NEW POLYPEPTIDES INDUCING APOPTOSIS AND USES THEREOF

The present invention concerns the use of a polypeptide comprising the sequence X-WXₙ-Xₙ-Rₓₙ-Xₙ-Xₙ-xₚ and derivatives thereof, as an apoptosis inducer for the treatment of cancer and wherein the polypeptide comprises no more than about 50 amino acid residues of the HPV E2 protein.
NEW POLYPEPTIDES INDUCING APOPTOSIS AND USES THEREOF

The present application claims the priority of Patent Application EP07301239.5 filed on July 16, 2007, which is incorporated therein by reference.

Field of the invention

The present invention relates to cancer treatment and, more specifically, to a polypeptide inducing apoptosis for treating cancer.

Background of the invention

Apoptosis is essential for the maintenance of tissue size and cell number homeostasis of multi-cellular organisms, and apoptotic abnormalities are thought to play an important role in the development of various neoplastic diseases as well as a number of neurodegenerative diseases.

Notably, many studies have established the implication of apoptosis defaults in tumor progression. For tumor treatment, it is thus necessary to develop new apoptosis inducers.

Viruses have evolved complex strategies to interact with the cellular apoptotic machinery, and exhibit both pro- and anti-apoptotic functions. Notably, viral anti-apoptotic activities enable viruses to escape immune surveillance, while pro-apoptotic activities serve specific functions such as release of viral particles at the end of the viral vegetative cycle. Oncogenic, or "high-risk", genital Human Papillomaviruses (HPV) display well-characterized anti-apoptotic activities notably mediated by the E6 oncogene, which interferes with both p53- and Fas-mediated apoptosis by targeting p53 and FADD for proteasomal degradation (SCHIFFNER et al., Cell, vol.75, p: 495-505, 1993; FILIPPOVA et al., J. Biol. Chem., vol.279, p: 25729-25744, 2004). HPV pro-apoptotic functions are provided by the E7 oncogene (JONES et al., Virol. vol.239, p: 97-107, 1997), as well as by the viral E2 transcription factor (BLACHON et al., J. Biol. Chem., vol.280, p:36088-36098, 2005; DEMERET et al., Oncogene, vol.22, p:168-175, 2003). However, the pro-apoptotic function of E2 is restricted to the oncogenic HPV types, and not to the low-risk types which are only associated with
benign lesions, indicating a putative link between the pro-apoptotic function of E2 and the oncogenic potential of HPVs (BLACHON et al., abovementioned, 2005).

More particularly, the present inventors have found that, surprisingly, the pro-apoptotic function of E2 is mediated through a small fragment of the E2-polypeptide (12 to 14 amino acids), comprising essentially the second α-helix of the E2-polypeptide. Indeed, this particulate peptide sequence, when issued from oncogenic and non-oncogenic HPV types, is able to induce the apoptosis of the cells in which it has been internalised.

Caspase 8 is a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of this caspase plays a central role in the execution-phase of cell apoptosis. Caspase 8, as the other caspases, exists as inactive proenzymes composed of a prodomain, a large protease subunit, and a small protease subunit. Activation of caspase 8 requires a proteolytic processing at conserved internal aspartic residues to generate a heterodimeric enzyme consisting of the large and small subunits. This protein is involved in the programmed cell death induced by Fas and various apoptotic stimuli (Kischkel FC, 1995, EMBO J.14, 5579-5588). Here is shown that a fragment of the E2-polypeptide of oncogenic and non oncogenic HPV interacts with caspase 8, leading to the apoptosis of the cells in which the fragment has been introduced.

As the pro-apoptotic properties of the E2 protein were known, several methods have been proposed to induce cell death in tumours using the HPV E2 protein. However, such methods used the whole protein, and relied therefore exclusively on E2 proteins from oncogenic HPV strains (see e.g. WO 03/010192 (UNIVERSITY OF BRISTOL) and WO 98/32861 (INSTITUT PASTEUR)). In these studies, there was no disclosure of the use of a fragment of the E2-protein to induce apoptosis of cells in which it is internalised.

WO 00/01720 (HARVARD COLLEGE) discloses a method for interfering with the proliferation of cells infected by HPV. This method consists in interfering with the formation of the E1-E2 complex in the nucleus of cells infected by HPV, in order to inhibit viral DNA replication. This document discloses therefore the use of a fragment
of E2 as peptidomimetic to interfere with the formation of the E1-E2 complex in the nucleus, as an inhibitor of viral replication. However, there is no disclosure of a use of this mimetic as an apoptotic inducer on cancer cells that are – or not – infected with HPV, through the delivery of the E2 fragment in the cytoplasm of the cells.

Therefore, even if the use of the entire E2-derived polypeptide has been described as an apoptotic inducer for treating HPV-induced cancer, there was no data concerning the pro-apoptotic properties of a fragment of the E2-polypeptide, and/or its use as an apoptotic inducer for the treatment of cancer, said cancer being induced by HPV or not.

Summary of the invention

The present invention relates to the use of a polypeptide comprising the sequence $X_1WX_2X_3X_4RX_5X_6X_7X_8X_9$ and derivatives thereof, optionally associated with a pharmaceutically acceptable vehicle, as an apoptosis inducer for the treatment of cancer, wherein:

- $X_1$ is an amino acid selected in the group consisting in histidine (H), tyrosine (Y), aspartic acid (D), glutamine (Q), and leucine (L), preferably histidine (H) or tyrosine (Y);

- $X_2$ is a polar amino acid, charged or not, and selected in the group consisting in serine (S) threonine (T), lysine (K), arginine (R), asparagine (N), aspartic acid (D), cysteine (C), glycine (G), glutamine (Q), histidine (H), and glutamic acid (E);

- $X_3$ is a polar amino acid, charged or not, and selected in the group consisting in serine (S), threonine (T), asparagine (N), cysteine (C), histidine (H), leucine (L), tyrosine (Y), alanine (A), and isoleucine (I);

- $X_4$ is an amino acid selected in the group consisting in threonine (T), arginine (R), asparagine (N), alanine (A), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tryptophane (W), valine (V) and tyrosine (Y);

- $X_5$ is an amino acid selected in the group consisting in leucine (L), methionine (M), isoleucine (I), tryptophane (W), tyrosine (Y), histidine (H), glutamine (Q), lysine (K), and arginine (R);
• \( X_6 \) is an amino acid selected in the group consisting in leucine (L), and glutamic acid (E);

• \( X_7 \) is an amino acid selected in the group consisting in cysteine (C), asparagine (N), alanine (A), tyrosine (Y), serine (S), glutamine (Q), and proline (P);

• \( X_8 \) is a hydrophobic amino acid selected in the group consisting in alanine (A), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tryptophane (W), valine (V) and tyrosine (Y); and

• \( X_9 \) is a threonine or an hydrophobic amino acid selected in the group consisting in alanine (A), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tryptophane (W), valine (V) and tyrosine (Y).

wherein the polypeptide comprises no more than about 50 amino acid residues of the HPV E2 protein.

In a second aspect, the present invention relates to the use of a nucleic acid encoding for a polypeptide as defined previously, optionally associated with a pharmaceutically acceptable vehicle, in a pharmaceutical composition for the treatment of cancer in a subject.

In a third aspect, the present invention relates to the use of a vector comprising said nucleic acid, optionally associated with a pharmaceutically acceptable vehicle, in a pharmaceutical composition for the treatment of cancer in a subject.

In a forth aspect, the present invention relates to the use of a host cell genetically engineered with said nucleic acid, or with said vector, in a pharmaceutical composition for the treatment of cancer in a subject.

Finally, the present invention relates to the use of an antibody directed against said polypeptide, optionally associated with a pharmaceutically acceptable vehicle, in a pharmaceutical composition for the treatment of cancer in a subject.
Brief description of the drawings

Figure 1 shows the inhibition of E2-induced apoptosis by caspase 8 inhibitors.

Figure 2 shows that the E2-caspase 8 filament formation precedes cell death.

Figure 3 shows that GFP-E2/caspase 8 filament formation occurs without caspase activation.

Figure 4 shows that E2 caspase 8 filament formation correlates with E2 pro-apoptotic activity and is mediated through direct binding of E2 to the DED-containing pro-domain of caspase 8.

Figure 5 shows that E2-caspase 8 filament formation is inhibited by MC159 and that E2 enhances Death-Receptor mediated apoptosis.

Figure 6 shows that the second helix of the amino-terminal domain of E2 recapitulates E2-induced filament formation and cell death.

Figure 7 shows the alignment of amino-terminal domain of E2 proteins from different types of HPV.

Figure 8 shows the percentage of apoptotic cells 48 hours after the infection with plasmids encoding for different part of the E2-terminal domain of HPV18, coupled with GFP.

Figure 9 shows the percentage of apoptotic cells 48 hours after the infection with plasmids encoding for wild-type and mutated sequences of the E2-terminal domain of HPV11, HPV16 and HPV18, coupled with GFP.

Detailed description

The present invention is based on the discovery that a small polypeptidic domain of E2 protein from HPV is able to induce apoptosis.
Thus, in a first aspect, the present invention provides a polypeptide comprising the sequence $X_1WX_2X_3X_4RX_5X_6X_7X_8X_9$ (SEQ ID NO:1), and derivatives thereof, wherein:

- $X_1$ is an amino acid (i.e., an $\alpha$ amino acid), preferably an amino acid selected in the group consisting in histidine (H), tyrosine (Y), aspartic acid (D), glutamine (Q), and leucine (L), preferably histidine (H) or tyrosine (Y).

- $X_2$ is an amino acid (i.e., an $\alpha$ amino acid), preferably a polar amino acid, charged or not, and selected in the group consisting in serine (S) threonine (T), lysine (K), arginine (R), asparagine (N), aspartic acid (D), cysteine (C), glycine (G), glutamine (Q), histidine (H), and glutamic acid (E).

Preferably, $X_2$ is selected in the group consisting in threonine (T), lysine (K), arginine (R), asparagine (N), glutamine (Q), histidine (H), glutamic acid (E), most preferably lysine (K) or glutamine (Q).

- $X_3$ is an amino acid (i.e., an $\alpha$ amino acid), preferably a polar amino acid, charged or not, and selected in the group consisting in serine (S), threonine (T), asparagine (N), cysteine (C), histidine (H), leucine (L), tyrosine (Y), alanine (A), isoleucine (I) most preferably selected in the group consisting in histidine (H), leucine (L), cysteine (C), or threonine (T).

- $X_4$ is an amino acid (i.e., an $\alpha$ amino acid), preferably selected in the group consisting in threonine (T), arginine (R), asparagine (N), alanine (A), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tryptophane (W), valine (V) and tyrosine (Y).

Preferably, $X_4$ is an hydrophobic amino acid selected in the group consisting in alanine (A), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tryptophane (W), valine (V) and tyrosine (Y), most preferably selected in the group consisting in isoleucine (I), valine (V), and leucine (L).

- $X_5$ is an amino acid (i.e., an $\alpha$ amino acid), preferably selected in the group consisting in leucine (L), methionine (M), isoleucine (I), tryptophane (W),
tyrosine (Y), histidine (H), glutamine (Q), lysine (K), and arginine (R),
most preferably selected in the group consisting in leucine (L), glutamine
(Q), lysine (K), and arginine (R).

- X₆ is an amino acid (i.e., an α amino acid), preferably selected in the group
  consisting in leucine (L), and glutamic acid (E), most preferably glutamic
  acid (E).

- X₇ is an amino acid (i.e., an α amino acid), preferably selected in the group
  consisting in cysteine (C), asparagine (N), alanine (A), tyrosine (Y), serine
  (S), glutamine (Q), and proline (P), most preferably selected in the group
  consisting in cysteine (C), asparagine (N), alanine (A), serine (S), and
  glutamine (Q).

- X₈ is an amino acid (i.e., an α amino acid), preferably X₈ is an
  hydrophobic amino acid selected in the group consisting in alanine (A),
  isoleucine (I), leucine (L), methionine (M), phenylalanine (F),
  tryptophane (W), valine (V) and tyrosine (Y), most preferably selected in
  the group consisting in isoleucine (I), valine (V), and alanine (A).

- X₀ is an amino acid (i.e., an α amino acid), preferably X₀ is a threonine or
  an hydrophobic amino acid selected in the group consisting in alanine (A),
  isoleucine (I), leucine (L), methionine (M), phenylalanine (F),
  tryptophane (W), valine (V) and tyrosine (Y), most preferably X₀ is an
  hydrophobic amino, and is selected, as an example, in the group consisting
  in isoleucine (I), leucine (L), valine (V), and methionine (M).

The polypeptide of the invention is able to induce apoptosis. In view of the
example, one of skill in the art can simply identify the claimed polypeptides which
induce apoptosis.

In the specification, each amino acid is represented follows:

- Serine (S)
- Threonine (T)
• Asparagine (N)
• Glutamine (Q)
• Lysine (K)
• Arginine (R)

5 • Histidine (H)
• Glutamic acid (E)
• Aspartic acid (D)
• Cysteine (C)
• Glycine (G)

10 • Proline (P)
• Alanine (A)
• Isoleucine (I)
• Leucine (L)
• Methionine (M)

15 • Phenylalanine (F)
• Tryptophane (W)
• Valine (V)
• Tyrosine (Y)

Advantageously, the polypeptide of the invention comprises an amino acid sequence having less than 50 amino acids in length derived from the E2 protein of a HPV virus, preferably less than 25 amino acids in length, and more preferably less than 20 amino acids in length.
More advantageously, the polypeptide of the invention is less than 100 amino acids in length, preferably less than 50 amino acids in length, and more preferably less than 25 amino acids in length.

As used herein, a “derivative” or “an amino acid sequence derived from” refers to an amino acid sequence having at least 70% of identity with the reference amino acid sequence, preferably at least 80% of identity, and most preferably at least 90% of identity.

In a preferred embodiment, such “derivative” is a polypeptide wherein amino acid tryptophan (W) in second position is substituted by an other hydrophobic amino acid, which is preferably selected in the group consisting in alanine (A), isoleucine (I), valine (V), leucine (L), phenylalanine (F), cysteine (C), and methionine (M).

In another preferred embodiment, such “derivative” is a polypeptide wherein the amino acid arginine (R) in position six is substituted by any amino acid except proline (P), which is preferably selected among lysine (K), alanine (A), valine (V), leucine (L), isoleucine (I), methionine (M), cysteine (C), and glycine (G).

In still another preferred embodiment, such derivatives is a polypeptide wherein amino acid tryptophan (W) in second position and amino acid arginine (R) in position six are both substituted as previously described.

