



(51) International Patent Classification:

C12N 15/113 (2010.01) C12N 15/115 (2010.01)

(21) International Application Number:

PCT/IT2019/050159

(22) International Filing Date:

05 July 2019 (05.07.2019)

(25) Filing Language:

Italian

(26) Publication Language:

English

(30) Priority Data:

102018000007017 06 July 2018 (06.07.2018) IT

(71) Applicant: ULISSE BIOMED S.R.L. [IT/IT]; Via Cavour, 20, 33100 UDINE (IT).

(72) Inventors: IPPODRINO, Rudy; Viale Miramare, 39, 34135 TRIESTE (IT). MARINI, Bruna; Salita di Contovello, 27, 34136 TRIESTE (IT). BERTOCCO, Martina; Via Rodolfo Rodolfi, 28, 33075 CORDOVADO (IT).

(74) Agent: PETRAZ, Davide Luigi et al.; GLP SRL, Viale Europa Unita, 171, 33100 UDINE (IT).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Published:

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: APTAMERS FOR USE IN THE THERAPY, PREVENTION, DIAGNOSIS AND DETECTION OF THE PAPILLOMAVIRUS

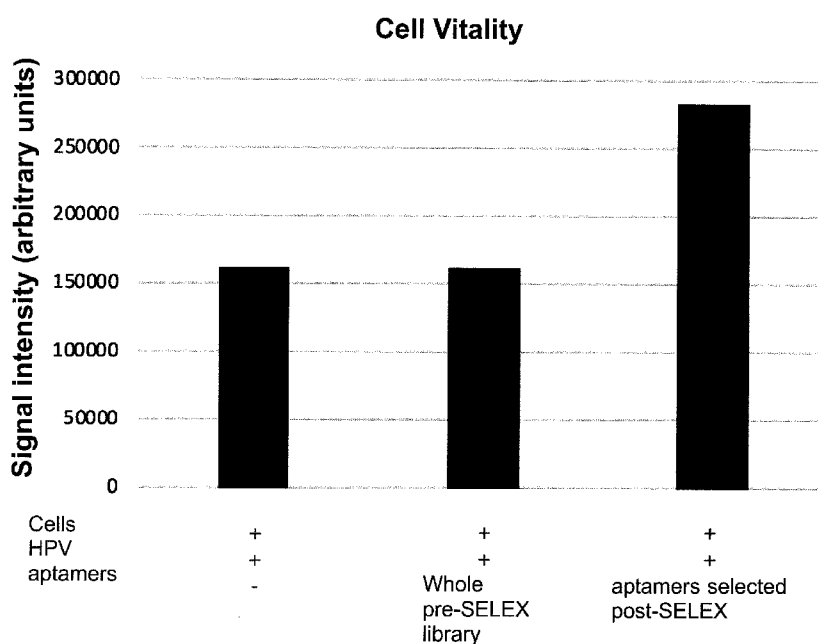


fig. 1

(57) Abstract: Aptamers for use in the therapy, prevention, diagnosis and detection of the Papillomavirus.



- 1 -

“APTAMERS FOR USE IN THE THERAPY, PREVENTION, DIAGNOSIS AND DETECTION OF THE PAPILLOMAVIRUS”

* * * * *

FIELD OF THE INVENTION

5 Embodiments described here concern aptamers for use in the therapy, prevention, diagnosis and detection of the Papillomavirus.

BACKGROUND OF THE INVENTION

Papillomavirus

10 It is known that Papillomaviruses belong to the Papillomaviridae family and comprise a heterogeneous group of viruses capable of infecting birds, reptiles, marsupials and mammals (Bravo, I. G., de Sanjosé, S. & Gottschling, M. *The clinical importance of understanding the evolution of Papillomaviruses* Trends Microbiol. 432–438 (2010)).

15 According to the database called Papillomavirus Episteme, (PaVe; <https://pave.niaid.nih.gov/>), more than 250 Papillomaviruses have been identified and over 100 of these can be found in humans (Bernard, H.U. et al. *Classification of Papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments*. Virology 70–79 (2010)).

