(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 January 2012 (19.01.2012)

PCT

(10) International Publication Number
WO 2012/006727 A1

(51) International Patent Classification:
C12N 15/37 (2006.01) *C07K 14/025* (2006.01)
A61K 39/12 (2006.01) *C12Q 1/70* (2006.01)
A61P 31/20 (2006.01) *G01N 33/564* (2006.01)
A61P 37/04 (2006.01) *G01N 33/569* (2006.01)

(21) International Application Number:
PCT/CA201 1/000823

(22) International Filing Date:
15 July 201 1 (15.07.201 1)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/364,750 15 July 2010 (15.07.2010) US

(71) Applicant *(for all designated States except US):* **BRITISH COLUMBIA CANCER AGENCY BRANCH** [CA/CA]; 675 West 10th Avenue, Vancouver, British Columbia V5Z 1L3 (CA).

(72) Inventors; and

(75) Inventors/ Applicants *(for US only):* **WEBB, John, R.** [CA/CA]; 1666 Georgia View Place, North Saanich, British Columbia V8L 4R6 (CA). **WICK, Darin, Arne** [CA/CA]; 1161 Bewdley Ave, Victoria, British Columbia V9A 5N2 (CA).

(74) Agent: **CHATTERJEE, Alakananda;** Borden Ladner Gervais LLP, 1200 Waterfront Centre, 200 Burrard Street, P.O. Box 48600, Vancouver, British Columbia V7X 1T2 (CA).

(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).

[Continued on next page]

(54) Title: HUMAN PAPILLOMAVIRUS E7 ANTIGEN COMPOSITIONS AND USES THEREOF

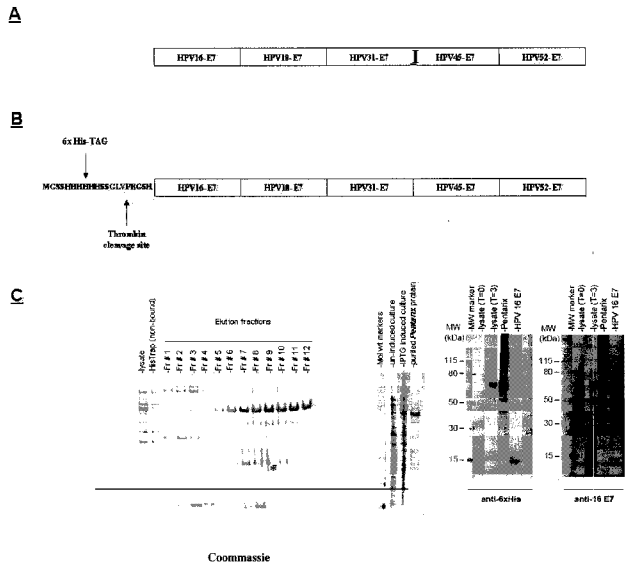


FIGURE 1

(57) Abstract: The present invention relates to human papillomavirus E7 antigen compounds and compositions for treating human papillomavirus infection and associated conditions. The invention provides, in part, polypeptide and nucleic acid molecules including sequences substantially identical to the sequences of two or more human papillomavirus (HPV) E7 antigens, where the E7 antigens are selected from at least two different HPV strains, and methods of using the same.

Published:

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

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

HUMAN PAPILLOMAVIRUS E7 ANTIGEN COMPOSITIONS AND USES THEREOF

FIELD OF INVENTION

[0001] The present invention relates to compounds and compositions for treating human papillomavirus infection and associated conditions. More specifically, the present invention relates to human papillomavirus E7 antigen compounds and compositions for treating human papillomavirus infection and associated conditions.

BACKGROUND OF THE INVENTION

[0002] Human papillomaviruses (HPV) are a group of more than 100 related, but genetically distinct, 'types' of virus, which can be broadly classified as low-risk and high-risk. Low risk HPV types are associated with common warts (or papillomas) that are generally benign and non-life threatening. In contrast, persistent infection with high-risk types of HPV is associated with pre-cancerous cervical dysplasia and cervical cancer (1,2). High risk HPV types are also associated with cancers of the anus, vulva, vagina, and penis (3) as well as certain subsets of head and neck cancers (4-6) and breast cancer (57).

[0003] High risk HPV types that cause cervical cancer are sexually transmitted and are highly prevalent in the normal, healthy population. It is estimated that a majority of people become infected shortly after becoming sexually active (7, 59). The vast majority of high risk HPV infections are thought to be self-limiting, with minimal associated pathology, however infection with high risk HPV may develop into malignancy (8).

[0004] While HPVl 6 and HPVl 8 are the most prevalent of the high risk HPV types, there are many additional high risk types of HPV. A recent comparative analysis of 1,918 cervical cancer patients and 1,928 healthy control women revealed that the most common HPV types in cancer patients (in descending order of frequency) were HPV types 16, 18, 45, 31, 33, 52, 58 and 35 (11). Likewise, a global analysis of the prevalence of HPV in cervical cancer revealed that HPVl 6 was present in 50% of cases, HPV 18 in 14%, HPV45 in 8%, and HPV31 in 5%, with other members of the thirteen "high risk" types making up the remaining cases (12).

[0005] Due to their prevalence, HPV16 and HPVl 8 have been the focus of a broad prophylactic immunization campaign aimed at preventing initial infection by HPV (9, 10) and currently approved prophylactic vaccines target the high risk strains HPVl 6 and HPVl 8 (as well as the

low risk strains HPV6 and HPVl 1) in anticipation that prophylactic vaccination will diminish or prevent the occurrence of HPV-associated malignancy later in life. It has also been suggested that HPVl 6 and HPVl 8 prophylactic vaccines may confer partial cross-protection against other high risk types of HPV (13-15).

[0006] Prophylactic HPV vaccines currently in clinical use are comprised of recombinant viral capsid glycoprotein (LI) that spontaneously forms synthetic virus-like particles (VLP) (60, 61). Immunization with VLP-based, prophylactic vaccines elicits a strong, neutralizing antibody response against the LI protein, which prevents viral infection from becoming established.

[0007] Prophylactic vaccines however appear to have minimal impact on established infection (16). For example, prophylactic cervical cancer vaccines are ineffective for those individuals who have already been exposed to HPV, since once the virus gains entry into the cell, it is protected from the neutralizing effects of extracellular antibody, allowing viral replication (and latent infection) to proceed unimpeded. Infection with high risk HPV may result in integration of the viral episome into host DNA, often resulting in deletion of several early *{E2, E4* and *E5)* and late *{LI* and *LI)* genes, leaving the HPV proteins, E6 and E7, as the only viral proteins that continue to be expressed in the infected cell (23, 24, 59). In this situation, vaccine-induced immunity against the LI capsid protein is ineffective for therapy.

[0008] In addition, because of the high prevalence of HPV infection in today's adult population combined with the slow progress of cervical carcinogenesis, it is anticipated that it will take 20 years, or more, until mass implementation of a prophylactic vaccine will have an impact on the incidence of cervical cancer. Also, prophylactic vaccines have met with resistance in some instances and the rate of vaccination is variable (17). In some areas, vaccine cost remains an issue (18).

[0009] Therapeutic HPV vaccines, designed to eradicate pre-existing lesions by generating cellular immunity against HPV-infected cells that express viral proteins, have been explored as an alternative for treatment of HPV-associated cancer (for review see 19, 20, 62-65) and many of these approaches have been aimed at the development of vaccines that elicit a robust CD8 T cell response since many vaccines currently approved are generally poor at eliciting CD8 immunity (66, 67). HPV E7 therapeutic vaccination approaches have included peptide immunization (26, 28-30), DNA immunization (31-33, 68), immunization with recombinant, E7-expressing Vaccinia virus (25, 34), adenovirus (35-37), *Salmonella typhimurium* (38, 39) or *Listeria monocytogenes* (40, 41), E7-pulsed dendritic cells (42-45) or E7-containing virus-like particles

(VLP) (46-49) and a number of these therapeutic vaccine strategies have advanced to early clinical trials.

[0010] Many of these therapeutic vaccine strategies are often logistically cumbersome and the responses elicited rarely reach the level of CD8 T cell expansion that is seen during the acute phase of an authentic anti-viral immune response (69). Strategies for enhancing CD8 immunity via booster vaccination, such as various types of heterologous prime-boost regimens, including DNA-peptide, DNA-virus or two distinct viral vehicles for prime-boost, have been used for eliciting CD8 immunity (70-72). However, these methods can be difficult to translate to the clinic and there is poor consensus regarding which methodologies are optimal.

[001 1] HPV vaccines have been described in a number of publications including PCT publications WO2005/089164 (published September 29, 2005), WO2007/121894 (published November 1, 2007), WO2007/121895 (published November 1, 2007), WO2008/049329 (published May 2, 2008), and WO2008/ 145745 (published December 4, 2008).

[0012] The TC-1 model tumor system, originally derived from mouse primary lung epithelial cells that were transformed with HPV1 6 E6 and E7 oncogenes, which are required for transformation and immortalization of infected cells and maintenance of the cells in a transformed state (21, 22), along with activated human c-Ha-ras (25), has become widely adopted as a test system for HPV therapeutic vaccines. Implantation of TC-1 tumor cells into immunocompetent C57B1/6 mice results in the formation of rapidly progressing tumors at the site of inoculation. However, specific cellular immunity against the HPV1 6 E7 protein can confer protection against TC- 1 tumor outgrowth. For example, CD8+ T cells specific for the H-2Db-restricted epitope (E749-57; RAHYNIVTF) of E7 have been reported to be capable of lysing E7-expressing tumor cells and causing regression of established TC-1 tumors (26, 27).

[0013] Immunization with whole exogenous protein has been suggested to be an inefficient means of eliciting MHC class I-restricted CD8+ T cell responses (50). However, immunization with recombinant (51) or synthetic (52) full length E7 protein has been reported to elicit CD8+ T cell immunity when delivered in combination with either QuilA or CpG-containing oligonucleotides, respectively. Likewise, recombinant proteins comprised of fusions between immunogenic heat shock proteins (HSP) and selected target antigens are also reported to elicit CD8+ immunity against the target antigen. It has been suggested that immunization with whole exogenous protein plus TLR3 or TLR9 agonists facilitates the process of cross-priming and promotes the development of antigen-specific CD8+ T cell responses (55, 56).

[0014] Currently, cervical dysplasia and early stage cervical cancer are most commonly treated using a surgical procedure known as LEEP (Loop electrosurgical excision procedure) in which abnormal tissue is removed using a thin wire loop charged with an electrocurrent. More advanced stages of cervical cancer are treated by surgery (partial or radical hysterectomy) combined with chemotherapy and or radiation therapy.

SUMMARY OF THE INVENTION

[0015] The invention provides, in part, human papillomavirus E7 antigen compounds and compositions. The compounds and compositions may be useful for treating or diagnosing human papillomavirus infection and associated conditions.

[0016] In one aspect, the invention provides a polypeptide including an amino acid sequence substantially identical to the amino acid sequence of two or more human papillomavirus (HPV) E7 antigens, where the E7 antigens are selected from at least two different HPV strains.

[0017] In alternative embodiments, the different HPV strains may be high risk strains, such as HPV 16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, or HPV82. In alternative embodiments, the E7 antigens may be selected from five different HPV strains, such as HPV 16, HPV 18, HPV31, HPV45, and HPV52.

[0018] In alternative embodiments, the polypeptide may include two or more of the amino acid sequences set forth in SEQ ID NOs: 1 to 15, or the amino acid sequences set forth in SEQ ID NOs: 1 to 5, such as the amino acid sequence set forth in SEQ ID NO: 16 or 17.

[0019] In alternative embodiments, the polypeptide may be encoded by a nucleotide sequence comprising two or more of the nucleotide sequences set forth in SEQ ID NOs: 18 to 32.

[0020] In alternative embodiments, the polypeptide may be encoded by a nucleotide sequence comprising two or more of the nucleotide sequences set forth in SEQ ID NOs: 18 to 22, such as SEQ ID NOs: 33 or 34.

[0021] In alternative embodiments, the E7 antigens may be capable of inducing an immune response to the two different HPV strains.

[0022] In other aspects, the invention provides a nucleic acid molecule including a sequence substantially identical to the nucleotide sequences of two or more human papillomavirus (HPV) E7 antigens, where the E7 antigens are selected from at least two different HPV strains.

[0023] In alternative embodiments, the different HPV strains maybe high risk strains, such as HPV 16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, or HPV82.

[0024] In alternative embodiments, the E7 antigens may be selected from five different HPV strains, such as HPV1 6, HPV1 8, HPV3 1, HPV45, and HPV52.

[0025] In alternative embodiments, the nucleic acid molecule may include two or more of the nucleic acid sequences set forth in SEQ ID NOs: 18 to 32.

[0026] In alternative embodiments, the nucleic acid molecule may include the nucleic acid sequences set forth in SEQ ID NOs: 18 to 22, such as SEQ ID NOs: 33 or 34.

[0027] In alternative aspects, the invention provides a nucleic acid molecule encoding a HPV E7 polypeptide.

[0028] In alternative aspects, the invention provides an expression vector including a nucleic acid sequence as described herein operably linked to a sequence that allows for expression of the nucleic acid sequence in a host cell.

[0029] In alternative aspects, the invention provides a host cell comprising a nucleic acid molecule or expression vector as described herein.

[0030] In alternative aspects, the invention provides a composition including a polypeptide, nucleic acid molecule, expression vector or host cell as described herein. The composition may include a carrier and/or an adjuvant. The adjuvant may be a Toll-like receptor (TLR) agonist such as a TLR3 agonist (e.g., poly(I.C)) or a TLR9 agonist (e.g., a CpG containing oligonucleotide). Alternatively or additionally, the adjuvant may be an interferon-alpha, an agonist of the 4-IBB receptor, an agonist of the CD40 receptor, or an anti-CD40 antibody.

[003 1] In alternative aspects, the invention provides a method of stimulating an immune response in a subject in need thereof by administering a polypeptide, nucleic acid molecule, expression vector or host cell as described herein, to the subject. In alternative aspects, the invention provides a method of treating or preventing a condition associated with HPV infection in a subject in need thereof, by administering a polypeptide, nucleic acid molecule, expression vector or host cell as described herein, to the subject. In alternative aspects, the invention provides a method of treating a HPV infection in a subject in need thereof, by administering a

polypeptide, nucleic acid molecule, expression vector or host cell as described herein, to the subject.

[0032] In alternative aspects, the invention provides a use of a polypeptide, nucleic acid molecule, expression vector or host cell as described herein, for stimulating an immune response in a subject in need thereof. In alternative aspects, the invention provides a use of a polypeptide, nucleic acid molecule, expression vector or host cell as described herein, for treating or preventing a condition associated with HPV infection in a subject in need thereof. In alternative aspects, the invention provides a use of a polypeptide, nucleic acid molecule, expression vector or host cell as described herein, for treating a HPV infection in a subject in need thereof.

[0033] The condition associated with HPV infection may be one or more of a cancer of the breast, cervix, anus, vulva, vagina, penis, head and neck, and lung, or pre-malignant lesion thereof, or may be a pre-cancerous cervical epithelial neoplasia (CIN I through CIN III) or a cervical cancer.

[0034] In alternative embodiments, the HPV infection may be by a high risk HPV type, such as HPV 16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, or HPV82. In alternative embodiments, the methods or uses may further include administering an adjuvant such as a Toll-like receptor (TLR) agonist (e.g., TLR3 agonist like poly(I:C) or a TLR9 agonist like a CpG containing oligonucleotide). Alternatively or additionally, the adjuvant may include an interferon-alpha, an agonist of the 4-IBB receptor, an agonist of the CD40 receptor, or an anti-CD40 antibody. The administering may include administration of multiple doses over a time frame of less than 14 days, or may include administration of multiple doses over one to four days, and/or may include administration of multiple daily doses.

[0035] In alternative aspects, the invention provides a peptide consisting essentially of one or more of the sequences TSNYNIVTF (SEQ ID NO: 35), AEPDTSNYNIVTFCC (SEQ ID NO: 36) or TSNYNIVTFCCQCKS (SEQ ID NO: 37). In alternative aspects, the invention provides a method of diagnosing a HPV3 1 infection comprising contacting a sample with a peptide consisting essentially of one or more of the sequences TSNYNIVTF (SEQ ID NO: 35), AEPDTSNYNIVTFCC (SEQ ID NO: 36) or TSNYNIVTFCCQCKS (SEQ ID NO: 37). In alternative aspects, the invention provides a method of determining the response of a subject to a HPV3 1 infection contacting a sample with a peptide consisting essentially of one or more of the

sequences TSNYNIVTF (SEQ ID NO: 35), AEPDTSNYNIVTFCC (SEQ ID NO: 36) or TSNYNIVTFCCQCKS (SEQ ID NO: 37).

[0036] This summary of the invention does not necessarily describe all features of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

[0038] **FIGURES 1A-B** are schematic diagrams showing the structural organization of the *Pentarix* protein **(A)** without an affinity tag or **(B)** with a cleavable 6x affinity tag and a thrombin cleavage site.

[0039] **FIGURE 1C** shows the preparation and purification of *Pentarix* protein with a cleavable 6x affinity tag. The Figure shows expression of recombinant Pentarix protein in *E coli* and an example of a typical purification using nickel affinity purification. Total protein contained within a lysate of IPTG-induced *E. coli* before and after passage over a HisTrap column (GE Healthcare) and protein eluted from the column (Fr#l-12) in the presence of increasing concentrations of imidazole were detected by Coommassie Blue staining (left panel). Identity of purified Pentarix protein was confirmed by Western blot analysis of lysates from un-induced and induced cultures as well as purified Pentarix and HPV16 E7 proteins using anti-6xHis tag antibody or anti-HPV 16E7 antibody.

[0040] **FIGURE 2A** is a graph showing **$OVA_{257-264}$**-specific CD8+ T cell responses elicited in response to immunization with whole exogenous OVA protein plus the TLR3 agonists poly(I:C) or polylC/LC. Naive C57B1/6 mice (2 mice per condition) were immunized with whole OVA protein (500 μg) plus or minus poly(I:C) (10 μg) or polylC/LC (10 μg/ml). Seven days post immunization mice were euthanized and the number of **$OVA_{257-264}$** (SIINFEKL)-specific CD8+ T cells in bulk splenocytes of immunized mice were quantitated by IFN-γ ELISPOT. Results are reported as the number of IFN-γ spot-forming cells per $1x10^6$ splenocytes after stimulation with media only, SIINFEKL peptide (10 μg/ml)(SEQ ID NO: 39) or irrelevant H2Db-binding peptide (10 μg/ml).

[0041] **FIGURE 2B** is a graph showing that **$OVA_{257-264}$**-specific CD8 T cell responses are elicited in dose-dependent manner after immunization with whole exogenous OVA protein plus the TLR3 agonist poly(I:C).

[0042] **FIGURES 3A-E** show **$OVA_{257-264}$** -specific $CD8_+$T cell or HPV16 E7 responses elicited in response to long or short interval (cluster) homologous prime-boost immunization. **A,** Naive C57B1/6 mice (2 mice per condition) were immunized with whole OVA protein (100 μ¾) plus poly(I:C) (10 μg) at day -7, day -21 or day -7 and day -21 and were euthanized at day 0. The number of **$OVA_{257-264}$** -specific CD8+ T cells in bulk splenocytes of immunized mice were quantitated by IFN-γ ELISPOT. **B,** Naive C57B1/6 mice (3 mice per condition) were immunized with the indicated number of sequential daily doses of whole, soluble OVA protein (100 μg) admixed with poly(I:C) (10 μg). One additional group of mice received a single immunization that was equivalent to four times the normal daily dose (i.e. 400 μg of OVA protein plus 40 μg of poly(I:C)). Seven days after the first immunization mice were euthanized and bulk splenocyte preparations were assessed by IFN-γ ELISPOT to quantitate the number of **$OVA_{257-264}$** -specific CD8+ T cells. Results in **A** and **B** are reported as the number of IFN-γ spot-forming cells per $1x10^6$ splenocytes after stimulation with media only or SIINFEKL peptide (10 μg/ml). **C,** Naive C57B1/6 mice were immunized with one dose or four consecutive daily doses of whole, soluble OVA protein (100 μg) plus poly(I:C) (10 μg) as indicated. **D,** Mice in panel c that received four consecutive daily doses of OVA protein plus poly(I:C) were reimmunized with another four consecutive daily doses of the same, starting at day 47 after imitation of the first round of immunization. Peripheral blood was obtained from the saphenous vein of individual mice that were serially bled on the indicated days post-immunization. RBC in peripheral blood were lysed and lymphocytes were stained with FITC-conjugated anti-CD8 and PE-conjugated H-2Kb/ **$OVA_{257-264}$** tetramer and analyzed by flow cytometry. Events shown in **C** and **D** are gated on CD8+ lymphocytes and are from a representative single animal to allow precise monitoring of the evolution of the antigen-specific T cell responses within a given animal over time. **E,** Naive C57B1/6 mice were immunized with one dose (left panel), four consecutive daily doses of whole, soluble HPV16 E7 protein (100 ug) plus poly(I:C) (10 ug), or four consecutive daily doses of whole, soluble HPV16 E7 protein (100 ug) only. Seven days post-immunization, peripheral blood was obtained from immunized mice. Lymphocytes were stained with FITC-conjugated anti-CD8 and PE-conjugated $D^b/E7_{49-57}$ tetramer and analyzed by flow cytometry. Events shown are gated on $CD8^+$ lymphocytes.

[0043] **FIGURE 4** is a series of graphs showing sequential daily immunization with whole, soluble protein plus the TLR3 agonist poly(I:C) induces regression of large, established tumors. C57B1/6 mice (3 mice per cohort) were implanted with OVA-expressing EG7 tumors cells ($1x10^5$) on day 0 and were left untreated (upper left), or were treated with 1 dose of poly(I:C) (10

μg) (upper right), 1 dose of whole, soluble OVA protein (100 μg) plus poly(I:C) (10 μg) (lower left) or four sequential daily doses of whole, soluble OVA protein (100 μg) plus poly(I:C) (10 μg)) (lower right). Time of treatment for each group is indicated by the arrowhead(s). Average tumor volume at time of treatment for each group was 224mm^3 (poly(I:C) only), 194mm^3 (1 dose of OVA + poly(I:C)) or 344mm^3 (4 doses of OVA + poly(I:C)). Mice in the last cohort were intentionally treated at a time when tumor size was larger in order to exemplify the beneficial effects of sequential daily immunization.

[0044] **FIGURES 5A and 5B** are a graphs showing HPV16 $E7_{49-57}$-specific CD8+ T cell responses elicited in response to a single immunization with whole *Pentarix* protein plus the TLR3 agonist poly(I:C). Naive C57B1/6 mice were left untreated or were immunized with 100 μg of whole, soluble *Pentarix* protein admixed with 10 μg of poly(I:C), or were treated with 10 μg of poly(I:C) only **(5B).** Seven days post-immunization mice were euthanized and bulk splenocyte preparations were assessed by IFN-γ ELISPOT to quantitate the number of HPV16 **$E7_{49-57}$**-specific CD8+ T cells. Briefly, splenocytes ($3x10^5$ per well, triplicate wells per condition) from individual animals were stimulated overnight with either media alone or with HPV16 **$E7_{49-57}$** peptide (10 ug/ml) or irrelevant control peptide (KAVYNFATM; SEQ ID NO: 40). Results from naïve (unimmunized) mice are included for comparison. Results are reported as the number of IFN-γ spot-forming cells per $lxlO^6$ splenocytes after stimulation with media only or HPV16 **$E7_{49-57}$**, or irrelevant peptide (10 μg/ml) **(5B).**

[0045] **FIGURES 6A and 6B** are graphs showing HPV16 $E7_{49-57}$-specific CD8+.T cell responses elicited in response to a single immunization with whole *Pentarix* protein plus the TLR9 agonist CpG oligonucleotide. Naive C57B1/6 mice were left untreated or were immunized with 100 μg of whole, soluble *Pentarix* protein admixed with 10 μg of CpG oligo #2395 (Invivogen) or with CpG oligo only. Seven days post-immunization mice were euthanized and bulk splenocyte preparations were assessed by IFN-γ ELISPOT to quantitate the number of HPV16 $E749-57$-specific CD8+ T cells. Briefly, splenocytes ($3xl0^5$ per well, triplicate wells per condition) from individual animals were stimulated overnight with either media alone or with HPV16 **$E7_{49-57}$** peptide (10 ug/ml) or irrelevant control peptide (KAVYNFATM; SEQ ID NO: 40). Results from naive (unimmunized) mice are included for comparison. Results are reported as the number of IFN-γ spot-forming cells per $lxl\ 0^6$ splenocytes after stimulation with media only, HPV16 $E7_{49-57}$ or irrelevant peptide (10 μg/ml). The data presented in **6B** are representative of three experiments; results are reported as the number of IFN-γ spot-forming cells per $lxlO^6$ splenocytes +/- SD for each triplicate.

[0046] **FIGURES 7A-B** are graphs showing HPV16 $E7_{49-57}$-specific CD8+$_+$T cell responses elicited in response to a single immunization with whole *Pentarix* protein plus the TLR3 agonist poly(I:C) or with 4 successive daily doses of *Pentarix* protein plus poly(I:C). Naive C57B1/6 mice (3 per cohort for Fig. **7B)** were left untreated (naive) or were immunized one time or 4 times (daily on days 1-4) with 100 μg of whole, soluble *Pentarix* protein admixed with 10 μg of poly(I:C). Seven days **(7**A) or eight days **(7B)** post-immunization mice were euthanized and bulk splenocyte preparations were assessed by IFN-γ ELISPOT to quantitate the number of HPV16 $E7_{49-57}$-specific CD8+ T cells. Results are reported as the number of IFN-γ spot-forming cells per $1x10^6$ splenocytes after stimulation with media only or HPV16 $E7_{49-57}$, or irrelevant peptide (10 μg/ml) **(7B).** Splenocytes ($3x10^5$ per well) from individual animals were stimulated overnight with either media alone or with HPV16 $E7_{49-57}$ peptide (10 ug/ml) or irrelevant control peptide (KAVYNFATM; SEQ ID NO: 40). The data presented in **7B** are representative of three experiments; results are reported as the number of IFN-γ spot-forming cells per $lxlO^6$ splenocytes +/- SD for each triplicate.

[0047] **FIGURE 7C** shows the results from a study where lymphocytes in spleen and peripheral blood of a mouse that was immunized for 4 successive days with 100 ug Pentarix protein plus 10 ug poly(I:C) (left two panels) or 100 ug Pentarix protein only (right panel) were stained with FITC-conjugated anti-CD8 and PE-conjugated D^b/16 $E7_{49-57}$ tetramer and analyzed by flow cytometry. Events shown are gated on CD8 lymphocytes and are representative of 4 such animals.

