to a C-terminal peptide of an PV L2 protein essentially consisting of from 5 to 50 contiguous amino acids selected from the 50 C-terminal

amino acids of PV L2 or a functional variant sequence derived therefrom for immunizing a subject against PV infection.

As used herein, the term "papillomavirus" (PV) relates to a DNA virus from the papillomaviridae family of viruses that infects the skin and mucous membranes of mammals, preferably livestock, more preferably cattle and horses, most preferably humans.

Bovine papillomaviruses (BPV) are associated with several forms of cutaneous and mucosal papilloma in cattle. Based on sequence relatedness, BPV types 1 to 10 have been characterized so far. Additional candidate types, including bovine alimentary papillomavirus-11 (BaPV-11), BPV1bis, BPV3bis, and BPV-BAA5-Japan have been described. BPV infections are common in cattle, with around 50% of cattle being estimated to bear lesions/warts in the UK (Campo, M.S., 1995. Infection by bovine papillomavirus and prospects for vaccination. Trends in microbiology 3, 92-7).

For human PV (HPV), more than 110 genotypes have been described (de Villiers, E. M., C. Fauquet, T. R. Broker, H. U. Bernard, and H. zur Hausen. 2004. Classification of papillomaviruses. Virology 324:17-27). Approximately 50 HPV genotypes are known to infect the mucosa. These mucosal genotypes are classified into three different groups based on their epidemiological association with cancer: "low-risk" human papillomaviruses (LR-HPV), "high-risk" human papillomaviruses (HR-HPV) and "putative high-risk" human papillomaviruses (pHR-HPV). Preferably, HPVs of the current invention are HR-HPVs, which are the main cause for the development of cervical cancer, more preferably HPVs are HPV 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82, most preferably HPV16. The nucleic acids encoding said viruses are well known in the art.

The term "L2 protein" or "L2" relates to the second late gene product of HPV besides the L1 protein. L2 is the minor capsid antigen and forms part of the HP-viral capsid. The coding sequence for the L2 protein starts upstream of the coding sequence for the L1 protein and overlaps with said L1 coding sequence. Preferably, L2 is an L2 protein of a HR-HPV, most preferably of HPV16.

The term "C-terminal peptide" relates to a peptide essentially consisting of between 5 and 50 contiguous amino acids in length from the C-terminal 50 amino acids of a PV L2 polypeptide, having the function of inducing an anti-PV L2 immune response in a suitable host, e.g. a mammal. The stretch of 50 C-terminal amino acids from which the C-terminal peptide essentially consists of 5 to 50 contiguous amino acids is exemplarily shown in SEQ ID No. 1-27 for BPV1, 1bis, 2, 3, 3bis, 5, 6, 7, 8, 9, 10 and BPV-BAA5-Japan (SEQ ID Nos. 1-12) and for HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 (SEQ ID Nos. 13-27). It will be understood that a stretch of corresponding amino acids in other PV types can be identified without further ado by counting 50 amino acids starting from the C-terminus of a PV L2 protein.

More preferably, the C-terminal peptide is a peptide consisting of between 5 and 41 amino acids corresponding to an amino acid sequence with a similar, preferably equal length comprised in amino acids 433 to 473 of the HPV16 L2 protein or a peptide consisting of

between 5 and 21 amino acids corresponding to an amino acid sequence with a similar, preferably equal length comprised in amino acids 450 to 470 of the HPV16 L2 protein. Most preferably, the C-terminal peptide is a peptide consisting of between 5 and 14 amino acids corresponding to an amino acid sequence with an equal length comprised in amino acids 450 to 463 of the HPV16 L2 protein. It is to be understood that amino acids of any PV L2 protein of interest corresponding to amino acids 433 to 473, to amino acids 450 to 470, or amino acids 450 to 463 of the HPV16 L2 protein can e.g. be determined by aligning the sequences of SEQ ID No. 13 (C-terminal 50 amino acids of the HPV16 L2 protein) to the amino acid sequence of the C-terminal 50 amino acids of said PV L2 protein of interest, as shown e.g. in Fig. 1. For said other PV L2 protein of interest, a C-terminal peptide according to the invention can be selected from the stretch of amino acids identified accordingly.

Also included are functional variant sequences of the C-terminal peptide of the invention specified above, said variants having an amino acid sequence being at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the amino acid sequence of one of the C-terminal peptides and retaining the function of the C-terminal peptides of the current invention, i.e. being capable of inducing an anti PV L2 immune response in a host. The conservation of activity can be tested by the one skilled in the art, e.g. by testing if the variant is able to induce anti-PV L2 antibodies in a suitable host. The percent identity values are, preferably, calculated over the entire amino acid sequence region. A series of programs based on a variety of algorithms is available to the skilled worker for comparing different sequences. In this context, the algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. To carry out the sequence alignments, the program PileUp (J. Mol. Evolution., 25, 351-360, 1987, Higgins et al., CABIOS, 5 1989: 151-153) or the programs Gap and BestFit (Needleman and Wunsch (J. Mol. Biol. 48; 443-453 (1970)) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981))), which are part of the GCG software packet (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991)), are to be used. The C-terminal peptide or the artificial fusion polypeptide of the present invention can be recombinantly manufactured or may be chemically synthesised.

In a preferred embodiment of the C-terminal peptide to be applied in accordance with the present invention, said C-terminal peptide is fused to a further peptide. Thereby, an artificial fusion peptide is generated.

In the context of the current invention, the term "artificial fusion polypeptide" relates to a polypeptide of between 6 and 1000 amino acids in length comprising at least two different domains, one of the domains being the C-terminal peptide of the current invention. The other domain is a domain not being the continuation of the C-terminal peptide with the sequence of the L2 protein. It is to be understood that the second domain may have an amino acid sequence occurring in the L2 amino acid sequence provided that the said sequence is not naturally flanking the C-terminal peptide in the L2 protein.

The artificial fusion polypeptide may comprise further amino acids which may serve as a tag for purification or detection or as a linker. In a preferred embodiment of the artificial fusion polypeptide of the present invention, said artificial fusion polypeptide further comprises a

detectable tag. The term "detectable tag" refers to a stretch of amino acids which are added to or introduced into the artificial fusion polypeptide of the invention. Preferably, the tag shall be added C- or N- terminally to the artificial fusion polypeptide of the present invention. The said stretch of amino acids shall allow for detection of the artificial fusion polypeptide by an antibody which specifically recognizes the tag or it shall allow for forming a functional conformation, such as a chelator or it shall allow for visualization by fluorescent tags. Preferred tags are the Myc-tag, FLAG-tag, 6-His-tag, HA-tag, GST-tag or GFP-tag. These tags are all well known in the art.

The terms C-terminal peptide and artificial fusion polypeptide also include chemically modified peptides or polypeptides, e.g., containing modified amino acids or being biotinylated or coupled to fluorophores, such as fluorescein, or Cy 3, being conformationally restricted, e.g. by disulfide bridging or by stapling (Walensky 2004, Science 305(5689): 1466-1470), or being linked to cell penetration polypeptides or protein transduction domains (Snyder 2004, Pharm Res 21(3): 389-393). Such modifications may improve the biological properties of the C-terminal peptide or of the artificial fusion polypeptides, e.g., cell penetration, binding, stability, or may be used as detection labels.