20 Human Papillomaviruses (HPVs) are double-stranded DNA viruses, which infect the epithelial tissues of the mucous membrane of the anogenital tract, the oral cavity and the upper digestive tract, as well as the cutaneous epithelial tissues of the hands, feet and torso (Van Doorslaer, K. et al. *Evolution of the Papillomaviridae*. Virology 11-20 (2013)). The largest genus is the α group, which comprises 64 HPV which mainly infect epithelia of the mucous membrane (mucosal HPV). The thirteen high-risk HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 25 56, 58 and 59) that have been classified as oncogenes and the six types that are probably high-risk (26, 53, 66, 68, 73 and 82) belong to this group.

30 HPV can cause different types of tumors, but certainly cervical cancer is the one most significantly associated with HPV (Venturoli, S. et al. *Correlation of high-risk human Papillomavirus genotypes persistence and risk of residual or recurrent cervical disease after surgical treatment*. J. Med. Virol. 1434–1440 (2008)).

In addition to mucosal genotypes, there are cutaneous α -HPVs considered to

- 2 -

be low risk, that is, they have a low oncogenic potential. HPV2 and 57 (which cause common warts), HPV3 and 10 (which cause flat warts) belong to this class. The next largest group is β , whose members primarily infect cutaneous epithelia (Tommasino, M. et al. *The biology of beta human Papillomaviruses*. Virus Research 128–138 (2017)). They can be associated with human tumors and, in particular, with non-melanoma skin cancers. The HPVs of the remaining groups (in particular γ , μ and ν) normally only cause lesions of the benign type.

HPV viral entry and cellular trafficking

The adhesion of the HPV virion to the target cells represents the first fundamental event in the viral infectious cycle. This process begins with the extracellular bonding of heparan sulphate proteoglycan (HSPG) which induces a conformational change of the capsid proteins L1 and L2; this leads to the subsequent transfer of virions to a complex of superficial membrane receptors necessary for the entry of molecules into the cell (Aksoy P. et al. *Rev. HPV entry into cells*. Mutat. Res. 13–22 (2017)). Although the Papillomavirus infectious cycle has been extensively studied, as well as its oncogenic capacity, the process of viral entry is, on the contrary, one of the least characterized biological mechanisms.

The Kallikrein-8 (KLK8) and furin proteins specifically bond the L1 and L2 proteins and this represents an important “activating” event that allows the subsequent intracellular trafficking of the viral particles. Viral endocytosis occurs through a process dependent on actin; tetraspanin protein CD151 and its associated $\alpha 3\beta 1$, $\alpha 6\beta 1$ and $\alpha 6\beta 4$, the growth factor tyrosine-kinase receptors, annexin A2 and the cytoskeletal adapter obscurin-like 1 (OBSL1) are involved in the HPV16 entry dependent on a microdomain enriched with tetraspanine (TEM). It is important to underline that most of the specific mechanisms that characterize the viral entry of Papillomavirus and the corresponding intracellular trafficking have been described only for a small population of Papillomavirus, mainly HPV16.

While the endocytosis of the individual viral particles linked to the cell surface is relatively rapid, the internalization of the particles with high m.o.i. (moiety of infection) is discontinuous and slow, and takes several hours (Schelhaas M. Helenius A. et al. *Entry of human Papillomavirus type 16 by actin-dependent*,

- 3 -

clathrin- and lipid raft-independent endocytosis. PLoS Pathog. (2012)).

The CD151 proteins and the associated TEMs probably coordinate the organization and assembly of the entry receptor complexes; once assembled and bound to the virion, these complexes facilitate rapid entry. A similar role played
5 by other tetraspanins has been found in the case of other viruses with or without envelopes (Feneant L. et al. *CD81 and hepatitis c virus (HCV) infection*. Viruses. 535–572. (2014)). The cleavage of L2 by furin occurs on the surface of the host cell and on the extracellular matrix (ECM) after the bonding of the virions with HSPG (Richards RM et al. *Cleavage of the Papillomavirus minor capsid protein, L2, at a furin consensus site is necessary for infection*. PNAS. 1522–1527
10 (2006)).