[0048] **FIGURES 8A and 8B** are a series of graphs showing immunization with whole, soluble *Pentarix* protein plus the TLR3 agonist poly(I:C) induces regression of large, established TCI tumors. A, C57B1/6 mice (3 mice per cohort) were implanted with E7-expressing TCI tumors cells ($lxl\ 0^5$) on day -14 and on day 0 (when tumors reached approximately 200 mm^5 in size) were left untreated (left), or were treated with 1 dose of poly(I:C) (10 μg) (middle) or 1 dose of whole, soluble *Pentarix* protein (100 μg) plus poly(I:C) (10 μg) (right). Tumors were measured every 2-3 days using an electronic digital caliper and size was calculated using the formula $width^2$ x length x 0.5. **B,** Naive C57B1/6 mice (8 per cohort) were implanted subcutaneously with $lxlO^5$ E7-expressing TC-1 tumors cells. Once tumors reached an average volume of 350 mm^3 mice were treated with either a single dose of Pentarix (100 ug) plus poly(I:C) (10 ug), 4 successive daily doses of Pentarix (100 ug) plus poly(I:C) (10 ug), 4 successive daily doses of poly(I:C) only (10 ug per dose) or were left untreated. Tumors were measured every 2 to 4 days with electronic calipers and tumor-bearing mice were euthanized when the tumor volume

exceeded approximately 2,000 mm^3 or when mice became moribund or lost >20% body weight. Data are presented as average tumor volume for all mice within a cohort (left panel) or survival (right panel).

[0049] **FIGURE 8C** is a series of graphs showing immunization of TCl-tumor bearing mice with *Pentarix* protein plus poly(I:C) elicits complete tumor regression and the establishment of E7-specific CD8+ memory cells that persist after tumor progression. Naive C57B1/6 mice were implanted subcutaneously with $1x10^5$ E7-expressing TC-1 tumor cells. Once tumors reached an average volume of 200 mm^3 mice were treated with a single dose of *Pentarix* (100 μg) plus poly(I:C) (10 μg) and tumors fully regressed within 15 days of immunization. A sample of peripheral blood was taken from the saphenous vein 21 days post-immunization and was stained with FITC-conjugated anti-CD8 and PE-conjugated $D^b/16\ E7_{49-57}$ tetramer as well as the memory phenotype markers CD62L, CD127 and KLRG1 and analyzed by flow cytometry. Events shown are gated on CD8+ lymphocytes.

[0050] **FIGURES** 9A **- B** are a series of graphs showing immunization with whole, soluble *Pentarix* protein combined with poly(I:C) or CpG oligonucleotide induces regression of established TCI tumors. A, C57B1/6 mice (4 mice per cohort) were implanted with E7-expressing TCI tumors cells ($1x10^5$) on day -21 and on day 0 were treated with 1 dose of whole, soluble *Pentarix* protein (100 μg) plus CpG oligo #2395 (10 μg) (upper left) or 1 dose of CpG oligo #2395 only (10 μg) (upper right) or were left untreated (lower left). Tumors were measured every 2-3 days using an electronic digital caliper and size was calculated using the formula $width^2$ x length x 0.5. The upper left, upper right and lower left 3 plots show regression of tumors in individual mice, whereas the lower right plot shows combined average tumor volume measurement for each cohort. **B,** Naive C57B1/6 mice (indicated number of mice per cohort) were implanted subcutaneously with $1x10^5$ E7-expressing TC-1 tumor cells. Once tumors reached an average volume of 200 mm^3 mice were treated (as indicated) with either a single dose of Pentarix (100 ug) plus poly(I:C) (10 ug), a single dose of Pentarix (100 ug) plus CpG oligonucleotide (10 ug) or poly(I:C) (10 ug), CpG oligonucleotide (10 ug) or Pentarix protein (100 ug) alone or were left untreated. Tumors were measured every 2 to 4 days with electronic calipers, and data are presented as tumor volume over time for individual animals within each cohort (upper 6 panels) or as survival for all mice within a cohort (lower 2 panels). Tumor-bearing mice were euthanized when the tumor volume exceeded approximately 2,000 mm^3 or when mice became moribund or lost >20% body weight). p values were calculated using the log rank (Mantel-Cox) test.

[0051] **FIGURE 10A** is a graph showing epitope-specific CD8+ T cell responses elicited in response to a single immunization with whole *Pentarix* protein plus the TLR3 agonist poly(I:C). Naive C57B1/6 mice were immunized with 100 μg of whole, soluble *Pentarix* protein admixed with 10 μg of poly(l:C) (Amersham). Seven days post-immunization mice were euthanized and bulk splenocyte preparations were assessed by IFN-γ ELISPOT to quantitate the number of CD8+ T cells specific for each of the peptides indicated. Results are reported as the number of IFN-γ spot-forming cells per $1x10^6$ splenocytes after stimulation with media only or the indicated peptide (10 μg/ml).

[0052] **FIGURES 10B-D** are graphs showing immunization with whole, soluble Pentarix protein plus poly(I:C) elicits immune responses against multiple genotypes of HPV. C57B1/6 **(B),** or HLA-A2/D^b transgenic mice **(C)** were immunized (s.c) daily for 4 successive days with 100 ug Pentarix protein combined with 10 ug poly(I:C). Eight days post-immunization mice were euthanized and bulk **(B** and **C)** or CD4-depleted splenocyte preparations **(B** only) were analyzed by IFN-γ ELISPOT (CD4 depletion was >99% as measured by FACS analysis post-depletion). Bulk and CD4-depleted splenocyte preparations were stimulated overnight with a panel of overlapping 15mer peptides (overlapping by 11 amino acids) that spanned the entire Pentarix protein. **D,** Splenocytes from C57B1/6 mice immunized (s.c) with Pentarix protein combined with 10 ug poly(I:C) were stimulated overnight with either media alone or with the minimal peptide epitopes HPV16 $E7_{49-57}$,HPV31 $E7_{49-57}$ or irrelevant control peptide (KAVYNFATM) and analyzed by IFN-γ ELISPOT. Results are reported as the number of IFN-γ spot-forming cells per $1x10^6$ splenocytes and are representative of 3 such experiments.

[0053] **FIGURE 11** is a graph showing HPV 16 $E7_{49-57}$-specific $CD8+^+$ T cell responses elicited in response to a single immunization with whole *Pentarix* protein only (no adjuvant) or with 4 successive daily doses of *Pentarix* protein only (no adjuvant). Naive C57B1/6 mice were immunized one time or 4 times (daily on days 1-4) with 100 μg of whole, soluble *Pentarix* protein in PBS. Seven days post-immunization mice were euthanized and bulk splenocyte preparations were assessed by IFN-γ ELISPOT to quantitate the number of HPV16 $E7_{49-57}$-specific CD8+ T cells. Results are reported as the number of IFN-γ spot-forming cells per $1x10^6$ splenocytes after stimulation with media only, HPV16 $E7_{49-57}$ or irrelevant negative control peptide (each at 10 μg/ml).

[0054] **FIGURES 12 A-O** show the amino acid sequences (SEQ ID NOs: 1-15) and the nucleotide sequences (SEQ ID NOs: 18-32) of E7 proteins from HPV 16, HPV 18, HPV31,

HPV45, HPV52, HPV33, HPV35, HPV39, HPV51, HPV56, HPV58, HPV59, HPV68, HPV73, and HPV82, respectively.

[0055] **FIGURES 12P-Q** show the amino acid sequences of the *Pentarix* protein with (SEQ ID NO: 16) and without (SEQ ID NO: 17) an amino-terminal 6 x His affinity tag.

[0056] **FIGURES 12R-S** show the nucleotide sequences of the *Pentarix* protein with (SEQ ID NO: 34) and without (SEQ ID NO: 33) an amino-terminal 6 x His affinity tag.

DETAILED DESCRIPTION

[0057] The invention provides, in part, human papillomavirus E7 antigen compounds and compositions. The compounds and compositions may be useful for treating or diagnosing human papillomavirus infection and associated conditions.

[0058] In some embodiments, compounds and compositions according to the invention are useful for targeting multiple HPV types, for example, at least two or more HPV genotypes, such as high risk HPV types. Accordingly, compounds and compositions according to the invention may be useful in inducing an immune response to one or more of the HPV types from which the HPV E7 antigens, or sequences substantially identical to the HPV E7 antigens that comprise the compounds or compositions, are derived. Such compounds and compositions with broad population coverage may be commercially useful as they are applicable to a larger group of people.

[0059] **Human Papillomavirus (HPV)**

[0060] By "human papillomavirus" or "HPV" is meant a virus belonging to a group of more than 100 related but genetically distinct "types" which can be broadly classified as "low risk" and "high risk."

[0061] "Low risk" HPV types include, without limitation, HPV types HPV1 1, HPV40, HPV42, HPV43, HPV44, HPV54, HPV61, HPV70, HPV72, and HPV81.

[0062] "High risk" HPV types include, without limitation, HPV 16, HPV 18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, and HPV82.

[0063] The HPV genome is generally a double-stranded circular DNA of about 7000-8000 base pairs surrounded by a protein capsid. The genome has an early (E) region encoding the early antigens E1-E7 and a late (L) region encoding the structural LI and L2 capsid proteins. The E6 and E7 proteins are required for transformation and immortalization of infected cells and continuous expression of these proteins is required to maintain cells in a transformed state. On occasion, HPV DNA becomes integrated into the DNA of the host cell and this process is associated with the loss of several viral genes. For example, integration commonly leads to deletion of several early ***(E2, E4*** and ***E5)*** and late ***(LI*** and ***L2)*** genes, leaving E6 and E7 as the only viral proteins that continue to be expressed in the infected cell. HPV genome sequences have been described and are available at various public databases. Such sequences may be found, for example, at GenBank Accession numbers K02718 (HPV 16), X05015 (HPV 18), J04353 (HPV31), M12732 (HPV33), M62849 (HPV39), X74479 (HPV45), NC_001533 (HPV51), X74481 (HPV52), X74483 (HPV56), D90400 (HPV58), NC_001635/X77858 (HPV59), X67161 (HPV68), ***etc.***

[0064] The sequences of E7 antigens from various HPV types have been described and are available at various public databases. Such sequences may be found, for example, at GenBank Accession numbers NP_041326 (HPV 16 E7), NP_04031 1 (HPV18 E7), AAA46951 (HPV31 E7), AAA46959 (HPV33 E7), AAA46967 (HPV35 E7), AAA47051 (HPV39 E7), P21736 (HPV45 E7), P26558 (HPV51 E7), P36831 (HPV52 E7), P36833 (HPV56 E7), P26557 (HPV58 E7), CAA54850 (HPV59 E7), P54668 (HPV68 E7), CAA63883 (HPV73 E7), and AAK28450 (HPV82 E7), ***etc.***

[0065] **Therapeutic Indications**

[0066] The compounds and compositions according to the invention may be used to treat HPV infection or a condition associated with such infection. HPV infection has been associated with a variety of conditions including, without limitation, common warts (or papillomas), cancer, ***etc.*** In general, low risk HPV types are associated with common warts (or papillomas) while high risk HPV types are associated with cancer.

[0067] By a "cancer" or "neoplasm" is meant any unwanted growth of cells serving no physiological function. In general, a cell of a neoplasm has been released from its normal cell division control, ***i.e.,*** a cell whose growth is not regulated by the ordinary biochemical and physical influences in the cellular environment. In most cases, a neoplastic cell proliferates to form a clone of cells which are either benign or malignant. Examples of cancers or neoplasms

include, without limitation, transformed and immortalized cells, tumours, and carcinomas such as breast cell carcinomas and cervical carcinomas. The term cancer includes cell growths that are technically benign but which carry the risk of becoming malignant. By "malignancy" is meant an abnormal growth of any cell type or tissue. The term malignancy includes cell growths that are technically benign but which carry the risk of becoming malignant. This term also includes any cancer, carcinoma, neoplasm, neoplasia, or tumor.

[0068] Accordingly, by "condition associated with HPV infection" is meant any condition, disease or disorder that has been correlated with the presence of an existing HPV infection, for example, any condition, disease or disorder that has been correlated with the presence of an existing high risk HPV infection. In some embodiments, a condition associated with HPV infection includes a condition, disease or disorder of the cervix, lower genital or anogenital tract, skin or oral cavity.

[0069] In some embodiments, a condition associated with HPV infection includes malignant and/or pre-malignant lesions of the cervix, lower genital or anogenital tract, for example, cancer of the cervix, anus, vulva, vagina, perineum, penis, *etc.* or pre-malignant lesions thereof. In alternative embodiments, a condition associated with HPV infection includes cancer of the lung, respiratory tract, epithelium, head and neck, breast cancer, oral cancer, *etc.* or pre-malignant lesions thereof.

[0070] In alternative embodiments, a condition associated with HPV infection includes a pre-malignant dysplastic condition, such as pre-cancerous cervical dysplasia, cervical intra-epithelial neoplasia (CIN) grade 1, 2, or 3, vulval intraepithelial neoplasia (VIN), vaginal intraepithelial neoplasia (VAIN), anal intraepithelial neoplasia (AIN), *etc.*

[0071] In some embodiments, the compounds and compositions according to the invention may be used to diagnose HPV infection. In alternative embodiments, a peptide including one or more of the sequences TSNYNIVTF (SEQ ID NO: 35), AEPDTSNYNIVTFCC (SEQ ID NO: 36) or TSNYNIVTFCCQCKS (SEQ ID NO: 37) maybe used to diagnose HPV31 infection, or to determine the immune response of a compound that includes a HPV3 1 E7 sequence to HPV3 1. Alternatively, one or more of the TSNYNIVTF, AEPDTSNYNIVTFCC or TSNYNIVTFCCQCKS sequences maybe used to rule out a HPV31 infection.

[0072] **HPV** E7 **Compounds, Test Compounds, And Methods of Making Same**

[0073] A compound according to the invention includes, without limitation, a polypeptide including the amino acid sequence of two or more HPV E7 antigens from different HPV genotypes, and analogues, variants, homologues and fragments thereof, as well as nucleic acid molecules encoding such polypeptides. In some embodiments, the two or more HPV E7 antigens will be capable of eliciting an immune response, such as a T cell CD8+ response, against the different HPV genotypes from which they are derived.

[0074] A "protein," "peptide" or "polypeptide" is any chain of two or more amino acids, including naturally occurring or non-naturally occurring amino acids or amino acid analogues, regardless of post-translational modification (e.g., glycosylation or phosphorylation). An "amino acid sequence", "polypeptide", "peptide" or "protein" of the invention may include peptides or proteins that have abnormal linkages, cross links and end caps, non-peptidyl bonds or alternative modifying groups. Such modifications are also within the scope of the invention. The term "modifying group" is intended to include structures that are directly attached to the peptidic structure (e.g., by covalent coupling), as well as those that are indirectly attached to the peptidic structure (e.g., by a stable non-covalent association or by covalent coupling to additional amino acid residues, or mimetics, analogues or derivatives thereof, which may flank the core peptidic structure). For example, the modifying group can be coupled to the amino-terminus or carboxy-terminus of a peptidic structure, or to a peptidic or peptidomimetic region flanking the core domain. Alternatively, the modifying group can be coupled to a side chain of at least one amino acid residue of a peptidic structure, or to a peptidic or peptidomimetic region flanking the core domain (e.g., through the epsilon amino group of a lysyl residue(s), through the carboxyl group of an aspartic acid residue(s) or a glutamic acid residue(s), through a hydroxy group of a tyrosyl residue(s), a serine residue(s) or a threonine residue(s) or other suitable reactive group on an amino acid side chain). Modifying groups covalently coupled to the peptidic structure can be attached by means and using methods well known in the art for linking chemical structures, including, for example, amide, alkylamino, carbamate or urea bonds.

[0075] The terms "nucleic acid" or "nucleic acid molecule" encompass both RNA (plus and minus strands) and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. The nucleic acid may be double-stranded or single-stranded. Where single-stranded, the nucleic acid may be the sense strand or the antisense strand. A nucleic acid molecule may be any chain of two or more covalently bonded nucleotides, including naturally occurring or non-naturally occurring nucleotides, or nucleotide analogs or derivatives. By "RNA" is meant a sequence of two or more covalently bonded, naturally occurring or modified

ribonucleotides. One example of a modified RNA included within this term is phosphorothioate RNA. By "DNA" is meant a sequence of two or more covalently bonded, naturally occurring or modified deoxyribonucleotides. By "cDNA" is meant complementary or copy DNA produced from an RNA template by the action of RNA-dependent DNA polymerase (reverse transcriptase). Thus a "cDNA clone" means a duplex DNA sequence complementary to an RNA molecule of interest, carried in a cloning vector. By "complementary" is meant that two nucleic acids, e.g., DNA or RNA, contain a sufficient number of nucleotides which are capable of forming Watson-Crick base pairs to produce a region of double-stranedness between the two nucleic acids. Thus, adenine in one strand of DNA or RNA pairs with thymine in an opposing complementary DNA strand or with uracil in an opposing complementary RNA strand. It will be understood that each nucleotide in a nucleic acid molecule need not form a matched Watson-Crick base pair with a nucleotide in an opposing complementary strand to form a duplex. A nucleic acid molecule is "complementary" to another nucleic acid molecule if it hybridizes, under conditions of high stringency, with the second nucleic acid molecule. A nucleic acid molecule according to the invention includes both complementary molecules.

[0076] In some embodiments, a compound according to the invention includes, without limitation, a polypeptide including an amino acid sequence substantially identical to the amino acid sequence of two or more E7 antigens from different HPV types, such as HPV 16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, or HPV82, or a nucleic acid molecule encoding such a polypeptide.

[0077] In alternative embodiments, a compound according to the invention includes, without limitation, a polypeptide including an amino acid sequence substantially identical to the amino acid sequence of three or more E7 antigens from different HPV types, such as HPV types, such as HPV 16, HPV 18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, or HPV82, or a nucleic acid molecule encoding such a polypeptide.

[0078] In alternative embodiments, a compound according to the invention includes, without limitation, a polypeptide including an amino acid sequence substantially identical to the amino acid sequence of four or more E7 antigens from different HPV types, such as HPV types, such as HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, or HPV82, or a nucleic acid molecule encoding such a polypeptide.

[0079] In alternative embodiments, a compound according to the invention includes, without limitation, a polypeptide including an amino acid sequence substantially identical to the amino acid sequence of five or more E7 antigens from different HPV types, such as HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, or HPV82, or a nucleic acid molecule encoding such a polypeptide.

[0080] In alternative embodiments, a compound according to the invention includes, without limitation, a polypeptide including an amino acid sequence substantially identical to the amino acid sequence of five E7 antigens from different HPV types, such as HPV16, HPV18, HPV31, HPV45, and HPV52, or a nucleic acid molecule encoding such a polypeptide.

[0081] In some embodiments, a compound according to the invention includes, without limitation, a polypeptide including an amino acid sequence substantially identical to the amino acid sequence of two or more of SEQ ID NOs: 1-15.

[0082] In some embodiments, a compound according to the invention includes, without limitation, a polypeptide including an amino acid sequence substantially identical to the amino acid sequence of three or more of SEQ ID NOs: 1-15.

[0083] In some embodiments, a compound according to the invention includes, without limitation, a polypeptide including an amino acid sequence substantially identical to the amino acid sequence of four or more of SEQ ID NOs: 1-15.

[0084] In some embodiments, a compound according to the invention includes, without limitation, a polypeptide including an amino acid sequence substantially identical to the amino acid sequence of five or more of SEQ ID NOs: 1-15.

[0085] For example, a compound according to the invention includes, without limitation, a polypeptide including an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 16 or 17.

[0086] In some embodiments, a compound according to the invention includes, without limitation, a nucleic acid molecule including a nucleotide sequence substantially identical to the nucleotide sequence of two or more of SEQ ID NOs: 18-32.

[0087] In some embodiments, a compound according to the invention includes, without limitation, a nucleic acid molecule including a nucleotide sequence substantially identical to the nucleotide sequence of three or more of SEQ ID NOs: 18-32.

[0088] In some embodiments, a compound according to the invention includes, without limitation, a nucleic acid molecule including a nucleotide sequence substantially identical to the nucleotide sequence of four or more of SEQ ID NOs: 18-32.

[0089] In some embodiments, a compound according to the invention includes, without limitation, a nucleic acid molecule including a nucleotide sequence substantially identical to the nucleotide sequence of all five of SEQ ID NOs: 18-22.

[0090] For example, a compound according to the invention includes, without limitation, a nucleic acid molecule including a nucleotide sequence substantially identical to the nucleotide sequence of SEQ ID NOs: 33 or 34.

[0091] A "substantially identical" sequence is an amino acid or nucleotide sequence that differs from a reference sequence only by one or more conservative substitutions, as discussed herein, or by one or more non-conservative substitutions, deletions, or insertions located at positions of the sequence that do not destroy or substantially reduce T cell recognition and/or HLA binding of the polypeptide expressed by the amino acid sequence or encoded by the nucleic acid molecule. Such a sequence can be any value from 50% to 99%, or more generally at least 50%, 55% or 60%, or at least 65%, 75%, 80%, 85%, 90%, or 95%, or as much as 96%, 97%, 98%, or 99% identical when optimally aligned at the amino acid or nucleotide level to the sequence used for comparison using, for example, the Align Program (Myers and Miller, CABIOS, 1989, 4:1 1-17) or FASTA. For polypeptides, the length of comparison sequences maybe at least 2, 5, 10, or 15 amino acids, or at least 20, 25, or 30 amino acids. In alternate embodiments, the length of comparison sequences may be at least 35, 40, or 50 amino acids, or over 60, 80, or 100 amino acids. For nucleic acid molecules, the length of comparison sequences may be at least 5, 10, 15, 20, or 25 nucleotides, or at least 30, 40, or 50 nucleotides. In alternate embodiments, the length of comparison sequences may be at least 60, 70, 80, or 90 nucleotides, or over 100, 200, or 500 nucleotides. Sequence identity can be readily measured using publicly available sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, or BLAST software available from the National Library of Medicine, or as described herein). Examples of useful software include the programs Pile-up and PrettyBox. Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, substitutions, and other modifications.

[0092] Alternatively, or additionally, two nucleic acid sequences may be "substantially identical" if they hybridize under high stringency conditions. In some embodiments, high stringency conditions are, for example, conditions that allow hybridization comparable with the hybridization that occurs using a DNA probe of at least 500 nucleotides in length, in a buffer containing 0.5 M $NaHP0_4$, pH 7.2, 7% SDS, 1 mM EDTA, and 1% BSA (fraction V), at a temperature of 65°C, or a buffer containing 48% formamide, 4.8x SSC, 0.2 M Tris-Cl, pH 7.6, 1x Denhardt's solution, 10% dextran sulfate, and 0.1% SDS, at a temperature of 42°C. (These are typical conditions for high stringency northern or Southern hybridizations.) Hybridizations may be carried out over a period of about 20 to 30 minutes, or about 2 to 6 hours, or about 10 to 15 hours, or over 24 hours or more. High stringency hybridization is also relied upon for the success of numerous techniques routinely performed by molecular biologists, such as high stringency PCR, DNA sequencing, single strand conformational polymorphism analysis, and in situ hybridization. In contrast to northern and Southern hybridizations, these techniques are usually performed with relatively short probes (e.g., usually about 16 nucleotides or longer for PCR or sequencing and about 40 nucleotides or longer for in situ hybridization). The high stringency conditions used in these techniques are well known to those skilled in the art of molecular biology, and examples of them can be found, for example, in Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y., 1998, which is hereby incorporated by reference.

[0093] In some embodiments, a compound according to the invention includes a compound that is substantially identical to a native HPV E7 antigen sequence. Accordingly, sequences for use in the compounds according to the invention may include sequences that are substantially identical to any of SEQ ID NOs: 1-34, or to any other HPV E7 sequences.

[0094] It is to be understood that the individual E7 antigen sequences may occur in any order in the amino acid or nucleotide sequence of a compound according to the invention, as long as at least two or more E7 antigen sequences from different HPV genotypes are present in a single molecule. In some embodiments, 3, 4, 5, 6, 7, 8, 9 or 10 or more different HPV E7 antigens from different HPV types may be used in a compound according to the invention.

[0095] Exemplary HPV E7 antigen sequence ordering include those set forth in Table 1.

Table 1: Exemplary permutations of E7 sequences from 5 HPV types

16,18,31,45,52	16,18,31,52,45	16,18,45,31,52
16,18,45,52,31	16,18,52,31,45	16,18,52,45,31
16,31,18,45,52	16,31,18,52,45	16,31,45,18,52
16,31,45,52,18	16,31,52,18,45	16,31,52,45,18
16,45,18,31,52	16,45,18,52,31	16,45,31,18,52
16,45,31,52,18	16,45,52,18,31	16,45,52,31,18
16,52,18,31,45	16,52,18,45,31	16,52,31,18,45
16,52,31,45,18	16,52,45,18,31	16,52,45,31,18
18,16,31,45,52	18,16,31,52,45	18,16,45,31,52
18,16,45,52,31	18,16,52,31,45	18,16,52,45,31
18,31,16,45,52	18,31,16,52,45	18,31,45,16,52
18,31,45,52,16	18,31,52,16,45	18,31,52,45,16
18,45,16,31,52	18,45,16,52,31	18,45,31,16,52
18,45,31,52,16	18,45,52,16,31	18,45,52,31,16
18,52,16,31,45	18,52,16,45,31	18,52,31,16,45
18,52,31,45,16	18,52,45,16,31	18,52,45,31,16
31,16,18,45,52	31,16,18,52,45	31,16,45,18,52
31,16,45,52,18	31,16,52,18,45	31,16,52,45,18
31,18,16,45,52	31,18,16,52,45	31,18,45,16,52
31,18,45,52,16	31,18,52,16,45	31,18,52,45,16
31,45,16,18,52	31,45,16,52,18	31,45,18,16,52
31,45,18,52,16	31,45,52,16,18	31,45,52,18,16
31,52,16,18,45	31,52,16,45,18	31,52,18,16,45
31,52,18,45,16	31,52,45,16,18	31,52,45,18,16
45,16,18,31,52	45,16,18,52,31	45,16,31,18,52
45,16,31,52,18	45,16,52,18,31	45,16,52,31,18
45,18,16,31,52	45,18,16,52,31	45,18,31,16,52
45,18,31,52,16	45,18,52,16,31	45,18,52,31,16
45,31,16,18,52	45,31,16,52,18	45,31,18,16,52
45,31,18,52,16	45,31,52,16,18	45,31,52,18,16
45,52,16,18,31	45,52,16,31,18	45,52,18,16,31

45, 52, 18, 31, 15	45, 52, 31, 16, 18	45, 52, 31, 18, 16
52, 16, 18, 31, 45	52, 16, 18, 45, 31	52, 16, 31, 18, 45
52, 16, 31, 45, 18	52, 16, 45, 18, 31	52, 16, 45, 31, 18
52, 18, 16, 31, 45	52, 18, 16, 45, 31	52, 18, 31, 16, 45
52, 18, 31, 45, 16	52, 18, 45, 16, 31	52, 18, 45, 31, 16
52, 31, 16, 18, 45	52, 31, 16, 45, 18	52, 31, 18, 16, 45
52, 31, 18, 45, 16	52, 31, 45, 16, 18	52, 31, 45, 18, 16
52, 45, 16, 18, 31	52, 45, 16, 31, 18	52, 45, 18, 16, 31
52, 45, 18, 31, 16	52, 45, 31, 16, 18	52, 45, 31, 18, 16

[0096] In some embodiments, the E7 antigen sequences are "naturally occurring" or "native" ***i.e.,*** isolated from a natural source rather than artificially modified. Such sources may include, without limitation, biological samples ***(e.g.,*** blood, serum, plasma, semen, mucus, urine, oral, vaginal and cervical fluids, gynecological sample, biopsies, etc.) obtained from infected subjects or from other source.