As used herein, the term "subject" relates to a metazoan organism with the capacity to generate an immune response to molecules foreign to the organism. Preferably, the subject is an animal, more preferably a mammal, most preferably a human being.

The term "immunizing" relates to an active immunization by the introduction of a C-terminal peptide into the body of a subject to be immunized, thereby causing activation of said body's protection mechanism. Preferably, immunization causes activation and expansion of T-cells and / or B-cells specifically recognizing the C-terminal peptide of the current invention. More preferably, immunization causes the production of antibodies preventing infection of body cells by PV; most preferably, immunization causes the production of antibodies preventing infection of body cells by at least two, at least three, or at least four different PV types.

Preferably, immunization results in preventing infection of a subject by PV. More preferably, immunization results in a long-lasting, most preferably life-long, immune-response against PV. It will be understood that the said period of time is dependent on the amount of the immunizing agent which has been administered and individual factors of the subject. It is to be understood that immunization may not be effective in all subjects treated. However, the term requires that a statistically significant portion of subjects of a cohort or population are effectively prevented from being infected by PV. Preferably, a cohort or population of subjects is envisaged in this context which normally, i.e. without immunization, would be infected with PV with a high probability. Whether prevention of infection by immunization is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student's t-test, Mann-Whitney test etc.. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the treatment shall be effective for at least 60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort or population.

The definitions made above apply *mutatis mutandis* to the following:

The present invention further relates to a polynucleotide comprising a nucleic acid sequence encoding the C-terminal peptide of the present invention for immunizing a subject against PV infection.

The term "polynucleotide" as used in accordance with the present invention relates to a polynucleotide comprising a nucleic acid sequence which encodes the C-terminal peptide as described above. Suitable assays for measuring the activities mentioned before are described in the accompanying Examples. A polynucleotide encoding a C-terminal peptide has been obtained in accordance with the present invention from HPV16. Thus, the polynucleotide, preferably, comprises the nucleic acid sequence shown in SEQ ID NO: 28 encoding the C-terminal peptide having an amino acid sequence as shown in SEQ ID NO: 13. It is to be understood that a C-terminal peptide according to the invention having e.g. an amino acid sequence as shown in SEQ ID NO: 13 may be also encoded due to the degenerated genetic code by other polynucleotides as well.

Moreover, the term "polynucleotide" as used in accordance with the present invention further encompasses variants of the aforementioned specific polynucleotides. The polynucleotide variants, preferably, comprise a nucleic acid sequence characterized in that the sequence can be derived from the aforementioned specific nucleic acid sequence shown in SEQ ID NO: 28 by at least one nucleotide substitution, addition and/or deletion whereby the variant nucleic acid sequence shall still encode a polypeptide having the activity as specified above. Variants also encompass polynucleotides comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. These stringent conditions are known to the skilled worker and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. A preferred example for stringent hybridization conditions are hybridization conditions in 6 ' sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more wash steps in 0.2 ' SSC, 0.1% SDS at 50 to 65°C. The skilled worker knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, under "standard hybridization conditions" the temperature differs depending on the type of nucleic acid between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 ' SSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42°C. The hybridization conditions for DNA:DNA hybrids are preferably for example 0.1 ' SSC and 20°C to 45°C, preferably between 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are preferably, for example, 0.1 ' SSC and 30°C to 55°C, preferably between 45°C and 55°C. The abovementioned hybridization temperatures are determined for example for a nucleic acid with approximately 100 bp (= base pairs) in length and a G + C content of 50% in the absence of formamide. The skilled worker knows how to determine the hybridization conditions required by referring to textbooks such as the textbook mentioned above, or the following textbooks: Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 1985, "Nucleic Acids Hybridization: A Practical

Approach", IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford. Moreover, also encompassed are polynucleotides which comprise nucleic acid sequences encoding amino acid sequences which are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequences shown in SEQ ID NO: 1-27. The percent identity values are, preferably, calculated over the entire amino acid or nucleic acid sequence region as specified above.

The polynucleotide of the present invention shall be provided, preferably, either as an isolated polynucleotide (i.e. isolated from its natural context) or in genetically modified form. The polynucleotide, preferably, is DNA, including cDNA, or RNA. The term encompasses single as well as double stranded polynucleotides. Moreover, comprised are also chemically modified polynucleotides including naturally occurring modified polynucleotides such as glycosylated or methylated polynucleotides or artificial modified one such as biotinylated polynucleotides.

The polynucleotides of the present invention may contain further nucleic acid sequences as well.

In another embodiment of the polynucleotide to be applied in accordance with the present invention the polynucleotide is comprised in a vector.

The term "vector", preferably, encompasses phage, plasmid, viral or retroviral vectors as well as artificial chromosomes, such as bacterial or yeast artificial chromosomes. Moreover, the term also relates to targeting constructs which allow for random or site-directed integration of the targeting construct into genomic DNA. Such target constructs, preferably, comprise DNA of sufficient length for either homologous or heterologous recombination as described in detail below. The vector encompassing the polynucleotides of the present invention, preferably, further comprises selectable markers for propagation and/or selection in a host. The vector may be incorporated into a host cell by various techniques well known in the art. For example, a plasmid vector can be introduced in a precipitate such as a calcium phosphate precipitate or rubidium chloride precipitate, or in a complex with a charged lipid or in carbon-based clusters, such as fullerenes. Alternatively, a plasmid vector may be introduced by heat shock or electroporation techniques. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host/cells.

More preferably, in the vector of the invention the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells or isolated fractions thereof. Expression of said polynucleotide comprises transcription of the polynucleotide, preferably into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known in the art. They, preferably, comprise regulatory sequences ensuring initiation of transcription and, optionally, poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers.

Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the lac, trp or tac promoter in *E. coli*, and examples for regulatory elements permitting expression in eukaryotic host cells are the AOX1 or GAL1 promoter in yeast or the CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells. Moreover, inducible expression control sequences may be used in an expression vector encompassed by the present invention. Such inducible vectors may comprise tet or lac operator sequences or sequences inducible by heat shock or other environmental factors. Suitable expression control sequences are well known in the art. Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pBluescript (Stratagene), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogen) or pSPORT1 (GIBCO BRL). Preferably, said vector is an expression vector and a gene transfer or targeting vector. Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the polynucleotides or vector of the invention into targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors; see, for example, the techniques described in Sambrook, *Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y. (1994).

In a further embodiment, the current invention relates to a vaccine comprising a C-terminal peptide or a polynucleotide of the present invention.

The term "vaccine" as used herein comprises the compounds of the present invention and optionally one or more pharmaceutically acceptable carrier. The compounds of the present invention can be formulated as pharmaceutically acceptable salts. Acceptable salts comprise acetate, methylester, HCl, sulfate, chloride and the like. The vaccines are, preferably, administered topically or systemically. Suitable routes of administration conventionally used for drug administration are oral, intravenous, or parenteral administration as well as inhalation. However, depending on the nature and mode of action of a compound, the vaccines may be administered by other routes as well. For example, polynucleotide compounds may be administered in a gene therapy approach by using viral vectors or viruses or liposomes.

Moreover, the compounds can be administered in combination with other vaccines either in a common vaccine or as separated vaccines wherein said separated vaccines may be provided in form of a kit of parts.