The C-terminal of furin is cleaved on the final residue (arginine) of a conserved consensus site (RTKR, residues 9-12 for HPV16); 12 N-terminal residues of the HPV16 L2 protein are removed. The biological significance of
15 this cleavage remains unknown, but the inhibition of the proteolysis by means of the mutation of the cleavage site or the biochemical inhibition of the furin causes alternations in the trafficking of the L2/vDNA (viral DNA) complex, and the viral infection is potentially inhibited.

The furin-mediated cleavage seems to cause a conformational change in the structure of the capsid and/or of the L2 protein, since the conserved and
20 neutralizing RG-1 epitope (residues 17-36 for HPV16) becomes accessible to staining with immunohistochemistry techniques shortly after the bonding of the virion in a furin-dependent manner (Day PM et al. *Mechanisms of human Papillomavirus type 16 neutralization by L2 cross-neutralizing and L1 type-specific antibodies*. J. Virol. 4638–4646. (2008)). The cell surface cyclophilins (peptidyl-prolyl isomerase, PPI) also seem to modulate the conformation of L2,
25 since the exposure of the RG-1 epitope is sensitive to cyclosporin A, a broad inhibitor of PPI (Broniarczyk J. et al. *Human Papillomavirus infection requires the TSG101 component of the ESCRT machinery*. Virology 460–461 (2014)).

30 Shortly after entry, initial HPV trafficking is modulated by tetraspanine CD63 and its partners syntenin/ALIX. These molecules are necessary for sorting the HPV virions from the early endosomes (EE) into late endosomal acids (LE) and multivesicular bodies (MVB), a prerequisite for the capsid disassembly,

- 4 -

uncoating and segregation of the L2/vDNA complex from the L1 protein (Broniarczyk J. et al. *The VPS4 component of the ESCRT machinery plays an essential role in HPV infectious entry and capsid disassembly*. Sci. Rep. (2017)).

5 If the MVB trafficking is interrupted by the knockdown of CD63 factor or syntenin, the subcellular transport of the L2/vDNA complex is altered and the infection is partially blocked; therefore these cofactors are an essential requirement for viral transport in MVBs.

Consequently, the components of the ESCRT (Endosomal Sorting Complexes Required for Transport) machinery, a group of cytosolic multisubunit complexes
10 that facilitate endosomal maturation and the biogenesis of the MVBs, guarantee an effective HPV infection.

Endosomal acidification is a necessary requirement for HPV16 infection, but it is unclear whether it is simply necessary for a correct endosomal maturation and biogenesis of the MBVs, or whether the acidification can also trigger
15 conformational changes in the capsid of the HPV, or in the host proteins that are required for downstream processes such as uncoating and vDNA trafficking.

Acid-dependent cathepsin proteases further cleave the L1 capsid by processing it inside the endosomal compartments.

The proteolysis and disassembly of the capsid can be visualized by means of
20 immunofluorescence with the monoclonal antibody L1-7, specific for the L1 303-313 residues, located in the central cavities below each of the L1 parameters (Bienkowska-Haba M. et al. *Cyclophilins facilitated dissociation of the human Papillomavirus type 16 capsid protein L1 from the L2/DNA complex following virus entry*. J. Virol 9875–9887 (2012)). This region is only available for the
25 bond to L1-7 after capsid degradation, about 8 hours after infection.

Although it is useful for staining the capsid disassembly, the reactivity of L1-7 does not reveal a true infectious uncoating, since the staining is blocked by the cathepsin inhibitors without any effect on infectivity. Within the MVBs the L2/vDNA complex is removed from the L1 capsid protein partially degraded in a
30 cyclophilin-dependent manner (Popa A. et al. *Direct bonding of retromer to human Papillomavirus type 16 minor capsid protein L2 mediates endosome exit during viral infection*. PLoS Pathog. (2015)).