[0097] In alternative embodiments, compounds can be prepared by, for example, replacing, deleting, or inserting an amino acid residue at any position of the E7 antigen sequences from any HPV type or polypeptide as described herein, with other conservative amino acid residues, i.e., residues having similar physical, biological, or chemical properties, and for example screening for the ability of the compound to elicit a CD8+ T cell response as described herein or known in the art. In some embodiments, fragments of native E7 antigens are contemplated within the scope of the invention, as long as the fragments do not exhibit no or substantially reduced T cell recognition and/or HLA binding.

[0098] As used herein, the term "conserved amino acid substitutions" refers to the substitution of one amino acid for another at a given location in the peptide, where the substitution can be made without substantial loss of the relevant function. In making such changes, substitutions of like amino acid residues can be made on the basis of relative similarity of side-chain substituents, for example, their size, charge, hydrophobicity, hydrophilicity, and the like, and such substitutions may be assayed for their effect on the function of the peptide by routine testing.

[0099] As used herein, the term "amino acids" means those L-amino acids commonly found in naturally occurring proteins, D-amino acids and such amino acids when they have been modified.

Accordingly, amino acids of the invention may include, for example: 2-Aminoadipic acid; 3-Aminoadipic acid; beta-Alanine; beta-Aminopropionic acid; 2-Aminobutyric acid; 4-Aminobutyric acid; piperidinic acid; 6-Aminocaproic acid; 2-Aminoheptanoic acid; 2-Aminoisobutyric acid; 3-Aminoisobutyric acid; 2-Aminopimelic acid; 2,4 Diaminobutyric acid; Desmosine; 2,2'-Diaminopimelic acid; 2,3-Diaminopropionic acid; N-Ethylglycine; N-Ethylasparagine; Hydroxylysine; allo-Hydroxylysine; 3-Hydroxyproline; 4-Hydroxyproline; Isodesmosine; allo-Isoleucine; N-Methylglycine; sarcosine; N-Methylisoleucine; 6-N-methyllysine; N-Methylvaline; Norvaline; Norleucine; and Ornithine.

[00100] In some embodiments, conserved amino acid substitutions may be made where an amino acid residue is substituted for another having a similar hydrophilicity value (e.g., within a value of plus or minus 2.0, or plus or minus 1.5, or plus or minus 1.0, or plus or minus 0.5), where the following may be an amino acid having a hydropathic index of about -1.6 such as Tyr (-1.3) or Pro (-1.6) assigned to the amino acid residues (as detailed in United States Patent No. 4,554,101, incorporated herein by reference): Arg (+3.0); Lys (+3.0); Asp (+3.0); Glu (+3.0); Ser (+0.3); Asn (+0.2); Gin (+0.2); Gly (0); Pro (-0.5); Thr (-0.4); Ala (-0.5); His (-0.5); Cys (-1.0); Met (-1.3); Val (-1.5); Leu (-1.8); Ile (-1.8); Tyr (-2.3); Phe (-2.5); and Trp (-3.4).

[00101] In alternative embodiments, conservative amino acid substitutions may be made where an amino acid residue is substituted for another having a similar hydropathic index (e.g., within a value of plus or minus 2.0, or plus or minus 1.5, or plus or minus 1.0, or plus or minus 0.5). In such embodiments, each amino acid residue may be assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics, as follows: Ile (+4.5); Val (+4.2); Leu (+3.8); Phe (+2.8); Cys (+2.5); Met (+1.9); Ala (+1.8); Gly (-0.4); Thr (-0.7); Ser (-0.8); Trp (-0.9); Tyr (-1.3); Pro (-1.6); His (-3.2); Glu (-3.5); Gin (-3.5); Asp (-3.5); Asn (-3.5); Lys (-3.9); and Arg (-4.5).

[00102] In alternative embodiments, conservative amino acid substitutions may be made using publicly available families of similarity matrices (73-79). The PAM matrix is based upon counts derived from an evolutionary model, while the Blosum matrix uses counts derived from highly conserved blocks within an alignment. A similarity score of above zero in either of the PAM or Blosum matrices may be used to make conservative amino acid substitutions.

[00103] In alternative embodiments, conservative amino acid substitutions may be made where an amino acid residue is substituted for another in the same class, where the amino acids are divided into non-polar, acidic, basic and neutral classes, as follows: non-polar: Ala, Val, Leu,

Ile, Phe, Trp, Pro, Met; acidic: Asp, Glu; basic: Lys, Arg, His; neutral: Gly, Ser, Thr, Cys, Asn, Gin, Tyr.

[00104] Conservative amino acid changes can include the substitution of an L-amino acid by the corresponding D-amino acid, by a conservative D-amino acid, or by a naturally-occurring, non-genetically encoded form of amino acid, as well as a conservative substitution of an L-amino acid. Naturally-occurring non-genetically encoded amino acids include beta-alanine, 3-amino-propionic acid, 2,3-diamino propionic acid, alpha-aminoisobutyric acid, 4-amino-butyric acid, N-methylglycine (sarcosine), hydroxyproline, ornithine, citrulline, t-butylalanine, t-butylglycine, N-methylisoleucine, phenylglycine, cyclohexylalanine, norleucine, norvaline, 2-napthylalanine, pyridylalanine, 3-benzothienyl alanine, 4-chlorophenylalanine, 2-fluorophenylalanine, 3-fluorophenylalanine, 4-fluorophenylalanine, penicillamine, l,2,3,4-tetrahydro-isoquinoline-3-carboxylix acid, beta-2-thienylalanine, methionine sulfoxide, homoarginine, N-acetyl lysine, 2-amino butyric acid, 2-amino butyric acid, 2,4,-diamino butyric acid, p-aminophenylalanine, N-methylvaline, homocysteine, homoserine, cysteic acid, epsilon-amino hexanoic acid, delta-amino valeric acid, or 2,3-diaminobutyric acid.

[00105] In alternative embodiments, conservative amino acid changes include changes based on considerations of hydrophilicity or hydrophobicity, size or volume, or charge. Amino acids can be generally characterized as hydrophobic or hydrophilic, depending primarily on the properties of the amino acid side chain. A hydrophobic amino acid exhibits a hydrophobicity of greater than zero, and a hydrophilic amino acid exhibits a hydrophilicity of less than zero, based on the normalized consensus hydrophobicity scale of Eisenberg *et* a/.(80). Genetically encoded hydrophobic amino acids include Gly, Ala, Phe, Val, Leu, Ile, Pro, Met and Trp, and genetically encoded hydrophilic amino acids include Thr, His, Glu, Gin, Asp, Arg, Ser, and Lys. Non-genetically encoded hydrophobic amino acids include t-butylalanine, while non-genetically encoded hydrophilic amino acids include citrulline and homocysteine.

[00106] Hydrophobic or hydrophilic amino acids can be further subdivided based on the characteristics of their side chains. For example, an aromatic amino acid is a hydrophobic amino acid with a side chain containing at least one aromatic or heteroaromatic ring, which may contain one or more substituents such as -OH, -SH, -CN, -F, -CI, -Br, -I, -N0 $_2$, -NO, -NH_2, -NHR, -NRR, -C(0)R, -C(0)OH, -C(0)OR, -C(0)NH_2, -C(0)NHR, -C(0)NRR, etc., where R is independently (Ci-C_6) alkyl, substituted (Ci-C_6) alkyl, (Ci-C_6) alkenyl, substituted (Ci-C_6) alkenyl, (C_1-C_6) alkynyl, substituted (Ci-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl,

(C_6-C_{26}) alkaryl, substituted (C_6-C26) alkaryl, 5-20 membered heteroaryl, substituted 5-20 membered heteroaryl, 6-26 membered alkheteroaryl or substituted 6-26 membered alkheteroaryl. Genetically encoded aromatic amino acids include Phe, Tyr, and Trp, while non-genetically encoded aromatic amino acids include phenylglycine, 2-napthylalanine, beta-2-thienylalanine, l,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid, 4-chlorophenylalanine, 2-fluorophenylalanine3-fluorophenylalanine, and 4-fluorophenylalanine.

[00107] An apolar amino acid is a hydrophobic amino acid with a side chain that is uncharged at physiological pH and which has bonds in which a pair of electrons shared in common by two atoms is generally held equally by each of the two atoms (i.e., the side chain is not polar). Genetically encoded apolar amino acids include Gly, Leu, Val, He, Ala, and Met, while non-genetically encoded apolar amino acids include cyclohexylalanine. Apolar amino acids can be further subdivided to include aliphatic amino acids, which is a hydrophobic amino acid having an aliphatic hydrocarbon side chain. Genetically encoded aliphatic amino acids include Ala, Leu, Val, and Ile, while non-genetically encoded aliphatic amino acids include norleucine.

[00108] A polar amino acid is a hydrophilic amino acid with a side chain that is uncharged at physiological pH, but which has one bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Genetically encoded polar amino acids include Ser, Thr, Asn, and Gin, while non-genetically encoded polar amino acids include citrulline, N-acetyl lysine, and methionine sulfoxide.

[00109] An acidic amino acid is a hydrophilic amino acid with a side chain pKa value of less than 7. Acidic amino acids typically have negatively charged side chains at physiological pH due to loss of a hydrogen ion. Genetically encoded acidic amino acids include Asp and Glu. A basic amino acid is a hydrophilic amino acid with a side chain pKa value of greater than 7. Basic amino acids typically have positively charged side chains at physiological pH due to association with hydronium ion. Genetically encoded basic amino acids include Arg, Lys, and His, while non-genetically encoded basic amino acids include the non-cyclic amino acids ornithine, 2,3,-diaminopropionic acid, 2,4-diaminobutyric acid, and homoarginine.

[001 10] Accordingly, conservative substitutions include, without limitation, the following substitutions:

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gin; asn	lys
Asn (N)	gin; his; asp, lys; gin	arg
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gin (Q)	asn; glu	asn
Glu (E)	asp; gin	asp
Gly (G)	ala	ala
His (H)	asn; gin; lys; arg	arg
lie (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gin; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

[001 11] It will be appreciated by one skilled in the art that the above classifications are not absolute and that an amino acid may be classified in more than one category. In addition, amino acids can be classified based on known behaviour and or characteristic chemical, physical, or biological properties based on specified assays or as compared with previously identified amino acids. Amino acids can also include bifunctional moieties having amino acid-like side chains.

[001 12] Conservative changes can also include the substitution of a chemically derivatised moiety for a non-derivatised residue, by for example, reaction of a functional side group of an amino acid. Thus, these substitutions can include compounds whose free amino groups have been derivatised to amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Similarly, free carboxyl groups can be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides, and side chains can be derivatized to form O-acyl or O-alkyl derivatives for free hydroxyl groups or N-im-benzylhistidine for the imidazole nitrogen of histidine. Peptide analogues also include amino acids that have been chemically altered, for example, by methylation, by amidation of the C-terminal amino acid by an alkylamine such as ethylamine, ethanolamine, or ethylene diamine, or acylation or methylation of an amino acid side chain (such as acylation of the epsilon amino group of lysine). Peptide analogues can also include replacement of the amide linkage in the peptide with a substituted amide (for example, groups of the formula -C(0)-NR, where R is **(C_1-C_6)** alkyl, (C_1-C_6) alkenyl, **(Ci-C_6)** alkynyl, substituted (d-C_6) alkyl, substituted (Ci-C_6) alkenyl, or substituted (Ci-C_6) alkynyl) or isostere of an amide linkage (for example, -CH_2NH-, -CH_2S, -**CH_2CH_2-,** -CH=CH- (cis and trans), -C(0)CH_2-, -CH(OH)CH_2-, or-CH_2SO-).

[001 13] The compound can be covalently linked, for example, by polymerisation or conjugation, to form homopolymers or heteropolymers. Spacers and linkers, typically composed of small neutral molecules, such as amino acids that are uncharged under physiological conditions, can be used. Linkages can be achieved in a number of ways. For example, cysteine residues can be added at the peptide termini, and multiple peptides can be covalently bonded by controlled oxidation. Alternatively, heterobifunctional agents, such as disulfide/amide forming agents or thioether/amide forming agents can be used. The compound can also be linked to another compound that can modulate an immune response. The compound can also be constrained, for example, by having cyclic portions.

[001 14] Polypeptides, peptides or peptide analogues can be synthesised by standard chemical techniques, for example, by automated synthesis using solution or solid phase synthesis methodology. Automated peptide synthesisers are commercially available and use techniques well known in the art. Polypeptides, peptides and peptide analogues can also be prepared from their corresponding nucleic acid molecules using recombinant DNA technology using standard methods such as those described in, for example, Sambrook, *et al.* (81) or Ausubel *et al.* (82).

[001 15] In some embodiments, a nucleic acid molecule may be operably linked. By "operably linked" is meant that a gene and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s). Such operably linked sequences may be in the form of vectors or expression constructs that can be transformed or transfected into host cells for expression. Any suitable vector can be used such as for example pET15b (ampicillin resistant) or pET24a (kanamycin resistant).

[001 16] The term "recombinant" means that something has been recombined, so that when made in reference to a nucleic acid construct the term refers to a molecule that is comprised of nucleic acid sequences that are joined together or produced by means of molecular biological techniques. The term "recombinant" when made in reference to a protein or a polypeptide refers to a protein or polypeptide molecule which is expressed using a recombinant nucleic acid construct created by means of molecular biological techniques. Recombinant nucleic acid constructs may include a nucleotide sequence which is ligated to, or is manipulated to become ligated to, a nucleic acid sequence to which it is not ligated in nature, or to which it is ligated at a different location in nature. Referring to a nucleic acid construct as 'recombinant' therefore indicates that the nucleic acid molecule has been manipulated using genetic engineering, i.e. by human intervention. Recombinant nucleic acid constructs may for example be introduced into a host cell by transformation. Such recombinant nucleic acid constructs may include sequences derived from the same host cell species or from different host cell species, which have been isolated and reintroduced into cells of the host species. Recombinant nucleic acid construct sequences may become integrated into a host cell genome, either as a result of the original transformation of the host cells, or as the result of subsequent recombination and/or repair events.

[001 17] Compounds identified as being useful may be subsequently analyzed using a TCI model, or any other animal model for HPV infection.

[001 18] **Pharmaceutical & Veterinary Compositions, Dosages, And Administration**

[001 19] Compounds of the invention can be provided alone or in combination with other compounds (for example, nucleic acid molecules, small molecules, peptides, or peptide analogues), in the presence of a liposome, an adjuvant, or any carrier, such as a pharmaceutically acceptable carrier, in a form suitable for administration to mammals, for example, humans, cattle, sheep, *etc.*

[001 20] As used herein "pharmaceutically acceptable carrier" or "excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Alternatively, the carrier can be suitable for intravenous, intraperitoneal, intramuscular, sublingual or oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00121] Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the compounds to subjects suffering from HPV infection or presymptomatic for a condition associated with HPV infection. Any appropriate route of administration may be employed, for example, parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intrathecal, intracisternal, intraperitoneal, intranasal, aerosol, topical, or oral administration. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

[00122] Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences" (83). Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel. In general, the compounds are administered to an individual in an amount sufficient to stop or slow a condition associated with HPV infection, or to treat HPV infection, depending on the disorder.

[00123] In the case of vaccine formulations, an effective amount of a compound of the invention can be provided, alone or in combination with other compounds, with an immunological adjuvant, for example, Freund's incomplete adjuvant, dimethyldioctadecylammonium hydroxide, or aluminum hydroxide.

[00124] In alternative embodiments, a compound according to the invention may be provided in combination with an adjuvant selected from a Toll-like receptor (TLR) agonist, such as a TLR3 agonist (e.g., poly(I:C) and derivatives thereof, polyA:U and derivatives thereof, synthetic RNA molecules, naturally occurring RNA molecules, double-stranded RNAs, microbial nucleic acids etc.) or a TLR9 agonist (e.g., a CpG containing oligonucleotide, microbial nucleic acids, etc.), an interferon-alpha, an agonist of the 4-IBB receptor, an agonist of the CD40 receptor, or an anti-CD40 antibody.

[00125] The compound may also be linked with a carrier or other molecule, such as bovine serum albumin or keyhole limpet hemocyanin to enhance immunogenicity. In some embodiments, the compound may be provided with calreticulin, Mycobacterium tuberculosis heat shock protein (HSP70), ubiquitin, bacterial toxin, cytokine (such as an interleukin), imidazoquimo lines, *etc.*

[00126] In some embodiments, compounds or compositions according to the invention may be provided in a kit, optionally with a carrier and/or an adjuvant, together with instructions for use.

[001 27] An "effective amount" of a compound according to the invention includes a therapeutically effective amount, immunologically effective amount, or a prophylactically effective amount. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as treatment of HPV infection or a condition associated with such infection. The outcome of the treatment may for example be measured by a decrease in HPV viremia, inhibition of viral gene expression, delay in development of a pathology associated with HPV infection, stimulation of the immune system, or any other method of determining a therapeutic benefit. A therapeutically effective amount of a compound may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the compound are outweighed by the therapeutically beneficial effects. By "immunogenically

effective amount" is meant an amount effective, at dosages and for periods of time necessary, to achieve the desired immune result, such as stimulation or elicitation of an immune response, such as a T cell CD8+ response. In some embodiments, by "stimulation of an immune response" or "stimulating an immune response" is meant an increase in the measured immune response, such as a T cell CD8+ response, of any value between about 5% and about 95%, or between about 10% and about 90%, or between about 30% and about 60%, or over 100% increase when compared with a control or reference sample or compound. In alternative embodiments, by "stimulation of an immune response" or "stimulating an immune response" is meant an increase in the measured immune response, such as a T cell CD8+ response, of any value between about a 2-fold and about a 1000-fold, or about a 10-fold to about a 500-fold, or about a 30-fold to about a 100-fold, or more than a 1000-fold increase when compared with a control or reference sample or compound. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as prevention of onset of a condition associated with HPV infection. Typically, a prophylactic dose is used in subjects prior to or at an earlier stage of disease, so that a prophylactically effective amount may be less than a therapeutically effective amount. A suitable range for effective amounts of a compound may for example be any integer from 0.1 nM-O.lM, 0.1 nM-0.05M, 0.05 ηM-15μM or 0.01 ηM-10μM.

[00128] It is to be noted that dosage values may vary with the severity of the condition to be alleviated. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. Dosage ranges set forth herein are exemplary only and do not limit the dosage ranges that may be selected by medical practitioners. The amount of active compound(s) in the composition may vary according to factors such as the disease state, age, sex, and weight of the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It may be advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage.

[00129] In alternative embodiments, a composition including a compound according to the invention, in combination with a TLR agonist or other adjuvant, may be provided in a "cluster" dosing regimen to elicit an enhanced CD8+ T cell response. By a "cluster" dosing regimen is

meant administration of the composition over a short period of time i.e., less than about 14 days, for example, about 1 day to about 4, 5, 6, 7 or 8 days. In some embodiments, a cluster dosing regimen includes administration of multiple daily doses of the composition over a short period of time i.e., less than about 14 days, for example, about 1 day to about 4, 5, 6, 7 or 8 days.

[001 30] If desired, treatment with a compound according to the invention may be combined with more traditional and existing therapies for HPV infection or a condition associated with such infection. For example, a compound according to the invention may be provided in combination with radiation therapy, chemotherapy or surgery (e.g., LEEP) as appropriate.

[001 3 1] Compounds according to the invention may be provided chronically or intermittently. "Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

[00132] In general, compounds of the invention should be used without causing substantial toxicity. Toxicity of the compounds of the invention can be determined using standard techniques, for example, by testing in cell cultures or experimental animals and determining the therapeutic index, i.e., the ratio between the LD50 (the dose lethal to 50% of the population) and the LD100 (the dose lethal to 100% of the population). In some circumstances however, such as in severe disease conditions, it may be necessary to administer substantial excesses of the compositions.

[00133] Throughout the following description, specific details are set forth in order to provide a more thorough understanding of the invention. However, the invention may be practiced without these particulars. In other instances, well known elements have not been shown or described in detail to avoid unnecessarily obscuring the invention. Accordingly, the specification and drawings are to be regarded in an illustrative, rather than a restrictive sense.

[001 34] The present invention will be further illustrated in the following examples.

[00135] EXAMPLE 1: Preparation and Purification of Pentarix Protein

[00136] The E7 oncoproteins of HPV strains 16, 18, 31, 45 and 52 were produced as a full length recombinant protein (termed *"Pentarix")* in *E. coli* **(Fig. 1A).** In one study, a single

contiguous DNA comprising the complete E7 protein from each of HPV **16, 18**, 3**1,** 45 and 52 plus an amino-terminal **6xHIS** affinity TAG and thrombin cleavage site was produced as a synthetic DNA construct **(Fig. IB).** The multi-gene sequence was subsequently cloned into the expression vectors pET17 and pET24a (Invitrogen) and full length protein (Pentarix) with a cleavable 6xHIS affinity TAG was expressed in *E. coli* genotype BL21 (DE3) pLysS.

[00137] Briefly, 5 ml of LB media containing ampicillin (100 μg/ml) plus chloramphenicol (34 μg/ml) in the case of pET17 constructs, or containing kanamycin (34 ug/ml) plus chloramphenicol (34 ug/ml) in the case of pET24a constructs, was inoculated with a single colony of recombinant *E.-coli* and allowed to grow to saturation overnight. The next morning the 5 ml culture was used to inoculate 1000 ml of LB media containing ampicillin (100 μg/ml) plus chloramphenicol (34 μg/ml in the case of pET17 constructs, or containing kanamycin (34 ug/ml) plus chloramphenicol (34 ug/ml) in the case of pET24a constructs. When the growth in the 1000 ml culture reached $OD6o_0$ of between 0.2 and 0.4, IPTG was added to the culture to a final concentration of 2 mM and growth was continued for another 2 to 3 hours to allow for expression of recombinant protein. Bacteria were subsequently pelleted by centrifugation and resuspended in 30 ml lysis buffer (20 mM sodium phosphate, pH 7.4, 500 mM sodium chloride, 10 mM imidazole, **6** M urea, 1 mM DTT). Bacteria were lysed by two successive cycles of freezing and thawing followed by four successive cycles of sonication (30 seconds per cycle). Debris and insoluble protein was removed by centrifugation for 15 minutes at 15,000 rpm to render a soluble lysate solution. *Pentarix* protein with a cleavable **6x** affinity tag was purified from soluble lysate by passage over an affinity column (HisTrap HP, GE Healthcare) attached to an AKTA column chromatography system. After extensive washing with lysis buffer, *Pentarix* protein with a cleavable 6x affinity tag was eluted from the column using lysis buffer containing 500 mM imidazole. Elution fractions containing *Pentarix* protein with a cleavable **6x** affinity tag were then pooled and dialyzed against 4 changes of tissue culture grade phosphate buffered saline to render the final solution of *Pentarix* protein. Protein expression and purification was monitored by running various in process and final fractions on SDS-PAGE and visualizing proteins via Coomassie Blue staining or Western blot using anti-6x HIS Tag antibody (ABM) or anti-HPV **16E7** antibody (Invitrogen) **(Fig. 1C).** The purified protein was fully soluble in PBS and migrated on SDS-PAGE gel in accordance with its predicted molecular weight of 59,037 Da **(Fig. 1C).**

[00138] **EXAMPLE 2: CD8 responses to exogenous protein antigen**

[00139] Studies to determine **$OVA_{257-264}$**-specific CD8+ T cell responses elicited in response to immunization with whole exogenous OVA protein plus the TLR3 agonists poly(I:C) or polylC/LC were performed. Naive C57B1/6 mice (2 mice per condition) were immunized with whole OVA protein (500 μg) plus or minus poly(I:C) (10 μg) or polylC/LC (10 μg/ml). Seven days post- immunization mice were euthanized and the number of **$OVA_{257-264}$** (SIINFEKL)-specific CD8+ T cells in bulk splenocytes of immunized mice were quantitated by IFN-γ ELISPOT. Results are reported as the number of IFN-γ spot-forming cells per $1x10^6$ splenocytes after stimulation with media only, SIINFEKL peptide (10 μg/ml) or irrelevant H2Db-binding peptide (10 μg/ml). The results suggest that poly(I:C) and polylC/LC have comparable adjuvant activity *in vivo* **(Fig. 2A).**

[00140] In another study, naïve C57B1/6 mice (2 mice per dose, 18 mice in total) were immunized with the indicated amounts of whole, soluble OVA protein plus poly(I:C). Seven days post-immunization mice were euthanized and bulk splenocyte preparations were assessed by IFN-γ ELISPOT. Splenocytes ($3x10^5$ per well, triplicate wells per condition) from individual animals were stimulated overnight with either media alone or with SIINFEKL peptide (10 ug/ml), and results are reported as the number of IFN-γ spot-forming cells per $1x10^6$ splenocytes +/- SD for each cohort **(Fig. 2B).**

[00141] **EXAMPLE 3: Dosing Regimens**

[00142] **$OVA_{257-264}$**-specific CD8+ T cell responses elicited in response to long or short interval (cluster) homologous prime-boost immunization were determined as follows. Naïve C57B1/6 mice (2 mice per condition) were immunized with whole OVA protein (100 μg) plus poly(I:C) (10 μg) at day -7, day -21 or day -7 and day -21 and were euthanized at day 0. The number of **$OVA_{257-264}$**-specific CD8+ T cells in bulk splenocytes of immunized mice were quantitated by IFN-γ ELISPOT. Naïve C57B1/6 mice (3 mice per condition) were also immunized with the indicated number of sequential daily doses of whole, soluble OVA protein (100 μg) admixed with poly(I:C) (10 μg). One additional group of mice received a single immunization that was equivalent to four times the normal daily dose (i.e. 400 μg of OVA protein plus 40 μg of poly(I:C)). Seven days after the first immunization mice were euthanized and bulk splenocyte preparations were assessed by IFN-γ ELISPOT to quantitate the number of **$OVA_{257-264}$**-specific CD8+ T cells. The results in **Figs. 3a** and **b** are reported as the number of IFN-γ spot-forming cells per $1x10^6$ splenocytes after stimulation with media only or SIINFEKL

peptide (10 μg/ml). Naive C57B1/6 mice were also immunized with one dose or four consecutive daily doses of whole, soluble OVA protein (100 μg) plus poly(I:C) (10 μg) as indicated in **Fig. 3c.** Mice that received 4 four consecutive daily doses of OVA protein plus poly(I:C) were reimmunized with another four consecutive daily doses of the same, starting at day 47 after imitation of the first round of immunization. Peripheral blood was obtained from the saphenous vein of individual mice that were serially bled on the indicated days post-immunization. RBC in peripheral blood were lysed and lymphocytes were stained with FITC-conjugated anti-CD8 and PE-conjugated H-2Kb/ $OVA_{257-264}$ tetramer and analyzed by flow cytometry. Events shown in **Figs. 3c** and **d** were gated on CD8+ lymphocytes and were from a representative single animal to allow precise monitoring of the evolution of the antigen-specific T cell responses within a given animal over time. After this second round of vaccination, OVA-specific CD8+ T cells expanded to levels that were even higher than what was achieved after primary immunization, reaching 52% of peripheral CD8+ T cells within 7 days of secondary immunization **(Fig. 3d).** Accordingly, the secondary response elicited at a later time point was considerably stronger than the primary response elicited by cluster vaccination.