As used herein, “percentage of identity” between two amino acids sequences, means the percentage of identical amino-acids, between the two sequences to be compared, obtained with the best alignment of said sequences, this percentage being purely statistical and the differences between these two sequences being randomly spread over the amino acids sequences. As used herein, “best alignment” or “optimal alignment”, means the alignment for which the determined percentage of identity (see below) is the highest. Sequences comparison between two amino acids sequences are usually realized by comparing these sequences that have been previously align according to the best alignment; this comparison is realized on segments of comparison in order to identify and compared the local regions of similarity. The best sequences alignment to perform comparison can be realized, beside by a manual way, by using the global homology algorithm developed by SMITH and WATERMAN (Ad. App. Math., vol.2, p:482, 1981), by using the local homology algorithm developed by NEDDELEMAN and WUNSCH (J. Mol. Biol., vol.48, p:443, 1970), by using the method of similarities developed by PEARSON and LIPMAN (Proc. Natl. Acad. Sci.
USA, vol.85, p:2444, 1988), by using computer softwares using such algorithms (GAP, BESTFIT, BLAST P, BLAST N, FASTA, TFASTA in the Wisconsin Genetics software Package, Genetics Computer Group, 575 Science Dr., Madison, WI USA), by using the MUSCLE multiple alignment algorithms (Edgar, Robert C., *Nucleic Acids Research*, vol. 32, p:1792, 2004 ). To get the best local alignment, one can preferably used BLAST software, with the BLOSUM 62 matrix, or the PAM 30 matrix. The identity percentage between two sequences of amino acids is determined by comparing these two sequences optimally aligned, the amino acids sequences being able to comprise additions or deletions in respect to the reference sequence in order to get the optimal alignment between these two sequences. The percentage of identity is calculated by determining the number of identical position between these two sequences, and dividing this number by the total number of compared positions, and by multiplying the result obtained by 100 to get the percentage of identity between these two sequences.

Preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWKhMRLECAI (position 32 to 42 of SEQ ID NO:2), YWKhirLECvl (position 32 to 42 of SEQ ID NO:3), YWKLirLECAV (position 32 to 42 of SEQ ID NO:4), HWKLTrMECVL (position 32 to 42 of SEQ ID NO:5), HWKliRMECAl (position 32 to 42 of SEQ ID NO:6), HWKliRMECAl (position 32 to 42 of SEQ ID NO:7), HWRLRRICAl (position 32 to 42 of SEQ ID NO:8), YWNCvRLEnAI (position 32 to 42 of SEQ ID NO:9), YWkyvRlENAI (position 32 to 42 of SEQ ID NO:10), YWKCvRMENAI (position 32 to 42 of SEQ ID NO:11), YWKLvRmENVI (position 32 to 42 of SEQ ID NO:12), YWQLIRWENAI (position 31 to 41 of SEQ ID NO:13), YWQLIRLENAI (position 31 to 41 of SEQ ID NO:14), YWKLvRYECAI (position 32 to 42 of SEQ ID NO:15), YWKLvRYECAI (position 32 to 42 of SEQ ID NO:16), YWTLLRYEAM (position 32 to 42 of SEQ ID NO:17), YWTLRLRYEAM (position 32 to 42 of SEQ ID NO:18), YWKAVREyvL (position 32 to 42 of SEQ ID NO:19), YWKAVRQEnVI (position 32 to 42 of SEQ ID NO:20), YWKAvRHenVV (position 32 to 42 of SEQ ID NO:21), HWHyVRLEnAML (position 32 to 42 of SEQ ID NO:22), HWKCIIRyECvl (position 32 to 42 of SEQ ID NO:23), HWKCIIRyECvl (position 32 to 42 of SEQ ID NO:24), HWKlClyESvl (position 35 to 45 of SEQ ID NO:25), HWKCMRHEsVL (position 32 to 42 of SEQ ID NO:26), HWKCMRHEsVL (position 32 to 42 of SEQ ID NO:27), HWKCIIRLesVL (position 32 to 42 of SEQ ID NO:28), HWKCIIRLEAAl (position 32 to 42 of SEQ ID NO:29), HWKCIIRMEAVV (position 32 to 42 of SEQ ID NO:30), HWKCIIRLeCAI (position 32 to 42 of SEQ ID NO:31), HWKCIIRLeCAI (position 32 to 42 of SEQ ID NO:32), HWKHVRHENVL (position 32 to 42 of SEQ ID NO:33),
HWKHKVRLENVL (position 32 to 42 of SEQ ID NO:34), HWQLLRQEIQII (position 32 to 42 of SEQ ID NO:35), HWKLLLRQEIQIL (position 32 to 42 of SEQ ID NO:36), HWKLLLRQEIQVL (position 32 to 42 of SEQ ID NO:37), HWQLLRQEIQIL (position 32 to 42 of SEQ ID NO:38), HWQLLRQEIQVL (position 32 to 42 of SEQ ID NO:39), HWQTLRQEIQIL (position 32 to 42 of SEQ ID NO:40), HWKLLLRQEIQAL (position 32 to 42 of SEQ ID NO:41), HWQTLRKEAVL (position 32 to 42 of SEQ ID NO:42), HWQTLRKEPVL (position 32 to 42 of SEQ ID NO:43), HWQTLRQEAQL (position 32 to 42 of SEQ ID NO:44), HWQTLRKEAVT (position 32 to 42 of SEQ ID NO:45), HWTLRRREAVL (position 32 to 42 of SEQ ID NO:46), HWLLLRKEAVL (position 32 to 42 of SEQ ID NO:47), HWQTLRKEAVL (position 32 to 42 of SEQ ID NO:48), HWQTLRKEAVL (position 32 to 42 of SEQ ID NO:49), HWQTLRKEAVL (position 32 to 42 of SEQ ID NO:50), HWQLLRREAVL (position 32 to 42 of SEQ ID NO:51), HWQLLRREAVL (position 32 to 42 of SEQ ID NO:52), HWQLLRREAVL (position 32 to 42 of SEQ ID NO:53), HWQALRREAVL (position 32 to 42 of SEQ ID NO:54), QWNLLLRQEQVL (position 32 to 42 of SEQ ID NO:55), QWNLLLRQEQVL (position 32 to 432 of SEQ ID NO:56), YWDLNRKLYVT (position 29 to 39 of SEQ ID NO:57), YWENVRKENAI (position 32 to 42 of SEQ ID NO:58), YWENIRKENAI (position 32 to 43 of SEQ ID NO:59), YWENIRKENAI (position 32 to 42 of SEQ ID NO:60), YWESMRKEQVL (position 31 to 41 of SEQ ID NO:61), YWKAVERLENVI (position 31 to 41 of SEQ ID NO:62), and LWNLRRRAINAI (position 32 to 42 of SEQ ID NO:63).

Said sequences correspond to amino acids 36 to 46 of the E2 protein from HPV-18 and amino acid sequences at equivalent position in E2 proteins from the different HPV viruses disclosed in figure 7.

More preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWQLIRWENAI (position 31 to 41 of SEQ ID NO:13), YWKHMRLECAI (position 32 to 42 of SEQ ID NO:2), HWKCIRESVL (position 32 to 42 of SEQ ID NO:28), and HWKCMRHEESVL (position 32 to 42 of SEQ ID NO:26).

According to a preferred embodiment, the polypeptide of the invention comprises the sequence X₁WX₂X₃X₄RX₅X₆X₇X₈X₉X₁₀ (SEQ ID NO:64), and derivatives thereof, wherein:

- X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, and X₉ are as described previously,
• X₁₀ is an amino acid (i.e., an α amino acid), preferably X₁₀ is a glutamine (Q) or an hydrophobic amino acid selected in the group consisting in alanine (A), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tryptophane (W), valine (V) and tyrosine (Y), most preferably X₁₀ is an hydrophobic amino, and is selected, as an example, in the group consisting in alanine (A), leucine (L), methionine (M), phenylalanine (F), tryptophane (W), valine (V) and tyrosine (Y).

Preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWKHMRLCAIY (position 32 to 43 of SEQ ID NO:2), YWKHRILECVLM (position 32 to 43 of SEQ ID NO:3), YWKLRLECAVIF (position 32 to 43 of SEQ ID NO:4), HWKLRMECVLFL (position 32 to 43 of SEQ ID NO:5), HWKLRMECAIM (position 32 to 43 of SEQ ID NO:6), HWKLRMECALL (position 32 to 43 of SEQ ID NO:7), HWRLRRIECALY (position 32 to 43 of SEQ ID NO:8), YWNCVRLENAIY (position 32 to 43 of SEQ ID NO:9), YWKYVRLENAIF (position 32 to 43 of SEQ ID NO:10), YWVKVRMENAIF (position 32 to 43 of SEQ ID NO:11), YWKLRVMENVIL (position 32 to 43 of SEQ ID NO:12), YWQLRWEENAIF (position 31 to 42 of SEQ ID NO:13), YWQLIRLENAIL (position 31 to 42 of SEQ ID NO:14), YWKLYRECAIF (position 32 to 43 of SEQ ID NO:15), YWKLYRECAIF (position 32 to 43 of SEQ ID NO:16), YWTLVRYECAMF (position 32 to 43 of SEQ ID NO:17), YWTLLRYEAAMF (position 32 to 43 of SEQ ID NO:18), YWKAVRHEYVLY (position 32 to 43 of SEQ ID NO:19), YWKAVRQENVIY (position 32 to 43 of SEQ ID NO:20), YWKAUVRHENVVL (position 32 to 43 of SEQ ID NO:21), HWYVRLENAML (position 32 to 43 of SEQ ID NO:22), HWKIRYECVLL (position 32 to 43 of SEQ ID NO:23), HWKCIYECVLL (position 32 to 43 of SEQ ID NO:24), HWKCLRYESVLL (position 35 to 46 of SEQ ID NO:25), HWKCMRHESVLL (position 32 to 43 of SEQ ID NO:26), HWKCMRHESVLL (position 32 to 43 of SEQ ID NO:27), HWKCIRLESVLL (position 32 to 43 of SEQ ID NO:28), HWKCLRIEAAAL (position 32 to 43 of SEQ ID NO:29), HWKCLRMEAVVL (position 32 to 43 of SEQ ID NO:30), HWKCIYRCALQ (position 32 to 43 of SEQ ID NO:31), HWKCIYRCALQ (position 32 to 43 of SEQ ID NO:32), HWKHYRHENVLL (position 32 to 43 of SEQ ID NO:33), HWKHYRLENVLL (position 32 to 43 of SEQ ID NO:34), HWQLLRQEQIY (position 32 to 43 of SEQ ID NO:35), HWKLLRQEQLL (position 32 to 43 of SEQ ID NO:36), HWKLRLRQEQLL (position 32 to 43 of SEQ ID NO:37), HWQLLRQEQIYL (position 32 to 43 of SEQ ID NO:38), HWQLLRQEQVLF (position 32 to 43 of SEQ ID NO:39), HWQTLRQEQLL (position 32 to 43 of SEQ ID
NO:40), HWKLLRSEQALL (position 32 to 43 of SEQ ID NO:41), HWQTLRKEAVLL (position 32 to 43 of SEQ ID NO:42), HWQTLRKEPVLL (position 32 to 43 of SEQ ID NO:43), HWQTLRQEAVLL (position 32 to 43 of SEQ ID NO:44), HWQTLRKEAVTL (position 32 to 43 of SEQ ID NO:45), HWTLRLREAVLL (position 32 to 43 of SEQ ID NO:46), HWLLLRLKEAVLL (position 32 to 43 of SEQ ID NO:47), HWQTLRKEAVLL (position 32 to 44 of SEQ ID NO:48), HWQTLRKEAVLL (position 32 to 43 of SEQ ID NO:49), HWQTLRKEAVLL (position 32 to 43 of SEQ ID NO:50), HWQILRREAVLL (position 32 to 43 of SEQ ID NO:51), HWQILRKEAVLL (position 32 to 43 of SEQ ID NO:52), HWRLLLKEAVLL (position 32 to 43 of SEQ ID NO:53), HWQLRRLEAVLL (position 32 to 43 of SEQ ID NO:54), QWNLLRSEQVLF (position 32 to 43 of SEQ ID NO:55), QWLIRQEQVLF (position 32 to 43 of SEQ ID NO:56), YWDLNRLKLYVTY (position 29 to 40 of SEQ ID NO:57), YWENVKRENAIM (position 32 to 43 of SEQ ID NO:58), YWENIRKENAIM (position 32 to 43 of SEQ ID NO:59), YWENIRKENAIM (position 32 to 43 of SEQ ID NO:60), YWESMRKEQVLA (position 31 to 42 of SEQ ID NO:61), YWKAVRLENVIA (position 31 to 42 of SEQ ID NO:62), and LWNLLRRENAIW (position 32 to 43 of SEQ ID NO:63).

Said sequences correspond to amino acids 36 to 47 of the E2 protein from HPV-18 and amino acid sequences at equivalent position in E2 proteins from the different HPV viruses disclosed in figure 7.

More preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWQLIRWENAIF (position 31 to 42 of SEQ ID NO:13), YWKHMRLLEAIY (position 32 to 43 of SEQ ID NO:2), HWKCIRLESVLL (position 32 to 43 of SEQ ID NO:28), and HWKCMRHEVLL (position 32 to 43 of SEQ ID NO:26).

According to another preferred embodiment, the polypeptide of the invention comprises the sequence $X_1WX_2X_3X_4RX_5X_6X_7X_8X_9X_{10}X_{11}$ (SEQ ID NO:65), and derivatives thereof, wherein:

- $X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}$ are as described previously,
- $X_{11}$ is an amino acid (i.e., an $\alpha$ amino acid), preferably $X_{11}$ is a histidine (H) or an aromatic amino acid selected in the group consisting in tyrosine
(Y), phenylalanine (F), and tryptophane (W), most preferably X_{11} is selected in the group consisting in histidine (H), tyrosine (Y), and phenylalanine (F).

Preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWKhmRLeCAIYY (position 32 to 44 of SEQ ID NO:2), YWKhIRLECVLMY (position 32 to 44 of SEQ ID NO:3), YWKLIRLECAVFY (position 32 to 44 of SEQ ID NO:4), HWKLTRMECVLFY (position 32 to 44 of SEQ ID NO:5), HWKLIRMECAIMY (position 32 to 44 of SEQ ID NO:6), HWKLIRMECALLY (position 32 to 44 of SEQ ID NO:7), HWRLRRIECAALYY (position 32 to 44 of SEQ ID NO:8), YWNCVRLENAILY (position 32 to 44 of SEQ ID NO:9), YWKYVRLENAIMY (position 32 to 44 of SEQ ID NO:10), YWKCVRMENAIFY (position 32 to 44 of SEQ ID NO:11), YWKLVRMVENILF (position 32 to 44 of SEQ ID NO:12), YWQLIRWENAIF (position 32 to 43 of SEQ ID NO:13), YWQLIRLENAILF (position 31 to 43 of SEQ ID NO:14), YWKLVRYECAIFY (position 32 to 44 of SEQ ID NO:15), YWKLVRYECAIFY (position 32 to 44 of SEQ ID NO:16), YWTLLRVCAMFY (position 32 to 44 of SEQ ID NO:17), YWTLLRVEAMFY (position 32 to 44 of SEQ ID NO:18), YWKAIVRHEYVLLY (position 32 to 44 of SEQ ID NO:19), YWKAIVRQENVIYY (position 32 to 44 of SEQ ID NO:20), YWKAIVRHENVVLV (position 32 to 44 of SEQ ID NO:21), HWYIVRLENAML (position 32 to 44 of SEQ ID NO:22), HWKCIIRYECVLLH (position 32 to 44 of SEQ ID NO:23), HWKCIIRYECVLLH (position 32 to 44 of SEQ ID NO:24), HWKCLRYESVLLH (position 35 to 47 of SEQ ID NO:25), HWKCMRHESVLLY (position 32 to 44 of SEQ ID NO:26), HWKCMRHESVLLY (position 32 to 44 of SEQ ID NO:27), HWKCIRLESVLLH (position 32 to 44 of SEQ ID NO:28), HWKCLRIEAA (position 32 to 44 of SEQ ID NO:29), HWKCLMEAVVLY (position 32 to 44 of SEQ ID NO:30), HWKCIRLECAQY (position 32 to 44 of SEQ ID NO:31), HWKCIRLECALQY (position 32 to 44 of SEQ ID NO:32), HWKHVRHENVVLH (position 32 to 44 of SEQ ID NO:33), HWKHVRLENVLH (position 32 to 44 of SEQ ID NO:34), HWQLLRQEQIIYH (position 32 to 44 of SEQ ID NO:35), HWKLRRQEQILLY (position 32 to 44 of SEQ ID NO:36), HWKLRRQEQVLFY (position 32 to 44 of SEQ ID NO:37), HWQLLRQEQILF (position 32 to 44 of SEQ ID NO:38), HWQLLRQEQVLFY (position 32 to 44 of SEQ ID NO:39), HWQTLRQEQILLH (position 32 to 44 of SEQ ID NO:40), HWKLRRQEQALLF (position 32 to 44 of SEQ ID NO:41), HWQTLRKEAVL (position 32 to 44 of SEQ ID NO:42), HWQTLRKEPVVL (position 32 to 44 of SEQ ID NO:43),
HWQTLRQEAVLLY (position 32 to 44 of SEQ ID NO:44), HWQTLRKEAVTLY (position 32 to 44 of SEQ ID NO:45), HWTLRRREAVLLY (position 32 to 44 of SEQ ID NO:46), HWLLLRKEAVLLY (position 32 to 44 of SEQ ID NO:47), HWQTLRKEAVLLY (position 32 to 44 of SEQ ID NO:48), HWQTLRKEAVLLY (position 32 to 44 of SEQ ID NO:49), HWQTLRKEAVLLY (position 32 to 44 of SEQ ID NO:50), HWQILRREAVLLY (position 32 to 44 of SEQ ID NO:51), HWQILRKEAVLLY (position 32 to 44 of SEQ ID NO:52), HWRLLRKEAVLLY (position 32 to 44 of SEQ ID NO:53), HWQLLRREAVLLY (position 32 to 44 of SEQ ID NO:54), QWNLLRQEQLF (position 32 to 44 of SEQ ID NO:55), QWNLRQEQLF (position 32 to 44 of SEQ ID NO:56), YWDLNRKLTVNYY (position 29 to 41 of SEQ ID NO:57), YWENVRKENAIMH (position 32 to 44 of SEQ ID NO:58), YWENIRKENAIMH (position 32 to 44 of SEQ ID NO:59), YWENIRKENAIMH (position 32 to 44 of SEQ ID NO:60), YWESMRKEQLAF (position 31 to 43 of SEQ ID NO:61), YWKAVERLNAVY (position 31 to 43 of SEQ ID NO:62), and LWNLLRRENAIWY (position 32 to 44 of SEQ ID NO:63).

Said sequences correspond to amino acids 36 to 48 of the E2 protein from HPV-18 and amino acid sequences at equivalent position in E2 proteins from the different HPV viruses disclosed in figure 7.

More preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWQLRWEAIFF (position 31 to 43 of SEQ ID NO:13), YWKHRLECAIYY (position 32 to 44 of SEQ ID NO:2), HWKCIRLESLVLLH (position 32 to 44 of SEQ ID NO:28), and HWKCMLHESVLLY (position 32 to 44 of SEQ ID NO:26).

According to still another preferred embodiment, the polypeptide of the invention comprises the sequence $X_1WX_2X_3X_4RX_5X_6X_7X_8X_9X_{10}X_{11}X_{12}$ (SEQ ID NO:66), and derivatives thereof, wherein:

- $X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}$, and $X_{11}$ are as described previously,

- $X_{12}$ is an amino acid (i.e., an $\alpha$ amino acid), preferably $X_{12}$ is selected in the group consisting in lysine (K), threonine (T), alanine (A), tyrosine (Y), phenylalanine (F), valine (V), and most preferably $X_{12}$ is selected in the group consisting in lysine (K), tyrosine (Y), and phenylalanine (F).
Preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWKHMRLCAIYYK (position 32 to 45 of SEQ ID NO:2), YWKHIRLECVMYK (position 32 to 45 of SEQ ID NO:3), YWKLIRLECAVFYK (position 32 to 45 of SEQ ID NO:4), HWKLTRMECVLFYK (position 32 to 45 of SEQ ID NO:5), HWKLIRMECAIMYT (position 32 to 45 of SEQ ID NO:6), HWKLIRMECALLTY (position 32 to 45 of SEQ ID NO:7), HWRLRRIECAILYYK (position 32 to 45 of SEQ ID NO:8), YWNCVRLENAIYYA (position 32 to 45 of SEQ ID NO:9), YWKYVRLENAIFYA (position 32 to 45 of SEQ ID NO:10), YWKVCRMENALIFYA (position 32 to 45 of SEQ ID NO:11), YWKLVRMENVILLEFA (position 32 to 45 of SEQ ID NO:12), YWQLIRWENAIFFA (position 31 to 44 of SEQ ID NO:13), YWQLIRLENAILFT (position 31 to 44 of SEQ ID NO:14), YWKLVRYECAIFYK (position 32 to 45 of SEQ ID NO:15), YWKLVRYECAIFYK (position 32 to 45 of SEQ ID NO:16), YWTLLVRYECAMFYT (position 32 to 45 of SEQ ID NO:17), YWTLLRVEAMFYA (position 32 to 45 of SEQ ID NO:18), YWKAVRHEYVLYYK (position 32 to 45 of SEQ ID NO:19), YWKAVRQENVIYYK (position 32 to 45 of SEQ ID NO:20), YWKAVRPHENVVLYK (position 32 to 45 of SEQ ID NO:21), HWHYVRLENAMLFK (position 32 to 45 of SEQ ID NO:22), HWKCIERYECVLHKLH (position 32 to 45 of SEQ ID NO:23), HWKCIRYECVLHKLH (position 32 to 45 of SEQ ID NO:24), HWKCLRYESVLHKLH (position 35 to 48 of SEQ ID NO:25), HWKCMRHSVELLYK (position 32 to 45 of SEQ ID NO:26), HWKCMRHSVELLYK (position 32 to 45 of SEQ ID NO:27), HWKCIRLESVLHKLH (position 32 to 45 of SEQ ID NO:28), HWKCLRIEAILFLK (position 32 to 45 of SEQ ID NO:29), HWKCIRMEAVVLYK (position 32 to 45 of SEQ ID NO:30), HWKCIRLECALQYK (position 32 to 45 of SEQ ID NO:31), HWKCIRLECALQYK (position 32 to 45 of SEQ ID NO:32), HWKHVRHENVLLHLH (position 32 to 45 of SEQ ID NO:33), HWKHVRLENVLLHLH (position 32 to 45 of SEQ ID NO:34), HWQLLRQEIQIHYY (position 32 to 45 of SEQ ID NO:35), HWKLRRQEIQILYY (position 32 to 45 of SEQ ID NO:36), HWKLRRQEIQVLFYY (position 32 to 45 of SEQ ID NO:37), HWQLLRQEIQIFHY (position 32 to 45 of SEQ ID NO:38), HWQLLRQEIQFLYY (position 32 to 45 of SEQ ID NO:39), HWQTLLRQEIQHLY (position 32 to 45 of SEQ ID NO:40), HWKLRRQEIQALLFY (position 32 to 45 of SEQ ID NO:41), HWQTLLRKEAVLLYY (position 32 to 45 of SEQ ID NO:42), HWQTLLRKEPVYLYY (position 32 to 45 of SEQ ID NO:43), HWQTLLRQEAVLLYF (position 32 to 45 of SEQ ID NO:44), HWQTLLRKEAVLYF (position 32 to 45 of SEQ ID NO:45), HWTLLRREAVLHY (position 32 to 45 of SEQ ID NO:46), HWLLLRKEAVLHY (position 32 to 45 of SEQ ID NO:47), HWQTLRKEAVLHYF
(position 32 to 45 of SEQ ID NO:48), HWQTLRKEAVLLYF (position 32 to 45 of SEQ ID NO:49), HWQTLRKEAVLLYF (position 32 to 45 of SEQ ID NO:50), HWQILRREAVLLYF (position 32 to 45 of SEQ ID NO:51), HWQILRKEAVLLYF (position 32 to 45 of SEQ ID NO:52), HWRLLRKEAVLLYF (position 32 to 45 of SEQ ID NO:53), HWQALRREAVLLYY (position 32 to 45 of SEQ ID NO:54), QWNLLRQEQLFYH (position 32 to 45 of SEQ ID NO:55), QWNLRQEQLFYH (position 32 to 45 of SEQ ID NO:56), YWDLNKRKLYVTYY (position 29 to 42 of SEQ ID NO:57), YWENVRKENAIMHY (position 32 to 45 of SEQ ID NO:58), YWENIRKENAIMHY (position 32 to 45 of SEQ ID NO:59), YWENIRKENAIMHY (position 32 to 45 of SEQ ID NO:60), YWESMRKEQVLAFY (position 31 to 44 of SEQ ID NO:61), YWKAVRLENVIAYY (position 31 to 44 of SEQ ID NO:62), and LWNLLRRENAIWYV (position 32 to 45 of SEQ ID NO:63).

Said sequences correspond to amino acids 36 to 49 of the E2 protein from HPV-18 and amino acid sequences at equivalent position in E2 proteins from the different HPV viruses disclosed in figure 7.

More preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWQLIRWENAIFFA (position 31 to 44 of SEQ ID NO:13), YWKHMRLECAIYYK (position 32 to 45 of SEQ ID NO:2), HWKCIIRLESHLLHK (position 32 to 45 of SEQ ID NO:28), and HWKCMRHESVLLLYK (position 32 to 45 of SEQ ID NO:26).

According to another preferred embodiment, the polypeptide of the invention comprises the sequence $X_1WX_2X_3X_4RX_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ (SEQ ID NO:67), and derivatives thereof, wherein:

- $X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11},$ and $X_{12}$ are as described previously,

- $X_{13}$ is an amino acid (i.e., an $\alpha$ amino acid), preferably $X_{13}$ is an hydrophobic amino acid selected in the group consisting in alanine (A), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tryptophane (W), valine (V) and tyrosine (Y), most preferably $X_{13}$ is an alanine (A) or a leucine (L).

Preferably, $X_{13}$ is an alanine (A).
Preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWKHMRLLECAIYYKA (position 32 to 46 of SEQ ID NO:2), YWKHIRLECVLMYKA (position 32 to 46 of SEQ ID NO:3), YWKLIRLECAVFYKA (position 32 to 46 of SEQ ID NO:4),

HWKLTRMECVLFYKA (position 32 to 46 of SEQ ID NO:5),
HWKLIRMECAIMYTA (position 32 to 46 of SEQ ID NO:6),
HWKLIRMECALLYTA (position 32 to 46 of SEQ ID NO:7),
HWRLRRIECALYYKA (position 32 to 46 of SEQ ID NO:8),
YWNCVRLENAIYYAA (position 32 to 46 of SEQ ID NO:9),

YWKYVRLENAIYYAA (position 32 to 46 of SEQ ID NO:10),
YWKCVRMENAIYYAA (position 32 to 46 of SEQ ID NO:11),
YWKLVRMENVILFAA (position 32 to 46 of SEQ ID NO:12),
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SEQ ID NO:15), YWKLVRYECAIFYKA (position 32 to 46 of SEQ ID NO:16),
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YWKAVRQENVIYYAA (position 32 to 46 of SEQ ID NO:20),

YWKAVERHEVVLKAA (position 32 to 46 of SEQ ID NO:21),
HWHYVRLENAMLFAA (position 32 to 46 of SEQ ID NO:22),
HKCIRCIECVLHHKA (position 32 to 46 of SEQ ID NO:23),
HWCIRCIECVLHHKA (position 32 to 46 of SEQ ID NO:24),
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HWQTLRKEPVLLYYYA (position 32 to 46 of SEQ ID NO:43),
HWQTLRQEAVLYYYA (position 32 to 46 of SEQ ID NO:44),
HWQTLRKEAVTLYYYA (position 32 to 46 of SEQ ID NO:45),
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QWLIRQEQLFYAA (position 32 to 46 of SEQ ID NO:56),
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YWENVKRAIMHYA (position 32 to 46 of SEQ ID NO:58),
YWENVKRAIMHYFA (position 32 to 46 of SEQ ID NO:59),
YWENVKRAIMHYA (position 32 to 46 of SEQ ID NO:60),
YWESMRKEVLYFA (position 31 to 45 of SEQ ID NO:61),
YWKAVERLNNVYYA (position 31 to 45 of SEQ ID NO:62), and
LWNLLRRENAYWVL (position 32 to 46 of SEQ ID NO:63).

Said sequences correspond to amino acids 36 to 50 of the E2 protein from HPV-18 and amino acid sequences at equivalent position in E2 proteins from the different HPV viruses disclosed in figure 7.

More preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWQLRWEAAIFFAA (position 31 to 45 of SEQ ID NO:13), YWKHMRLCAIYYKA (position 32 to 46 of SEQ ID NO:2), HWKCPRLAEVLLHKA (position 32 to 46 of SEQ ID NO:28), and HWKCMRMHLSSVLLYKA (position 32 to 46 of SEQ ID NO:26).
According to another preferred embodiment, the polypeptide of the invention comprises the sequence $X_1WX_2X_3X_4RX_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}$ (SEQ ID NO:68), and derivatives thereof, wherein:

- $X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12}$, and $X_{13}$ are as described previously,

- $X_{14}$ is an amino acid (i.e., an $\alpha$ amino acid), preferably $X_{14}$ is a positively charged amino acid selected in the group consisting in lysine (K), arginine (R), and histidine (H), and most preferably, $X_{14}$ is a lysine (K) or an arginine (R).

Preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWKHMRLCAIYVKAR (position 32 to 47 of SEQ ID NO:2), YWKHIRLECVLHYKAR (position 32 to 47 of SEQ ID NO:3), YWKLMRLCAVYFKAR (position 32 to 47 of SEQ ID NO:4), HWKLTRMELVFYKAK (position 32 to 47 of SEQ ID NO:5), HWKLIRMCAIMYTAR (position 32 to 47 of SEQ ID NO:6), HWKLIRMCALLYTAK (position 32 to 47 of SEQ ID NO:7), HWRLRIECALEYKAK (position 32 to 47 of SEQ ID NO:8), YWNKVRLENAIYKAR (position 32 to 47 of SEQ ID NO:9), YWKYVRLENAIYKAR (position 32 to 47 of SEQ ID NO:10), YWKCVRMENAIFYKAR (position 32 to 47 of SEQ ID NO:11), YWKLVRMENVILAAR (position 32 to 47 of SEQ ID NO:12), YWQLIRWENAIVFAAR (position 31 to 46 of SEQ ID NO:13), YWQLIRLENAILFTAR (position 31 to 46 of SEQ ID NO:14), YWKLVRVCAIFYKAR (position 32 to 47 of SEQ ID NO:15), YWKLVRVCAIFYKAR (position 32 to 47 of SEQ ID NO:16), YWTLLRYEAMFYAAR (position 32 to 47 of SEQ ID NO:17), YWKLVRVYECAMFYTAR (position 32 to 47 of SEQ ID NO:18), YWKAVRHEYVLYKAR (position 32 to 47 of SEQ ID NO:19), YWKAVRQENVYKKAR (position 32 to 47 of SEQ ID NO:20), YWKAVRQENVYKAR (position 32 to 47 of SEQ ID NO:21), HWHYVRLENMLFKAR (position 32 to 47 of SEQ ID NO:22), HWKCIRYECVLLHAK (position 32 to 47 of SEQ ID NO:23), HWKCIRYECVLLHAK (position 32 to 47 of SEQ ID NO:24), HWKCIRYECVLLHAK (position 35 to 50 of SEQ ID NO:25),
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YWKAVRLENVIAYYAR (position 31 to 46 of SEQ ID NO:62), and LWNLLRRENAIWYVLR (position 32 to 47 of SEQ ID NO:63).