The compounds are, preferably, administered in conventional dosage forms prepared by combining the drugs with standard pharmaceutical carriers according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount

of active ingredient with which it is to be combined, the route of administration and other well-known variables.

The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formulation and being not deleterious to the recipient thereof. The pharmaceutical carrier employed may be, for example, either a solid, a gel or a liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are phosphate buffered saline solution, syrup, oil such as peanut oil and olive oil, water, emulsions, various types of wetting agents, sterile solutions and the like.

The diluent(s) is/are selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the vaccine or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like.

A therapeutically effective dose refers to an amount of the compounds to be used in a vaccine of the present invention which induces immunization according to the present specification. The dosage regimen will be determined by the attending physician and other clinical factors; preferably in accordance with any one of the above described methods. As is well known in the medical arts, dosages for any one patient may depend upon several factors, including the patient's age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Immunization can be monitored by periodic assessment. A typical dose can be, for example, in the range of 1 to 1000 μg ; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors.

The vaccines and formulations referred to herein are administered at least once in order to treat or ameliorate or prevent a disease or condition recited in this specification. However, the said vaccines may be administered more than one time, for example up to four times.

Specific vaccines are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound referred to herein above in admixture or otherwise associated with a pharmaceutically acceptable carrier or diluent. For making those specific vaccines, the active compound(s) will usually be mixed with a carrier or the diluent, or enclosed or encapsulated in a capsule, sachet, cachet, paper or other suitable containers or vehicles. The resulting formulations are to be adopted to the mode of administration, i.e. in the forms of tablets, capsules, suppositories, solutions, suspensions or the like. Dosage recommendations shall be indicated in the prescribers or users instructions in order to anticipate dose adjustments depending on the considered recipient.

The term "treating" relates to ameliorating the diseases or disorders referred to herein or the symptoms accompanied therewith to a significant extent. Said treating as used herein also includes an entire restoration of the health with respect to the diseases or disorders referred to herein. It is to be understood that treating as used in accordance with the present invention

may not be effective in all subjects to be treated. However, the term shall require that a statistically significant portion of subjects suffering from a disease or disorder referred to herein can be successfully treated. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student's t-test, Mann-Whitney test etc. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the treatment shall be effective for at least 60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort or population.

The term "preventing" refers to retaining health with respect to the diseases or disorders referred to herein for a certain period of time in a subject. It will be understood that the said period of time is dependent on the amount of the drug compound which has been administered and individual factors of the subject discussed elsewhere in this specification. It is to be understood that prevention may not be effective in all subjects treated with the compound according to the present invention. However, the term requires that a statistically significant portion of subjects of a cohort or population are effectively prevented from suffering from a disease or disorder referred to herein or its accompanying symptoms. Preferably, a cohort or population of subjects is envisaged in this context which normally, i.e. without preventive measures according to the present invention, would develop a disease or disorder as referred to herein. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools discussed elsewhere in this specification.

As used herein, the term "PV-related disease" relates to all disorders caused by the infection of a subject by PV. Preferably, PV-related diseases are warts, including common warts, plantar warts, flat warts, anogenital warts, Epidermodysplasia verruciformis, focal epithelial hyperplasia, oral papillomas, or laryngeal papillomatosis. More preferably, PV-related disease is HPV-related cancer, most preferably cervical cancer, anal cancer, penile cancer, vulvar cancer, or head and neck cancer.

All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

The following Examples shall merely illustrate the invention. They shall not be construed, whatsoever, to limit the scope of the invention.

Figures:

Fig. 1: Alignments of PV L2 Proteins

PV L2 proteins were aligned using the Kalign algorithm (Lassmann T. and Sonnhammer E.L.L. (2005); Kalign - an accurate and fast multiple sequence alignment algorithm; BMC Bioinformatics 6: 298). Numbers relate to amino acid positions in the full-length HPV16 L2 protein.

Fig. 2: Overview L2 peptides expressed as Trx fusion proteins

Indicated amino acids of the HPV16 L2 were expressed as Trx fusions (Rubio et al., 2009, Potent anti-HPV immune responses induced by tandem repeats of the HPV16 L2 (20 -- 38) peptide displayed on bacterial thioredoxin, Vaccine 27(13):1949-56), e.g. a 433_473 Trx fusion antigen is a Trx-L2 fusion protein comprising amino acids 433 to 473 of the HPV16 L2 protein.

Fig. 3: SDS-Page of purified Trx fusion proteins

Purified Trx fusion peptides were resolved by SDS-Page and stained with Coomassie.

Fig. 4: Immunization scheme used for immunizing mice with Trx-L2 fusion proteins

Fig. 5: Titration of anti HPV16 L2 antibody titers in mice

5 mice per Trx-L2 fusion were immunized according to a standard immunization scheme (Fig. 4). After immunization, sera were titrated for the presence of anti HPV-16 L2 antibodies using an anti GST-L2 ELISA as described in Example 2. Most Trx-L2 fusion proteins elicit anti-L2 antibodies.

Fig. 6: HPV16 Neutralization assay:

Sera from mice immunized against Trx-L2 fusion proteins were assayed for HPV16-neutralizing activity as described in Examples 3 and 4. A high percentual neutralization indicates the presence of neutralizing anti-HPV16 antibodies. Only the 433_473 Trx fusion antigen elicits neutralizing antibodies.

Fig. 7: Titration Neutralization assay

Serum from mouse 5 from the 433_473 Trx fusion antigen group was titrated in the neutralization assay of Examples 3 and 4. IC50 is 6400.

Fig. 8: Cross-neutralization assay

Serum from mouse 5 from the 433_473 Trx fusion antigen group was used in a 1:50 dilution in a cross-neutralization assay against various PV. With the exception of HPV6, all PV tested were inhibited by at least 50%.

Example 1

Trx-L2 Expression, Extraction and Purification:

Expression:

Growth of 400 ml Rosetta culture to OD 0.5-0.6 in LB

Induction with 1mM IPTG à over night 30 °C

Centrifuge culture 10 min 5000 rpm

Decant supernatant and wash pellet in 100 ml 10 mM Tris, pH 8

Decant supernatant and store pellet at -20 °C or proceed with lysis

Extraction:

Resuspend pellet in 40 ml lysis buffer (300 mM NaCl, 25 mM Tris, 0.16% Tween20, 0.5 mM PMSF, 0.1 mg/ml lysozyme, pH 8)

Incubate suspension at room temp for 10 min

Incubate suspension on ice for 20 min

French Press lysis: 3-4 passes with at least 15,000 psi à collect 100 µl sample

Centrifugation at 17,000 rpm, 1h 4 °C

Collect supernatant and store on ice until purification à collect 100 µl sample

Purification:

Prepare 1 ml HiTrap columns (GE Healthcare) for purification:

Wash columns with 10 CVs (column volumes) water

Apply ~ 1.5 ml of a 100 mM NiSO₄ solution to each column (incubate 5 min)

Wash columns with 10 CVs water

Wash columns with 10 CVs binding buffer (300 mM NaCl, 25 mM Tris, 50 mM Imidazole, pH 7.5)

Connect protein-containing supernatant to peristaltic pumps and columns; circulate over night at 0.5 ml/min