[00143] In another study, as observed with the model antigen OVA, immunization with four doses of HPV16 E7 protein plus poly(I:C) elicited a robust CD8 immune response compared to immunization with HPV16 E7 protein alone **(Fig. 3e).**

[00144] In another study, C57B1/6 mice (3 mice per cohort) were implanted with OVA-expressing EG7 tumors cells (lxl 0^5) on day 0 and were left untreated, or were treated with 1 dose of poly(I:C) (10 μg), 1 dose of whole, soluble OVA protein (100 μg) plus poly(I:C) (10 μg) or four sequential daily doses of whole, soluble OVA protein (100 μg) plus poly(I:C) (10 μg) for specified periods of time, as shown in **Fig.** 4. Average tumor volume at time of treatment for each group was 224mm^3 (poly(I:C) only), 194mm^3 (1 dose of OVA + poly(I:C)) or 344mm^3 (4 doses of OVA + poly(I:C)). Mice in the last cohort were intentionally treated at a time when tumor size was larger in order to exemplify the beneficial effects of sequential daily immunization.

[00145] **EXAMPLE** 4: **Immunization with single or multiple doses of Pentarix protein**

[00146] Mice (naive C57B1/6) were left untreated or were immunized subcutaneously with 100 μg of recombinant *Pentarix* protein admixed with 10 μg of the TLR3 agonist polyl-C (Amersham or Sigma) **(Fig. 5A)** or the TLR9 agonist CpG-2395 (oligo #2395, Invivogen), or

with CpG oligo only **(Fig. 6A).** Seven days post-immunization mice were euthanized and spleens were excised. Single cell suspensions of splenocytes were prepared in 10 ml of cRPMI (RPMI 1640, 10% FCS, 2 mM L-glutamine, 50 uM 2-mercaptoethanol, 10 mM HEPES and 10 mM sodium pyruvate) by mashing spleens through a 70 uM filter using the plunger from a 5 ml syringe. ELISPOT plates (MSIP, Millipore) were pre-coated overnight with 10 μg/ml anti-IFN-γ capture antibody (AN18-Mabtech) and then blocked for 2 hours at 37°C with cRPMI. Splenocytes (3xl0 ^{5} cells per well) were plated in triplicate in the absence of any stimulus (media only), in the presence of 10 μg/ml of the H-2D^{b} restricted $\mathbf{E7_{49\text{-}57}}$ peptide from HPV16, or an irrelevant H2-D^{b}-binding control peptide (KAVYNFATC). After overnight incubation at 37°C, ELISPOT plates were washed and incubated for 2 hours at 37°C with 1 μg/ml biotinylated anti-mouse IFN-γ (mAb R4-6A2, Mabtech) followed by development with Vectastain ABC Elite kit and Vectastain AEC substrate reagent according to manufacturers' instructions (Vector Labs). Spots were quantitated using a commercial ELISPOT counting service (Zellnet). Results are presented as the number of IFN-γ spot forming cells per 1x10^{6} splenocytes when cultured in the presence of media only, media plus HPV16 $\mathbf{E7_{49\text{-}57}}$ peptide or irrelevant peptide (10 μg/ml).

[00147] In another study **(Fig. 7A),** mice (naive C57B1/6) were left untreated or were immunized subcutaneously one time or 4 times (daily on days 1-4) with 100 μg of recombinant *Pentarix* protein admixed with 10 μg of the TLR3 agonist polyl-C (Amersham or Sigma). Mice receiving multiple consecutive daily immunizations were immunized at approximately 24 hour intervals. Seven days after the initial immunization mice were euthanized and spleens were excised. Single cell suspensions of splenocytes were prepared in 10 ml of cRPMI (RPMI 1640, 10% FCS, 2 mM L-glutamine, 50 uM 2-mercaptoethanol, 10 mM HEPES and 10 mM sodium pyruvate) by mashing spleens through a 70 uM filter using the plunger from a 5 ml syringe. ELISPOT plates (MSIP, Millipore) were pre-coated overnight with 10 μg/ml anti-IFN-γ capture antibody (ANl 8-Mabtech) and then blocked for 2 hours at 37°C with cRPMI. Splenocytes (3xl0 ^{5} cells per well) were plated in triplicate in the absence of any stimulus (media only), or in the presence of 10 μg/ml $E7_{49\text{-}57}$ peptide. After overnight incubation at 37°C, ELISPOT plates were washed and incubated for 2 hours at 37°C with 1 μg/ml biotinylated anti-mouse IFN-γ (mAb R4-6A2, Mabtech) followed by development with Vectastain ABC Elite kit and Vectastain AEC substrate reagent according to manufacturers' instructions (Vector Labs). Spots were quantitated using a commercial ELISPOT counting service (Zellnet). Results are presented as the number of IFN-γ spot forming cells per lxl 0^{6} splenocytes when cultured in the presence of media only or media plus HPV16 $\mathbf{E7_{49\text{-}57}}$ peptide.

[00148] In another study, we found that in mice receiving 4 successive doses of Pentarix protein plus poly(I:C), up to 11% of CD8 T cells in peripheral blood and up to 22% of CD8 T cells in the spleen stained positively with H-2D^b HPV16 $E7_{49-57}$ tetramer **(Fig. 7C).** In this study, HPV-specific T cells were not detectable in the spleens of mice immunized with 4 doses of Pentarix protein only, indicating the importance of adjuvant for CD8 T cell expansion under the study conditions.

[00149] In another study **(Fig. 8A),** naive C57B1/6 mice (3 mice per cohort) were implanted with E7-expressing TCI tumors cells ($1x10^5$ per mouse), subcutaneously into the left flank, on day -14. On day 0 (when tumors reached approximately 200 mm^3 in size) mice were either left untreated, or were treated with a single inoculation (subcutaneous in the scruff of the neck) of polyl-C only (10 μg) or *Pentarix* protein (100 μg) plus the TLR3 agonist polyl-C (10 μg). Mice immunized with *Pentarix* protein (100 μg) plus the TLR3 agonist polyl-C (10 μg) **(Fig. 8A,** right panel) fully regressed tumors and remained tumor free for the duration of the study (60 days). Tumors were measured every 2-3 days using an electronic digital caliper and volumes were calculated using the formula width^2 x length x 0.5 and tumor-bearing mice were euthanized when the tumor volume reached approximately 2000 mm^3 according to the CCAC (Canadian Council on Animal Care) guidelines.

[00150] In another study, TC-1 tumor cells were grown in cRPMI containing 0.4 mg/ml G418 to 60-80% confluency and were harvested by a brief exposure to 0.25% trypsin followed by neutralization with cRPMI. TC-1 tumor cells (1×10^5 per mouse) were implanted subcutaneously into the left flank of naive C57B1/6 mice and tumor growth was monitored by measuring the tumor every two to three days using electronic calipers. Tumor volumes were calculated using the formula width^2 x length x 0.5. Tumor-bearing mice were euthanized when the tumor volume exceeded 2000 mm^3 according to the CCAC (Canadian Council on Animal Care) guidelines.

[00151] In this study, the increased level of E7-specific CD8 T cells evoked by cluster vaccination also conferred an improved ability to regress E7-expresing TCI tumors, which was demonstrated when tumors were allowed to grow to a larger size than normal prior to the initiation of treatment **(Fig. 8B).** Mice that harbored tumors with an average volume of 350 mm^3 at time of treatment were immunized with either a single dose or 4 successive daily doses of Pentarix plus poly(I:C). Five of 8 mice receiving a single dose of vaccine exhibited transient (but incomplete) tumor regression and significantly improved time of survival compared to untreated

mice or mice treated with poly(I:C) only. However, all mice receiving a single dose of vaccine eventually succumbed to progressive tumor growth. In contrast, of mice that received 4 successive doses of Pentarix plus poly(I:C), 100% (8 of 8) fully regressed these large tumors to the point that they were no longer palpable. Although some tumors began to recur 4-5 weeks after treatment, 75% of mice (6 of 8) in the 4-dose cohort were still alive at day 38 and 50% remained tumor free. All mice in all other cohorts had been euthanized due to progressive tumor growth by this time point.

[001 52] In another study, in which naïve C57B1/6 mice were implanted with E7-expressing TCI tumors cells, immunization of TCI -tumor bearing mice with Pentarix protein plus polyI:C elicited complete tumor regression and the establishment of E7-specific CD8 memory cells that persisted after tumor regression **(Fig. 8C).**

[00153] In another study **(Fig. 9A),** HPV E7-expressing TC-1 tumor cells (1×10^5 cells per mouse) were implanted subcutaneously into the left flank of naïve C57B1/6 mice (4 mice per cohort) on day -2 1. On day 0 (when tumors reached approximately 200 mm^3 in size) mice were treated with a single inoculation (subcutaneous in the scruff of the neck) of *Pentarix* protein (100 μg) plus 10 μg of the TLR9 agonist CpG 2395 (Invivogen) or 10 μg of the TLR9 agonist CpG 2395 only (no *Pentarix)* or were left untreated. Mice immunized with *Pentarix* protein (100 μg) plus 10 μg of the TLR9 agonist CpG 2395 fully regressed tumors and remained tumor free for the duration of the study. Tumor growth was monitored by measuring the tumor every two to three days using calipers and tumor volumes were calculated using the formula $width^2 \times length \times 0.5$ and tumor-bearing mice were euthanized when the tumor volume reached approximately 2000 mm^3 according to the CCAC (Canadian Council on Animal Care) guidelines.

[00154] In another study to assess the effector function of CD8 T cells elicited by a single immunization with Pentarix protein plus these adjuvants, in terms of their ability to regress established, E7-expressing tumors, HPV16 E7-expressing TC-1 tumors were implanted subcutaneously in naive recipient mice and were allowed to grow until they reached a volume of approximately 200 mm^3. Animals immunized subcutaneously with Pentarix protein admixed with either poly(I:C) or CpG oligonucleotide began to regress these tumors, generally within one week of immunization **(Fig. 9B).** In this study, all animals immunized with Pentarix protein plus adjuvant had complete tumor regression by three weeks post-immunization and remained tumor free for at least three months. In contrast, mice that were either untreated or that were treated

with adjuvant only or Pentarix protein only displayed progressive tumor growth and were euthanized (generally within 28 days of tumor implantation) due to excessive tumor burden.

[001 55] In summary, as was observed with the OVA protein, *Pentarix* elicited strong CD8+ T-cell mediated immune responses when admixed with agonists of either TLR3 **(Figs. 5A and 5B)** or TLR9 **(Figs. 6A and 6B).** Also as was observed with the model antigen OVA, application of the sequential daily (cluster) immunization strategy substantially enhanced the CD8+ immune response elicited by *Pentarix* **(Figs. 7A-C).** *Pentarix* also elicited CD8+ T-cell mediated immune responses without adjuvant **(Fig. 11).**

[001 56] Immune responses elicited by vaccination with *Pentarix* protein were capable of regressing established TC-1 tumors within days of vaccination **(Figs. 8A, 8B, 9A and 9B).** This was true when *Pentarix* was combined with either poly(I:C) **(Figs. 8A, 8B and 9B)** or CpG oligonucleotide **(Figs. 9A and 9B).** In two different experiments, complete or near complete regression of established TC-1 tumors was achieved in all mice receiving *Pentarix* whereas 100% of control mice (no treatment or adjuvant only) succumbed to progressively growing tumors.

[00157] **EXAMPLE 5: Pentarix elicits immunity to a HPV31 E7 epitope**

[001 58] Naive C57B1/6 mice were immunized with 100 μg of whole, soluble *Pentarix* protein admixed with 10 μg of poly(I:C) (Amersham). Seven days post-immunization mice were euthanized and bulk splenocyte preparations were assessed by IFN-γ ELISPOT to quantitate the number of CD8+ T cells specific for peptides identified using two different predictive MHC-binding algorithms (SYFPEITHI and IEDB). Results are reported as the number of IFN-γ spot-forming cells per $1x10^6$ splenocytes after stimulation with media only or the indicated peptide (10 μg/ml). Both the long (14mer and 20mer) and short (9mer) versions of the HPV16 $E7_{49-57}$ peptide were found to elicit responses from immunized mice, as well as the HPV31-derived candidate peptide (HPV31 $E7_{252-260}$-TSNYNIVTF; SEQ ID NO: 35) with the highest H2Db score on both algorithms **(Fig. 10A).** This finding represents a new epitope for HPV31 E7 and demonstrates that *Pentarix* elicits immunity to a HPV31 E7 epitope.

[00159] **EXAMPLE 6: Pentarix elicits immune responses against multiple genotypes of HPV**

[001 60] To assess the scope of the cellular immune response elicited by Pentarix, bulk splenocytes and CD4-depleted splenocytes from mice immunized with Pentarix plus poly(I:C)

were analyzed directly ex vivo by ELISPOT with a library of overlapping 15mer peptides that spanned the entire Pentarix protein sequence. Where indicated, CD4 cells were depleted from bulk splenocytes using magnetic depletion. Briefly, bulk splenocytes were stained with PE-conjugated anti-CD4 antibody (clone L3T4; BD Biosciences) and labeled cells were depleted using anti-PE microbeads according to manufacturer's instructions (Miltenyi). As shown in **Fig 10B,** the response elicited in C57B1/6 mice by Pentarix encompassed all five of the HPV strains contained within the vaccine. Peptides containing the well-characterized H-2D^b-restricted epitope HPV16 **E7**$_{49-57}$ (RAHYNIVTF; SEQ ID NO: 38) comprised the strongest response in terms of absolute numbers of antigen-specific CD8 T cells. The next strongest response was elicited by 15mer peptides from HPV31 encompassing a related peptide (TSNYNIVTF; SEQ ID NO: 35) that is predicted by algorithm analyses to be an even stronger binder to H-2D^b than HPV16 E7$_{49-57}$. A variety of 15mer peptides from other HPV E7 protein sequences also elicited responses of varying intensity from both bulk and CD4-depleted splenocytes, confirming that Pentarix is capable of eliciting a broad scope cellular immune response, even in inbred mice with a limited repertoire of MHC molecules. In addition, HLA-A2 transgenic mice (HLA-A2/D^b, Jackson Labs stock # 004191) were also immunized with Pentarix plus poly(I:C) and assessed by ELISPOT using the same library of overlapping 15mer peptides. Interestingly, although the general strength of the response was greater in C57B1/6 than HLA-A2/D^b mice, the overall complexity of the response was very similar **(Fig IOC),** suggesting that an H2^b-restricted response had been elicited but that an HLA-A2 restricted response had not. Furthermore, as has been observed in a number of other studies using HPV 16 E7 antigen, we were unable to detect a response against the HLA-A2 restricted 11-20 or 86-93 minimal peptide epitopes of HPV16 E7 in mice immunized with Pentarix plus poly(I:C).

[00161] To confirm that TSNYNIVTF (SEQ ID NO: 35) was the precise minimal epitope within the strongly reactive HPV31 15mer peptides AEPDTSNYNIVTFCC (SEQ ID NO: 36) and TSNYNIVTFCCQCKS (SEQ ID NO: 37) splenocytes from mice immunized with Pentarix plus poly(LC) were assessed by ELISPOT and were found to be responsive to this 9mer minimal peptide **(Fig 10D).**

[00162] **OTHER EMBODIMENTS**

[00163] Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the spirit and scope of the invention in accordance with the common general knowledge of those skilled in this art. Such modifications

include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Accession numbers, as used herein, may refer to Accession numbers from multiple databases, including GenBank, the European Molecular Biology Laboratory (EMBL), the DNA Database of Japan (DDBJ), or the Genome Sequence Data Base (GSDB), for nucleotide sequences, and including the Protein Information Resource (PIR), SWISSPROT, Protein Research Foundation (PRF), and Protein Data Bank (PDB) (sequences from solved structures), as well as from translations from annotated coding regions from nucleotide sequences in GenBank, EMBL, DDBJ, or RefSeq, for polypeptide sequences. Numeric ranges are inclusive of the numbers defining the range, and of sub-ranges encompassed therein. As used herein, the terms "comprising", "comprises", "having" or "has" are used as an open-ended terms, substantially equivalent to the phrase "including, but not limited to". Terms such as "the," "a," and "an" are to be construed as indicating either the singular or plural. Citation of references herein shall not be construed as an admission that such references are prior art to the present invention. All publications are incorporated herein by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein and as though fully set forth herein. The invention includes all embodiments and variations substantially as hereinbefore described and with reference to the examples and drawings.

[001 64] The present invention has been described with regard to one or more embodiments. However, it will be apparent to persons skilled in the art that a number of variations and modifications can be made without departing from the scope of the invention as defined in the claims.

REFERENCES

1. Schiffman, M., P. E. Castle, J. Jeronimo, A. C. Rodriguez, and S. Wacholder. 2007. Human papillomavirus and cervical cancer. *Lancet* 370:890-907.
2. Bosch, F. X., A. Lorincz, N. Munoz, C. J. Meijer, and K. V. Shah. 2002. The causal relation between human papillomavirus and cervical cancer. *Journal of clinical pathology* 55:244-265.
3. Castellsague, X. 2008. Natural history and epidemiology of HPV infection and cervical cancer. *Gynecol Oncol* U0-.S4-7.
4. Psyrri, A., and D. DiMaio. 2008. Human papillomavirus in cervical and head-and-neck cancer. *Nature clinical practice. Oncology* 5:24-3 1.
5. Devaraj, K., M. L. Gillison, and T. C. Wu. 2003. Development of HPV vaccines for HPV-associated head and neck squamous cell carcinoma. *Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists* 14:345-362.
6. Syrjanen, S. 2005. Human papillomavirus (HPV) in head and neck cancer. *J Clin Virol* 32 Suppl l:S59-66.
7. Winer, R. L., S. K. Lee, J. P. Hughes, D. E. Adam, N. B. Kiviat, and L. A. Koutsky. 2003. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. *American Journal of Epidemiology* 157:218-226.
8. Frazer, I. H. 2004. Prevention of cervical cancer through papillomavirus vaccination. *Nature reviews.Immunology* 4:46-54.
9. Cutts, F. T., S. Franceschi, S. Goldie, X. Castellsague, S. de Sanjose, G. Garnett, W. J. Edmunds, P. Claeys, K. L. Goldenthal, D. M. Harper, and L. Markowitz. 2007. Human papillomavirus and HPV vaccines: a review. *Bull World Health Organ* 85:719-726.
10. Leggatt, G. R., and I. H. Frazer. 2007. HPV vaccines: the beginning of the end for cervical cancer. *Curr Opin Immunol* 19:232-238.
11. Munoz, N., **F.** X. Bosch, S. de Sanjose, R. Herrero, X. Castellsague, K. V. Shah, P. J. Snijders, C. J. Meijer, and **G.** International Agency for Research on Cancer Multicenter Cervical Cancer Study. 2003. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *The New England journal of medicine* 348:5 18-527.
12. Bosch, F. X., M. M. Manos, N. Munoz, M. Sherman, A. M. Jansen, J. Peto, M. H. Schiffman, V. Moreno, R. **Kurman,** and **K.** V. Shah. **1995.** Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *Journal of the National Cancer Institute* 87:796-802.
13. Jenkins, D. 2008. A review of cross-protection against oncogenic HPV by an HPV-16/18 AS04-adjuvanted cervical cancer vaccine: importance of virological and clinical endpoints and implications for mass vaccination in cervical cancer prevention. *Gynecol Oncol* 110:S18-25.
14. Ault, K. A. 2007. Human papillomavirus vaccines and the potential for cross-protection between related HPV types. *Gynecol Oncol* 107:S31-33.
15. Harper, D. M., E. L. Franco, C. M. Wheeler, A. B. Moscicki, B. Romanowski, C. M. Roteli-Martins, D. Jenkins. A. Schuind, S. A. Costa Clemens, and G. Dubin. 2006. Sustained efficacy up to 4.5 years of a bivalent LI virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet* 367:1247-1255.

16. Hildesheim, A., R. Herrero, S. Wacholder, A. C. Rodriguez, D. Solomon, M. C. Bratti, J. T. Schiller, P. Gonzalez, G. Dubin, C. Porras, S. E. Jimenez, and D. R. Lowy. 2007. Effect of human papillomavirus 16/18 L I viruslike particle vaccine among young women with preexisting infection: a randomized trial. *Jama* 298:743-753.

17. Herzog, T. J., W. K. Huh, L. S. Downs, J. S. Smith, and B. J. Monk. 2008. Initial lessons learned in HPV vaccination. *Gynecol Oncol* 109:S4-1 1.

18. Goldie, S. J., M. O'Shea, M. Diaz, and S. Y. Kim. 2008. Benefits, cost requirements and cost-effectiveness of the HPV 16, 18 vaccine for cervical cancer prevention in developing countries: policy implications. *Reprod Health Matters* 16:86-96.

19. Monie, A., C. F. Hung, and T. C. Wu. 2007. Preventive and therapeutic HPV vaccines. *Curr Opin Investig Drugs* 8:1038-1050.

20. Hung, C. F., B. Ma, A. Monie, S. W. Tsen, and T. C. Wu. 2008. Therapeutic human papillomavirus vaccines: current clinical trials and future directions. *Expert Opin Biol Ther* 8:421-439.

21. Narisawa-Saito, M., and T. Kiyono. 2007. Basic mechanisms of high-risk human papillomavirus-induced carcinogenesis: roles of E6 and **E7** proteins. *Cancer science* 98: 1505-15 11.

22. Crook, T., J. P. Morgenstem, L. Crawford, and L. Banks. 1989. Continued expression of HPV-16 **E7** protein is required for maintenance of the transformed phenotype of cells co-transformed by HPV- 16 plus EJ-ras. *Embo J* 8:513-5 19.

23. Smotkin, D., and F. O. Wettstein. 1987. The major human papillomavirus protein in cervical cancers is a cytoplasmic phosphoprotein. *J Virol* 6 1:1686-1689.

24. Munger, K., A. Baldwin, K. M. Edwards, H. Hayakawa, C. L. Nguyen, M. Owens, M. Grace, and K. Huh. 2004. Mechanisms of human papillomavirus-induced oncogenesis. *J Virol* 78:1 1451-1 1460.

25. Lin, K. Y., F. G. Guarnieri, K. F. Staveley-O'Carroll, H. I. Levitsky, J. T. August, D. M. Pardoll, and T. C. Wu. 1996. Treatment of established tumors with a novel vaccine that enhances major histocompatibility class II presentation of tumor antigen. *Cancer research* 56:2 1-26.

26. Feltkamp, M. C , H. L. Smits, M. P. Vierboom, R. P. Minnaar, B. M. de Jongh, J. W. Drijfhout, J. ter Schegget, C. J. Melief, and W. M. Kast. 1993. Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. *European journal of immunology* 23:2242-2249.

27. Vierboom, M. P., M. C. Feltkamp, A. Neisig, J. W. Drijfhout, J. ter Schegget, J. J. Neefjes, C. J. Melief, and W. M. Kast. 1998. Peptide vaccination with an anchor-replaced CTL epitope protects against human papillomavirus type 16-induced tumors expressing the wild-type epitope. *Journal of immunotherapy (Hagerstown, Md: 1997)* 2 1:399-408.

28. Manuri, P. R., B. Nehete, P. N. Nehete, R. Reisenauer, S. Wardell, A. N. Courtney, R. Gambhira, D. Lomada, A. K. Chopra, and K. J. Sastry. 2007. Intranasal immunization with synthetic peptides corresponding to the E6 and **E7** oncoproteins of human papillomavirus type 16 induces systemic and mucosal cellular immune responses and tumor protection. *Vaccine* 25:3302-33 10.

29. Chen, W., W. Yan, and L. Huang. 2008. A simple but effective cancer vaccine consisting of an antigen and a cationic lipid. *Cancer immunology, immunotherapy : CII* 57:5 17-530.

30. Berraondo, P., C. Nouze, X. Preville, D. Ladant, and C. Leclerc. 2007. Eradication of large tumors inmice by a tritherapy targeting the innate, adaptive, and regulatory components of the immune system. *Cancer research*

67:8847-8855.

31. Peng, S., H. Ji, C. Trimble, L. He, Y. C. Tsai, J. Yeatermeyer, D. A. Boyd, C. F. Hung, and T. C. Wu. 2004. Development of a DNA vaccine targeting human papillomavirus type 16 oncoprotein E6. *Journal of virology* 78:8468-8476.

32. Lin, C. T., Y. C. Tsai, L. He, R. Calizo, H. H. Chou, T. C. Chang, Y. K. Soong, C. F. Hung, and C. H. Lai. 2006. A DNA vaccine encoding a codon-optimized human papillomavirus type 16 E6 gene enhances CTL response and anti-tumor activity. *Journal of Biomedical Science* 13:481-488.

33. Cheng, W. F, C. F. Hung, S. I. Pai, K. F. Hsu, L. He, M. Ling, and T. C. Wu. 2002. Repeated DNA vaccinations elicited qualitatively different cytotoxic T lymphocytes and improved protective antitumor effects. *Journal of Biomedical Science* 9:675-687.