Said sequences correspond to amino acids 36 to 51 of the E2 protein from HPV-18 and amino acid sequences at equivalent position in E2 proteins from the different HPV viruses disclosed in figure 7.

More preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWQLIRWENAIIFFAAAR (position 31 to 46 of SEQ ID NO:13), YWKMHRLECAIIYYKAR (position 32 to 47 of SEQ ID NO:2), HWCRCIRLESVLLHKAK (position 32 to 47 of SEQ ID NO:28), and HWKCMREJHSVLLYKAK (position 32 to 47 of SEQ ID NO:26).

According to another preferred embodiment, the polypeptide of the invention comprises the sequence X1WX2X3X4RX5X6X7X8X9X10X11X12X13X14X15 (SEQ ID NO:69), and derivatives thereof, wherein:

- X1, X2, X3, X4, X5, X6, X7, X8, X9, X10, X11, X12, X13, and X14 are as described previously,
- X15 is an amino acid (i.e., an α amino acid), preferably X15 is a polar amino acid selected in the group consisting in lysine (K), arginine (R), glutamine (Q), and glutamic acid (E), and most preferably, X15 is a glutamine (Q), or glutamic acid (E).

Preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWKMHRLECAIIYYKARE (position 32 to 48 of SEQ ID NO:2), YWKHRRLECVLMYKARE (position 32 to 48 of SEQ ID NO:3), YWKLIRLECAVFYKARE (position 32 to 48 of SEQ ID NO:4), HWKLTRMECVLFYKAKE (position 32 to 48 of SEQ ID NO:5), HWKLREMECAIMYTARQ (position 32 to 48 of SEQ ID NO:6), HWKLREMECALLYTAKQ (position 32 to 48 of SEQ ID NO:7), HWRLRRIECAIIYYKAK (position 32 to 48 of SEQ ID NO:8), YWNCVRLENAIIYYAARE (position 32 to 48 of SEQ ID NO:9), YWKSVRLENAIIYYAARE (position 32 to 48 of SEQ ID NO:10), YWKCVRMENAIIYYAARE (position 32 to 48 of SEQ ID NO:11),
YWKLVRMENVILFAARE (position 32 to 48 of SEQ ID NO:12),
YWQLIRWENAIFFAARE (position 31 to 47 of SEQ ID NO:13),
YWQLIRLENAILFTARE (position 31 to 47 of SEQ ID NO:14),
YWKLVRYECAIFYKARE (position 32 to 48 of SEQ ID NO:15),
YWKLVRYECAIFYKARE (position 32 to 48 of SEQ ID NO:16),
YWTLVRYECAMFTARE (position 32 to 48 of SEQ ID NO:17),
YWTLRLEYAMFYAARE (position 32 to 48 of SEQ ID NO:18),
YWKAVRHEVLYYKARE (position 32 to 48 of SEQ ID NO:19),
YWKAVRQENVYIYKARE (position 32 to 48 of SEQ ID NO:20),
YWKAVRHENVYLYKARQ (position 32 to 48 of SEQ ID NO:21),
HWHYVRLENAMLKARQ (position 32 to 48 of SEQ ID NO:22),
HWKCIRYECVLLHKAQK (position 32 to 48 of SEQ ID NO:23),
HWKCIRYECVLLHKAQK (position 32 to 48 of SEQ ID NO:24),
HWKCIRYECVLLHKAQK (position 35 to 51 of SEQ ID NO:25),
HWKCIRMRHESVLYKAKQ (position 32 to 48 of SEQ ID NO:26),
HWKCIRMRHESVLYKAKQ (position 32 to 48 of SEQ ID NO:27),
HWKCIRLESVLLHKAQK (position 32 to 48 of SEQ ID NO:28),
HWKCLRISEAALLKARE (position 32 to 48 of SEQ ID NO:29),
HWKCLRMEAVVLYKARQ (position 32 to 48 of SEQ ID NO:30),
HWKCIRLECALQYKARE (position 32 to 48 of SEQ ID NO:31),
HWKCIRLECALQYKARE (position 32 to 48 of SEQ ID NO:32),
HWKHVRHENVLLHKARE (position 32 to 48 of SEQ ID NO:33),
HWKHVRLENVLLHKARE (position 32 to 48 of SEQ ID NO:34),
HWQLLRQEIQIYHYARR (position 32 to 48 of SEQ ID NO:35),
HWKLRRQEIQILYYARK (position 32 to 48 of SEQ ID NO:36),
HWKLRRQEIQVLYYARR (position 32 to 48 of SEQ ID NO:37),
HWQLRRQEIQILHYARK (position 32 to 48 of SEQ ID NO:38),
HWQLRRQEIQVLYYARK (position 32 to 48 of SEQ ID NO:39),
HWQTRLRQEIQILHYARK (position 32 to 48 of SEQ ID NO:40),
HWKLRRQEIQALLFFARK (position 32 to 48 of SEQ ID NO:41),
HWQTRLREAVLILYARQ (position 32 to 48 of SEQ ID NO:42),
HWQLRRKEPVLILYARE (position 32 to 48 of SEQ ID NO:43),
HWQTRLREAVLILYARQ (position 32 to 48 of SEQ ID NO:44),
HWQTRLREAVLILYARQ (position 32 to 48 of SEQ ID NO:45),
HWQTRLREAVLILYARQ (position 32 to 48 of SEQ ID NO:46),
HWQTRLREAVLILYARQ (position 32 to 48 of SEQ ID NO:47),
HWQTRLREAVLILYARQ (position 32 to 48 of SEQ ID NO:48),
Said sequences correspond to amino acids 36 to 52 of the E2 protein from HPV-18 and amino acid sequences at equivalent position in E2 proteins from the different HPV viruses disclosed in figure 7.

More preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWQLRWEAIFFAARE (position 31 to 47 of SEQ ID NO:13), YWKHMRLEAIYYKARE (position 32 to 48 of SEQ ID NO:2), HWKCIRLESVLHAKQ (position 32 to 48 of SEQ ID NO:28), and HWKCMRHESSVLKYAKQ (position 32 to 48 of SEQ ID NO:26).

According to another preferred embodiment, the polypeptide of the invention comprises the sequence X₁WX₂X₃X₄RX₅X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃X₁₄X₁₅X₁₆ (SEQ ID NO:70), and derivatives thereof, wherein:

- X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, X₁₀, X₁₁, X₁₂, X₁₃, X₁₄, and X₁₅ are as described previously,
- X₁₆ is an amino acid (i.e., an α amino acid), preferably X₁₅ is selected in the group consisting in lysine (K), arginine (R), glutamine (Q), glutamic acid (E), methionine (M), leucine (L), glycine (G), histidine (H), alanine (A), and asparagine (N).
Preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWKMHRLECAIYYKAREM (position 32 to 49 of SEQ ID NO:2), YWKHIRECVLMYYKAREM (position 32 to 49 of SEQ ID NO:3), YWKLIRLECAVFYKAREM (position 32 to 49 of SEQ ID NO:4), HWKLTRMECVLFYYKAKEL (position 32 to 49 of SEQ ID NO:5), HWKLIRMECAIMYTARQM (position 32 to 49 of SEQ ID NO:6), HWKLIRMECALLYTAKQM (position 32 to 49 of SEQ ID NO:7), HWRLRRIECALYYKAKEL (position 32 to 49 of SEQ ID NO:8), YWNCVRLENAIYYAARRER (position 32 to 49 of SEQ ID NO:9), YWKYVRLENAYFAAARER (position 32 to 49 of SEQ ID NO:10), YWKCVRMENAIFYAARER (position 32 to 49 of SEQ ID NO:11), YWKLVREMENVILFAAREN (position 32 to 49 of SEQ ID NO:12), YWQLIRWENAIFFAAREH (position 31 to 48 of SEQ ID NO:13), YWQLIRLENAILFTAREH (position 31 to 48 of SEQ ID NO:14), YWKLIVRYECAIFYKAREG (position 32 to 49 of SEQ ID NO:15), YWKLIVRYECAIFYKAREG (position 32 to 49 of SEQ ID NO:16), YWTLVRYECAMFYARER (position 32 to 49 of SEQ ID NO:17), YWTLRLYEEAMFYAARER (position 32 to 49 of SEQ ID NO:18), YWKAVRHEYVLYKAREN (position 32 to 49 of SEQ ID NO:19), YWKAVRQPENVIIYYKAREN (position 32 to 49 of SEQ ID NO:20), YWKAVRHENVVLKARQON (position 32 to 49 of SEQ ID NO:21), HWHYVRLENAMLFKARQA (position 32 to 49 of SEQ ID NO:22), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:23), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:24), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:25), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:26), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:27), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:28), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:29), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:30), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:31), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:32), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:33), HWKCIYRCVLLHKAKQM (position 32 to 49 of SEQ ID NO:34), HWQQLRQEQIIHYARRH (position 32 to 49 of SEQ ID NO:35), HWQQLRQEQIIHYARRH (position 32 to 49 of SEQ ID NO:36), HWQQLRQEQIIHYARRH (position 32 to 49 of SEQ ID NO:37),
HWQLLRQEQILFHYARKN (position 32 to 49 of SEQ ID NO:38),
HWQLLRQEQVLFYYARKN (position 32 to 49 of SEQ ID NO:39),
HWQTLRQEQILLYHARKN (position 32 to 49 of SEQ ID NO:40),
HWKLLRQEQALLFFARKH (position 32 to 49 of SEQ ID NO:41),
5 HWQTLRKEAVLLYYAREK (position 32 to 49 of SEQ ID NO:42),
HWQTLRKEPVLLYYYAREK (position 32 to 49 of SEQ ID NO:43),
HWQTLRQEAVLFFARQK (position 32 to 49 of SEQ ID NO:44),
HWQTLRKEAVTLYFARQK (position 32 to 49 of SEQ ID NO:45),
HWTLRRREAVLLLYYARQK (position 32 to 49 of SEQ ID NO:46),
10 HWLLLRKEAVLLLYFARQK (position 32 to 49 of SEQ ID NO:47),
HWQTLRKEAVLLYFARQK (position 32 to 49 of SEQ ID NO:48),
HWQTLRKEAVLLYFARQN (position 32 to 49 of SEQ ID NO:49),
HWQTLRKEAVLLYFARQH (position 32 to 49 of SEQ ID NO:50),
HWQLLRREAVLLYFARQK (position 32 to 49 of SEQ ID NO:51),
15 HWQILRKEAVLLYFARRK (position 32 to 49 of SEQ ID NO:52),
HWRLLRKEAVLLYFARQN (position 32 to 49 of SEQ ID NO:53),
HWQALRREAVLFFHYARKK (position 32 to 49 of SEQ ID NO:54),
QWNLLRQEQVLFHARKK (position 32 to 49 of SEQ ID NO:55),
QWNLIRQEQLFHFARKN (position 32 to 49 of SEQ ID NO:56),
20 YWDLNRKLYVTTYYARKE (position 29 to 46 of SEQ ID NO:57),
YWENVKRNAIMHYARKK (position 32 to 49 of SEQ ID NO:58),
YWERNRKENAIMFHKAKQ (position 32 to 49 of SEQ ID NO:59),
YWERNRKENAIMHYARKK (position 32 to 49 of SEQ ID NO:60),
YWESMRKEVQVLAFYAKKE (position 31 to 48 of SEQ ID NO:61),
25 YWKAVLRLENVIAYYARKE (position 31 to 48 of SEQ ID NO:62), and
LWNLLRRENAIWHYVLQRE (position 32 to 49 of SEQ ID NO:63).

Said sequences correspond to amino acids 36 to 53 of the E2 protein from HPV-18 and amino acid sequences at equivalent position in E2 proteins from the different HPV viruses disclosed in figure 7.

More preferably, the polypeptide of the invention comprises an amino acid sequence which is selected in the group comprising YWQLIRWENAIAFFAAREH (position 31 to 48 of SEQ ID NO:13), YWKHMRLCAIYYKAREM (position 32 to 49 of SEQ ID NO:2), HWKCIRESVLLHKAKQM (position 32 to 49 of SEQ ID NO:28), and HWKCMRHESVLLYKAKQM (position 32 to 49 of SEQ ID NO:26).
Preferably, the polypeptide of the invention also comprises an amino acid sequence coding for a cell penetrating peptide.

The term "cell penetrating peptide" (CPP) is defined as a carrier peptide that is capable of crossing biological membrane or a physiological barrier. Cell penetrating peptides are also called cell-permeable peptides, protein-transduction domains (PTD) or membrane- translocation sequences (MTS). CPPs have the ability to translocate \textit{in vitro} and/or \textit{in vivo} the mammalian cell membranes and enter into cells, and directs the apoptosis domain of interest to the cytoplasm.

It is important to note that, in cells infected with a virus, the polypeptide of the invention will not interfere with virus replication since it will not reach the nucleus of the targeted cells.

Thus, in another embodiment of the invention, the polypeptide of the invention is linked with a cell penetrating peptide which favours its internalisation into the cytoplasm but not in the nucleus of targeted cells.

Such CCP are known for the man skilled in the art. Examples are hydrophilic peptides, pore-forming peptides, RGD-containing peptides, etc... As general references on CPPs it can be cited: CELL PENETRATING PEPTIDES: PROCESSES AND APPLICATIONS, edited by UIO LANGEL (2002); or Advanced Drug Delivery Reviews (vol.57, p:489-660, 2005); or DIETZ and BAHR (Moll. Cell. Neurosci., vol.27, p:85-131, 2004).

In another embodiment of the invention, the polypeptide of the invention is derivatized at one or more backbone or side-chain points e.g. peptides, nucleic acids, carbohydrates, that enhance cellular uptake. Such modifications are known for the man skilled in the art.

In one embodiment, the membrane barrier can be overcome by associating the polypeptide of the invention in complexes with lipid formulations closely resembling the lipid composition of natural cell membranes. In particular, the polypeptide of the invention may be encapsulated in liposomes to form pharmaceutical preparations suitable for administration to living cells and, in particular, suitable for topical administration to human skin. Liposomes offer several advantages: they are non-toxic and biodegradable in composition; they display long circulation half-lives, and
recognition molecules can be readily attached to their surface for targeting to tissues. Liposomes have been described in the art as in vivo delivery vehicle (US5190762).

Other types of lipid aggregates may be used to formulate the polypeptide of the invention. Such aggregates include liposomes, unilamellar vesicles, multilamellar vesicles, micelles and the like, having particle sizes in the nanometer to micrometer range. Methods of making lipid aggregates are by now well-known in the art.

In another embodiment, the polypeptide of the invention is formulated with a positively charged synthetic (cationic) lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), in the form of liposomes or small vesicles. Another cationic lipid which can be used is the DOTMA analog, 1,2-bis(oleoyloxy)-3-(trimethyl-ammonio)propane (DOTAP) in combination with a phospholipid to form delivery vesicles.

LIPOFECTINTM and/or LIPOFECTAMINE™, commercially available reagents, can be used to deliver the polypeptide of the invention directly into cells.

Also, quaternary ammonium surfactant is another suitable lipid vesicles in the context of the invention.