The complex then transfers to the trans-Golgi (TGN) thanks to the retromer

(Popa A. et al. *Direct bonding of retromer to human Papillomavirus type 16 minor capsid protein L2 mediates endosome exit during viral infection*. PLoS Pathog. (2015)), where it resides until the beginning of mitosis (Di Giuseppe S. et al. *Human Papillomavirus major capsid protein L1 remains associated with the incoming viral genome throughout the entry process*. J. Virol. (2017)).

Recent studies have shown that a fraction of the L1 capsid, in the form of conformationally intact pentamers, accompanies the L2/vDNA complex to the TGN and nucleus, but the functional role of these pentamers of L1 remains unclear (Lipovsky A. et al. *Application of the proximity-dependent assay and fluorescence imaging approaches to study viral entry pathways*. Methods Mol. Biol. 437–451. (2015)).

The co-localization of L2/vDNA with TGN markers has been confirmed by numerous studies, but several groups have reported the transport of vDNA also in more distal retrograde sites. The co-localization of some partial vDNA markers with cis-Golgi markers has been reported, such as the GM130 factor and giantine (Zhang W et al. *Vesicular trafficking of incoming human Papillomavirus 16 to the Golgi apparatus and endoplasmic reticulum requires γ -secretase activity*. mBio. (2014)). In the same way, sensitive techniques such as the Proximity Ligation Assay have suggested that the L2/vDNA retrograde traffickings beyond the Golgi head toward the endoplasmic reticulum (ER) (Villeneuve J. et al. *Golgi enzymes do not cycle through the endoplasmic reticulum during protein secretion or mitosis*. Mol. Biol. Cell. 141–151 (2017)).

Whether these represent primary or alternative routes of infection, or also unproductive dead ends, is not yet clear. It is worth noting that the dynamic flow of proteins within the secretory compartments makes it difficult to determine precisely where the co-localization occurs by means of microscopy.

Therapeutic treatments of HPV infections

The therapeutic treatments for HPV infections are different depending on the site of the infection; the therapeutic choice differs according to the type of lesion, its position, the depth, the number and the extension of the affected area.

For example, with regard to infections to the uterine cervix, surgical removal (conization), laser therapy, cauterization or cryotherapy are performed, which are performed only when the lesion is considered to be of high grade and more prone

- 6 -

to the subsequent tumor development.

With regard to genital condyloma and cutaneous warts, surgical laser treatments, based on cauterization, or treatments with immunomodulators (such as Inquimod, produced by Aldara) are performed.

5 It is also possible to attack the warts with acids or destroy them with laser, with the diathermocoagulation technique or with liquid nitrogen, however all these methods cause damage to healthy tissue and there is a non-negligible frequency in the appearance of relapses. Furthermore, tissue repair is slow, and the wound can become infected.

10 Some isolated warts can be removed with the cutting scoop, called Curette, which is able to remove the wart from the rest of the skin. There are also some non-specific treatments that induce necrosis, coagulation of tissue proteins or superficial cytolysis (podophyllotoxin, trichloroacetic acid, nitrizinc complex, salicylic acid). There is also a derivative of green tea based on sinecatechins, an
15 activator of the immune system.

However, there is no specific therapy that has demonstrably proven to be effective in determining a complete recovery in all patients.

When warts regress spontaneously (about 65% of cases) they do not leave scars or other remains, while most chemical, physical, pharmacological or
20 surgical treatments entail a more or less high risk of side effects, adverse reactions and/or dichromatic or cicatricial outcomes.

However, warts can cause significant pain and discomfort to the patient, and spontaneous regression can occur very slowly (between one and five years). Current therapies have a very high rate of recurrence or re-infection; the high
25 contagiousness of infection and self-inoculation are elements that lead to a choice in favor of treatment.

Another important factor to be considered in favor of treatment of cutaneous warts is the possibility of eliminating the sources of contagion, and therefore for preventive purposes, for example with school-age children or with subjects living
30 in/frequenting communities.

For prevention purposes, it is recommended to protect small skin lesions that can facilitate the entry of the infective agent; observing basic hygiene rules, such as not sharing towels, slippers and nail care tools, contributes to prevention, but

does not reduce the risk.