Wash columns with 10 CVs binding buffer

Elute protein with 125 mM, 175 mM, 300 mM Imidazole step gradient (all in 300 mM NaCl, 25 mM Tris, pH 7.5 buffers) on FPLC or elute by hand by washing with 10 CVs 100 mM Imidazole and then eluting with ~ 5 ml 300 mM Imidazole à collect 100 µ sample of flow through

Determine protein-containing fractions by Bradford and Coomassie (SDS gel)

Dialyze proteins against 1 x PBS (with 500 mM NaCl) over night at 4 °C

Collect dialyzed samples, centrifuge 15 min 12,000 rpm 4 °C and collect supernatant

Bradford assay to determine protein content

Aliquot samples, shock freeze in liquid nitrogen and store at -70 °C

Example 2: GST-L2 ELISA

according to Rubio et al., 2009, Potent anti-HPV immune responses induced by tandem repeats of the HPV16 L2 (20 -- 38) peptide displayed on bacterial thioredoxin, Vaccine 27(13):1949-56:

treat ELISA plates with:

- purified Glutathione-casein diluted 1:500 in carbonate coating buffer, 50 µl/well à 4 °C overnight (ON)
- 3 x PBS/0.3 % Tween20 wash
- 0.2 % casein, 100 µl/well à 1 h 37 °C
- 3 x PBS/0.3 % Tween20 wash
- GST-L2 antigen diluted 1:30 - 1:50 in 0.2 % casein, 50 µl/well à 1 h 37 °C
- 3 x PBS/0.3 % Tween20n wash
- Serial 1:2 dilutions of sera in 0.2 % casein: 1:50 à 1:102400, 50 µl/well à 1 h 37 °C
- 3 x PBS/0.3 % Tween20 wash
- GAMPO (goat anti mouse peroxidase secondary antibody) diluted 1:3000 in 0.2 % casein, 50 µl/well à 1 h 37 °C
- 4 x PBS/0.3 % Tween20 wash
- 100 µl/well of developing solution
- > read in ELISA reader at 405 nm

Example 3

Pseudovirion Production and Purification

Pseudovirion production and purification and plasmids used in that procedure have been described before (e.g. Buck et al. 2005, Maturation of Papillomavirus Capsids; Journal of Virology, Vol. 79, No. 5, p. 2839-2846).

Day 1: Prepare a solution of 293TT cells (3×10^5 cells/ml) in supplemented D-MEM and seed 20 ml per 10 cm dish plate. To make a big preparation of PSV use 10 dishes per PSV.

Day 2: Transfect 293TT cells using Turbofect® like this per plate:

1 ml of un-supplemented D-MEM

Add 30 µg of total DNA (including plasmids codifying for L1/L2 and Gaussia)

Add 60 µL of Turbofect®

Mix the DNA and the Turbofect® by vortex and incubate at room temperature for 15 minutes

Add the mix to the cells drop by drop

Incubate the cells for three days at 37°C/5%CO₂ during 3 to 4 days.

Day 5 or 6: Pseudovirion extraction:

Cells are resuspended by pipetting up and down several times the medium, transfer the cell suspension to a 50 ml falcon.

Centrifuge the cells at 1900 rpm for 5 minutes, discard the supernatant and re-suspend the cells in 1 ml D-PBS (Invitrogen, 14040-141).

Transfer the cells to a 1.5 ml eppendorf tube (low binding tube)

Centrifuge the cells at 5000 rpm for 5 min at 4°C.

Discard the supernatant and re-suspend the cells in equal volume of lyses buffer.

To prepare lyses buffer: 300 µL of DPBS + 17,5 µL Brij 58(10%) + 2 µL RNaseA/T cocktail (Fermentas)

Incubate the cells in lyses buffer for 24 hours at 37°C under rotation.

Optiprep gradient preparation:

Prepare three initial solutions at 27%, 33% and 39% by dilution of the original solution (60%) using D-PBS/0.8M NaCl to dilute the optiprep. In tubes for SW41TI rotor add 3.2 ml of previous solutions starting with 39% and on top the 27% solution. Leave the tubes overnight at 4°C to allow the formation of the gradient.

Day 6 or 7: Pseudovirion maturation and purification:

Place the eppis on ice for 5 minutes

Add 0.17 volumes of NaCl 5M to the eppis (final concentration 0.8M NaCl in every tube)

Repeat the incubation on ice for 5 minutes

Centrifuge the eppi at 10000 rpm for 10 minutes at 4°C

Collect the supernatants in other eppi (Eppi # 2) and resuspend the pellets in 300 µL of D-PBS/0.8M NaCl.

Repeat centrifugation at 10000 rpm for 10 minutes at 4°C

Add the supernatant to the Eppi # 2 (estimated volume of 600 µL)

Add 2 µL of Benzonase and incubate for 1 hour at 37°C

Centrifuge at 10000 rpm for 10 minutes at 4°C

Add the supernatant on top of Optiprep gradient (balance the tubes by weight using D-PBS/0.8M NaCl solution)

Centrifuge the gradients at 37000 rpm for 5 hours at 16°C

Collect 2 ml of fractions starting from lower gradient

Aliquot the purified pseudovirions and freeze at -80°C

Example 4

Neutralization assay

This assay is used to detect neutralizing antibodies in sera against human papillomavirus. For this assay, pseudovirions containing the two capsid proteins L1 and L2 from HPV of different types are used to infect 293TT cells. As a result of the infection, the PSV release in the cell a plasmid encoding for a reporter gene Gaussia Luciferase. Infected cells will be detected by the presence of the luciferase on the culture medium. On the other hand, when neutralizing antibodies are present in the sera, they prevent the infection by pseudovirions and thus expression of the reporter gene.

Protocol

Prepare dilutions of (mouse) sera starting from 1:50. To dilute the sera use supplemented D-MEM. Add 50µL in duplicates in 96 well plates.

Add 50µL of PSV (HPV16, HPV18, BPV 1 etc) diluted 1:10,000- 1:100,000 in supplemented D-MEM to the wells where the sera dilutions was previously added and incubate for 15 minutes at room temperature.

Prepare a solution of 2.5×10^5 cells/ml and seed 50µL per well in 96 well/plates.

Incubate for two days at 37°C, 5% CO₂

Detection of Gaussia luciferase

Remove plates from the incubator; leave them at room temperature for 15 minutes.

Adjust Gaussia juice to room temperature.

Add in white plates 10 μ L of cell supernatant

Prepare the substrate: 100 μ L of Coelenterazine (previously resuspended with 1 ml of absolute ethanol) per 10 ml of Gaussia juice.

Add 100 μ L/well of substrate previously prepared

Read in Luminometer at minute 1. (program: copy of luminescence)

Repeat the read at minute 10.