In yet another embodiment of the invention, the polypeptide of the invention may have such attributes as being non-hydrolyzable, thereby increasing the stability against proteases or other physiological conditions which degrade the corresponding peptide. For example, peptide analogs can be generated using benzodiazepines, substituted gama lactam rings, C7 mimics, keto-methylene pseudopeptides, β-turn dipeptides cores, β-aminoalcohols, diaminoketones, and methyleneamino-modified. Also, several surrogates of the amide bond, including in the group of trans-olefins, fluoroalkylene, methyleneamino, phosphonamides or sulfonamides can be used in order to increase the half-life of the polypeptide.

In a second aspect the present invention relates to a nucleic acid encoding for a polypeptide as described above.

Said nucleic acid corresponds to RNA or DNA, preferably to DNA.

Preferably, said nucleic acid corresponds to the insert of the expression plasmid contained in the bacteria CNCM-3743 or CNCM-3744.
According to a preferred embodiment, the nucleic acid encoding the polypeptide of the invention is operatively linked to a gene expression sequence, which directs the expression of nucleic acid within a prokaryotic or an eukaryotic cell, preferably an eukaryotic cell. The "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the peptidic antagonist nucleic acid to which it is operatively linked. The gene expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, beta-actin promoter, muscle creatine kinase promoter, human elongation factor promoter and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the simian virus (e.g., SV40), papilloma virus, adenovirus, human immunodeficiency virus (HIV), cytomegalovirus (CMV), Rous sarcoma virus (RSV), hepatitis B virus (HBV), the long terminal repeats (LTR) of Moloney leukemia virus and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Others constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein promoter is induced to promote transcription and translation in the presence of certain metal ions. Others inducible promoters are known to those of ordinary skill in the art.

In general, the gene expression sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined antigen nucleic acid. The gene expression sequences optionally include enhancer sequences or upstream activator sequences as desired.

As used herein, the nucleic acid sequence encoding the polypeptide of the invention and the gene expression sequence are said to be "operably linked" when they are covalently linked in such a way as to place the expression or transcription and/or translation of the polypeptide of the invention coding sequence under the influence or control of the gene expression sequence. Two DNA sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the
transcription of the polypeptide of the invention and if the nature of the linkage between
the two DNA sequences does not (1) result in the introduction of a frame-shift mutation,
(2) interfere with the ability of the promoter region to direct the transcription of the
polypeptide of the invention, or (3) interfere with the ability of the corresponding RNA
transcript to be translated into a protein. Thus, a gene expression sequence would be
operably linked to a nucleic acid sequence coding for the polypeptide of the invention if
the gene expression sequence were capable of effecting transcription of that antigen
nucleic acid sequence such that the resulting transcript is translated into the desired
polypeptide.

10 The nucleic acid coding for the polypeptide of the invention may be delivered in
vivo alone or in association with a vector. In its broadest sense, a "vector" is any vehicle
capable of facilitating the transfer of the nucleic acid coding for the polypeptide of the
invention to the cells. Preferably, the vector transports the nucleic acid to cells with
reduced degradation relative to the extent of degradation that would result in the
absence of the vector. In general, the vectors useful in the invention include, but are not
limited to, plasmids, phagmids, viruses, other vehicles derived from viral or bacterial
sources that have been manipulated by the insertion or incorporation of the peptidic
antagonist nucleic acid sequences. Viral vectors are a preferred type of vector and
include, but are not limited to nucleic acid sequences from the following viruses:
retrovirus, such as moloney murine leukemia virus, harvey murine sarcoma virus,
murine mammary tumor virus, and rouse sarcoma virus; adenovirus, adeno-associated
virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses;
herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can
readily employ other vectors not named but known to the art.

25 Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which
non-essential genes have been replaced with the gene of interest. Non-cytopathic
viruses include retroviruses, the life cycle of which involves reverse transcription of
genomic viral RNA into DNA with subsequent proviral integration into host cellular
DNA. Retroviruses have been approved for human gene therapy trials. Most useful are
those retroviruses that are replication-deficient (i.e., capable of directing synthesis of the
desired proteins, but incapable of manufacturing an infectious particle). Such
genetically altered retroviral expression vectors have general utility for the high-
efficiency transduction of genes in vivo. Standard protocols for producing replication-
deficient retroviruses (including the steps of incorporation of exogenous genetic
material into a plasmid, transfection of a packaging cell lined with plasmid, production
of recombinant retroviruses by the packaging cell line, collection of viral particles from

tissue culture media, and infection of the target cells with viral particles) are provided in

KRIEGLER (A Laboratory Manual," W.H. Freeman C.O., New York, 1990) and in

MURRY ("Methods in Molecular Biology," vol.7, Humana Press, Inc., Clifton, N.J.,

Preferred viruses for certain applications are the adeno-viruses and adeno-

associated viruses, which are double-stranded DNA viruses that have already been

approved for human use in gene therapy. The adeno-associated virus can be engineered
to be replication deficient and is capable of infecting a wide range of cell types and

species. It further has advantages such as, heat and lipid solvent stability; high
transduction frequencies in cells of diverse lineages, including hemopoietic cells; and
lack of superinfection inhibition thus allowing multiple series of transductions.
Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-
specific manner, thereby minimizing the possibility of insertional mutagenesis and
variability of inserted gene expression characteristic of retroviral infection. In addition,
wild-type adeno-associated virus infections have been followed in tissue culture for
greater than 100 passages in the absence of selective pressure, implying that the adeno-
associated virus genomic integration is a relatively stable event. The adeno-associated
virus can also function in an extrachromosomal fashion.

Other vectors include plasmid vectors. Plasmid vectors have been extensively
described in the art and are well known to those of skill in the art. See e.g.,
Spring Harbor Laboratory Press, 1989. In the last few years, plasmid vectors have been
used as DNA vaccines for delivering antigen-encoding genes to cells in vivo. They are
particularly advantageous for this because they do not have the same safety concerns as
with many of the viral vectors. These plasmids, however, having a promoter compatible
with the host cell, can express a peptide from a gene operatively encoded within the
plasmid. Some commonly used plasmids include pBR322, pUC18, pUC19, pRC/CMV,
SV40, and pBlueScript. Other plasmids are well known to those of ordinary skill in the
art. Additionally, plasmids may be custom designed using restriction enzymes and
ligation reactions to remove and add specific fragments of DNA. Plasmids may be
delivered by a variety of parenteral, mucosal and topical routes. For example, the DNA
plasmid can be injected by intramuscular, intradermal, subcutaneous, or other routes. It
may also be administered by intranasal sprays or drops, rectal suppository and orally. It
may also be administered into the epidermis or a mucosal surface using a gene-gun. The
plasmids may be given in an aqueous solution, dried onto gold particles or in association with another DNA delivery system including but not limited to liposomes, dendrimers, cochleate and microencapsulation.

The nucleic acid vector can include selectable markers that are active both in bacteria and in mammalian cells.

According to a first specific embodiment, the nucleic acid vector of the present invention corresponds to “naked DNA” like plasmids, cosmids or phagemids. Such naked DNA can be associated with non-lipid cationic polymers (WU and WU, J. Biol. Chem., vol.263, p: 14621-4, 1988) or liposomes (BRIGHMAN et al., Am. J. Med. Sci., vol.298, p: 278-81, 1989) to form complexes enhancing cellular uptake.

Preferably, said nucleic acid vector is a plasmid vector, and corresponds to one of the vectors contained in bacteria CNCM-3743 or CNCM-3744.

According to a second specific embodiment, the nucleic acid vector is a viral vector adapted for in vivo gene therapy protocols. Examples of appropriate viral vectors includes retroviral vectors as described in EP 0871459, EP 0386882 and EP 1222300 and adenovirus vectors as described in US 2004/ 265273 and US 6,638,502. In this case, the internalization of virus occurs through the specific interaction of the viral envelope with a cell surface receptor, followed by receptor-mediated endocytosis of the virus/receptor complex.

In a third aspect, the present invention relates to a host cell genetically engineered with the polynucleotide or with the vector described previously

As used herein, the term “host cell genetically engineered” relates to host cells which have been transduced, transformed or transfected with the polynucleotide or with the vector described previously.

As representative examples of appropriate host cells, one can cites bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium, fungal cells such as yeast, insect cells such as Sf9, animal cells such as CHO or COS, plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.
Preferably, said host cell is a bacterial cell, most preferably a bacterial cell corresponding to the bacteria CNCM-3743 or CNCM-3744.

The introduction of the polynucleotide or of the vector described previously into the host cell can be effected by method well known from one of skill in the art such as calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation.

In a forth aspect the present invention, the present invention relates to an antibody directed against a polypeptide as described previously.

Polyclonal antibodies can be prepared by immunizing a suitable animal, such as mouse, rabbit or goat, with a polypeptide as described previously. The antibody titer in the immunized animal can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide. At an appropriate time after immunization, e.g., when the specific antibody titers are highest, antibody producing cells can be obtained from the animal and used to prepare monoclonal antibodies (mAb) by standard techniques, such as the hybridoma technique originally described by KOHLER and MILSTEIN (Nature, vol.256, p:495-497, 1975), the human B cell hybridoma technique (KOZBOR et al., Immunol., vol.4, p: 72, 1983), the EBV- hybridoma technique (COLE et al., In Monoclonal Antibodies and Cancer Therapy, Alan R. Liss,Inc., p: 77-96, 1985) or trioma techniques. The technology for producing hybridomas is well known (see generally Current Protocols in Immunology, COLIGAN et al. ed., John Wiley & Sons, New York, 1994). Hybridoma cells producing the desired monoclonal antibody are detected by screening the hybridoma culture supernatants for antibodies that bind the polypeptide of interest, e.g., using a standard ELISA assay.

In another embodiment, the present invention relates to a composition comprising a polypeptide as described above, a nucleic acid encoding thereof, or a nucleic acid vector comprising said nucleic acid, eventually associated with a pharmaceutically acceptable vehicle.
The phrase "pharmacologically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. Preferably, as used herein, the term "pharmacologically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The composition of the invention can comprise liposomes, micelles and other lipid formulations as described above.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

The polypeptides of the invention, nucleic acids coding therefore or nucleic acid vectors may be solubilized in a buffer or water or incorporated in emulsions and microemulsions. Suitable buffers include, but are not limited to, phosphate buffered saline Ca\(^{++}\)/Mg\(^{++}\) free (PBS), phosphate buffered saline (PBS), normal saline (150 mM NaCl in water), Tris buffer and surfactants.

There are numerous causes of peptide instability or degradation, including hydrolysis and denaturation. Hydrophobic interaction may cause clumping of molecules together (i.e. aggregation). This result may entail diminution of the induction of a Treg response. Stabilizers may be added to lessen or prevent such problems.

Stabilizers include cyclodextrine and derivatives thereof (see U.S. Pat. No.5,730,969). Suitable preservatives such as sucrose, mannitol, sorbitol, trehalose, dextran and glycerin can also be added to stabilize the final formulation. A stabilizer selected from ionic and non-ionic surfactants, D-glucose, D-galactose, D-xylene, D-galacturonic acid, trehalose, dextrans, hydroxyethyl starches, and mixtures thereof may be added to the formulation. Addition of alkali metal salt or magnesium chloride may stabilize a peptide. The peptide may also be stabilized by contacting it with a saccharide
selected from the group consisting of dextran, chondroitin sulphuric acid, starch, glycogen, dextrin, and alginic acid salt. Other sugars that can be added include monosaccharides, disaccharides, sugar alcohols, and mixtures thereof (E.g., glucose, mannose, galactose, fructose, sucrose, maltose, lactose, mannitol, xylitol). Polyols may stabilize a peptide, and are water-miscible or water-soluble. Suitable polyols may be polyhydroxy alcohols, monosaccharides and disaccharides including mannitol, glycerol, ethylene glycol, propylene glycol, trimethyl glycol, vinyl pyrrolidone, glucose, fructose, arabinose, mannose, maltose, sucrose, and polymers thereof. Various excipients may also stabilize peptides, including serum albumin, amino acids, heparin, fatty acids and phospholipids, surfactants, metals, polyols, reducing agents, metal chelating agents, polyvinyl pyrrolidone, hydrolysed gelatin, and ammonium sulfate.

In a preferred aspect, the present invention relates to the use of a polypeptide as described above, a nucleic acid sequence coding therefore, a vector comprising such a nucleic acid sequence, or a composition as described previously for treating cancer in a subject.

In fact, the apoptosis activity of the polypeptide of the invention enables to induce the death of the cells wherein it is internalized.

In particular, the polypeptide of the invention is efficient even in tumour cells that have not been or are not infected by HPV.

Thus, more particularly, the present invention relates to the use of the polypeptide as defined previously, optionally associated with a pharmaceutically acceptable vehicle, for the treatment of cancer in a subject, said polypeptide inducing the apoptosis of cancer cells in which it is internalised, and comprising no more than about 50 amino acid residues of the HPV E2 protein.

In a preferred embodiment, said cancer is not an HPV-induced cancer.

In another preferred embodiment, said cancer is a solid cancer, e.g. breast cancer, prostate cancer, melanomas, carcinomas, etc…

As used herein, the term “subject” denotes a mammal, such as a rodent, a feline, a canine and a primate. The subject is an animal such as cow, pig, horse, chicken, cat, dog and most preferably a human.
As the polypeptide of the invention triggers the apoptosis of cells in which it is internalised, systemic administration is not convenient. However, topical administration of the therapeutic composition is advantageous since it allows localized concentration at the site of administration with minimal systemic adsorption. Furthermore, the amount of material to be applied is far less than required for other administration routes. Topical administrations encompass intratumoral and subcutaneous injections or application to the skin or mucosa. Target mucosa can be that of the vaginal, cervical, vulvar, penile or anorectal mucosa, or can be that of the gastrointestinal tract, comprising the mouth, larynx, esophagus and stomach.

Thus, the composition according to the invention is formulated either under liquid form, or in a cream, gel, emulsion, or foam.

In a final aspect, the present invention relates to a method for treating cancer comprising administering to a subject a therapeutically effective amount of a polypeptide as described above, a nucleic acid sequence coding therefore, a vector comprising such a nucleic acid sequence, or a composition as described previously.

According to the present invention, an "effective amount" of a composition is one which is sufficient to achieve a desired biological effect, in this case inducing apoptosis in tumor cells. It is understood that the effective dosage will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The ranges of effective doses provided below are not intended to limit the invention and represent preferred dose ranges. However, the preferred dosage can be tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation.

In the following, the invention is described in more detail with reference to amino acid sequences, nucleic acid sequences and the examples. Yet, no limitation of the invention is intended by the details of the examples. Rather, the invention pertains to any embodiment which comprises details which are not explicitly mentioned in the examples herein, but which the skilled person finds without undue effort.

**Examples**

1) Cell death induced by E2 is associated to caspase 8 activation
The role of caspase 8 as the initiator caspase of E2-induced apoptosis was investigated using specific inhibitors of the apical caspase 8; i.e. the MC159 vFLIP protein (viral FLICE Inhibitory protein) from *Molluscum Contagiosum* and the Cowpox virus serpin crmA.