The same concept can be applied to the management of genital infections, with regards to both condylomas and also to lesions to the cervix, vagina or penis. In this context, the use of condoms that act as a mechanical barrier is advised.

- 5 The condom offers a certain level of protection, but it cannot totally prevent infection, since it does not cover the genital region in its entirety.

Therefore, to date none of the known preventive tools is 100% effective. It is important to emphasize that there are no preventive or therapeutic pharmacological treatments that act directly on the infection of the
10 Papillomavirus.

Aptamers

Aptamers are synthetic nucleic acids consisting of oligonucleotides, for example DNA or RNA or other secondary structures of nucleic acids, of variable length (generally between 20 and 100 base pairs), having the property of bonding
15 to a molecule or a protein. They exploit the natural interactions that occur between nucleic acids and other molecules (proteins, peptides, sugars, lipids, small inorganic molecules, metal ions, or macrostructures such as whole viruses and bacteria, etc.).

Like other natural oligonucleotides, aptamers are able to establish strong
20 interactions with non-nucleic targets, thanks to the formation of secondary structures (hairpin structures, loops, quadruplex structures, etc.) that oligonucleotides can assume in addition to hydrogen bonds for Watson-Crick base-pairing.

Thanks to this high affinity they have potential applications in many fields,
25 such as the therapeutic one, drug-delivery, in biosensors and as diagnostic systems. The main advantage, in addition to the high affinity, lies in the fact that aptamers are chemically synthesized at a very low cost.

It is known that aptamers can bond different portions of the target molecule, and can have a functional influence on the target itself; for example, they can
30 inhibit the functionality of a protein, or they can mask its catalytic sites or those of interaction with other molecules.

Aptamers were developed in 1990 in parallel by two research groups: that of Dr. Larry Gold and that of Szostak lab, who coined the name.

- 8 -

Aptamers are generally produced by means of an in vitro selection called SELEX (*Systematic Evolution of Ligands by Exponential Enrichment*), which since 2001 can be performed in an automated manner, considerably reducing the selection time (from a few weeks to a few days).

5 In the last decades the use of aptamers as substitutes for antibodies has had a wide development, among the multiple applications of aptamers it is possible to mention their use as biological receptors having analytical and diagnostic purposes and as therapeutic molecules.

10 Aptamers offer many advantages with respect to proteins or small pharmacological molecules, since they are synthetic (high reproducibility of effect with respect to antibodies) and specific (higher specificity with respect to small molecules). Aptamers can be used directly in biological or synthetic fluids, however, often chemical modifications have to be made to guarantee their stability and the efficiency of their target recognition. To date, both DNA and
15 RNA aptamers have been selected for a variety of targets including: lysozyme, thrombin, HIV transactive responsive element, haemin, interferon gamma, Prostate Specific Antigen (PSA), VEGF, etc.

Aptamers are not only a valid alternative to antibodies but they also represent a peculiar class of molecules with unique specifications and a very broad
20 application potential.

Document MX-A-2010/012604 is known, which describes RNA aptamers for the detection and diagnosis of lesions of the cervix associated with human HPV, obtained by SELEX technique with ARN library, that is, a library of RNA aptamers. The E2, E7 and L1 proteins have been used individually as targets to
25 select RNA aptamers similar to them. These aptamers show affinity and specificity in bonding to the recombinant proteins E2, E7 and L1 of HPV16 produced in bacteria, and are used to detect HPV infections in biological samples. This known document describes the production in bacteria of VLPs (*Virus Like Particles*) which consist only of the L1 protein, but the aptamer
30 selection is not made using VLPs. Even if VLPs were used for the aptamer selection, aptamers against the L2 protein that is contained in the Papillomavirus capsid occurring in nature would not be selected.