34. Borysiewicz, L. K., A. Fiander, M. Nimako, S. Man, G. W. Wilkinson, D. Westmoreland, A. S. Evans, M. Adams, S. N. Stacey, M. E. Boursnell, E. Rutherford, J. K. Hickling, and S. C. Inglis. 1996. A recombinant vaccinia virus encoding human papillomavirus types 16 and 18, E6 and E7 proteins as immunotherapy for cervical cancer. *Lancet* 347: 1523-1 527.

35. Li, J., Y. Sun, and A. Garen. 2002. Immunization and immunotherapy for cancers involving infection by a human papillomavirus in a mouse model. *Proceedings of the National Academy of Sciences of the United States of America* 99: 16232-16236.

36. Zhang, L., Y. Tang, H. Akbulut, D. Zelterman, P. J. Linton, and A. B. Deisseroth. 2003. An adenoviral vector cancer vaccine that delivers a tumor-associated antigen/CD40-ligand fusion protein to dendritic cells. *Proceedings of the National Academy of Sciences of the United States of America* 100: 15 10 1-15 106.

37. Jin, H. S., E. K. Park, J. M. Lee, S. E. NamKoong, D. G. Kim, Y. J. Lee, H. J. Jun, B. D. Han, S. M. Bae, and W. S. Ann. 2005. Immunization with adenoviral vectors carrying recombinant IL-12 and E7 enhanced the antitumor immunity to human papillomavirus 16-associated tumor. *Gynecologic oncology* 97:559-567.

38. Londono, L. P., S. Chatfield, R. W. Tindle, K. Herd, X. M. Gao, I. Frazer, and G. Dougan. 1996. Immunisation of mice using Salmonella typhimurium expressing human papillomavirus type 16 E7 epitopes inserted into hepatitis B virus core antigen. *Vaccine* 14:545-552.

39. Krul, M. R., E. J. Tijhaar, J. A. Kleijne, A. M. Van Loon, M. G. Nievers, H. Schipper, L. Geerse, M. Van der Kolk, P. A. Steerenberg, F. R. Mooi, and W. Den Otter. 1996. Induction of an antibody response in mice against human papillomavirus (HPV) type 16 after immunization with HPV recombinant Salmonella strains. *Cancer immunology, immunotherapy : CII* 43:44-48.

40. Gunn, G. R., A. Zubair, C. Peters, Z. K. Pan, T. C. Wu, and Y. Paterson. 2001 . Two Listeria monocytogenes vaccine vectors that express different molecular forms of human papilloma virus-16 (HPV-16) E7 induce qualitatively different T cell immunity that correlates with their ability to induce regression of established tumors immortalized by HPV-16. *Journal of immunology (Baltimore, Md.: 1950)* 167:6471-6479.

41. Lin, C. W., J. Y. Lee, Y. P. Tsao, C. P. Shen, H. C. Lai, and S. L. Chen. 2002. Oral vaccination with recombinant Listeria monocytogenes expressing human papillomavirus type 16 E7 can cause tumor growth in mice to regress. *International journal of cancer.Journal international du cancer* 102:629-637.

42. Mackova, J., L. Kutinova, P. Hainz, J. Krystofova, V. Sroller, P. Otahal, P. Gabriel, and S. Nemeckova. 2004. Adjuvant effect of dendritic cells transduced with recombinant vaccinia virus expressing HPV16-E7 is inhibited by co-expression of IL12. *International journal of oncology* 24: 1581-1 588.

43. Hauser, H., L. Shen, Q. L. Gu, S. Krueger, and S. Y. Chen. 2004. Secretory heat-shock protein as a dendritic

cell-targeting molecule: a new strategy to enhance the potency of genetic vaccines. ***Gene therapy*** 11:924-932.

44. Kang, T. H., J. H. Lee, H. C. Bae, K. H. Noh, J. H. Kim, C. K. Song, B. C. Shin, C. F. Hung, T. C. Wu, J. S. Park, and T. W. Kim. 2006. Enhancement of dendritic cell-based vaccine potency by targeting antigen to endosomal/lysosomal compartments. ***Immunology letters*** 106:126-134.

45. Chandy, A. G., M. Nurkkala, A. Josefsson, and K. Eriksson. 2007. Therapeutic dendritic cell vaccination with Ag coupled to cholera toxin in combination with intratumoural CpG injection leads to complete tumour eradication in mice bearing HPV 16 expressing tumours. ***Vaccine*** 25:6037-6046.

46. Greenstone, H. L., J. D. Nieland, K. E. de Visser, M. L. De Bruijn, R. Kirnbauer, R. B. Roden, D. R. Lowy, W. M. Kast, and J. T. Schiller. 1998. Chimeric papillomavirus virus-like particles elicit antitumor immunity against the E7 oncoprotein in an HPV16 tumor model. ***Proceedings of the National Academy of Sciences of the United States of America*** 95:1800-1805.

47. Da Silva, D. M., J. T. Schiller, and W. M. Kast. 2003. Heterologous boosting increases immunogenicity of chimeric papillomavirus virus-like particle vaccines. ***Vaccine*** 21:3219-3227.

48. Warrino, D. E., W. C. Olson, M. I. Scarrow, L. J. D'Ambrosio-Brennan, R. S. Guido, D. M. Da Silva, W. M. Kast, and W. J. Storkus. 2005. Human papillomavirus L1L2-E7 virus-like particles partially mature human dendritic cells and elicit E7-specific T-helper responses from patients with cervical intraepithelial neoplasia or cervical cancer in vitro. ***Human immunology*** 66:762-772.

49. Kaufmann, A. M., J. D. Nieland, I. Jochmus, S. Baur, K. Friese, J. Gabelsberger, F. Gieseking, L. Gissmann, B. Glasschroder, T. Grubert, P. Hillemanns, R. Hopfl, H. Ikenberg, J. Schwarz, M. Karrasch, A. Knoll, V. Kuppers, M. Lechmann, R. J. Lelle, H. Meissner, R. T. Muller, M. Pawlita, K. U. Petry, H. Pilch, E. Walek, and A. Schneider. 2007. Vaccination trial with HPV 16 L1E7 chimeric virus-like particles in women suffering from high grade cervical intraepithelial neoplasia (CIN 2/3). ***Internationaljournal of cancer.Journal international du cancer*** 121:2794-2800.

50. Carbone, F. R., and M. J. Bevan. 1990. Class I-restricted processing and presentation of exogenous cell-associated antigen in vivo. ***The Journal of experimental medicine*** 171:377-387.

51. Hallez, S., J. M. Brulet, C. Vandooren, F. Maudoux, S. Thomas, M. Heinderickx, A. Bollen, R. Wattiez, and A. Jacquet. 2004. Pre-clinical immunogenicity and anti-tumour efficacy of a deleted recombinant human papillomavirus type 16 E7 protein. ***Anticancer Research*** 24:2265-2275.

52. Welters, M. J., D. V. Filippov, S. J. van den Eeden, K. L. Franken, J. Nouta, A. R. Valentijn, G. A. van der Marel, H. S. Overkleeft, G. Lipford, R. Offringa, C. J. Melief, J. H. van Boom, S. H. van der Burg, and J. W. Drijfhout. 2004. Chemically synthesized protein as tumour-specific vaccine: immunogenicity and efficacy of synthetic HPV16 E7 in the TC-1 mouse tumour model. ***Vaccine*** 23:305-311.

53. Tobian, A. A., D. H. Canaday, W. H. Boom, and C. V. Harding. 2004. Bacterial heat shock proteins promote CD91-dependent class I MHC cross-presentation of chaperoned peptide to CD8+ T cells by cytosolic mechanisms in dendritic cells versus vacuolar mechanisms in macrophages. ***Journal of immunology (Baltimore, Md.: 1950)*** 172:5277-5286.

54. Tobian, A. A., C. V. Harding, and D. H. Canaday. 2005. Mycobacterium tuberculosis heat shock fusion protein enhances class I MHC cross-processing and -presentation by B lymphocytes. ***Journal of immunology (Baltimore, Md.: 1950)*** 174:5209-5214.

55. Salio, M., and V. Cerundolo. 2005. Viral immunity: cross-priming with the help of TLR3. ***Current biology : CB*** 15:R336-339.

56. Zaks, K., M. Jordan, A. Guth, K. Sellins, R. Kedl, A. Izzo, C. Bosio, and S. Dow. 2006. Efficient immunization and cross-priming by vaccine adjuvants containing TLR3 or TLR9 agonists complexed to cationic liposomes. *Journal of immunology (Baltimore, Md.: 1950)* 176:7335-7345.

57. Heng, B. *et al.* Human papilloma virus is associated with breast cancer. *Br J Cancer* (2009).

58. Frazer, I.H. Prevention of cervical cancer through papillomavirus vaccination. *Nature reviews.Immunology* **4,** 46-54 (2004).

59. Collins, S. *et al.* High incidence of cervical human papillomavirus infection in women during their first sexual relationship. *Bjog* **109,** 96-98 (2002).

60. Cutts, F.T. *et al.* Human papillomavirus and HPV vaccines: a review. *Bull World Health Organ* **85,** 719-726 (2007).

61. Leggatt, G.R. & Frazer, I.H. HPV vaccines: the beginning of the end for cervical cancer. *Curr Opin Immunol* **19,** 232-238 (2007).

62. Moscicki, A.B. HPV Vaccines: today and in the Future. *J Adolesc Health* **43,** S26-40 (2008).

63. Monie, A., Tsen, S.W., Hung, C.F. & Wu, T.C. Therapeutic HPV DNA vaccines. *Expert Rev Vaccines* **8,** 1221-1235 (2009).

64. Bermudez-Humaran, L.G. & Langella, P. Perspectives for the development of human papillomavirus vaccines and immunotherapy. *Expert Rev Vaccines* **9,** 35-44 (2010).

65. Cid-Arregui, A. Therapeutic vaccines against human papillomavirus and cervical cancer. *Open Virol J* **3,** 67-83 (2009).

66. Zinkernagel, R.M. & Hengartner, H. Protective 'immunity' by pre-existent neutralizing antibody titers and preactivated T cells but not by so-called 'immunological memory'. *Immunol Rev* **211,** 310-319 (2006).

67. Pantaleo, G. & Koup, R.A. Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. *Nat Med* **10,** 806-810 (2004).

68. Peng, S. *et al.* Cluster intradermal DNA vaccination rapidly induces E7-specific CD8+ T-cell immune responses leading to therapeutic antitumor effects. *Gene Ther* **15,** 1156-1166 (2008).

69. Murali-Krishna, K. *et al.* Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* **8,** 177-187 (1998).

70. Lu, S. Heterologous prime-boost vaccination. *Curr Opin Immunol* **21,** 346-351 (2009).

71. Radosevic, K., Rodriguez, A., Lemckert, A. & Goudsmit, J. Heterologous prime-boost vaccinations for poverty-related diseases: advantages and future prospects. *Expert Rev Vaccines* **8,** 577-592 (2009).

72. Ranasinghe, C. & Ramshaw, LA. Genetic heterologous prime-boost vaccination strategies for improved systemic and mucosal immunity. *Expert Rev Vaccines* **8,** 1171-1181 (2009).

73. Altschul, S.F. 1991. "Amino acid substitution matrices from an information theoretic perspective." Journal of Molecular Biology, 219: 555-665.

74. Dayhoff, M.O., Schwartz, R.M., Orcutt, B.C. 1978. "A model of evolutionary change in proteins." In "Atlas of Protein Sequence and Structure" 5(3) M.O. Dayhoff (ed.), 345 - 352, National Biomedical Research Foundation, Washington;

75. States, D.J., Gish, W., Altschul, S.F. 1991. "Improved Sensitivity of Nucleic Acid Database Search Using Application-Specific Scoring Matrices" Methods: A companion to Methods in Enzymology 3(1): 66 - 77;

76. Steven Henikoff and Jorja G. Henikoff. 1992 "Amino acid substitution matrices from protein blocks." Proc. Natl. Acad. Sci. USA. 89(biochemistry): 10915 - 10919;

77. M.S. Johnson and J.P. Overington. 1993. "A Structural Basis of Sequence Comparisons: An evaluation of scoring methodologies." Journal of Molecular Biology. 233: 716 - 738.

78. Steven Henikoff and Jorja G. Henikoff. 1993. "Performance Evaluation of Amino Acid Substitution Matrices." Proteins: Structure, Function, and Genetics. 17: 49- 61;

79. Karlin, S. and Altschul, S.F. 1990. "Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes" Proc. Natl. Acad. Sci. USA. 87: 2264 - 2268.)

80. Eisenberg *et al {J. Mol. Bio.* 179: 125-142, 184.

81. Sambrook, *et al.* Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

82. Ausubel *et al.* Current Protocols in Molecular Biology, John Wiley & Sons, 1994).

83. Remington's Pharmaceutical Sciences" (19th edition), ed. A. Gennaro, 1995, Mack Publishing Company, Easton, Pa.

WHAT IS CLAIMED IS:

1. A polypeptide comprising an amino acid sequence substantially identical to the amino acid sequence of two or more human papillomavirus (HPV) E7 antigens, wherein the E7 antigens are selected from at least two different HPV strains.

2. The polypeptide of claim 1, wherein the different HPV strains are high risk strains.

3. The polypeptide of claim 2, wherein the high risk strains are selected from two or more of the group consisting of HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, and HPV82.

4. The polypeptide of claim 1, wherein the E7 antigens are selected from five different HPV strains.

5. The polypeptide of claim 4, wherein the five different HPV strains comprise HPV 16, HPV18, HPV31, HPV45, and HPV52.

6. The polypeptide of claim 1, wherein the polypeptide comprises two or more of the amino acid sequences set forth in SEQ ID NOs: 1 to 15.

7. The polypeptide of claim 6, wherein the polypeptide comprises the amino acid sequences set forth in SEQ ID NOs: 1 to 5.

8. The polypeptide of claim 7, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 16 or 17.

9. The polypeptide of claim 6, wherein the polypeptide is encoded by a nucleotide sequence comprising two or more of the nucleotide sequences set forth in SEQ ID NOs: 18 to 32.

10. The polypeptide of claim 8, wherein the polypeptide is encoded by a nucleotide sequence comprising SEQ ID NO: 33 or 34.

11. The polypeptide of any one of claims 1 to 10 wherein the polypeptide is capable of inducing an immune response to the at least two different HPV strains.

12. A nucleic acid molecule comprising a sequence substantially identical to the nucleotide sequences of two or more human papillomavirus (HPV) E7 antigens, wherein the E7 antigens are selected from at least two different HPV strains.

13. The nucleic acid molecule of claim 12, wherein the different HPV strains are high risk strains.

14. The nucleic acid molecule of claim 13, wherein the high risk strains are selected from two or more of the group consisting of HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, and HPV82.

15. The nucleic acid molecule of claim 12, wherein the E7 antigens are selected from five different HPV strains.

16. The nucleic acid molecule of claim 15, wherein the five different HPV strains comprise HPV16, HPV18, HPV31, HPV45, and HPV52.

17. The nucleic acid molecule of claim 12, wherein the nucleic acid molecule comprises two or more of the nucleic acid sequences set forth in SEQ ID NOs: 18 to 32.

18. The nucleic acid molecule of claim 17, wherein the nucleic acid molecule comprises the nucleic acid sequences set forth in SEQ ID NOs: 18 to 22.

19. The nucleic acid molecule of claim 18, wherein the nucleic acid molecule comprises the nucleic acid sequence set forth in SEQ ID NO: 33 or 34.

20. A nucleic acid molecule encoding the polypeptide of any one of claims 1 to 11.

21. An expression vector comprising the nucleic acid sequence of any one of claims 12 to 20 operably linked to a sequence that allows for expression of the nucleic acid sequence in a host cell.

22. A host cell comprising the nucleic acid molecule of any one of claims 12 to 20 or the expression vector of claim 21.

23. A composition comprising the polypeptide of any one of claims 1 to 11 or the nucleic acid molecule of any one of claims 12 to 20 or the expression vector of claim 21 or the host cell of claim 22.

24. The composition of claim 23 further comprising a carrier.

25. The composition of claim 23 or 24 further comprising an adjuvant.

26. The composition of claim 25 wherein the adjuvant comprises a Toll-like receptor (TLR) agonist.

27. The composition of claim 26 wherein the TLR agonist is a TLR3 agonist or a TLR9 agonist.

28. The composition of claim 27 wherein the TLR3 agonist is poly(I:C).

29. The composition of claim 27 wherein the TLR9 agonist is a CpG containing oligonucleotide.

30. The composition of claim 25 wherein the adjuvant comprises an interferon-alpha, an agonist of the 4-IBB receptor, an agonist of the CD40 receptor, or an anti-CD40 antibody.

31. A method of stimulating an immune response in a subject in need thereof, the method comprising administering an effective amount of the polypeptide of any one of claims 1 to 11 or the nucleic acid molecule of any one of claims 12 to 20 or the expression vector of claim 21 or the host cell of claim 22, to the subject.

32. A method of treating or preventing a condition associated with HPV infection in a subject in need thereof, the method comprising administering an effective amount of the polypeptide of any one of claims 1 to 11 or the nucleic acid molecule of any one of claims 12 to 20 or the expression vector of claim 21 or the host cell of claim 22, to the subject.

33. The method of claim 32 wherein the condition associated with HPV infection is selected from the group consisting of one or more of a cancer of the breast, cervix, anus, vulva, vagina, penis, head and neck, and lung, or pre-malignant lesion thereof.

34. The method of claim 32 wherein the condition associated with HPV infection is a pre¬cancerous cervical epithelial neoplasia (CIN I through CIN III) or a cervical cancer.

35. A method of treating a HPV infection in a subject in need thereof, the method comprising administering an effective amount of the polypeptide of any one of claims 1 to 11 or the nucleic acid molecule of any one of claims 12 to 20 or the expression vector of claim 21 or the host cell of claim 22, to the subject.

36. The method of any one of claims 31 to 35 wherein the HPV infection is by a high risk HPV type.

37. The method of 36 wherein the high risk HPV type is selected from one or more of the group consisting of HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, and HPV82.

38. The method of any one of claims 31 to 37 further comprising administering an adjuvant.

39. The method of claim 38 wherein the adjuvant comprises a Toll-like receptor (TLR) agonist.

40. The method of claim 39 wherein the TLR agonist is a TLR3 agonist or a TLR9 agonist.

41. The method of claim 40 wherein the TLR3 agonist is poly(I:C).

42. The method of claim 40 wherein the TLR9 agonist is a CpG containing oligonucleotide.

43. The method of claim 38 wherein the adjuvant comprises an interferon-alpha, an agonist of the 4-IBB receptor, an agonist of the CD40 receptor, or an anti-CD40 antibody.

44. The method of any one of claims 31 to 43 wherein the administering comprises administration of multiple doses over a time frame of less than 14 days.

45. The method of any one of claims 31 to 43 wherein the administering comprises administration of multiple doses over one to four days.

46. The method of claim 44 or 45 wherein the administering comprises administration of multiple daily doses.

47. Use of an effective amount of the polypeptide of any one of claims 1 to 11 or the nucleic acid molecule of any one of claims 12 to 20 or the expression vector of claim 21 or the host cell of claim 22, for stimulating an immune response in a subject in need thereof.

48. Use of an effective amount of the polypeptide of any one of claims 1 to 11 or the nucleic acid molecule of any one of claims 12 to 20 or the expression vector of claim 21 or the host cell of claim 22 for treating or preventing a condition associated with HPV infection in a subject in need thereof.

49. The use of claim 48 wherein the condition associated with HPV infection is selected from the group consisting of one or more of a cancer of the breast, cervix, anus, vulva, vagina, penis, head and neck, and lung, or pre-malignant lesion thereof.

50. The use of claim 48 wherein the condition associated with HPV infection is a pre¬cancerous cervical epithelial neoplasia (CIN I through CIN III) or a cervical cancer.

51. Use of an effective amount of the polypeptide of any one of claims 1 to 11 or the nucleic acid molecule of any one of claims 12 to 20 or the expression vector of claim 21 or the host cell of claim 22, for treating a HPV infection in a subject in need thereof.

52. A peptide consisting essentially of one or more of the amino acid sequences TSNYNIVTF (SEQ ID NO: 35), AEPDTSNYNIVTFCC (SEQ ID NO: 36) or TSNYNIVTFCCQCKS (SEQ ID NO: 37).

53. A method of diagnosing a HPV31 infection comprising contacting a sample with a peptide consisting essentially of one or more of the amino acid sequences TSNYNIVTF (SEQ ID NO: 35), AEPDTSNYNIVTFCC (SEQ ID NO: 36) or TSNYNIVTFCCQCKS (SEQ ID NO: 37) .

54. A method of determining the response of a subject to a HPV31 infection contacting a sample with a peptide consisting essentially of the one or more of the amino acid sequences TSNYNIVTF (SEQ ID NO: 35), AEPDTSNYNIVTFCC (SEQ ID NO: 36) and TSNYNIVTFCCQCKS (SEQ ID NO: 37).

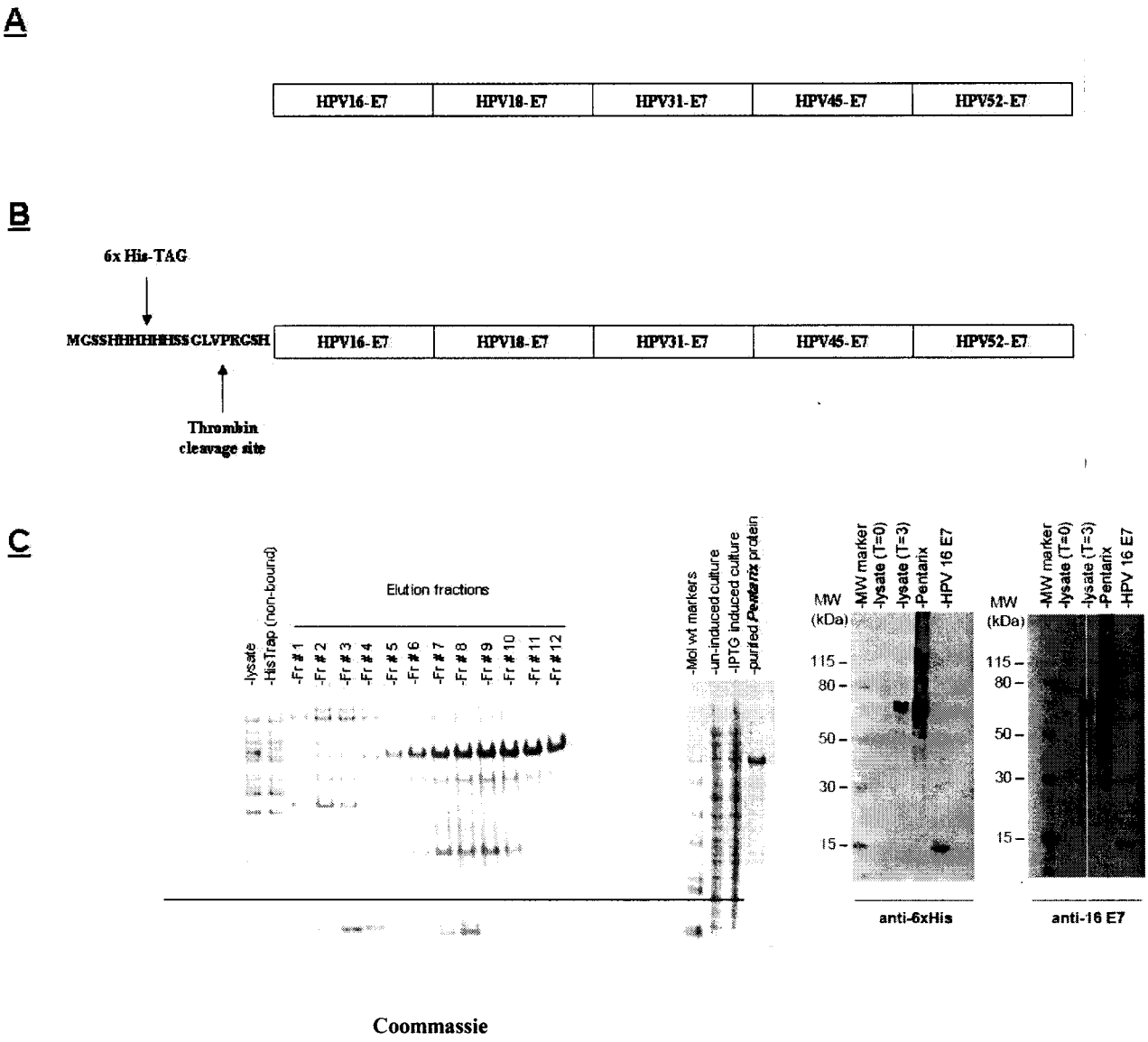

A
HPV16-E7
HPV18-E7
HPV31-E7
HPV45-E7
HPV52-E7
B
6x His-TAG
MGSSHHHHHHSSGLVPRGSH
Thrombin cleavage site
C
Elution fractions
-lysate
-HisTrap (non-bound)
-Fr # 1
-Fr # 2
-Fr # 3
-Fr # 4
-Fr # 5
-Fr # 6
-Fr # 7
-Fr # 8
-Fr # 9
-Fr # 10
-Fr # 11
-Fr # 12
-Mol wt markers
-un-induced culture
-IPTG induced culture
-purified Pentavix protein
MW (kDa)
-MW marker
-lysate (T=0)
-lysate (T=3)
-Pentavix
-HPV 16 E7
115
80
50
30
15
anti-6xHis
anti-16 E7
Coommassie

FIGURE 1

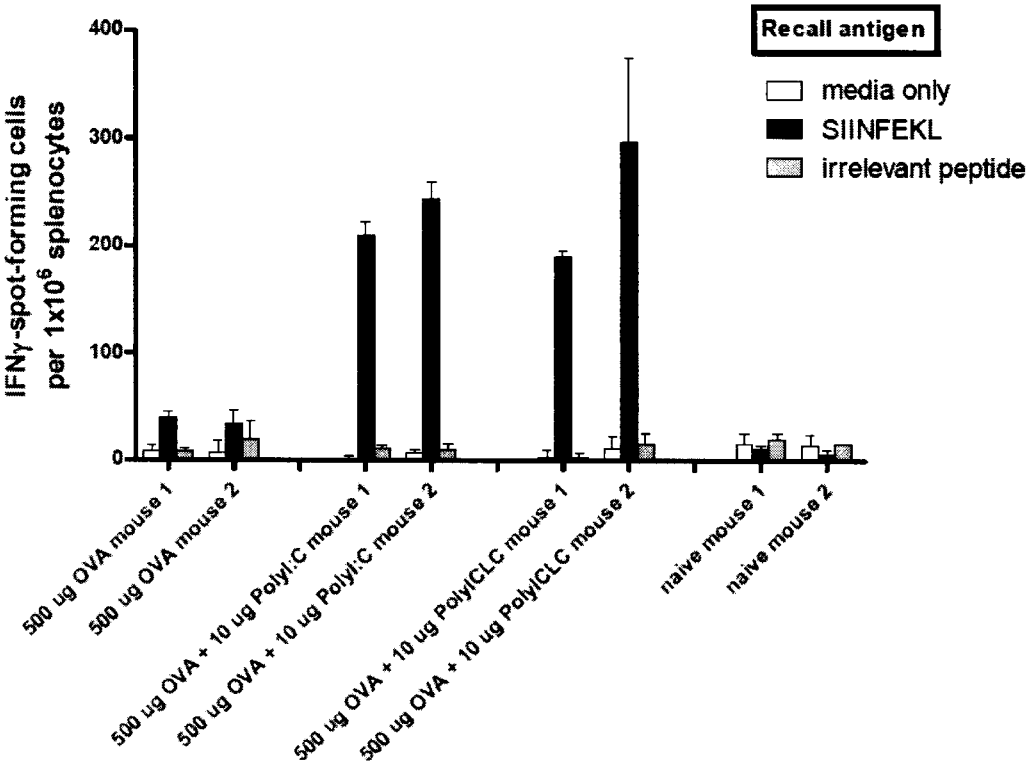
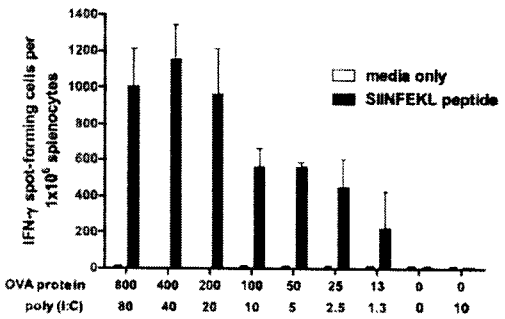


FIGURE 2A

Immunization with whole soluble exogenous antigen (Ova) with the TLR3 agonist polyI:C elicits strong, dose-dependent CD8 immunity.