293 cells were transfected by standard calcium phosphate co-precipitation technique into 6 well-plates with typically 1 to 2 μg plasmid coding for GFP or for GFP-E2 fusion protein as described in BELLANGER et al. (*J. Virol.*, vol.75(16), p:7244-7251, 2001), wherein said E2 protein corresponds to HPV18 E2 protein, with or without 1 μg of plasmid coding for HA-MC 159 provided by Richard SIEGEL (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda) or coding for crmA (provided by Visha DIXIT). 48 hours after transfection, cells were trypsinized, washed, resuspended in PBS-10% serum containing 25 μg/ml propidium iodide (PI)(SIGMA), and analyzed by flow cytometry for GFP fluorescence and PI content. Finally, the GFP-positive dead cells were estimated as the PI positive cells /GFP positive cells.

The figure 1 A) shows the dead cells percentage for 293 cells transfected with plasmids expressing GFP (GFP), GFP-E2 fusion protein (GFP-E2), with or without the caspase-8 inhibitors MC 159 (MC159) or crmA (crm A).

The figure 1 B) shows the phase contrast and GFP fluorescence of 293 cells 48 hours after transfection with plasmids expressing GFP-E2 fusion protein (GFP-E2), with or without the caspase-8 inhibitors MC 159 (MC159) or crmA (crm A).

The results confirm the pro-apoptotic activity of the protein E2 (fusion protein GFP-E2) of oncogenic HPV, notably HPV16 and 18, which has been shown to be linked to the presence of E2 in the cytoplasm and active nucleo-cytoplasmic shuttling (BLACHON et al., *J. Biol. Chem.*, vol.280, p: 36088-36098, 2005). The results also show that the caspase-8 inhibitors MC 159 (MC159) or crmA (crm A) efficiently abrogated E2-induced cell death when co-expressed with GFP-E2 (Figure 1), firmly establishing that caspase 8 activation is indeed the initiating event of the apoptotic pathway triggered by the HPV18 E2 protein.
2) Cell death follows E2 assembly into filaments which recruit and activate caspase 8

Saos cells were infected at a multiplicity of infection of 500 particles/cell with recombinant adenoviruses expressing either the fusion protein HPV18 GFP-E2 (Ad GFP-E2) or a fusion protein GFP-E2ΔNT with a non-apoptotic form of HPV18 E2 (Ad GFP-E2ΔNT) as described in DEMERET et al. (abovementioned, 2003). A video microscopy was performed on recombinant adenovirus-infected Saos-2 cells from 7 to 25 hours post infection with an AXIOVERT ZEISS microscope, and selected images were processed by the METAMORPH software.

The figure 2 A) shows the fluorescent and phase contrast images of the same field, selected at different time points for Saos cells infected with either Ad GFP-E2 or Ad GFP-E2ΔNT adenovirus. White arrows show dying cells.

The results show that after a lag time of about 12 hours of nuclear accumulation, GFP-E2 progressively redistributed to the cytoplasm where it formed perinuclear filamentous structures (Figure 2A). Then, apoptosis occurred specifically in the cells containing these filaments at variable times after their formation. Under similar conditions, the cells expressing GFP fused with a non-apoptotic mutant form of HPV18 E2 (GFP-E2ΔNT) did not show cell death, despite high levels of nuclear accumulation (Figure 2A). Finally, the results established that E2-induced cell death is preceded by assembly of GFP-E2 into cytoplasmic filaments.

Saos cells were infected with Ad GFP-E2 or Ad GFP-E2ΔNT as described previously, and then cultured on cover slides. Cells grown on cover slides were rinsed in PBS, fixed in 2% paraformaldehyde, permeabilized with 0.1% Triton, saturated in PBS-10% SVF, and incubated for 1 hr with rabbit anti-caspase 8 (PHARMINGEN) according to manufacturer's protocol. Finally, the slides are incubated with anti-rabbit antibody Texas-Red (TR) conjugated (AMERSHAM) and then nuclei were stained with DAPI (SIGMA), at 0.15 μg/ml. Pictures were taken with a ZEISS AXIOPHOT microscope coupled to a CCD camera 20 hours post infection for GFP and Texas-red fluorescence.

Simultaneously, some Ad GFP-E2 or Ad GFP-E2ΔNT infected cells were extracted 30 hours post-infection in 1% TRITON P300 buffer (20 mM NaH₂PO₄, 300
mM NaCl, 5 mM EDTA, 5 mM DTT) supplemented with protease inhibitors (ROCHE Diagnostics) for 30 min at 4°C followed by centrifugation for 30 min at 13,000 rpm. Dead (floating) and living (attached) GFP-E2 expressing cells were processed separately. Pellets containing TRITON-insoluble material were denatured in 5X LAEMMLI sample buffer and boiled for 20 min. Equivalent fractions of soluble and insoluble material in 1X sample buffer were separated on SDS-PAGE and processed for immunoblotting with rabbit anti-caspase 8 antibody (PHARMINGEN) according to manufacturer’s instructions.

The figure 2 B) shows the GFP fluorescence (GFP fluo), Texas-Red fluorescence (αC8-TR) and both fluorescence (Merge) for Saos-2 cells infected with Ad GFP-E2 (GFP-E2) or Ad GFP-E2ΔNT (GFP-E2ΔNT).

The figure 2 C) shows the immunoblot performed with anti-caspase 8 antibody on equivalent soluble (Sol) and insoluble (Ins) fractions of Saos-2 cells non infected (ni), infected with Ad GFP-E2ΔNT (GFP-E2ΔNT) or of dead or living Saos-2 cells infected with Ad GFP-E2 (GFP-E2). The black arrows indicate the positions of the p50/55 caspase precursor (proC8) and of the 26 Kd DED (Death Effector Domain) containing pro-domain.

The immunofluorescence analysis indicated that the GFP-E2 filaments contained endogenous caspase 8 that co-localized with E2 (Figure 2B). Formation of GFP-E2/caspase 8 cytoplasmic filaments correlated with translocation of both proteins to a detergent-insoluble fraction, containing processed caspase 8 (Figure 2C). Thus, E2-expressing dead cells contained only cleaved caspase 8, with the 26 Kd pro-domain present in a detergent-insoluble fraction (Figure 2C), suggesting that caspase 8 self-catalytic processing had occurred within filaments. In contrast, in all living cells expressing GFP-E2ΔNT or GFP-E2, only the inactive pro-form of caspase 8 was detected in soluble fractions (Figure 2C). This suggests that self-catalytic processing of caspase 8 had occurred within filaments, reflecting its enzymatic activation. Indeed, the analysis of caspase 8 activity has established that such enzymatic activity was readily detectable in extracts of GFP-E2 expressing cells but not in non infected cells or in cells
expressing the non apoptotic form of E2, providing a direct evidence of caspase 8 activation (data not shown).

In order to determine if the filament formation results from caspase activation, Saos cells were infected with Ad GFP-E2 recombinant adenovirus as described previously. Then the infected cells were treated with 40 μM Z-VAD-fmk just after infection, which abrogates cell death (data not shown and DEMERET et al., abovementioned, 2003); and processed for anti-caspase 8 immunofluorescence 24 hours post infection.

The figure 3 shows the GFP fluorescence (GFP fluo), texas-Red fluorescence (αC8-TR) and both fluorescence (Merge) for Saos-2 cells infected with Ad GFP-E and tretad with Z-VAD-fmk.

The results establish that E2/caspase 8 filaments can assemble in the presence of the caspase inhibitor Z-VAD-fmk, indicating that filament formation is independent of caspase activity and is an upstream event of E2-induced apoptosis (Figure 3).

Overall, these experiments show that the HPV18 E2 protein induces apoptosis through assembly into filaments that efficiently recruit and activate endogenous caspase 8.

3) The pro-apoptotic E2 protein induces caspase 8 oligomerization within filaments through direct interaction

Oligomerization of apical caspase 8 has been shown to be strictly dependent upon its homotypic binding to oligomerization-competent DFD (Death Fold Domain) containing adaptors, such as FADD which recruits caspase 8 to the Death Inducing Signaling Complex (DISC) through DED-mediated interactions. Actually, only few other caspase 8 adaptors have been described, among which the HIP-1/Hippi proteins which activate caspase 8 through pseudo-DEDs when expanded-poly Q Hungtintin is expressed in the cells (GERVAIS et al., Nat. Cell Biol., vol.4, p: 95-105, 2002). Nevertheless, analysis of the E2 protein crystal structure does not reveal any obvious
DFD (ANTSON et al., Nature, vol.403, p: 805-809, 2000), and therefore E2 appears not to act as a typical DFD adaptor for caspase 8 oligomerization.

In order to better understand the interaction between E2 protein and caspase 8, 293 cells were transfected as previously described with typically 1 to 1.5 µg plasmid coding for GFP, GFP-E2 fusion protein or GFP-E2ΔNT, with or without 0.5 µg of pcDNA plasmid coding for caspase 8, and then cultured on cover slides. 24 hours after transfection, cells grown on cover slides were rinsed in PBS, fixed in 2% paraformaldehyde, permeabilized with 0.1 % Triton, saturated in PBS-10% SVF, and incubated for 1 hr with rabbit anti-caspase 8 (PHARMINGEN) according to manufacturer’s protocol. Finally, the slides are incubated with anti-rabbit antibody Texas-Red (TR) conjugated (AMERSHAM) and then nuclei were stained with DAPI (SIGMA), at 0.15 µg/ml. Pictures were taken with a ZEISS AXIOPHOT microscope coupled to a CCD camera 24 hours post transfection for GFP and Texas-red fluorescence.

The figure 4 A) shows the GFP fluorescence of 293 living cells 24 hours after transfection with expression plasmids for GFP fusion proteins as indicated, in the presence or absence of a plasmid expressing caspase 8 (C8).

The figure 4 B) shows the GFP (GFP fluo), Texas Red (αC8-TR) and combined (Merge) fluorescence of 293 cells co-transfected with caspase 8 (C8) and GFP-E2 or GFP-E2ΔNT as indicated.

The results established that co-expression of caspase 8 with GFP-E2 in 293 cells induced a massive redistribution of the GFP-E2 into cytoplasmic filaments, under conditions where GFP-E2 alone was predominantly nuclear, indicating that ectopic expression E2 and caspase 8 is sufficient to induce filament formation (Figure 4A). Immunofluorescence analysis with anti-caspase 8 antibodies showed that the GFP-E2 filaments contained transfected caspase 8, with full co-localization of both proteins in the same structures (Figure 4B). Consequently, these results indicate that increased levels of caspase 8 improved GFP-E2/caspase filament assembly, and suggest that
caspase 8 plays an active role in filament formation. In contrast, caspase 8 expression did not affect the distribution of GFP-E2ΔNT or GFP alone (Figure 4A).

Since many HPV do not induce apoptosis, the interaction of caspase-8 from E2 proteins of HPV species inducing or not apoptosis has been investigated. For this purpose, transfection experiments on 293 cells with or without caspase-8 expression have been done as described previously with different GFP-E2 fusion proteins, wherein E2 polypeptides correspond either to HPV species inducing (i.e., HPV16 and HPV18) or not (i.e., HPV6 and HPV11) apoptosis.

One part of the transfected cells were analysed 24 hours after transfection for GFP fluorescence as described previously.

Simultaneously, the other part of transfected cells were analysed by immunoblot. These cells were extracted 24 hours post transfection in 1% TRITON P300 buffer (20 mM NaH₂PO₄, 300 mM NaCl, 5 mM EDTA, 5 mM DTT) supplemented with protease inhibitors (ROCHE DIAGNOSTICS) for 30 min at 4°C followed by centrifugation for 30 min at 13,000 rpm. Pellets containing TRITON-insoluble material were denatured in 5X LAEMMLI sample buffer and boiled for 20 min. Equivalent fractions of soluble and insoluble material in 1X sample buffer were separated on SDS-PAGE and processed for immunoblotting with rabbit anti-caspase 8 (PHARMINGEN) and rabbit anti-GFP (TORREY PINES) according to manufacturers’ instructions.

The figure 4 C) shows the GFP fluorescence of 293 living cells with expression plasmids for GFP fusion proteins as indicated, in the presence or absence of a plasmid expressing caspase 8.

The figure 4 D) shows the immunoblots with anti-GFP or anti-caspase 8 antibodies realized on equivalent fractions of detergent-soluble and insoluble extracts of cells co-expressing caspase 8 and the different GFP-E2 fusion proteins as indicated.

The results established that the non-apoptotic E2 proteins from the low-risk HPV types 6 and 11 (BLACHON et al., abovementioned, 2005) did not form filaments when
co-expressed with caspase 8, whereas the pro-apoptotic E2 protein from oncogenic HPV16 did induce filament formation (Figure 4C). Moreover, the assembly of GFP-E2 from HPV18 or HPV16 and caspase 8 into filaments correlated with translocation of both proteins into a detergent-insoluble fraction and caspase self-processing (Figure 4D). In the presence of the non-apoptotic E2 proteins from HPV 6 and 11, or with HPV18 E2ΔNT, ectopically expressed caspase 8 remained as the full-length pro-form predominantly present in the soluble fractions (Figure 4D). These results point to a clear correlation between filament forming properties of HVP18 and HPV 16 E2 proteins and their pro-apoptotic activities. Conversely, the assembly of caspase 8 into filaments was specific for the presence of pro-apoptotic E2 proteins, as the expression of caspase 8 with the non-apoptotic forms of E2 or with GFP alone led to a diffuse, distribution (Figure 4B). Filaments therefore represent a particular oligomerization state of caspase 8 that it is unable to implement itself.

Altogether, the results indicate that the pro-apoptotic E2 proteins and caspase 8 are necessary and sufficient to form filaments, suggesting that the oligomerization of caspase 8 is not driven by a typical DFD adaptor, which would be required at stochiometric levels with caspase 8. Indeed, the FADD adaptor was not detected as associated with insolubilized E2/caspase 8 filaments in either 293 or Saos cells (data not shown), indicating that E2-induced caspase 8 oligomerization was independent of FADD. In contrast, E2 and caspase 8 were both present at equally high levels in the insolubilized filaments, indicating that E2 plays a structural role in their formation despite its lack of an obvious DFD. Therefore, the caspase 8-containing filaments forming with pro-apoptotic E2 proteins may represent a novel type of intracellular death complexes independent of classical DFD adaptors.

In order to better determine the nature of the interaction between E2 protein and caspase-8, GST-pull-down experiments have been performed. For these experiments, equivalent amounts of purified GST-E2 or GST fixed on Glutation conjugated beads were incubated for 1 hr with \(^{35}\)S in vitro translated proteins (i.e., Caspase-8 (Casp 8), caspase 8 pro-domain (C8 DED), caspase 8 catalytic domain (C8 CD), vFLIP MC159 (MC159) or FADD) produced in TNT, in binding buffer (10 mM HEPES [pH 7.7], 200 mM NaCl, 1 mM MgCl\(_2\), 2 mM DTT, 50 mM sucrose, 0.1% TRITON X-100). Beads
were washed four times with binding buffer containing 1% TRITON X-100, bound proteins were separated by SDS-PAGE, and revealed by autoradiography.

The figure 4 E) shows the results of GST pull-down experiments of \textit{in vitro} translated $^{35}$S-labelled proteins with GST alone or GST-E2 fusion proteins as indicated. Bound (B) and 1/10$^{th}$ input (I) $^{35}$S-labelled proteins were loaded on SDS-PAGE and revealed by autoradiography.

The results established that HPV18 E2 interacted with caspase 8 through the caspase pro-domain that contains tandem DEDs, whereas no interaction was detected with the caspase catalytic domain (Figure 4E). In similar experiments, the E2 protein only marginally bound to the DED-containing vFLIP MC159 or FADD proteins (Figure 4E), indicating that E2 selectively binds to the DEDs domains of caspase 8.