Furthermore, in this known document the proteins E2, E7 and L1 for the

- 9 -

aptamer selection are used in a denatured form and therefore without a tertiary structure, which does not allow to obtain aptamers that bond the conformational epitopes of the Papillomavirus capsids occurring in nature. Furthermore, a further factor that limits the effectiveness of these aptamers in the detection of the virions occurring in nature is due to the production of proteins E2, E7 and L1 in prokaryotic systems (bacteria). In fact, since Papillomaviruses have the infectious cycle in eukaryotic cells, they normally have capsid proteins with post-translational modifications that the bacteria are not able to make. These post-translational modifications have a biological meaning in Papillomavirus since they contribute to cause its tropism, infectivity and immunogenicity (Fang et al. *Differences in the post-translational modifications of human papillomavirus type 6b major capsid protein expressed from a baculovirus system compared with a vaccinia virus system*. Biotechnol Appl Biochem. (2000)).

Therefore, in general the known document describes a system that is not able to effectively select epitopes exposed by Papillomaviruses occurring in nature.

Therefore, there is a need to perfect aptamers for use in the therapy, prevention, diagnosis and detection of the Papillomavirus that can overcome at least one of the disadvantages of the state of the art as described above.

The Applicant has devised, tested and embodied the present invention to overcome the shortcomings of the state of the art and to obtain these and other purposes and advantages.

Other limitations and disadvantages of conventional solutions and technologies will be clear to a person of skill after reading the remaining part of the present description with reference to the drawings and the description of the embodiments that follow, although it is clear that the description of the state of the art connected to the present description has to not be considered an admission that what is described here is already known from the state of the prior art.

SUMMARY OF THE INVENTION

The present invention is set forth and characterized in the independent claim. The dependent claims describe other characteristics of the invention or variants to the main inventive idea.

In accordance with some embodiments, aptamers are provided for use in the therapy and/or prevention of the Papillomavirus. According to one embodiment,

- 10 -

the aptamers are configured to specifically bond with high affinity conformational epitopes with post-translational modifications belonging to the entire capsid of the Papillomavirus.

5 A product for topical application on skin and/or mucous membranes, and/or a drug or medical device comprising aptamers in accordance with the present description are also included in the embodiments described here.

Further embodiments also concern a method to diagnose the Papillomavirus. According to one embodiment, the method provides to use, in a biological sample of body fluid, aptamers according to the present description, wherein the
10 aptamers alternatively:

- are bonded with a suitable marker whose activity is monitored in order to detect the presence of the Papillomavirus in body fluids, wherein the marker is a fluorescence reporter, or an enzyme, whose activity can be detectable electrochemically, or via absorbance or luminescence;
- 15 - act as primary antibody in immunochemical techniques;
- bond with a nano-switch for the detection of protein analytes;
- are used in biosensor techniques that comprise measurements in amperometry, impedance, voltammetry, capacitance, electrochemiluminescence, chemiluminescence.

20 Other embodiments concern a kit for the diagnosis of Papillomavirus; the kit comprises aptamers in accordance with the present description.

Further embodiments concern a method to select aptamers for the therapy, prevention, diagnosis and detection of the Papillomavirus, the method providing to generate aptamers which, as well as being configured to specifically bond with
25 high affinity conformational epitopes and with post-translational modifications belonging to the entire capsid of the Papillomavirus, also have specific characteristics that also at the same time satisfy the following conditions:

- i) high affinity bond with the epitopes defined using the SELEX technique;
- ii) bond with the epitopes having a defined inhibitory function (functional
30 assay);
- iii) stability in body fluids and/or iv) stability in products for topical application or use on the skin or mucous membranes, for example, creams, gels and oils;

- 11 -

wherein the conditions iii) and iv) can be both satisfied or alternative, depending on whether the aptamers are provided respectively for diagnostic use in a biological sample or for use in topical application on the skin and/or mucous membranes.

5 Further embodiments concern a method for the identification of novel epitopes responsible for infectivity of the Papillomaviruses. In one embodiment, the method provides to use aptamers selected by an aptamer selection method according to the present description to obtain an initial demonstration of a site relevant for infectivity that, once identified, can be used as a target of drugs or
10 monoclonal antibodies.