OVA-specific CD8+ T cell responses measured by IFN-γ ELISPOT assay 7 days post-immunization.

FIGURE 2B

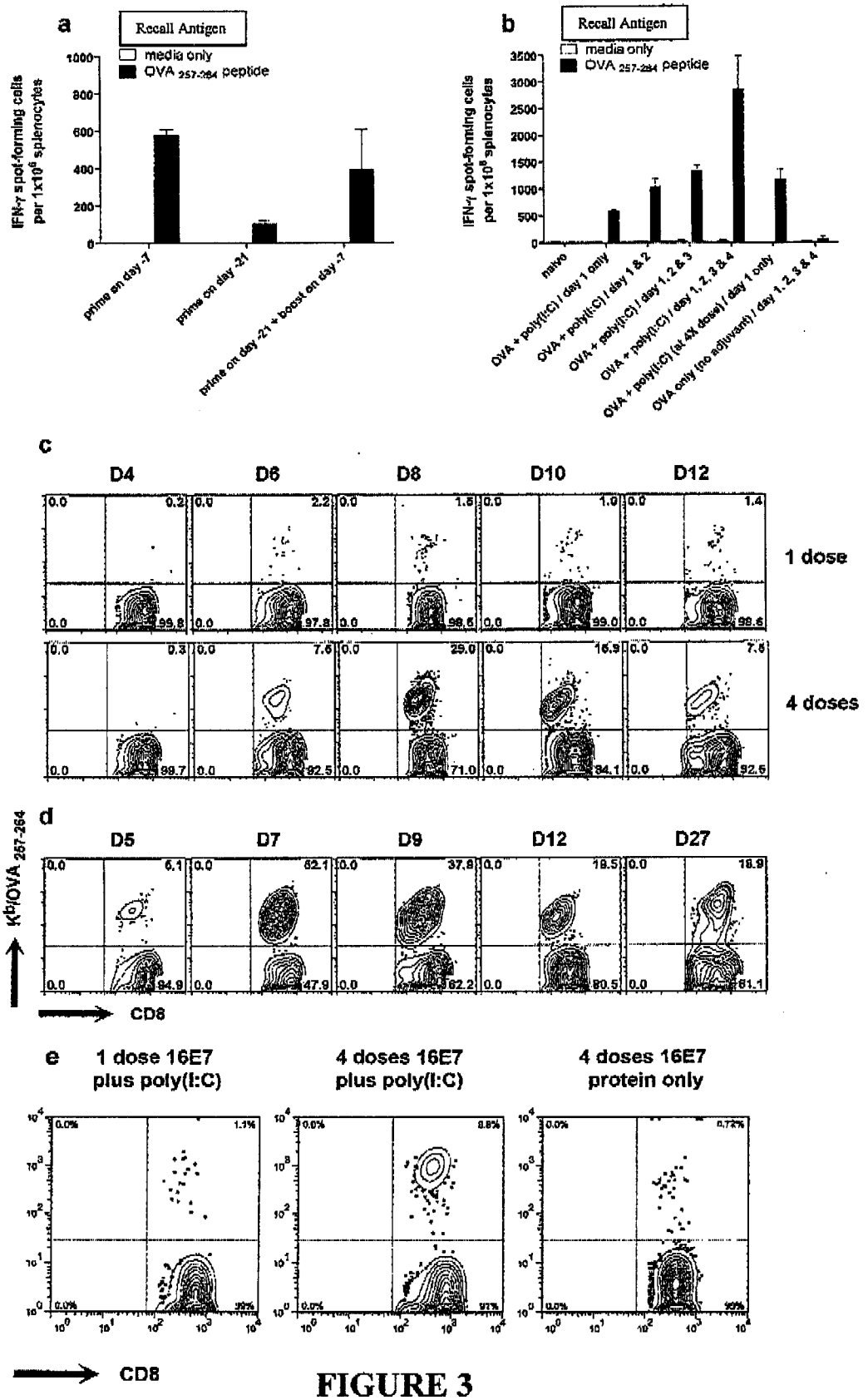

a
Recall Antigen
media only
OVA 257-264 peptide
IFN-γ spot-forming cells per 1x10^6 splenocytes
prime on day -7
prime on day -21
prime on day -21 + boost on day -7
b
Recall Antigen
media only
OVA 257-264 peptide
IFN-γ spot-forming cells per 1x10^6 splenocytes
naive
OVA + poly(I:C) / day 1 only
OVA + poly(I:C) / day 1 & 2
OVA + poly(I:C) / day 1, 2 & 3
OVA + poly(I:C) / day 1, 2, 3 & 4
OVA + poly(I:C) (at 4X dose) / day 1 only
OVA only (no adjuvant) / day 1, 2, 3 & 4
c
D4
D6
D8
D10
D12
1 dose
4 doses
d
D5
D7
D9
D12
D27
Kb/OVA 257-264
CD8
e
1 dose 16E7 plus poly(I:C)
4 doses 16E7 plus poly(I:C)
4 doses 16E7 protein only
Db/E7 49-57
CD8

FIGURE 3

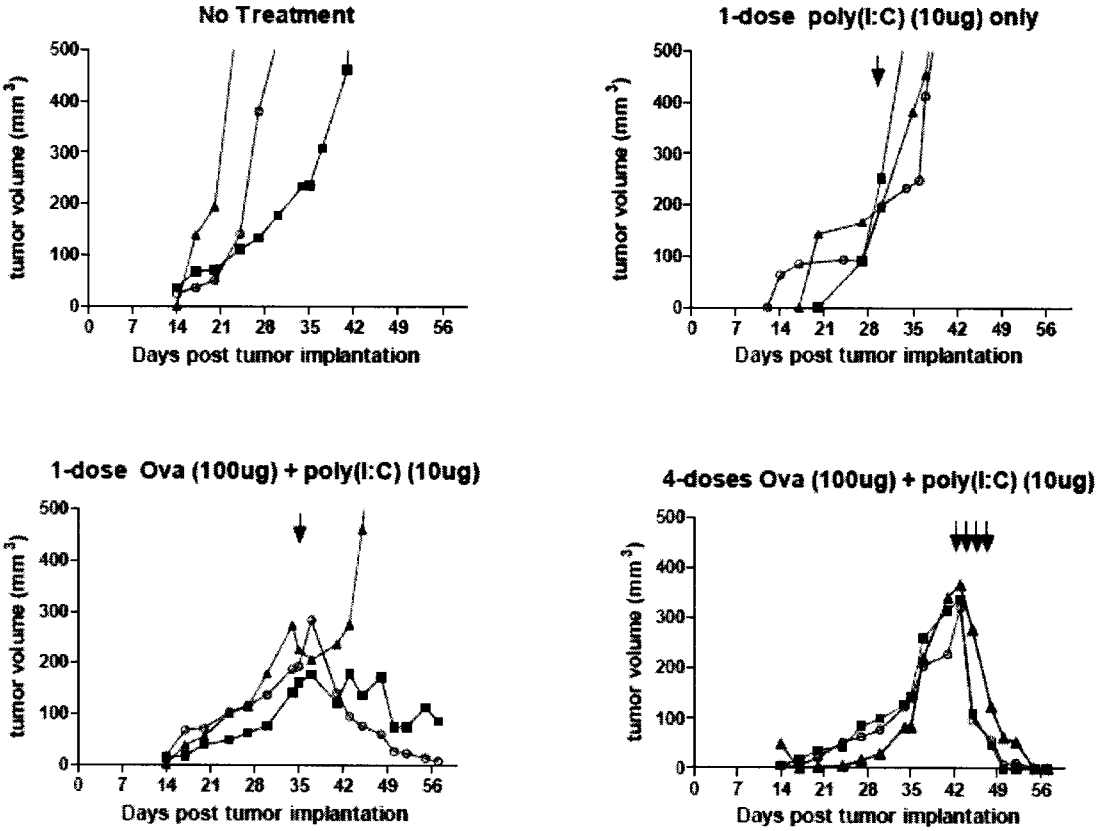

No Treatment
1-dose poly(I:C) (10ug) only
1-dose Ova (100ug) + poly(I:C) (10ug)
4-doses Ova (100ug) + poly(I:C) (10ug)
tumor volume (mm3)
Days post tumor implantation
0
100
200
300
400
500
0 7 14 21 28 35 42 49 56

FIGURE 4

08I004DW: The frequency of MHC class I-restricted $E7_{49-57}$ -specific Tcells elicted in response to immunization with one dose of Pentarix (100ug) plus polyI:C (10ug)
(measured 7 days post-immunization)

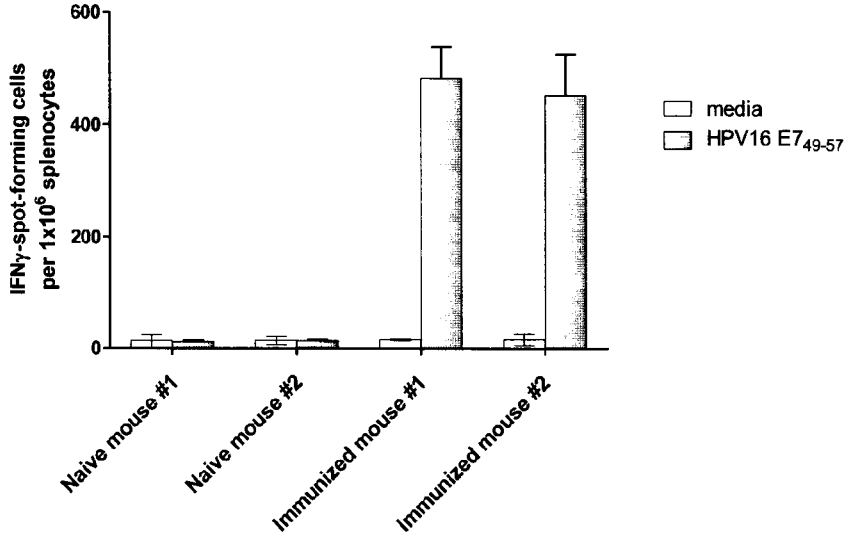


FIGURE 5A

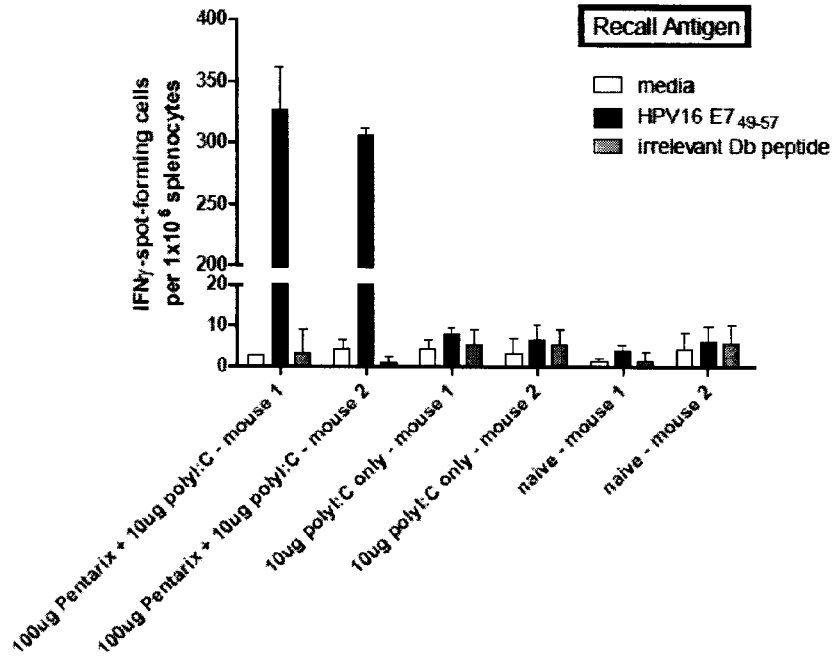


FIGURE 5B

09A004DW: The frequency of MHC class I-restricted $E7_{49-57}$ -specific Tcells elicted in response to immunization with one dose of Pentarix (100ug) plus CpG-2395 (10ug)
(measured 7 days post-immunization)

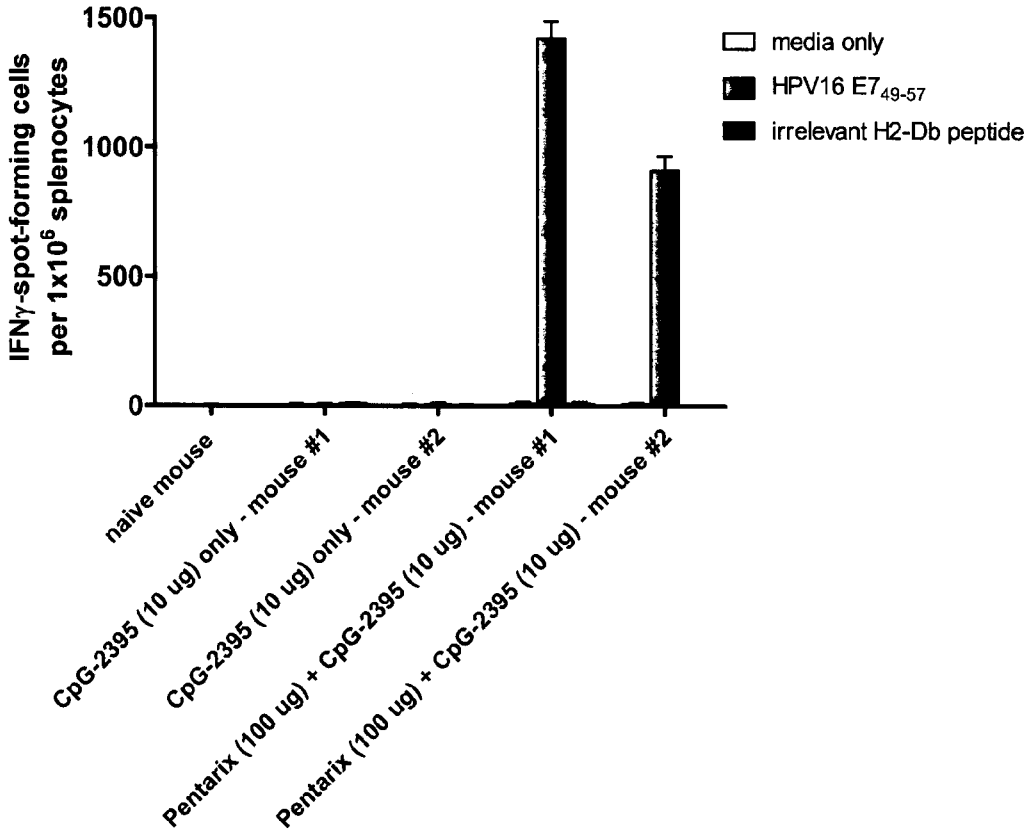


FIGURE 6A

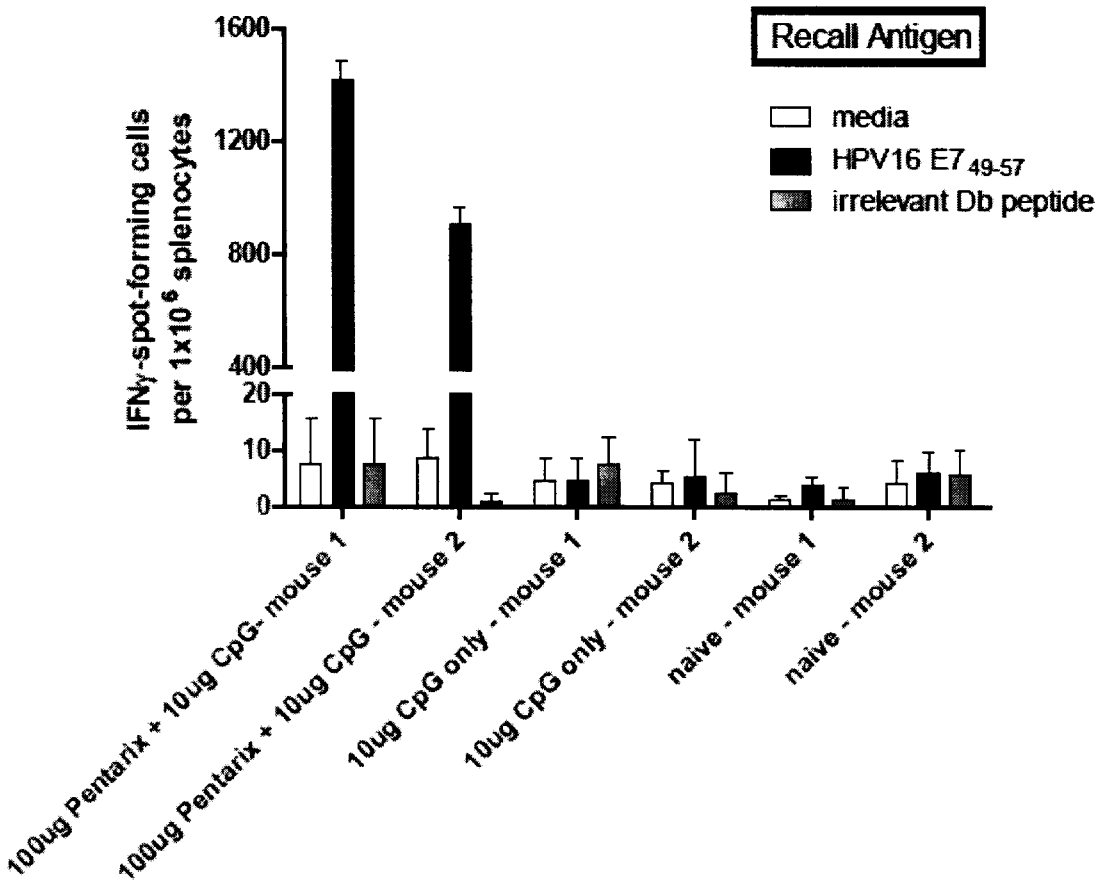

Recall Antigen
media
HPV16 E7 49-57
irrelevant Db peptide
IFNγ-spot-forming cells per 1x10^6 splenocytes
1600
1200
800
400
20
10
0
100ug Pentarix + 10ug CpG- mouse 1
100ug Pentarix + 10ug CpG - mouse 2
10ug CpG only - mouse 1
10ug CpG only - mouse 2
naive - mouse 1
naive - mouse 2

FIGURE 6B

08I004DW: The frequency of MHC class I-restricted $E7_{49-57}$ -specific Tcells elicted in response t immunization with four succesive daily doses of Pentarix (100ug) plus polyI:C (10ug)
(measured 7 days after first immunization)