In order to confirm said interaction, co-immunoprecipitation experiments have been performed. 293 cells were transfected as previously by plasmids expressing either GFP-E2 and caspase 8 or GFP-E2 and HA-MC159. Transfected cells were extracted in 1% TRITON P300 buffer (20 mM Na$_2$HPO$_4$, 300 mM NaCl, 5 mM EDTA, 5 mM DTT) supplemented with protease inhibitors (ROCHEDIAGNOSTICS) for 30 min at 4°C followed by centrifugation for 30 min at 13,000 rpm. Equal amounts of soluble proteins were then immunoprecipitated with anti-E2 polyclonal rabbit antibodies for 16 hours at 4°C, the immunoprecipitates were washed 3 times in 0.1% Triton P300 buffer, and processed for Western blotting with anti-GFP (TORREY PINES BIOLABS), anti-HA (SIGMA) or anti-caspase 8 antibodies.

The figure 4 F) shows the immunoblots realized with caspase 8, HA and GFP antibodies on anti-E2 immunoprecipitates (IPξE2) and 1/10 whole cell extracts (Inp) from 293 cells expressing GFP-E2 and caspase 8 or GFP-E2 and HA-MC159.

The results confirm that HPV18 E2 interact with caspase 8 but not with MC159 (Figure 4F).
Finally, and in order to confirm the interaction of E2 with the caspase catalytic domain of caspase 8, 293 cells were transfected as previously by plasmids expressing either GFP-caspase 8 (GFP-C8) and E2, GFP- catalytic domain of caspase 8 (GFP-C8 CD; aa 180 to 480 of caspase 8)), or GFP-E2 and caspase 8 pro-domain (C8 DED; aa 1 to 181 of caspase 8). The transfected cells were processed for immunofluorescence as described previously with anti-E2 or anti-caspase 8 antibodies, followed by secondary Texas Red (TR)-coupled antibodies.

The figure 4 G) upper panel shows the immunofluorescence pictures of 293 cells transfected with E2 and GFP fusion proteins with either full-length caspase 8 (GFP-C8) or its catalytic Caspase Domain alone (GFP-C8CD) and processed for immunofluorescence with anti E2-antibodies.

The figure 4 G) lower panel shows the immunofluorescence pictures of 293 cells transfected with GFP-E2 and the DED–containing pro-domain of caspase 8 (C8-DED) and processed for immunofluorescence using caspase 8 antibody.

Finally, the results show that HPV18 E2 engages direct, non-homotypic interactions with the DED of caspase 8 that are likely to be involved in filament formation. Indeed, the caspase pro-domain of caspase 8, as well as each of the DED domain, could mediate assembly into filaments with E2 (Figure 4G and data not shown).

4) E2/caspase 8 filaments are antagonized by the MC159 vFLIP protein but can include FADD

The present study has established that the assembly of GFP-E2/caspase 8 filaments occurs through direct contacts between E2 and the DED-containing domain of caspase 8, i.e. the same homotypic interaction domain involved in recruitment by FADD. However, interactions between E2 and caspase 8 are not homotypic (as discussed above) and an investigation on how they could be affected by typical DED-mediated interactions. For these experiments, the viral anti-apoptotic FLICE Inhibitory Protein (FLIP) MC159 and the FADD adaptor, both of which are known to engage
homotypic interactions with the DED of caspase 8, have been used. In contrast to FADD, which drives caspase oligomerization, MC159 negatively interferes with these interactions thereby repressing caspase activation (GARVEY et al., J. Virol., vol.76, p:697-706, 2002; SIEGEL et al., abovementioned, 1998).

293 cells were co-transfected as described previously with GFP-E2, caspase 8, HA-MC159 and FADD expression plasmids. 24 hrs post-transfection, the transfected cells were then processed for immunofluorescence as described previously with anti-caspase 8 (PHARMINGEN), anti-FADD (PHARMINGEN), or anti HA antibodies (SIGMA).

The figure 5 A) shows the pictures for GFP fluorescence (GFP fluo), Texas-Red fluorescence corresponding to caspase 8 cellular location (αC8-TR) or both (Merge) of 293 cells co-transfected with GFP-E2, caspase 8, HA-MC159 and FADD expression plasmids.

The figure 5 B) shows the pictures for GFP fluorescence (GFP fluo), Texas-Red fluorescence corresponding to FADD cellular location (αC8-TR) or corresponding to MC159 location (αHA-TR) or both (Merge) of 293 cells co-transfected with GFP-E2, caspase 8, HA-MC159 and FADD expression plasmids.

The results shows that that in the presence of MC159, GFP-E2/caspase 8 filaments disappeared, with caspase 8 expression reverting to a diffuse distribution and the E2 protein shuttling back to the nucleus (Figure 5A). MC159 itself consistently showed a diffuse cytoplasmic distribution as expected (SIEGEL et al., abovementioned, 1998 and Figure 3-B). In contrast to MC159, co-expressed FADD is efficiently incorporated within filaments that contain both GFP-E2 and caspase 8 (Figure 5A and 5B). All filaments stained positive for GFP-E2 and either caspase 8 or FADD, indicating that they simultaneously contained the three proteins (Figure 5A and 5B). FADD spontaneously self-oligomerized to form filaments when it was ectopically expressed as previously reported (P PEREZ and WHITE, J. Cell Biol., vol.141, p:1255-66, 1998; SIEGEL et al., J. Cell Biol., vol.141, p:1243-53, 1998). In the absence of co-expressed caspase 8 however, FADD filaments only marginally recruited GFP-E2, which
remained mainly nuclear (Figure 3B), as predicted by the GST pull-down experiments that show no specific interaction between E2 and FADD (Figure 4E).

In order to confirm the inhibition of E2/caspase-8 DED interaction by MC159, GST pull-down experiments have been performed as previously with incubation of GST or GST-E2 with $^{35}$S TNT-produced caspase 8 and MC159 mixed in binding buffer 1 hour before addition of GST or GST-E2, or with $^{35}$S TNT-produced caspase 8. Bound (B) and 1/10$^{th}$ input (I) $^{35}$S-labelled proteins were loaded on SDS-PAGE and revealed by autoradiography.

The figure 5 C) shows the GST pull-down experiments realized after incubation of GST-E2 or GST as indicated with $^{35}$S TNT-produced caspase 8 with or without $^{35}$S TNT-produced MC159. The lanes B and I correspond to bound and 1/10$^{th}$ input $^{35}$S-labelled proteins respectively.

The results effectively confirm that the binding of caspase 8 to E2 was reduced by the presence of MC159 (Figure 5C), indicating that the DED-mediated binding of MC159 to caspase 8 competes with caspase 8-E2 interactions and inhibits filament formation.

In conclusion, the E2/caspase 8/FADD filaments are made by caspase 8 interacting simultaneously with FADD and E2, leading to the assembly of multimerized protein complexes that contain the three proteins. The DED homotypic interactions between oligomerized FADD and caspase 8 therefore do not compete with E2-caspase 8 interaction, implying that the DEDs of caspase 8 could simultaneously engage with both partners. So, pro-apoptotic E2 and the FADD adaptor may be able to collaborate to induce caspase 8 oligomerization, through distinct and independent mechanisms.

To corroborate functionally such collaboration, 293 cells were transfected as described previously with expression plasmids coding for GFP, GFP-E2, and the Death Receptor Fas. For Fas-induced cell death, the agonistic APO 1-3 anti-Fas antibody (2 µg/ml; ALEXIS BIOCHEMICALS) was added for 16 hours. Dead cells were quantified by propidium iodide uptake 48 hours after transfection as described previously.
The figure 5 D) shows the cell death percentage after induction of Fas-induced cell death with APO 1-3 antibody on 293 cells transfected with expression plasmids for GFP, GFP-E2, Fas, or GFP-E2 and Fas.

The results established that HPV18 E2 enhanced Fas-induced apoptosis, probably by cooperating with FADD in the induction of caspase 8 oligomerization (Figure 5D). In this regard, the E2 protein counteracts the effects of the E6 oncoprotein of Human Papillomaviruses, which inhibits Fas-mediated apoptosis by accelerating the degradation of FADD (FILIPPOVA et al., abovementioned, 2004). Inhibition of caspase 8 catalytic activity by crmA prevented caspase self-activation following filament assembly, while the MC159 vFLIP reduced E2-induced cell death by abrogating the assembly of GFP-E2 filaments (Figure 1). The HPV18 E2 protein therefore represents a new type of viral pro-apoptotic factor, acting conversely to the viral FLIP anti-apoptotic MC159 protein. HPV18 E2 uses a novel mechanism to activate caspase 8, by direct induction of caspase 8 oligomerization within filaments through non-DFD interactions with caspase 8 DEDs. This process might ensure efficient cell destruction even though they contain altered Death Receptor apoptotic pathways, as it is often the case in infected cells.

5) A short α-helix of the E2 amino-terminal domain mediates filament formation and cell death

The figure 6 A) shows a schematic representation of the crystal structure of the HPV16 E2 amino-terminal domain, which is composed of three α-helix and a β-sheet bundle separated by a rigid fulcrum (ANTSON et al., abovementioned, 2000).

DEMERET et al. (abovementioned, 2003) has established that the N-terminal domain of pro-apoptotic E2 protein from HPV18 is sufficient to induce apoptosis. In this N-terminal domain, the second helix H2 of pro-apoptotic E2 protein from HPV18 corresponds to a signal peptide enabling the delocalization of a nuclear protein to the cytoplasm, said cellular localization being critical for the apoptosis induction (BLACHON, abovementioned, 2005).
To obtain further insight into the type of interaction between E2 and caspase 8, GFP fusion proteins comprising different domains of E2 have been made and analyzed their ability to interact with caspase 8.

For these experiments, helix H1 (aa 1-26) or H3 (aa 54-88) from the E2 amino-terminal domain from HPV 18 were cloned by a conventional PCR-based technique in frame with GFP. The helix H2 (aa 27-53) corresponding to the cytoplasmic localization domain of E2 protein from HPV18 was also cloned in frame with GFP in the same way.

293 cells were transfected as previously described with plasmids expressing caspase 8 and the GFP fusion proteins comprising the α-helix 1 and 3 of E2 (E2H1spanning aa 1 to 26, E2H3 spanning aa 54 to 88). As a control, 293 cells were also transfected with plasmids expressing caspase 8 and the GFP fusion protein comprising the α-helix 2 of E2 (E2H2 spanning aa 27 to 53), since no modification of apoptosis has been observed in BLACHON et al. (abovementioned, 2005) after the cell expression of a fusion protein comprising the same domain fused to GFP and a NLS signal peptide.

24 hrs post-transfection, one part of the transfected cells were then processed for immunofluorescence as described previously with anti-caspase 8 antibody (PHARMINGEN). Simultaneously, the dead cells were quantified in the other part of transfected cells by propidium iodide uptake 48 hours after transfection as described previously.

The figure 6 B) shows the pictures for GFP fluorescence (GFP fluo), Texas-Red fluorescence corresponding to caspase 8 cellular location (αC8-TR) or both (Merge) of 293 cells co-transfected with GFP-E2H1, GFP-E2H3 and the control (GFP-E2H2) expression plasmids.

The figure 6 C) shows the viability of HeLa cells expressing each GFP-E2 α-helix as indicated, determined 48 hours post-transfection by propidium iodide uptake.
The results established that the polypeptides corresponding to either the first or the third α-helixs of the amino terminal domain of E2 were not able to induce caspase 8 filaments. Nevertheless, the results show surprisingly that the second α-helix (amino acids 27 to 53) of E2, which is able to localize the E2 protein in the cytoplasm, is also able to induce caspase 8 filaments.

In accordance with filaments formation, the second α-helix of E2 alone was able to induce apoptosis similarly to the full-length protein (Figure 4D), confirming that the E2 pro-apoptotic activity is based on induction of caspase 8 oligomerization. Such a result was unexpected since no apoptosis has been observed in BLACHON et al. (abovementioned, 2005) after the cell expression of a fusion protein comprising the same domain fused to GFP and a NLS signal peptide.

Consequently, these results definitively exclude the involvement of a pseudo “Death Fold Domain” within E2 in the interaction with caspase 8. Based on these observations, we propose that the E2 protein of HPV18 induces caspase 8 oligomerization through direct interactions involving only the second α–helix of E2. Assembly of caspase 8 and E2 within filaments may provide the caspase with adequate oligomerization state to be efficiently self-activated.

6) The short α-helix of the E2 amino-terminal domain mediating cell death also pre-exists in E2 protein from non-apoptotic HPV6 and HPV11

In order to investigate the existence of apoptotic activity associated with helix H2 from other HPV type, helix H2 (aa 27-53 from HPV 18, and aa 22-49 from HPV 6, HPV 11 and HPV 16)) from the E2 amino-terminal domain from pro-apoptotic HPV 16 and HPV 18, and from non-apoptotic HPV 6 and HPV 11 were cloned by a conventional PCR-based technique in frame with GFP.

The apoptotic activity of the fusion proteins were assayed as described previously.
The results have shown surprisingly that the second α-helix (amino acids 22 to 49) of E2 protein from the non-apoptotic HPV such as the apoptotic HPV were able to induce caspase 8 filaments and apoptosis.

Consequently, it seems that the second α-helix (amino acids 22 to 49) of E2 protein has conserved its function of apoptosis induction in the different HPV types. The figure 7 shows the alignment of amino-terminal domain of E2 proteins from different types of HPV.

7) The short α-helix of the E2 amino-terminal domain mediating cell death also pre-exists in E2 protein from non-apoptotic HPV6 and HPV11

In order to better identify the fragment of the second helix H2 implicated in apoptosis induction, different fragments from E2 protein from HPV18 (aa 27-53, 30-53, 30-44, 34-46, 36-53, 36-44, 36-46, 36-48, and 36-50) were cloned by a conventional PCR-based technique in frame with GFP.

The apoptotic activity of the fusion proteins were assayed as described previously.

The results are presented in figure 8.

Results show that, surprisingly, amino acids 36 to 48 coupled with GFP are able to induce the cell death alone. On the contrary, amino acids 30-44, 36-44, 34-46, and 44-53, coupled with GFP, are poorly capable to induce cell death in 293 cells that have been transitorily infected with plasmids encoding such fusion proteins.

8) Study of punctual mutants in the short α-helix of the E2-amino terminal domain

We have previously shown that the region comprising amino acids 36 to 48 of the protein of HPV18 is highly proapoptotic.

In order to better characterize which amino acid(s) is(are) essential for the proapoptotic activity of this domain, point mutations have been introduced in the nucleotide sequence encoding for it.
For example, the Tryptophan W37 has been replaced by another hydrophobic non aromatic amino acid, the Valine (V) or by an hydrophilic amino acid, the Glycine (G).

Also, the Arginine R41 was replaced by an Alanine (A).

Results show that W37V does not affect the pro-apoptotic properties of amino acid 36-48, showing that the aromaticity of the amino acids is not at stake in this effect. On the contrary, the replacement of the hydrophobic amino acid by a hydrophilic one drastically diminishes the pro-apoptotic capacity of this domain, highlighting the importance of an hydrophobic amino acid in position 37 (figure 9).

The replacement of the Arginine 41 by an Alanine has no consequence on the apoptosis of the cells infected with the plasmidic vectors (figure 9). As this amino acid is implicated in the interaction with the transcription factor BrD4, there is no relationship between apoptosis induction of the helix 2 of the E2 domain of HPV18 and its capacity to regulate transcription.