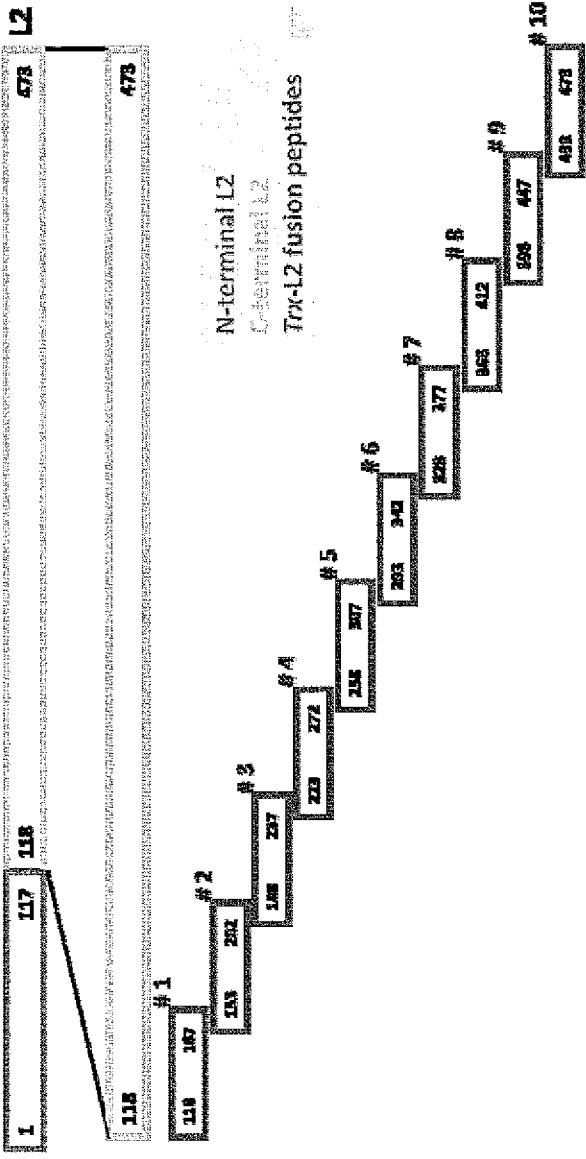
Claims

1. A C-terminal peptide of a PV L2 protein essentially consisting of from 5 to 50 contiguous amino acids selected from the 50 C-terminal amino acids of a PV L2 or a functional variant sequence derived therefrom for immunizing a subject against PV infection.
2. The C-terminal peptide polypeptide of claim 1, comprising an amino acid sequence corresponding to amino acids 433 to 473 of the HPV16 L2 protein.
3. The C-terminal peptide of claim 1 or 2, comprising an amino acid sequence corresponding to amino acids 450 to 463 of the HPV16 L2 protein.
4. The C-terminal peptide of any one of claims 1 to 3, wherein said peptide is fused to a further peptide.
5. The C-terminal peptide of any one of claims 1 to 4, wherein said subject is a human.
6. A polynucleotide comprising a nucleic acid sequence encoding the C-terminal peptide of any one of claims 1 to 4 for immunizing a subject against PV infection.
7. The polynucleotide of claim 6, wherein said subject is a human.
8. The polynucleotide of claim 6 or 7, wherein said polynucleotide is comprised in a vector.
9. A C-terminal peptide as defined in any one of claims 1 to 5 for use in treating or preventing a PV-related disease.
10. A polynucleotide as defined in any one of claims 6 to 8 for use in treating or preventing a PV-related disease.
11. The C-terminal peptide of claim 9 or the polynucleotide of claim 10, wherein said PV-related disease is cancer.
12. A vaccine comprising a C-terminal peptide as defined in any one of claims 1 to 5 or a polynucleotide as defined in any one of claims 6 to 8.

Fig. 1

AA in full-length HPV16 L2:		425	440	450	460	470
HPV16/1-50	-----	APSLIP--IVGSPQYIIA--DA-----	-----	GDFYL--	-----	HPSYMLRKRKRRLPYFFSDVSLAA
HPV18/1-50	-----	VWIVSP--TAPASTQYIGIH--GT-----	-----	HYYL--	-----	WPLYFIPKKRKRVPYFFADGFVAA
HPV31/1-50	-----	QVFPFLAPTTPQVSIFV--DG-----	-----	GDFYL--	-----	HPSYMLKRRRKRVSYYFTTDSVAA
HPV33/1-50	-----	TSSPVP--ISFPFPDTIVV--DG-----	-----	ADFVL--	-----	HPSYFILRRRKRFPYFFTDVRAA
HPV35/1-50	-----	ITNTVLP--VPTGPYXIIA--DG-----	-----	GDFYL--	-----	HPSYLLKRRRKRIPYFFADVSAV
HPV39/1-50	-----	QLPLVP--SGPIDTTYAITI--QG-----	-----	SNYYL--	-----	LPLLYFLLKRRKRIPYFFESDGYVAV
HPV45/1-50	-----	MWPSTSP--TNASTTYIGIH--GT-----	-----	QYVL--	-----	WPWYFFPKRKRKRIPYFFADGFVAA
HPV51/1-50	-----	VMPYVP--HTSIDTKHSIVI--LG-----	-----	GDXYL--	-----	WPTHLLRKRKRKRIPYFFTDGIVAH
HPV52/1-50	-----	HTPFVP--IAPTAPSTSIIV--DG-----	-----	TDFIL--	-----	HPSYFLLRRRKRFPYFFTDVRAA
HPV56/1-50	-----	STWPFVP--QSPYDVTHDVI--QG-----	-----	SSPAL--	-----	WPVYFFRRRRKRKRIPYFFADGDVA-
HPV58/1-50	-----	SSPFIP--ISPLTPFNTIIV--DG-----	-----	ADFML--	-----	HPSYFILRRRKRFPYFFADVRAA
HPV59/1-50	-----	VEPTYs--TTPFTTIQSINI--EG-----	-----	TNYFL--	-----	WPIYFLLPRKRKRVPYFFTDGSMAF
HPV68/1-50	-----	QLPLTP--STPIDTTYAITI--YG-----	-----	THXYL--	-----	LPLLEFLLKRRKRRLPYFFADGIVAL
HPV73/1-50	-----	TETIFT--IVPLQAPAGPIYI--YG-----	-----	SGFIL--	-----	HPSYLLKRRKRRLSYSTFDVATY-
HPV82/1-49	-----	WPFIP--HTSIDTQHAIVI--QG-----	-----	GDYYL--	-----	WPTYLLRKRKRKRIPYFFADGFVAY
BPV1/1-50	-----	DPDSTSPSLV--IDDTTTPIIII--DGHTVDLYSSNYTL--	-----	HPSLLRKRKRKHA--	-----	-----
BPV1bis/1-50	-----	DPDSTSPSLV--IDDTTTPIIII--DGHTVDLYSSNYTL--	-----	HPSLLRKRKRKHA--	-----	-----
BPV2/1-50	-----	GPDSTSPSLV--IDDNTPPIIIII--DGHTVDLYSSNYSL--	-----	HPSLLRKRKRKHA--	-----	-----
BPV3/1-50	-----	ESNDTDR--YQPFIP--VTPAATPDIIITFEEG-T-----	-----	ATFFL--	-----	KRHKHKHWF--
BPV3bis/1-50	-----	ESNDTDR--YQPFIP--VTPAATPDIIITFEEG-T-----	-----	ATFFL--	-----	KRHKHKHWF--
BPV5/1-50	-----	INIQN--GPVDVD--LQDSILPGIIT--DS-GVD-----	-----	GTYFLNTYLHPSLH--	-----	KRKKRRFS--
BPV6/1-50	-----	NTVPN--VP-PHTPFTP--LDPALPDIIVNL--EE-NT-----	-----	ATFFL--	-----	RKHRHKHWF--
BPV7/1-50	-----	NKDSK-SQ--IPVKIP--VHVDPSPDVVVI--SY-TADV--SIFSL--	-----	F-EPSLY--	-----	RKKYSYLY--
BPV8/1-50	-----	ITMQN--GPVHVD--GQDNIVPDIIII--DF-SVE-----	-----	GTYFLNTYAHPSLH--	-----	KRKKRRLS--
BPV9/1-50	PAHEEGTDR	-----	DI-P--LSPTLPPAIIIDFDED-T-----	ATFFL--	-----	KKKHKHWF--
BPV10/1-50	NAFTPTPRF	-----	PLTP--LSPAYTPTVIIDFEDG-S-----	ATFFL--	-----	RKKHKHWF--
BPV-BAA5-Japan/1-50	-AFTPTITPRV	-----	PLTP--LSPAYTPTIIIDFEDG-S-----	ATFFL--	-----	HPSLL--RKKHKHWF--