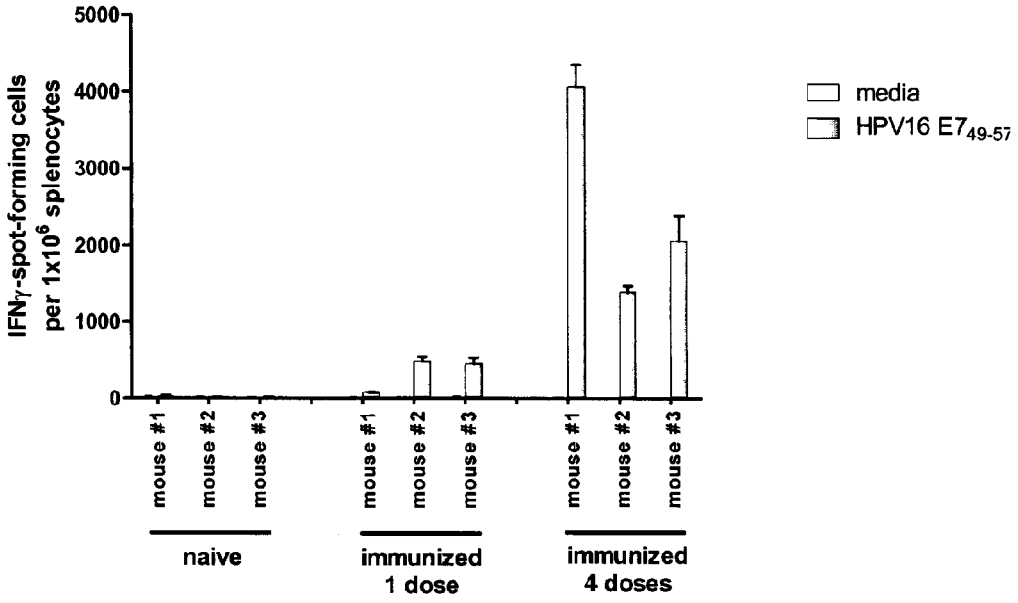


FIGURE 7A

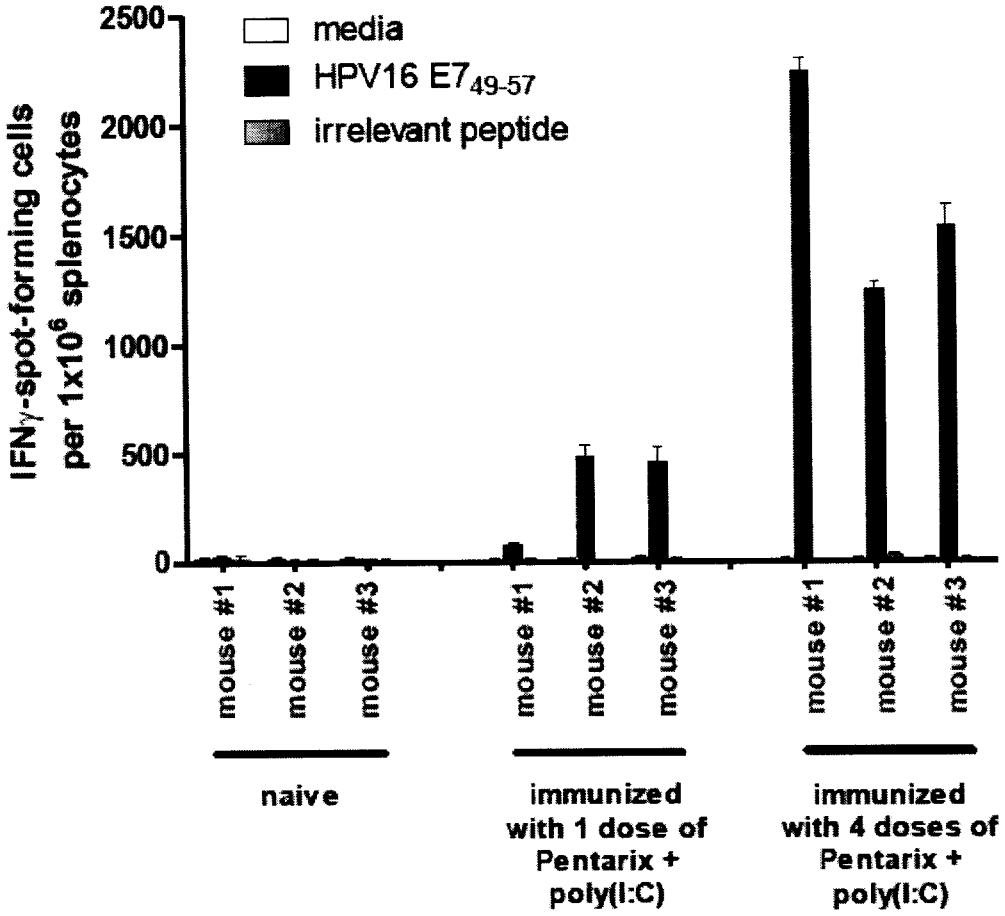


FIGURE 7B

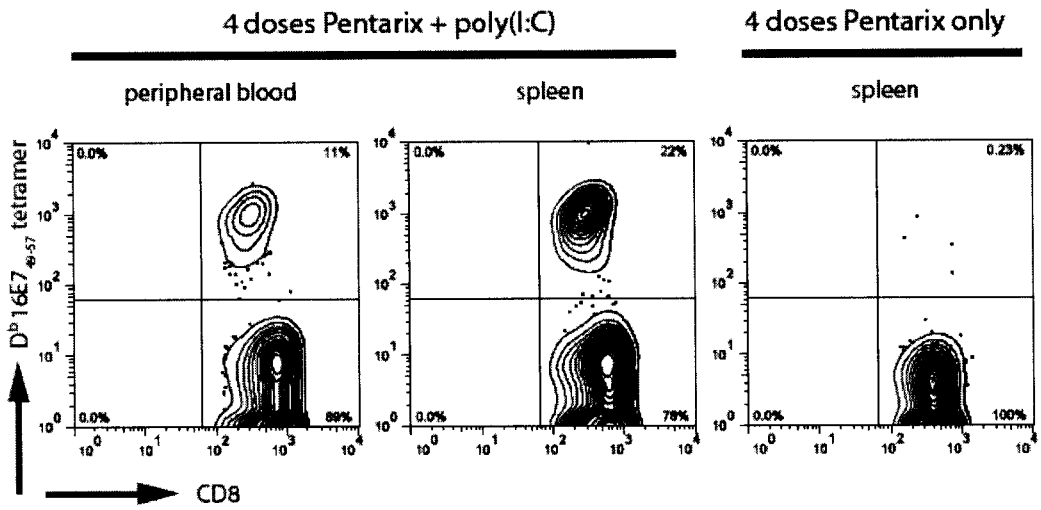


FIGURE 7C

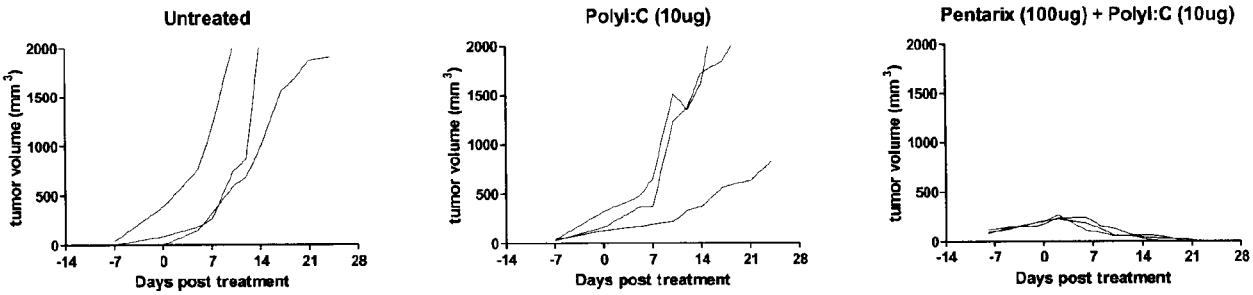


FIGURE 8A

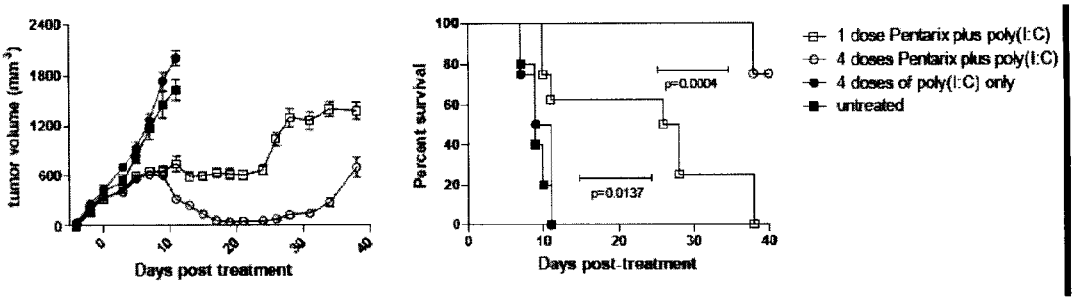


FIGURE 8B

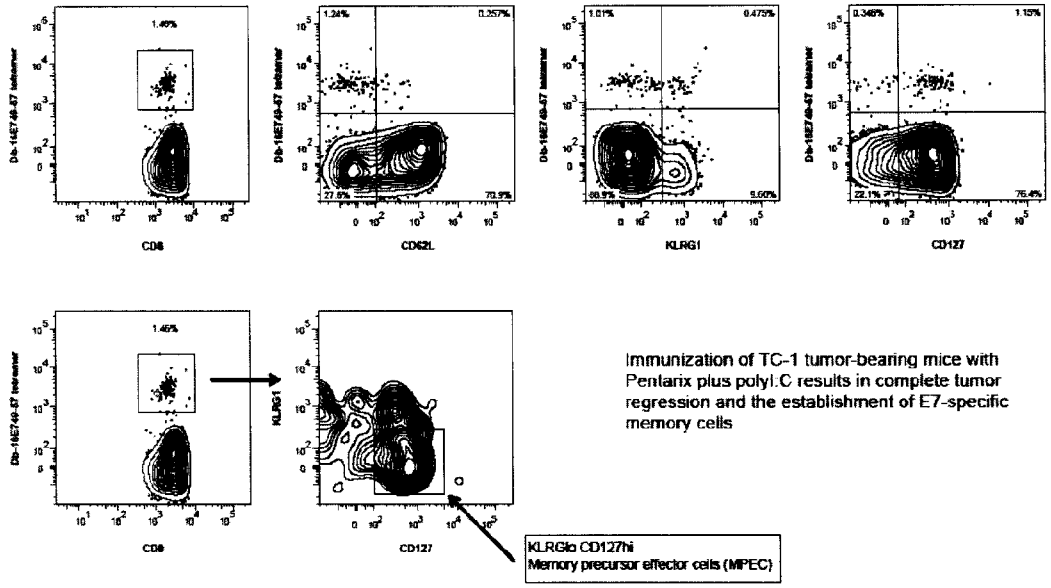


FIGURE 8C

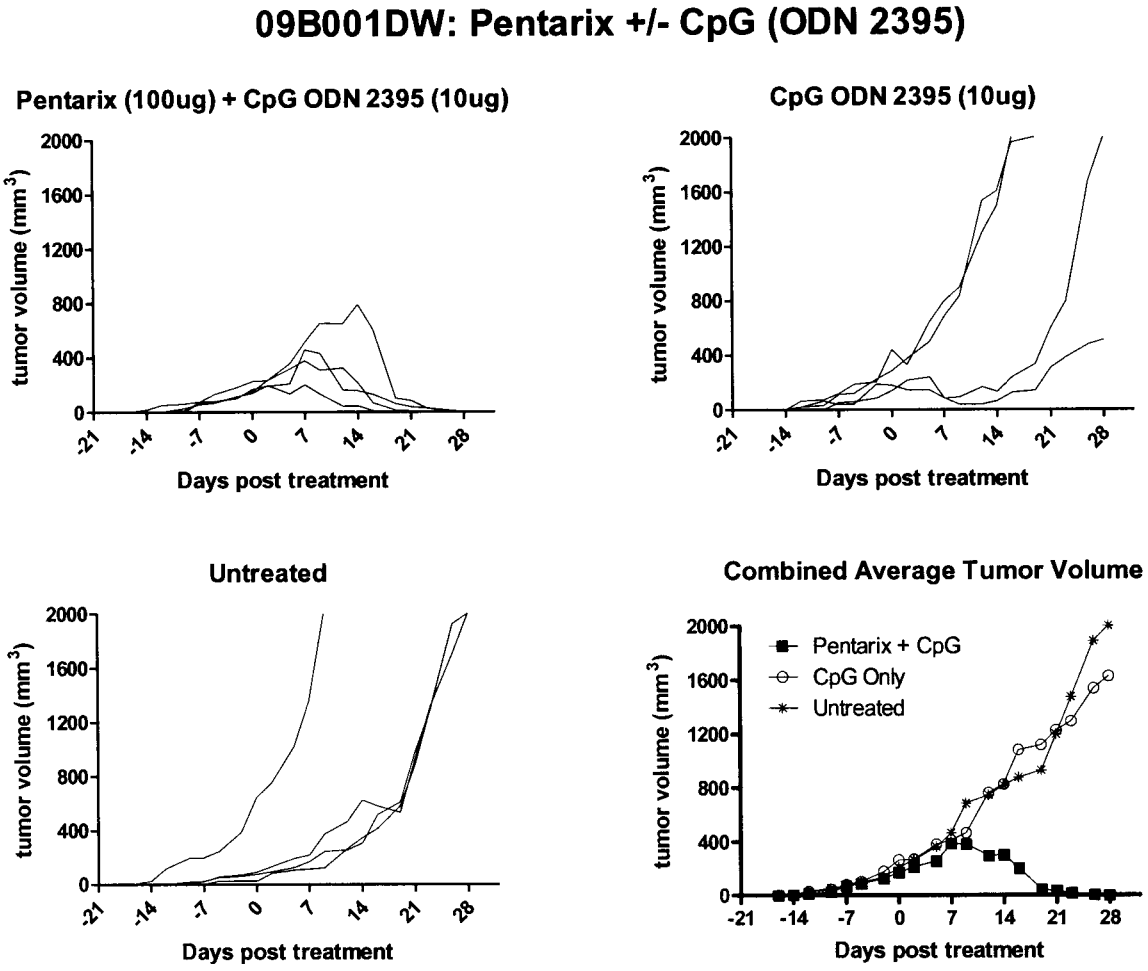

09B001DW: Pentarix +/- CpG (ODN 2395)
Pentarix (100ug) + CpG ODN 2395 (10ug)
CpG ODN 2395 (10ug)
Untreated
Combined Average Tumor Volume
tumor volume (mm3)
Days post treatment
Pentarix + CpG
CpG Only
Untreated
2000
1600
1200
800
400
0
-21
-14
-7
0
7
14
21
28

FIGURE 9A

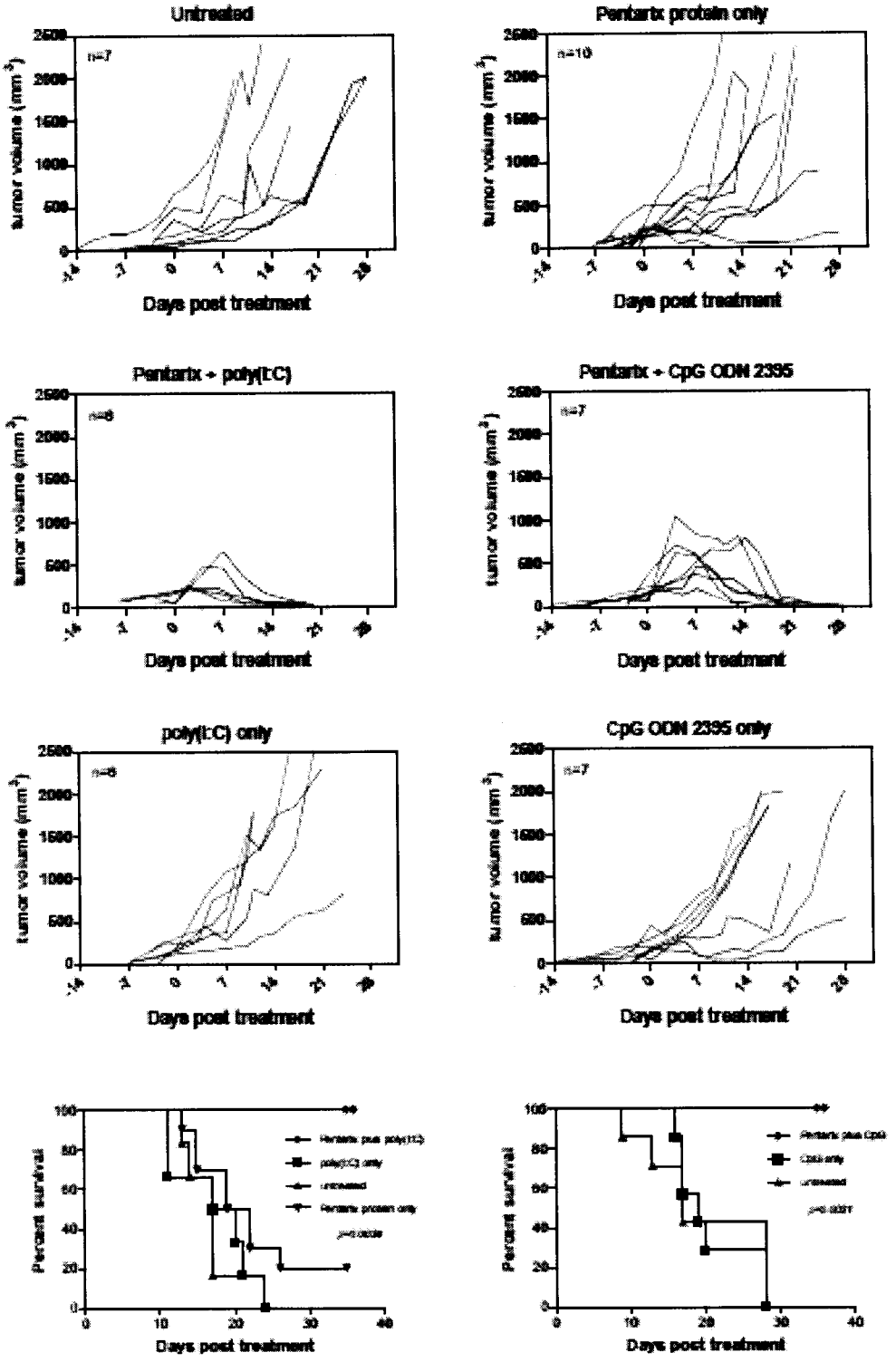

Untreated
Pentarix protein only
Pentarix + poly(I:C)
Pentarix + CpG ODN 2395
poly(I:C) only
CpG ODN 2395 only
Tumor volume (mm³)
Days post treatment
Percent survival

FIGURE 9B

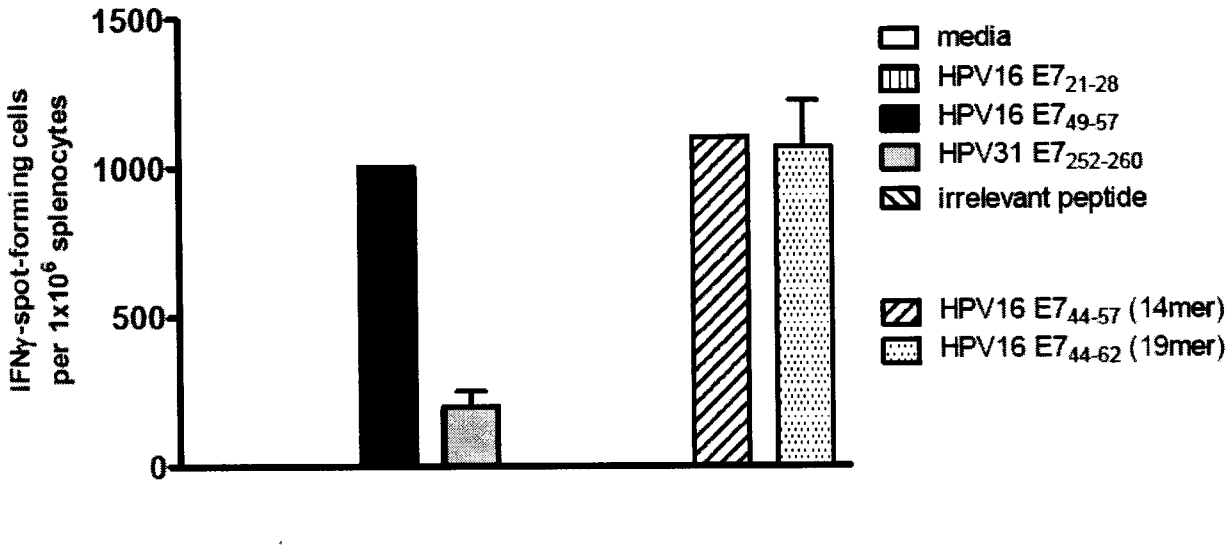

1500
1000
500
0
IFNγ-spot-forming cells per 1x10^6 splenocytes
media
HPV16 E7 21-28
HPV16 E7 49-57
HPV31 E7 252-260
irrelevant peptide
HPV16 E7 44-57 (14mer)
HPV16 E7 44-62 (19mer)

FIGURE 10A

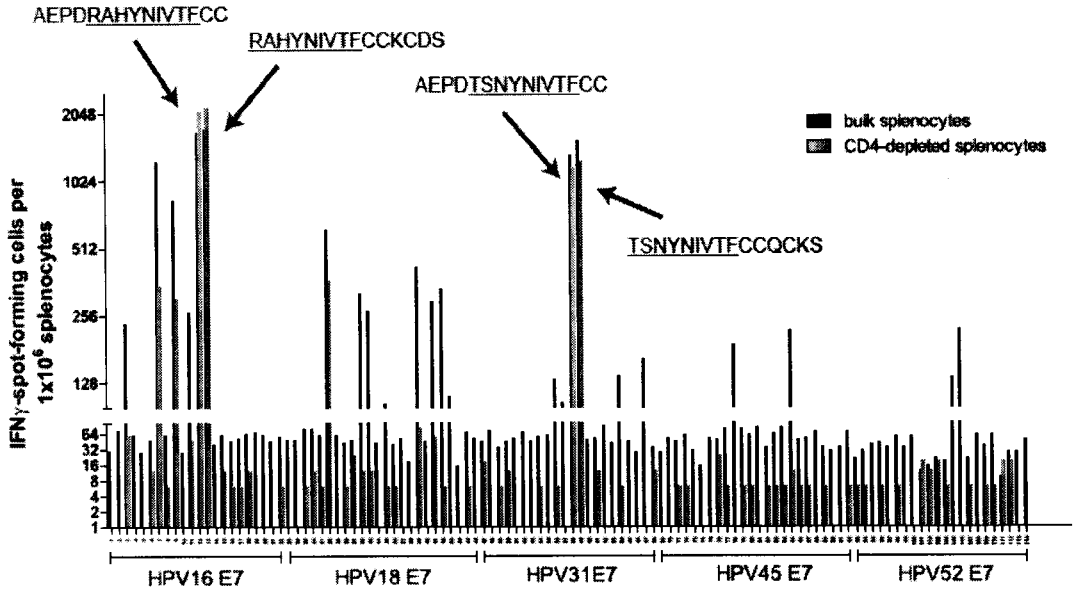


FIGURE 10B

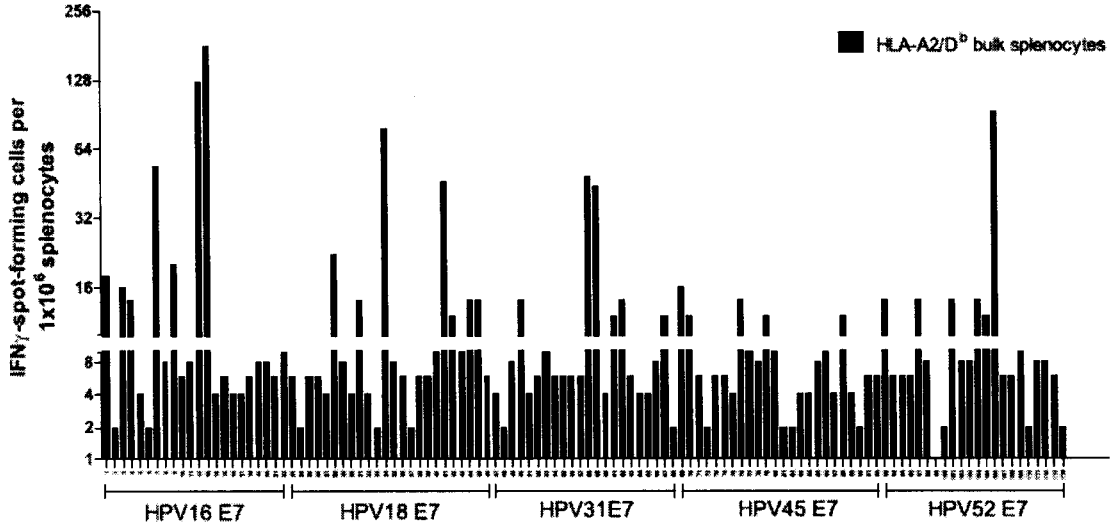


FIGURE 10C

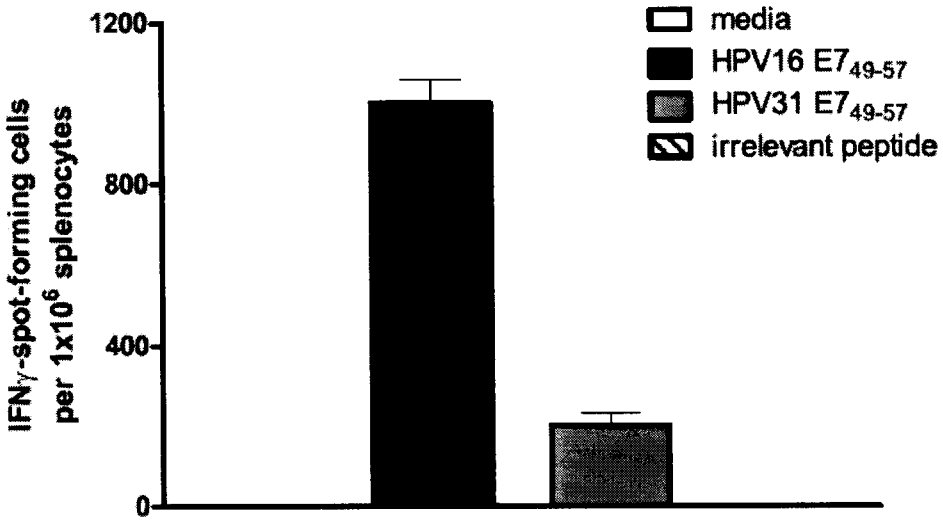

1200
800
400
0
IFNγ-spot-forming cells per 1x10^6 splenocytes
media
HPV16 E7 49-57
HPV31 E7 49-57
irrelevant peptide

FIGURE 10D

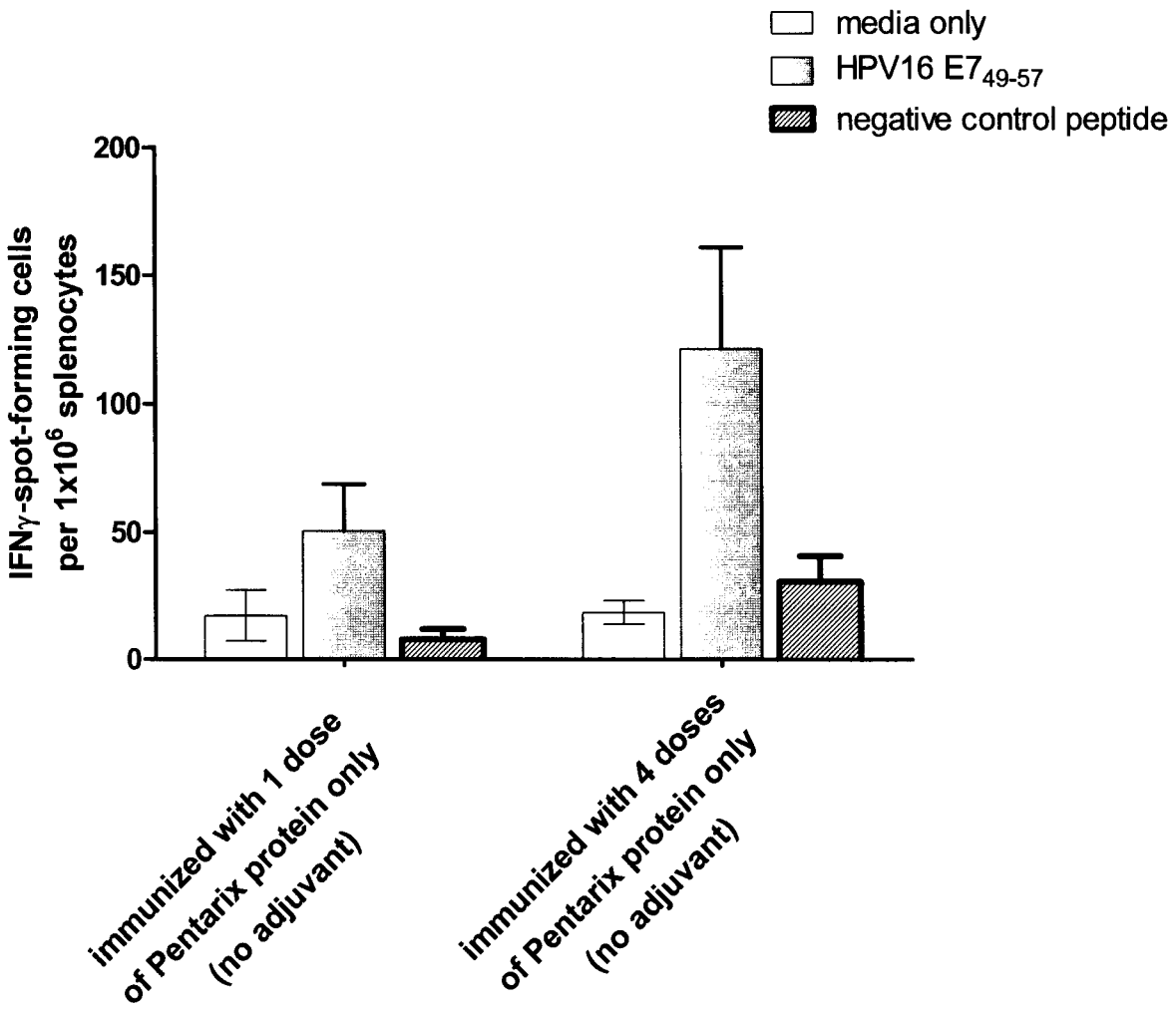

media only
HPV16 E7 49-57
negative control peptide
IFNγ-spot-forming cells per 1x10^6 splenocytes
200
150
100
50
0
immunized with 1 dose of Pentarix protein only (no adjuvant)
immunized with 4 doses of Pentarix protein only (no adjuvant)

FIGURE 11

A. HPV16 E7

NP_041326

MHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLRLCVQ

STHVDIRTLEDLLMGTLGIVCPICSQKP (SEQ ID NO: 1)

NC_001526

1 atgcatggag atacacctac attgcatgaa tatatgttag atttgcaacc agagacaact

61 gatctctact gttatgagca attaaatgac agctcagagg aggaggatga aatagatggt

121 ccagctggac aagcagaacc ggacagagcc cattacaata ttgtaacctt ttgttgcaag

181 tgtgactcta cgcttcggtt gtgcgtacaa agcacacacg tagacattcg tactttggaa

241 gacctgttaa tgggcacact aggaattgtg tgccccatct gttctcagaa accataa (SEQ ID

NO: 18)

B. HPV18 E7

NP_040311

MHGPKATLQDIVLHLEPQNEIPVDLLCHEQLSDSEEENDEIDGVNHQHLPARRAEPQRHTMLCMCCKCEA

RIELVVESSADDLRAFQQLFLNTLSFVCPWCASQQ (SEQ ID NO: 2)

NC_001357

1 atgcatggac ctaaggcaac attgcaagac attgtattgc atttagagcc ccaaaatgaa

61 attccggttg accttctatg tcacgagcaa ttaagcgact cagaggaaga aaacgatgaa

121 atagatggag ttaatcatca acatttacca gcccgacgag ccgaaccaca acgtcacaca

181 atgttgtgta tgtgttgtaa gtgtgaagcc agaattgagc tagtagtaga aagctcagca

241 gacgaccttc gagcattcca gcagctgttt ctgaacaccc tgtcctttgt gtgtccgtgg

301 tgtgcatccc agcagtaa (SEQ ID NO: 19)

FIGURE 12-1

C. HPV31 E7

AAA46951

MRGETPTLQDYVLDLQPEATDLHCYEQLPDSSDEEDVIDSPAGQAEPDTSNYNIVTFCCQCKSTLRLCVQ

STQVDIRILQELLMGSFGIVCPNCSTRL (SEQ ID NO: 3)