9) Study of the pro-apoptotic properties of short $\alpha$-helix of the E2-amino terminal domain of non different HPV types

We have previously shown that the region comprising amino acids 36 to 48 of the protein of HPV18 is highly pro-apoptotic.

In order to better characterize the pro-apoptotic properties of different amino acid sequences of short $\alpha$-helix of the E2-amino terminal domain, we analysed the death of cells infected with vectors carrying the $\alpha$-helix 2 of the E2-amino terminal domain of either HPV 11 (amino acids 23 to 49), or HPV16 (amino acids 23 to 49) coupled with GFP.

Results show that, surprisingly, the $\alpha$-helix 2 of the E2-amino terminal domain of HPV 11 is pro-apoptotic (figure 9), whereas the complete protein was not (figure 4D).

Moreover, the $\alpha$-helix 2 of the E2-amino terminal domain of the oncogenic HPV 16 is also pro-apoptotic (figure 9).
### 0-1 Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)

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| 1-5 Designated States for Which Indications are Made | all designations |

### 2 The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:

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CLAIMS

1. Use of a polypeptide comprising the sequence \(X_1\text{WX}_2\text{X}_3\text{X}_4\text{RX}_5\text{X}_6\text{X}_7\text{X}_8\text{X}_9\) and derivatives thereof, optionally associated with a pharmaceutically acceptable vehicle, as an apoptosis inducer for the treatment of cancer, wherein:

- \(X_1\) is an amino acid selected in the group consisting in histidine (H), tyrosine (Y), aspartic acid (D), glutamine (Q), and leucine (L), preferably histidine (H) or tyrosine (Y);

- \(X_2\) is a polar amino acid, charged or not, and selected in the group consisting in serine (S) threonine (T), lysine (K), arginine (R), asparagine (N), aspartic acid (D), cysteine (C), glycine (G), glutamine (Q), histidine (H), and glutamic acid (E);

- \(X_3\) is a polar amino acid, charged or not, and selected in the group consisting in serine (S), threonine (T), asparagine (N), cysteine (C), histidine (H), leucine (L), tyrosine (Y), alanine (A), and isoleucine (I);

- \(X_4\) is an amino acid selected in the group consisting in threonine (T), arginine (R), asparagine (N), alanine (A), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tryptophane (W), valine (V) and tyrosine (Y);

- \(X_5\) is an amino acid selected in the group consisting in leucine (L), methionine (M), isoleucine (I), tryptophane (W), tyrosine (Y), histidine (H), glutamine (Q), lysine (K), and arginine (R);

- \(X_6\) is an amino acid selected in the group consisting in leucine (L), and glutamic acid (E);

- \(X_7\) is an amino acid selected in the group consisting in cysteine (C), asparagine (N), alanine (A), tyrosine (Y), serine (S), glutamine (Q), and proline (P);

- \(X_8\) is a hydrophobic amino acid selected in the group consisting in alanine (A), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tryptophane (W), valine (V) and tyrosine (Y); and
- $X_0$ is a threonine or an hydrophobic amino acid selected in the group consisting in alanine (A), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tryptophane (W), valine (V) and tyrosine (Y), wherein the polypeptide comprises no more than about 50 amino acid residues of the HPV E2 protein.

2. The use according to claim 1, wherein said polypeptide comprises no more than about 50 amino acid residues.

3. The use according to claims 1 and 2, wherein said polypeptide is directed to the cytoplasm of the cancer cells.

4. The use according to claims 1 and 3, wherein said polypeptide comprises an amino acid sequence which is selected in the group comprising YWKHMRLLECAI (position 32 to 42 of SEQ ID NO:2), YWKHIRLECVL (position 32 to 42 of SEQ ID NO:3), YWKLRLECAV (position 32 to 42 of SEQ ID NO:4), HWKLTRMECVL (position 32 to 42 of SEQ ID NO:5), HWKLIRMTECAI (position 32 to 42 of SEQ ID NO:6), HWKLIRMTECAL (position 32 to 42 of SEQ ID NO:7), HWLRRIEICAL (position 32 to 42 of SEQ ID NO:8), YWNCVRLENAI (position 32 to 42 of SEQ ID NO:9), YWKYVRLENAI (position 32 to 42 of SEQ ID NO:10), YWKCVRMEAI (position 32 to 42 of SEQ ID NO:11), YWKLVRMENVI (position 32 to 42 of SEQ ID NO:12), YWQLIRWENAI (position 31 to 41 of SEQ ID NO:13), YWQLIRLENAI (position 31 to 41 of SEQ ID NO:14), YWKLVRYECAI (position 32 to 42 of SEQ ID NO:15), YWKLVRYECAI (position 32 to 42 of SEQ ID NO:16), YWTLVRYECAI (position 32 to 42 of SEQ ID NO:17), YWTTLRYECAAI (position 32 to 42 of SEQ ID NO:18), YWKAVRHEYVL (position 32 to 42 of SEQ ID NO:19), YWKAVRQENVI (position 32 to 42 of SEQ ID NO:20), YWKAVRHHENVV (position 32 to 42 of SEQ ID NO:21), HWHYVRLENAML (position 32 to 42 of SEQ ID NO:22), HWKCIYRVECVL (position 32 to 42 of SEQ ID NO:23), HWKCIYRVECVL (position 32 to 42 of SEQ ID NO:24), HWKCLRYESVL (position 35 to 45 of SEQ ID NO:25), HWKCMRHESVL (position 32 to 42 of SEQ ID NO:26), HWKCMRHESVL (position 32 to 42 of SEQ ID NO:27), HWKCIYRLESVL (position 32 to 42 of SEQ ID NO:28), HWKCLRLEAAL (position 32 to 42 of SEQ ID NO:29), HWKCLRMEAVV (position 32 to 42 of SEQ ID NO:30), HWKCIYRLECAI (position 32 to 42 of SEQ ID NO:31), HWKCIYRLECAI (position 32 to 42 of SEQ ID NO:32), HWHKVRHENVL (position 32 to 42 of SEQ ID NO:33), HWHKVRLENVL (position 32 to 42 of SEQ ID NO:34), HWQLLRSEQII (position 32 to 42 of SEQ ID NO:35), HWQLLRSEQIL (position 32 to 42 of SEQ ID NO:36), HWQLLRSEQVL (position 32 to 42 of SEQ ID NO:37), HWQLLRSEQIL
(position 32 to 42 of SEQ ID NO:38), HWQLRQEQVL (position 32 to 42 of SEQ ID NO:39), HWQTLRQEQL (position 32 to 42 of SEQ ID NO:40), HWKLLRQEQL (position 32 to 42 of SEQ ID NO:41), HWQTLRKEAV (position 32 to 42 of SEQ ID NO:42), HWQTLRKEPVL (position 32 to 42 of SEQ ID NO:43), HWQTLRQEAVL (position 32 to 42 of SEQ ID NO:44), HWQTLRKEAVT (position 32 to 42 of SEQ ID NO:45), HWTLLRREAVL (position 32 to 42 of SEQ ID NO:46), HWLLLRLKEAVL (position 32 to 42 of SEQ ID NO:47), HWQTLRKEAVL (position 32 to 42 of SEQ ID NO:48), HWQTLRKEAVL (position 32 to 42 of SEQ ID NO:49), HWQTLRKEAVL (position 32 to 42 of SEQ ID NO:50), HWQILRREAVL (position 32 to 42 of SEQ ID NO:51), HWQILRKEAVL (position 32 to 42 of SEQ ID NO:52), HWRLLRKEAVL (position 32 to 42 of SEQ ID NO:53), HWQALRREAVL (position 32 to 42 of SEQ ID NO:54), QWNLLRQEQL (position 32 to 42 of SEQ ID NO:55), QWNLIRQEQL (position 32 to 42 of SEQ ID NO:56), YWDLDNRLKLYVT (position 29 to 39 of SEQ ID NO:57), YWENVRKENAI (position 32 to 42 of SEQ ID NO:58), YWENIRKENAI (position 32 to 42 of SEQ ID NO:59), YWESMRKEQV (position 31 to 41 of SEQ ID NO:60), YWKAVRLLENVI (position 31 to 41 of SEQ ID NO:61), and LWNLLRRENENAI (position 32 to 42 of SEQ ID NO:63).

5. The use according to claim 1 to 4, wherein said polypeptide comprises an amino acid sequence which is selected in the group comprising YWQLRWEAII (position 31 to 41 of SEQ ID NO:13), YWKhMLRLECAII (position 32 to 42 of SEQ ID NO:2), HWKCRLRESVL (position 32 to 42 of SEQ ID NO:28), and HWKCMRHESEL (position 32 to 42 of SEQ ID NO:26).

6. The use according to claim 1 to 5, wherein said polypeptide comprises an amino acid sequence which is selected in the group comprising YWKMRLRECAII (position 32 to 44 of SEQ ID NO:2), YWKHMLRCEMLMY (position 32 to 44 of SEQ ID NO:3), YWKLIRLCAVGY (position 32 to 44 of SEQ ID NO:4), HWKLMRCECRLMY (position 32 to 44 of SEQ ID NO:5), HWKLIRMLCARMY (position 32 to 44 of SEQ ID NO:6), HWKLIRMCALLY (position 32 to 44 of SEQ ID NO:7), HWRLRRIECALYY (position 32 to 44 of SEQ ID NO:8), YWNCVRLREAIY (position 32 to 44 of SEQ ID NO:9), YWCKYVRLENAIY (position 32 to 44 of SEQ ID NO:10), YWKCVRMENAIFY (position 32 to 44 of SEQ ID NO:11), YWKLVRMENVILF (position 32 to 44 of SEQ ID NO:12), YWQLRWEAIIFF (position 31 to 43 of SEQ ID NO:13), YWQLRLENAII (position 31 to 43 of SEQ ID NO:14), YWKLVRYECAIFY (position 32 to 44 of SEQ
ID NO:15), YWKLVRYECAIFY (position 32 to 44 of SEQ ID NO:16), YWTLVRYECAMFY (position 32 to 44 of SEQ ID NO:17), YWTLLRYEAAAMFY (position 32 to 44 of SEQ ID NO:18), YWKAVRHEYVLYY (position 32 to 44 of SEQ ID NO:19), YWKAVRQENVIYY (position 32 to 44 of SEQ ID NO:20), YWKAVRHEENVVLY (position 32 to 44 of SEQ ID NO:21), HWHYVRLENAMLLF (position 32 to 44 of SEQ ID NO:22), HWKCIKYECVLLH (position 32 to 44 of SEQ ID NO:23), HWKCIKYECVLLH (position 32 to 44 of SEQ ID NO:24), HWKCLRYEESVLLH (position 35 to 47 of SEQ ID NO:25), HWKCMRHEVSLY (position 32 to 44 of SEQ ID NO:26), HWKCMRHEVSLY (position 32 to 44 of SEQ ID NO:27), HWKCIYRELVSLH (position 32 to 44 of SEQ ID NO:28), HWKCIYRELVSLH (position 32 to 44 of SEQ ID NO:29), HWKCIYRELVSLH (position 32 to 44 of SEQ ID NO:30), HWKCIYRELVSLH (position 32 to 44 of SEQ ID NO:31), HWKCIYRELVSLH (position 32 to 44 of SEQ ID NO:32), HWKCIYRELVSLH (position 32 to 44 of SEQ ID NO:33), HWKCIYRELVSLH (position 32 to 44 of SEQ ID NO:34), HWQILLRQEIQIYH (position 32 to 44 of SEQ ID NO:35), HWQILLRQEIQIYH (position 32 to 44 of SEQ ID NO:36), HWQILLRQEIQIYH (position 32 to 44 of SEQ ID NO:37), HWQILLRQEIQIYH (position 32 to 44 of SEQ ID NO:38), HWQILLRQEIQIYH (position 32 to 44 of SEQ ID NO:39), HWQILLRQEIQIYH (position 32 to 44 of SEQ ID NO:40), HWQILLRQEIQIYH (position 32 to 44 of SEQ ID NO:41), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:42), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:43), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:44), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:45), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:46), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:47), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:48), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:49), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:50), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:51), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:52), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:53), HWQILLRKEAVLLY (position 32 to 44 of SEQ ID NO:54), QWNLLRQUEQVLFH (position 32 to 44 of SEQ ID NO:55), QWNLLRQUEQVLFH (position 32 to 44 of SEQ ID NO:56), YWDLNRLYVTTY (position 29 to 41 of SEQ ID NO:57), YWENVRKENAIMH (position 32 to 44 of SEQ ID NO:58), YWENVRKENAIMH (position 32 to 44 of SEQ ID NO:59), YWENVRKENAIMH (position 32 to 44 of SEQ ID NO:60), YWESMRKEQVLFH (position 31 to 43 of SEQ ID NO:61), YWKAVRLENIYAF (position 31 to 43 of SEQ ID NO:62), and LWNLLRRENAIWY (position 32 to 44 of SEQ ID NO:63).
7. The use according to claim 1 to 6, wherein said polypeptide comprises an amino acid sequence which is selected in the group comprising YWQLIRWENAIF (position 31 to 43 of SEQ ID NO:13), YWKHMRLECAIYY (position 32 to 44 of SEQ ID NO:2), HWKCIRESVLLH (position 32 to 44 of SEQ ID NO:28), and HWKCMRHESVLGY (position 32 to 44 of SEQ ID NO:26).

8. The use according to claims 1 to 7, wherein said polypeptide further comprises an amino acid sequence coding for a cell penetrating peptide which favours its internalisation into the cytoplasm.

9. The use according to claims 1 to 7, wherein said polypeptide is formulated in lipid vesicles such as liposomes.

10. Use of a nucleic acid encoding for a polypeptide as defined in any one of claims 1 to 8, optionally associated with a pharmaceutically acceptable vehicle, in a pharmaceutical composition for the treatment of cancer in a subject.

11. The use according to claim 10, wherein said nucleic acid corresponds to the insert of the expression plasmid contained in the bacteria CNCM-3743 or CNCM-3744.

12. Use of a vector comprising a nucleic acid as defined in claim 10, optionally associated with a pharmaceutically acceptable vehicle, in a pharmaceutical composition for the treatment of cancer in a subject.

13. The use according to claim 12, wherein said vector corresponds to the vector contained in the bacteria CNCM-3743 or CNCM-3744.

14. Use of a host cell genetically engineered with the nucleic acid as defined in any one of claims 10 or 11, with the vector as defined in any one of claims 12 or 13, in a pharmaceutical composition for the treatment of cancer in a subject.

15. The use according to claim 14, wherein said host cell corresponds to the bacteria CNCM-3743 or CNCM-3744.

16. Use of an antibody directed against a polypeptide as defined in any one claims 1 to 8, optionally associated with a pharmaceutically acceptable vehicle, in a pharmaceutical composition for the treatment of cancer in a subject.

17. The use according to any previous claims, wherein said cancer is a solid cancer.
Figure 1
Figure 2
Figure 4
Figure 6
Figure 9

% apoptotic cells

pcgf-H2-18  W37V  W37G  R41A  pcgf-H2-11  pcgf-H2-16

H2 of E2 domain of HPV11 (aa 23-49)  H2 of E2 domain of HPV16 (aa 23 à 49)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K 14/025, C12N 15/37, A61K 38/16

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K C12N

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

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)
EPO-Internal, Sequence Search, CHEM ABS Data, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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See patent family annex.

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Date of the actual completion of the international search: 26 September 2008

Date of mailing of the international search report: 13/10/2008

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