Fig. 2:



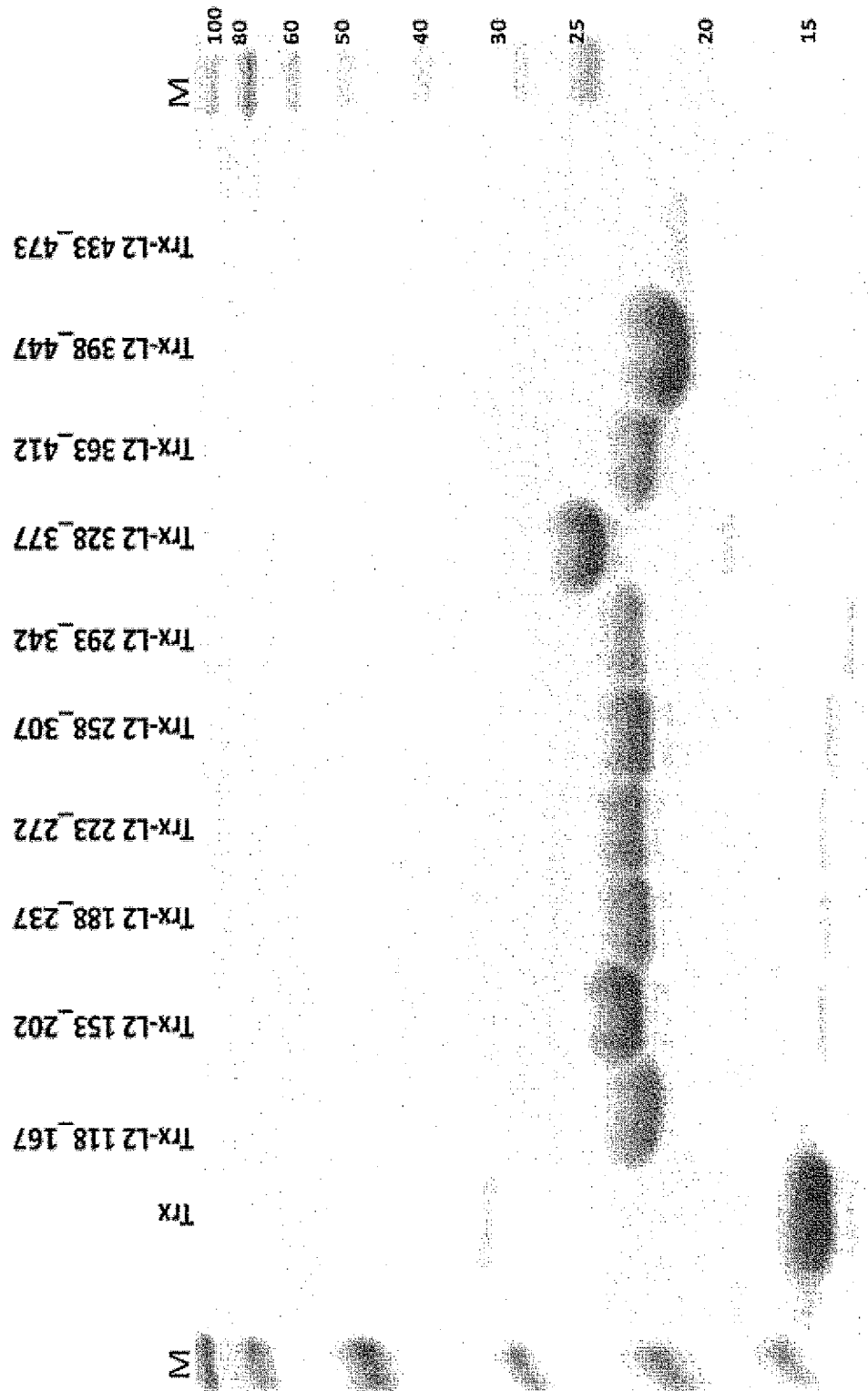


Fig. 3:

Fig. 4:

Immunization scheme (s.c.):

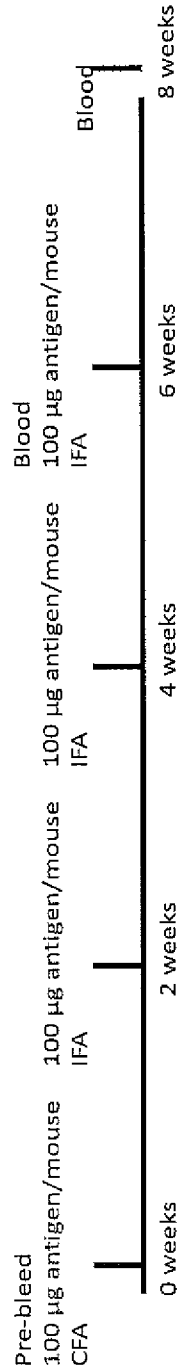


Fig. 5:

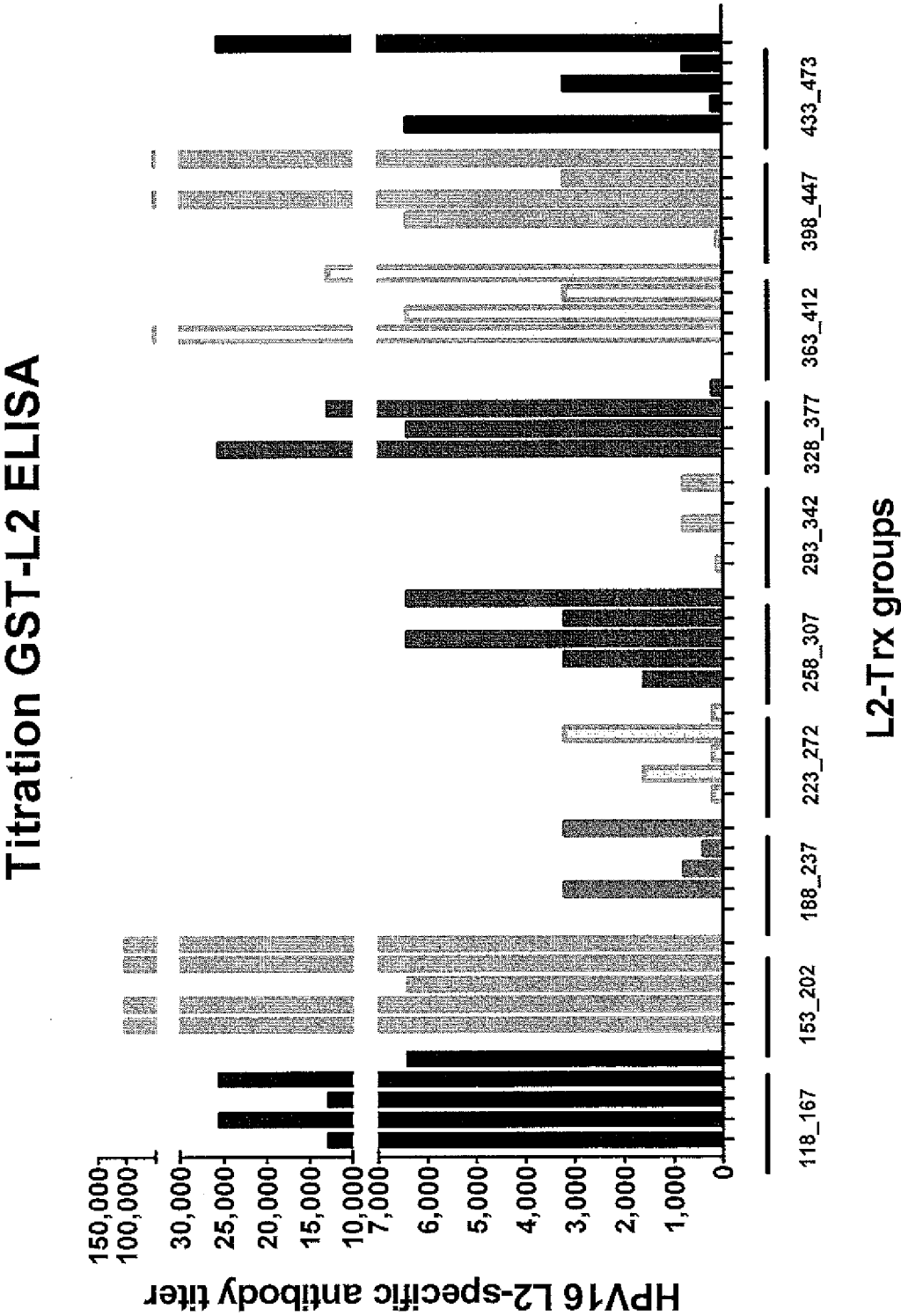
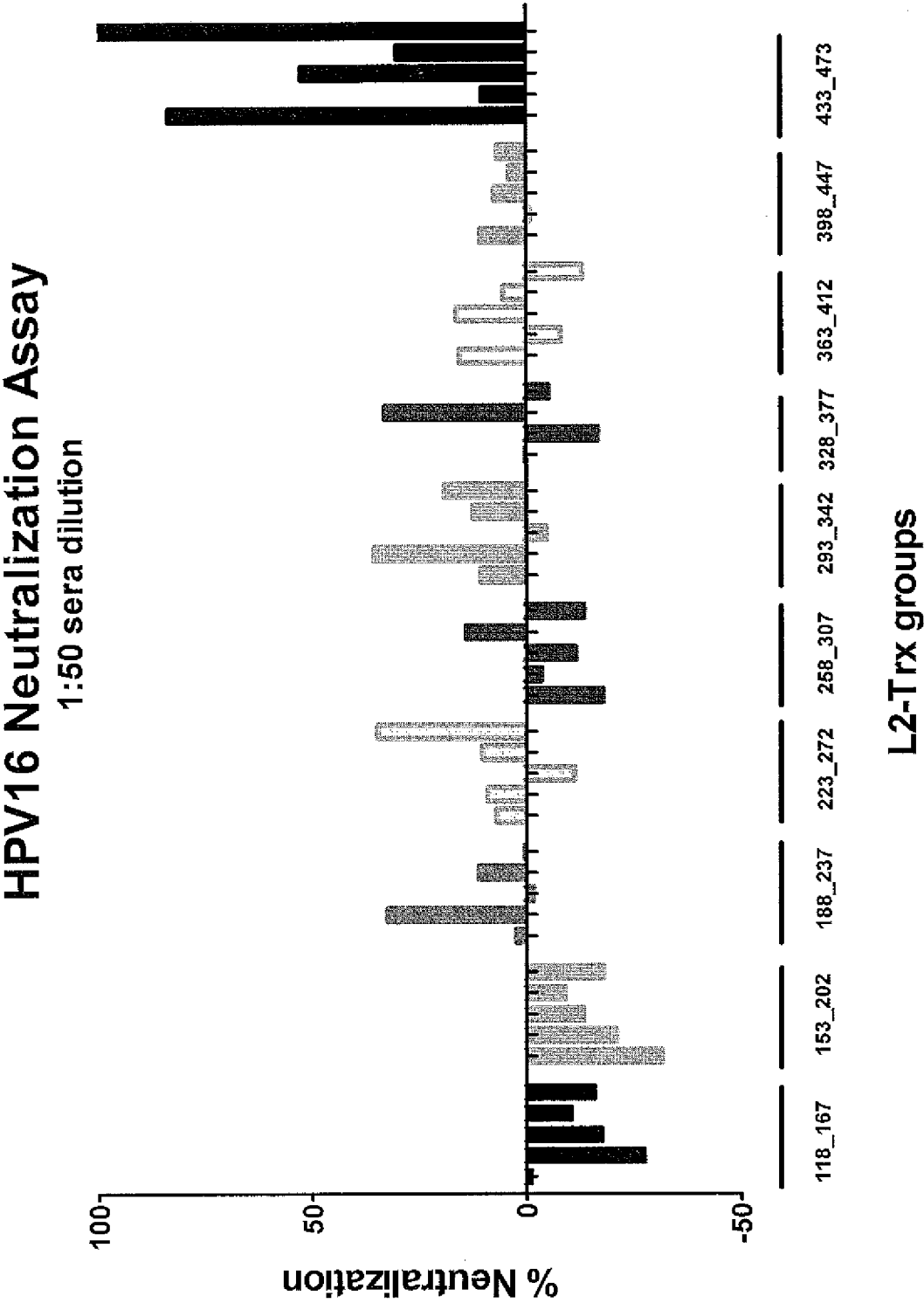


Fig. 6:



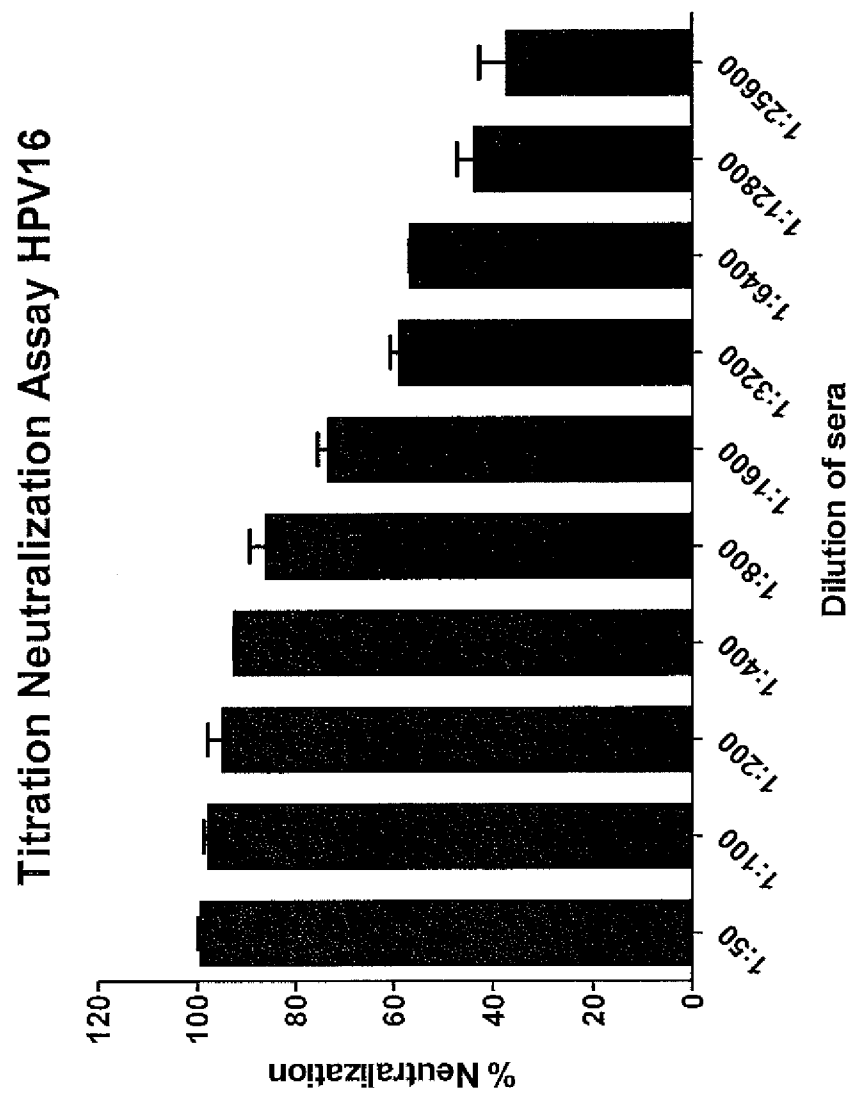
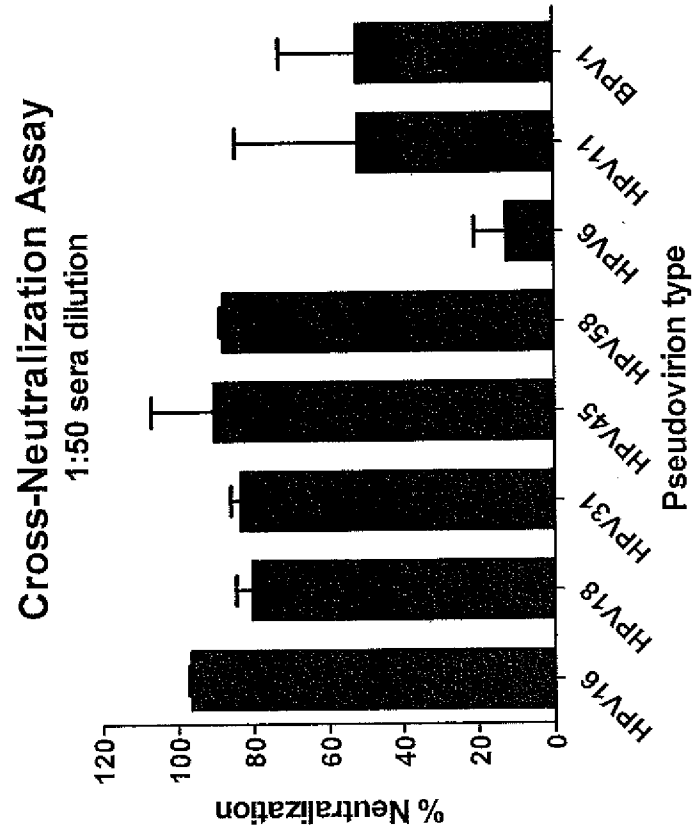


Fig. 7:

Fig. 8



INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2011/058963

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)

☐

 on paper
 - ☒

 in electronic form
 - b. (time)

☒

 in the international application as filed
 - ☐

 together with the international application in electronic form
 - ☐

 subsequently to this Authority for the purpose of search
2.

☐

 In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/058963

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/12 C07K14/01
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KARANAM BALASUBRAMANYAM; JAGU SUBHASHINI; HUH WARNER K; RODEN RICHARD B S: "Developing vaccines against minor capsid antigen L2 to prevent papillomavirus infection", IMMUNOLOGY AND CELL BIOLOGY, vol. 87, no. 4, 2009, pages 287-299, XP9148963, whole document esp. page 289 -----	1-12
Y	WO 2009/059328 A2 (UNIV JOHNS HOPKINS [US]; ALPHS HANNAH H; GAMBHIRA RATISH; RODEN RICHAR) 7 May 2009 (2009-05-07) whole document esp. paragraphs [7-14] and claim 1 ----- -/-	1-12



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

5 July 2011

Date of mailing of the international search report

15/07/2011

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Brück, Marianne

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2011/058963

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 2 184 368 A1 (DKFZ KREBSFORSCHUNGSZENTRUM [DE]) 12 May 2010 (2010-05-12) whole document esp. sequence 1 and paragraph [204] -----	1-12
Y	WO 00/09157 A1 (MERCK & CO INC [US]; LOWE ROBERT S [US]; JANSEN KATHRIN U [US]; JOYCE) 24 February 2000 (2000-02-24) whole document esp. pages 2 and 6 -----	1-12
Y	KONDO ET AL: "Neutralization of HPV16, 18, 31, and 58 pseudovirions with antisera induced by immunizing rabbits with synthetic peptides representing segments of the HPV16 minor capsid protein L2 surface region", VIROLOGY, ACADEMIC PRESS, ORLANDO, US, vol. 358, no. 2, 25 January 2007 (2007-01-25), pages 266-272, XP005853978, ISSN: 0042-6822, DOI: DOI:10.1016/J.VIROL.2006.08.037 whole document esp page 267, and Figure 2 -----	1-12
Y	HITZEROTH I I ET AL: "Immunogenicity of an HPV-16 L2 DNA vaccine", VACCINE, ELSEVIER LTD, GB, vol. 27, no. 46, 30 October 2009 (2009-10-30), pages 6432-6434, XP026704478, ISSN: 0264-410X, DOI: DOI:10.1016/J.VACCINE.2009.06.015 [retrieved on 2009-06-24] whole document esp. pages 6432 and 6434 -----	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2011/058963

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009059328	A2	07-05-2009	NONE
EP 2184368	A1	12-05-2010	WO 2010052317 A1 14-05-2010
WO 0009157	A1	24-02-2000	AT 344052 T 15-11-2006
		AU 753391 B2	17-10-2002
		AU 5469999 A	06-03-2000
		CA 2339324 A1	24-02-2000
		DE 69933875 T2	31-05-2007
		EP 1105157 A1	13-06-2001
		ES 2275350 T3	01-06-2007
		JP 4563582 B2	13-10-2010
		JP 2002522056 A	23-07-2002