PPH31A

1 atgcgtggag aaacacctac gttgcaagac tatgtgttag atttgcaacc tgaggcaact

61 gacctccact gttatgagca attacccgac agctcagatg aggaggatgt catagacagt

121 ccagctggac aagcagaacc ggacacatcc aattacaata tcgttacctt ttgttgtcag

181 tgtaagtcta cacttcgttt gtgtgtacag agcacacaag tagatattcg catattgcaa

241 gagctgttaa tgggctcatt tggaatcgtg tgccccaact gttctactag actgtaa (SEQ ID

NO: 20)

D. HPV45 E7

P21736

MHGPRETLQEIVLHLEPQNELDPVDLLCYEQLSESEEENDEADGVSHAQLPARRAEPQRHKILCVCCKCD

GRIELTVESSAEDLRTLQQLFLSTLSFVCPWCATNQ (SEQ ID NO: 4)

X74479

1 atgcatggac cccgggaaac actgcaagaa attgtattgc atttggaacc tcagaatgaa

61 ttagatcctg ttgacctgtt gtgttacgag caattaagcg agtcagagga ggaaaacgat

121 gaagcagatg gagttagtca tgcacaacta ccagcccgac gagccgaacc acagcgtcac

181 aaaatttttgt gtgtatgttg taagtgtgac ggcagaattg agcttacagt agagagctcg

241 gcagaggacc ttagaacact acagcagctg tttttgagca ccttgtcctt tgtgtgtccg

301 tggtgtgcaa ctaaccaata a (SEQ ID NO: 21)

FIGURE 12-2

E. HPV52 E7

P36831

MRGDKATIKDYILDLQPETTDLHCYEQLGDSSDEEDTDGVDRPDGQAEQATSNYYIVTYCHSCDSTLRLC IHSTATDLRTLQQMLLGTLQVVCPGCARL (SEQ ID NO: 5)

X74481

1 atgcgtggag acaaagcaac tataaaagat tatatattag atctgcaacc tgaaacaact
61 gacctacact gctatgagca attaggtgac agctcagatg aggaggatac agatggtgtg
121 gaccggccag atggacaagc agaacaagcc acaagcaatt actacattgt gacatattgt
181 cacagttgtg atagcacact acggctatgc attcatagca ctgcgacgga ccttcgtact
241 ctacagcaaa tgctgttggg cacattacaa gttgtgtgcc ccggctgtgc acggctataa
(SEQ ID NO: 22)

F. HPV33 E7

AAA46959

MRGHKPTLKEYVLDLYPEPTDLYCYEQLSDSSDEDEGLDRPDGQAQPATADYYIVTCCHTCNTTVRLCVN STASDLRTIQQLLMGTVNIVCPTCAQQ (SEQ ID NO: 6)

PPH33CG

1 atgagaggac acaagccaac gttaaaggaa tatgttttag atttatatcc tgaaccaact
61 gacctatact gctatgagca attaagtgac agctcagatg aggatgaagg cttggaccgg
121 ccagatggac aagcacaacc agccacagct gattactaca ttgtaacctg ttgtcacact
181 tgtaacacca cagttcgttt atgtgtcaac agtacagcaa gtgacctacg aaccatacag
241 caactactta tgggcacagt gaatattgtg tgccctacct gtgcacaaca ataa (SEQ ID NO: 23)

FIGURE 12-3

G. HPV35 E7

AAA46967

MHGEITTLQDYVLDLEPEATDLYCYEQLCDSSEEEEDTIDGPAGQAKPDTSNYNIVTSCCKCEATLRLCV

QSTHIDIRKLEDLLMGTFGIVCPGCSQRA (SEQ ID NO: 7)

PPH35CG

1 atgcatggag aaataactac attgcaagac tatgttttag atttggaacc cgaggcaact

61 gacctatact gttatgagca attgtgtgac agctcagagg aggaggaaga tactattgac

121 ggtccagctg gacaagcaaa accagacacc tccaattata atattgtaac gtcctgttgt

181 aaatgtgagg cgacactacg tctgtgtgta cagagcacac acattgacat acgtaaattg

241 gaagatttat taatgggcac atttggaata gtgtgccccg gctgttcaca gagagcataa (SEQ

ID NO: 24)

H. HPV39 E7

AAA47051

MRGPKPTLQEIVLDLCPYNEIQPVDLVCHEQLGESEDEIDEPDHAVNHQHQLLARRDEPQRHTIQCSCCK

CNNTLQLVVEASRDTLRQLQQLFMDSLGFVCPWCATANQ (SEQ ID NO: 8)

PPHT39

1 atgcgtggac caaagcccac cttgcaggaa attgtattag atttatgtcc ttacaatgaa

61 atacagccgg ttgaccttgt atgtcacgag caattaggag agtcagagga tgaaatagat

121 gaacccgacc atgcagttaa tcaccaacat caactactag ccagacggga tgaaccacag

181 cgtcacacaa tacagtgttc gtgttgtaag tgtaacaaca cactgcagct ggtagtagaa

241 gcctcacggg atactctgcg acaactacag cagctgttta tggactcact aggatttgtg

301 tgtccgtggt gtgcaactgc aaaccagtaa (SEQ ID NO: 25)

FIGURE 12-4

I. HPV51 E7

P26558

MRGNVPQLKDVVLHLTPQTEIDLQCYEQFDSSEEEDEVDNMRDQLPERRAGQATCYRIEAPCCRCSSVVQ

LAVESSGDTLRVVQQMLMGELSLVCPCCANN (SEQ ID NO: 9)

M62877 (partial)

a tgtaccacaa ttaaaagatg tagtattgca tttaacacca cagactgaaa ttgacttgca

atgctacgag caatttgaca gctcagagga ggaggatgaa gtagataata tgcgtgacca

gctaccagaa agacgggctg gacaggctac gtgttacaga attgaagctc cgtgttgcag

gtgttcaagt gtagtacaac tggcagtgga aagcagtgga gacacccttc gcgttgtaca

gcagatgtta atgggcgaac taagcctggt ttgcccgtgt tgtgcgaaca actagcaacg gc (SEQ

ID NO: 26)

J. HPV56 E7

P36833

MHGKVPTLQDVVLELTPQTEIDLQCNEQLDSSEDEDEDEVDHLQERPQQARQAKQHTCYLIHVPCCECKF

VVQLDIQSTKEDLRVVQQLLMGALTVTCPLCASSN (SEQ ID NO: 10)

X74483

1 atgcatggta aagtaccaac gctgcaagac gttgtattag aactaacacc tcaaacagaa

61 attgacctac agtgcaatga gcaattggac agctcagagg atgaggatga ggatgaagta

121 gaccatttgc aggagcggcc acagcaagct agacaagcta aacaacatac gtgttaccta

181 atacacgtac cttgttgtga gtgtaagttt gtggtgcagt tggacattca gagtaccaaa

241 gaggacctgc gtgttgtaca acagctgctt atgggtgcgt taacagtaac gtgcccactc

301 tgcgcatcaa gtaactaa (SEQ ID NO: 27)

FIGURE 12-5

K. HPV58 E7

P26557

MRGNNPTLREYILDLHPEPTDLFCYEQLCDSSDEDEIGLDGPDGQAQPATANYYIVTCCYTCGTTVRLCI

NSTTTDVRTLQQLLMGTCTIVCPSCAQQ (SEQ ID NO: 11)

PPH58

1 atgagaggaa acaacccaac gctaagagaa tatattttag atttacatcc tgaaccaact

61 gacctattct gctatgagca attatgtgac agctcagacg aggatgaaat aggcttggac

121 gggccagatg gacaagcaca accggccaca gctaattact acattgtaac ttgttgttac

181 acttgtggca ccacggttcg tttgtgtatc aacagtacaa caaccgacgt acgaacccta

241 cagcagctgc ttatgggcac atgtaccatt gtgtgcccta gctgtgcaca gcaataa (SEQ ID

NO: 28)

L. HPV59 E7

CAA54850

MHGPKATLCDIVLDLEPQNYEEVDLVCYEQLPDSDSENEKDEPDGVNHPLLLARRAEPQRHNIVCVCCKC

NNQLQLVVETSQDGLRALQQLFMDTLSFVCPLCAANQ (SEQ ID NO: 12)

X77858

1 atgcatggac caaaagcaac actttgtgac attgttttag atttggaacc acaaaattat

61 gaggaagttg accttgtgtg ctacgagcaa ttacctgact ccgactccga gaatgaaaaa

121 gatgaaccag atggagttaa tcatcctttg ctactagcta gacgagctga accacagcgt

181 cacaacattg tgtgtgtgtg ttgtaagtgt aataatcaac ttcagctagt agtagaaacc

241 tcgcaagacg gattgcgagc cttacagcag ctgtttatgg acacactatc ctttgtgtgt

301 cctttgtgtg cagcaaacca gtaa (SEQ ID NO: 29)

FIGURE 12-6

M. HPV68 E7

P54668

MHGPKPTVQEIVLELCPYNEIQPVDLVCHEQLGDSDDEIDEPDHAVNHHQHLLLARRDEQQRHRIQCLCC

KCNKALQLVVEASRDNLRTLQQLFMDSLNFVCPWCATETQ (SEQ ID NO: 13)

X67160

1 atgcatggac caaagcccac cgtgcaggaa attgtgttag agctatgtcc atacaatgaa

61 atacagccgg ttgaccttgt atgtcacgag caattaggag attcagacga tgaaatagat

121 gaacccgacc atgcagttaa tcaccaccaa catctactac tagccagacg ggacgaacaa

181 cagcgtcaca gaattcagtg tctgtgttgt aagtgtaaca aggcactgca actagtagta

241 gaagcgtcgc gggacaacct gcggacacta caacagctgt ttatggactc actaaatttt

301 gtgtgtccgt ggtgtgcaac tgaaacccag taa (SEQ ID NO: 30)

N. HPV73 E7

CAA63883

MHGKKTTLQDITLDLKPTTEIDLTCYESLDNSEDEDETDSHLDRQAERECYRIVTDCTKCQCTVCLAIES

NKADLRVIEELLMGTLGIVCPNCSRNL (SEQ ID NO: 14)

X94165

1 atgcatggaa aaaaaacaac cttgcaggac attactttag acctgaaacc aacaaccgaa

61 attgacctta catgttacga gtcattggac aactcagagg atgaggatga aacagacagc

121 catctagaca gacaagctga acgagagtgt tacagaatag ttactgactg cacgaagtgt

181 cagtgcacag tatgccttgc cattgaaagc aacaaagctg atttaagagt gatagaagag

241 ttgcttatgg gtacactagg tattgtgtgc cccaactgtt ccagaaacct ataa (SEQ ID NO:

31)

FIGURE 12-7

O. HPV82 E7

AAK28450

MRGNVPQVKDIVLELTPQPEIDLQCYEQFDSSDEEDEVDNMRDQPARQAGQATCYRIKVQCCRCSSLLQL

AVESSGDNLRIFQQLLMGDLSLVGPCCANN (SEQ ID NO: 15)

AF293961

1 atgcgtggta atgtaccaca agtaaaggac atagtgttgg agttaacacc acaacctgaa

61 attgacttgc aatgctacga gcaatttgac agctcagacg aggaggatga agtagataat

121 atgcgtgacc agccagccag acaagctgga caggctacgt gttacagaat taaagtgcag

181 tgttgcaggt gttcaagttt gctacagttg gcagtggaaa gcagtggaga caaccttcgc

241 atatttcagc aactgttaat gggtgaccta agcctggtgg gcccgtgttg cgcgaacaac

301 taa (SEQ ID NO: 32)

P.

MGSSHHHHHHSSGLVPRGSHMHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIV
TFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKPMHGPKATLQDIVLHLEPQNEIPVDLLCHEQLS
DSEEENDEIDGVNHQHLPARRAEPQRHTMLCMCCKCEARIELVVESSADDLRAFQQLFLNTLSFVCPWCASQQMR
GETPTLQDYVLDLQPEATDLHCYEQLPDSSDEEDVIDSPAGQAEPDTSNYNIVTFCCQCKSTLRLCVQSTQVDIR
ILQELLMGSFGIVCPNCSTRLMHGPRATLQEIVLHLEPQNELDPVDLLCYEQLSESEEENDEADGVSHAQLPARR
AEPQRHKILCVCCKCDGRIELTVESSADDLRTLQQLFLSTLSFVCPWCATNQMRGDKATIKDYILDLQPETTDLH
CYEQLGDSSDEEDTDGVDRPDGQAEQATSNYYIVTYCHSCDSTLRLCIHSTATDLRTLQQMLLGTLQVVCPGCAR
LTT (SEQ ID NO: 16)

Q.

MHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVD
IRTLEDLLMGTLGIVCPICSQKPMHGPKATLQDIVLHLEPQNEIPVDLLCHEQLSDSEEENDEIDGVNHQHLPAR
RAEPQRHTMLCMCCKCEARIELVVESSADDLRAFQQLFLNTLSFVCPWCASQQMRGETPTLQDYVLDLQPEATDL
HCYEQLPDSSDEEDVIDSPAGQAEPDTSNYNIVTFCCQCKSTLRLCVQSTQVDIRILQELLMGSFGIVCPNCSTR
LMHGPRATLQEIVLHLEPQNELDPVDLLCYEQLSESEEENDEADGVSHAQLPARRAEPQRHKILCVCCKCDGRIE
LTVESSADDLRTLQQLFLSTLSFVCPWCATNQMRGDKATIKDYILDLQPETTDLHCYEQLGDSSDEEDTDGVDRP
DGQAEQATSNYYIVTYCHSCDSTLRLCIHSTATDLRTLQQMLLGTLQVVCPGCARLTT- (SEQ ID NO: 17)

FIGURE 12-8

R.

ATGCATGGCGACACCCCGACGCTGCACGAATACATGCTGGATCTGCAACCGGAGACGACGGATCTGTATTGCTAT
GAGCAGCTGAACGACAGCAGCGAAGAGGAAGATGAGATCGACGGTCCGGCGGGTCAGGCGGAGCCGGATCGTGCG
CACTACAATATCGTTACCTTTTGTTGTAAATGCGATAGCACCCTGCGTCTGTGTGTTCAGAGCACGCACGTGGAT
ATTCGTACCCTGGAGGACCTGCTGATGGGTACCCTGGGTATCGTTTGCCCGATTTGTAGCCAGAAGCCGATGCAT
GGTCCGAAAGCGACGCTGCAGGATATCGTGCTGCACCTGGAACCGCAGAATGAGATCCCGGTGGATCTGCTGTGT
CATGAGCAGCTGAGCGACAGCGAAGAGGAAAACGATGAAATCGATGGTGTGAATCACCAGCATCTGCCGGCGCGC
CGCGCGGAACCGCAACGCCATACGATGCTGTGTATGTGCTGTAAATGTGAAGCGCGTATCGAGCTGGTGGTTGAA
AGCAGCGCCGATGACCTGCGTGCGTTCCAGCAGCTGTTCCTGAACACCCTGAGCTTCGTGTGCCCGTGGTGTGCC
AGCCAACAGATGCGTGGCGAAACCCCGACCCTGCAGGACTATGTGCTGGATCTGCAGCCGGAGGCCACCGATCTG
CACTGTTACGAACAACTGCCGGACAGCAGCGATGAGGAAGACGTGATCGATAGCCCGGCGGGCCAAGCCGAACCG
GACACCAGCAATTATAATATTGTGACCTTTTGTTGCCAGTGCAAGAGCACCCTGCGTCTGTGTGTTCAGAGCACG
CAAGTTGACATTCGTATCCTGCAGGAACTGCTGATGGGTAGCTTCGGTATTGTGTGTCCGAATTGCAGCACCCGC
CTGATGCACGGTCCGCGTGCCACGCTGCAAGAGATCGTTCTGCATCTGGAACCGCAGAACGAACTGGATCCGGTT
GATCTGCTGTGTTATGAACAACTGAGCGAGAGCGAAGAAGAGAATGATGAGGCGGATGGCGTTAGCCACGCCCAA
CTGCCGGCGCGCCGCGCGGAACCGCAGCGCCATAAAATCCTGTGCGTTTGTTGCAAGTGCGATGGCCGTATTGAA
CTGACCGTTGAAAGCAGCGCCGATGACCTGCGTACGCTGCAGCAGCTGTTTCTGAGCACGCTGAGCTTCGTTTGT
CCGTGGTGTGCGACGAATCAGATGCGCGGCGACAAAGCCACGATTAAGGATTATATTCTGGATCTGCAGCCGGAA
ACGACCGACCTGCATTGTTACGAACAGCTGGGCGATAGCAGCGACGAAGAAGATACCGACGGCGTTGACCGCCCG
GACGGTCAAGCGGAACAAGCCACCAGCAACTATTATATCGTTACGTACTGCCATAGCTGTGATAGCACCCTGCGC
CTGTGTATCCACAGCACGGCGACCGACCTGCGCACCCTGCAGCAGATGCTGCTGGGCACCCTGCAAGTTGTGTGC
CCGGGTTGCGCGCGTCTGACAACCTGA (SEQ ID NO : 33)

S.

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGCATGGCGACACC
CCGACGCTGCACGAATACATGCTGGATCTGCAACCGGAGACGACGGATCTGTATTGCTATGAGCAGCTGAACGAC
AGCAGCGAAGAGGAAGATGAGATCGACGGTCCGGCGGGTCAGGCGGAGCCGGATCGTGCGCACTACAATATCGTT
ACCTTTTGTTGTAAATGCGATAGCACCCTGCGTCTGTGTGTTCAGAGCACGCACGTGGATATTCGTACCCTGGAG
GACCTGCTGATGGGTACCCTGGGTATCGTTTGCCCGATTTGTAGCCAGAAGCCGATGCATGGTCCGAAAGCGACG
CTGCAGGATATCGTGCTGCACCTGGAACCGCAGAATGAGATCCCGGTGGATCTGCTGTGTCATGAGCAGCTGAGC
GACAGCGAAGAGGAAAACGATGAAATCGATGGTGTGAATCACCAGCATCTGCCGGCGCGCCGCGCGGAACCGCAA
CGCCATACGATGCTGTGTATGTGCTGTAAATGTGAAGCGCGTATCGAGCTGGTGGTTGAAAGCAGCGCCGATGAC
CTGCGTGCGTTCCAGCAGCTGTTCCTGAACACCCTGAGCTTCGTGTGCCCGTGGTGTGCCAGCCAACAGATGCGT
GGCGAAACCCCGACCCTGCAGGACTATGTGCTGGATCTGCAGCCGGAGGCCACCGATCTGCACTGTTACGAACAA
CTGCCGGACAGCAGCGATGAGGAAGACGTGATCGATAGCCCGGCGGGCCAAGCCGAACCGGACACCAGCAATTAT
AATATTGTGACCTTTTGTTGCCAGTGCAAGAGCACCCTGCGTCTGTGTGTTCAGAGCACGCAAGTTGACATTCGT
ATCCTGCAGGAACTGCTGATGGGTAGCTTCGGTATTGTGTGTCCGAATTGCAGCACCCGCCTGATGCACGGTCCG
CGTGCCACGCTGCAAGAGATCGTTCTGCATCTGGAACCGCAGAACGAACTGGATCCGGTTGATCTGCTGTGTTAT
GAACAACTGAGCGAGAGCGAAGAAGAGAATGATGAGGCGGATGGCGTTAGCCACGCCCAACTGCCGGCGCGCCGC
GCGGAACCGCAGCGCCATAAAATCCTGTGCGTTTGTTGCAAGTGCGATGGCCGTATTGAACTGACCGTTGAAAGC
AGCGCCGATGACCTGCGTACGCTGCAGCAGCTGTTTCTGAGCACGCTGAGCTTCGTTTGTCCGTGGTGTGCGACG
AATCAGATGCGCGGCGACAAAGCCACGATTAAGGATTATATTCTGGATCTGCAGCCGGAAACGACCGACCTGCAT
TGTTACGAACAGCTGGGCGATAGCAGCGACGAAGAAGATACCGACGGCGTTGACCGCCCGGACGGTCAAGCGGAA
CAAGCCACCAGCAACTATTATATCGTTACGTACTGCCATAGCTGTGATAGCACCCTGCGCCTGTGTATCCACAGC
ACGGCGACCGACCTGCGCACCCTGCAGCAGATGCTGCTGGGCACCCTGCAAGTTGTGTGCCCGGGTTGCGCGCGT
CTGACAACCTGA (SEQ ID NO : 34)

FIGURE 12-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA20 11/000823

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 15/37*** (2006.01) , ***A61K 39/12*** (2006.01) , ***A61P 31/20*** (2006.01) , ***A61P 37/04*** (2006.01) , ***C07K 14/025*** (2006.01) , ***C12Q 1/70*** (2006.01), ***G01N 33/564*** (2006.01) , *G01N 33/569* (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation seaixhed (classification system followed by classification symbols)
IPC: ***C12N 15/37*** (2006.01) . ***A61K 39/12*** (2006.01) . ***A6IP 31/20*** (2006.01) . ***A61P 3 7/04*** (2006.01) , ***C07K 14/025*** (2006.01) , ***C12Q 1/70*** (2006.01). ***G01N 33/564*** (2006.01) , *G01N 33/569* (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, TotalPatent, PubMed, Google Scholar, GenomeQuest
keywords:HPV16, HPV18, HPV3 l, HPV45, HPV52, E7, vaccine, antigen

C. DOCUMENTS CONSE3ERED TO BE RELEVANT

Category'*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/089792 A1 (TNSTITUT PASTEUR; PREVTLLE, X. ET AL.) 29-September-2005	8, 10, 11, 19-25, 31-38, 47-5 1
Y	example 3	26-30, 39-46
Y	WO 2007/137427 A1 (NVENTA BIOPHARMACEUTICALS CORPORATION; ROWSE ET AL.) 6-December-2007 see entire document	26-30, 39-46
P,X	WICK, D ET AL. : "A novel, broad spectrum therapeutic LTPV vaccine targeting the E7 proteins of HPV16, 18, 31, 45 and 52 that elicits potent E7-specific CD8 T cell immunity and regression of latge, established, E7-expressing TC-1 tumors." EUROGTN 20 11 Congress, 8-May-20 11, Abstract SS 18-6	
A	LIN, Y. ET AL.: "Vaccines agamst human papillomaviruses." Frontiers in Bioscience, l-.Tanuary-2007 vol. 12, pp. 246-264	

[ ] Further documents are listed in the continuation of Box C. [X] 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 application or patent but published on or after the international filing date

"L" document which may throw doubts on priority clami(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 m conflict with the application but citecTto understand the principle or theorv 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 rath one or more other such documents, such combination being obvious to a person skilled m the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 201 1 (14-10-201 1)	18 November 201 1 (18-1 1-201 1)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C I 14 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer Stephen Misener (8 19) 934-4548

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA20 11/000823

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 31-46

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 31-46 are directed to a method for treatment of the human or annual body by surgery or therapy which the hiternational Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims 1-22.

2. [X] Claim Nos. : 1-7, 9, 12-18

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

See supplemental sheet

3. [ ] Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. Ill Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This hiternational Searching Authority found multiple inventions in this international application, as follows :

See supplemental sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. : see supplemental sheet

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

Continuation of Box II:

Claims 1-7, 9 and 12-18, as directed to polypeptides or nucleic acid combinations of two or more HPV E7 antigens selected from two or more different HPV strains, fail to comply with the prescribed requirements to such an extent that a meaningful search can not be carried out (PCT Article 17(2)(a)(ii)). The claims encompass a vast number of possible combinations of E7 antigens while the specification provides support for a specific combination only, and therefore the claims are not fully supported by the description contrary to Article 6 of the PCT.

Continuation of Box III:

Group A - Claims 8, 10 and 19 (all completely); 1I and 20-5 1 (all partially) are directed to a fusion of E7 antigenic peptides, a nucleotide encoding same, constructs and compositions comprising same and therapeutic methods and uses employing same.

Group B - Claims 52-54 are directed to peptides derived from the HPV3 1 E7 protein and methods for using same in the diagnosis of HPV3 1 infection.

The only technical feature shared by the antigenic compounds and diagnostic peptides is the shared sequence of HPV3 1 E7. This sequence, however, is well known in the art from Goldsborough et al. (Virology vol. 171, pages 306-3 11 1989 "Nucleotide sequence of human papillomavirus type 31: a cervical neoplasia-associated vims."). Therefore, Groups A and B do not share a common inventive feature and lack unit}· of invention *a posteriori.*

Continuation of Box III part 4:

The international search report is restricted to claims 8, 10 and 19 (all completely): 11 and 20-51 (all partially).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA20 11/000823

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2005089792A1	29 September 2005 (29-09-2005)	AT372784T	15 September 2007 (15-09-2007)
		AU2005224036A1	29 September 2005 (29-09-2005)
		BRPI0508722A	14 August 2007 (14-08-2007)
		CA2559235A1	29 September 2005 (29-09-2005)
		CN1956730A	02 May 2007 (02-05-2007)
		DE602004008874D1	25 October 2007 (25-10-2007)
		DE602004008874T2	12 June 2008 (12-06-2008)
		DK1576967T3	2 1 January 2008 (21-01-2008)
		EP1576967A1	2 1 September 2005 (21-09-2005)
		EP1576967B1	12 September 2007 (12-09-2007)
		EP1725259A1	29 November 2006 (29-1 1-2006)
		EP2351580A1	03 August 201 1 (03-08-201 1)
		ES2293178T3	16 March 2008 (16-03-2008)
		JP2007533307A	22 November 2007 (22-1 1-2007)
		MXPA06010469A	04 July 2007 (04-07-2007)
		PT1576967E	03 December 2007 (03-12-2007)
		RU2006131598A	27 April 2008 (27-04-2008)
		SI1576967T1	29 February 2008 (29-02-2008)
		US2007072266A1	29 March 2007 (29-03-2007)
		US201 1171244A1	14 July 201 1 (14-07-201 1)
WO2007137427A1	06 December 2007 (06-12-2007)	AU2007266235A1	06 December 2007 (06-12-2007)
		CA2653474A1	06 December 2007 (06-12-2007)
		CN101489588A	22 July 2009 (22-07-2009)
		EP2032165A1	11 March 2009 (11-03-2009)
		EP2032165A4	09 September 2009 (09-09-2009)
		JP2009538836A	12 November 2009 (12-1 1-2009)
		KR20090021295A	02 March 2009 (02-03-2009)
		MX2008015293A	13 March 2009 (13-03-2009)
		RU2008151516A	10 July 2010 (10-07-2010)
		US201 1142873A1	16 June 201 1 (16-06-201 1)
		ZA200810740A	30 June 2010 (30-06-2010)