These and other aspects, characteristics and advantages of the present disclosure will be better understood with reference to the following description, drawings and attached claims. The drawings, which are integrated and form part of the present description, show some embodiments of the present invention, and
15 together with the description, are intended to describe the principles of the disclosure.

The various aspects and characteristics described in the present description can be applied individually where possible. These individual aspects, for example aspects and characteristics described in the attached dependent claims, can be the
20 object of divisional applications.

It is understood that any aspect or characteristic that is discovered, during the patenting process, to be already known, shall not be claimed and shall be the object of a disclaimer.

BRIEF DESCRIPTION OF THE DRAWINGS

25 These and other characteristics of the present invention will become apparent from the following description of some embodiments, given as a non-restrictive example with reference to the attached drawings wherein:

- Fig. 1 is an exemplifying and representative graph of the effect on HaCaT cells of the addition of HPV-specific aptamers selected by SELEX, measured in terms
30 of cellular vitality;
- Fig. 2 is a flow chart of a technique used to select aptamers according to embodiments described here.

DETAILED DESCRIPTION OF SOME EMBODIMENTS

- 12 -

We will now refer in detail to the various embodiments of the present invention, of which one or more examples are shown in the attached drawings. Each example is supplied by way of illustration of the invention and shall not be understood as a limitation thereof. For example, the characteristics shown or
5 described inasmuch as they are part of one embodiment can be adopted on, or in association with, other embodiments to produce another embodiment. It is understood that the present invention shall include all such modifications and variants.

Before describing these embodiments, we have to also clarify that the present
10 description is not limited in its application to details of the construction and disposition of the components as described in the following description using the attached drawings. The present description can provide other embodiments and can be obtained or executed in various other ways. We have to also clarify that the phraseology and terminology used here is for the purposes of description only,
15 and cannot be considered as limitative.

Unless otherwise defined, all the technical and scientific terms used here and hereafter have the same meaning as commonly understood by a person with ordinary experience in the field of the art to which the present invention belongs. Even if methods and materials similar or equivalent to those described here can
20 be used in practice and in the trials of the present invention, the methods and materials are described hereafter as an example. In the event of conflict, the present application shall prevail, including its definitions. The materials, methods and examples have a purely illustrative purpose and shall not be understood restrictively.

25 Embodiments described here concern the use of aptamers composed of secondary structures of synthetic nucleic acids and aptamers composed of secondary structures of nucleic acids chemically modified to prevent, limit, treat, detect and diagnose infections caused by Papillomavirus. These aptamers have been selected to be stable in body fluids, to bond both linear and also
30 conformational epitopes present in the entire Papillomavirus capsid inhibiting its cellular infectivity.

In particular, one aspect of the present description concerns aptamers for use in the therapy and/or prevention of the Papillomavirus, the aptamers being

configured to specifically bond with high affinity conformational epitopes and with post-translational modifications belonging to the whole Papillomavirus capsid.

Here and in the present description, with the term “post-translational
5 modifications” we mean, for example, glycosylations, phosphorylations, mannosylations, sumoylations, ubiquitinations, methylations, acylations, prenylation, conjugation with glycosylphosphatidylinositol, lipoylation, conjugation with flavin-based compounds, conjugation to EME groups, phosphopantetheinylation, Schiff bases, alkylations, amidation,
10 polyglutamylation, polyglycylation, butyrylation, gamma-carboxylation, hydroxylation, iodination, nucleotide addition (ADP-ribosylation), adenylation, uridylation, propionylation, conjugation with S-glutathione, S-nitrosylation, S-sulphenylation, S-sulfinylation, succinylation, carbamylation, carbonylation, biotinylation, oxidation, PEGylation, ISGylation, neddylation, pupylation,
15 citrullination, deamidation, eliminylation, addition of disulfide bridges, racemization.

The aptamers or mixture of aptamers that can be used in association with the embodiments described here can be selected from one or more of the following types of aptamers:

- 20 - DNA aptamers: they are more economical to produce, stable up to one year at ambient temperature without loss of performance, and they have a half-life in terms of hours in complex biological samples such as blood, containing nucleases;
- RNA aptamers: they are more expensive to produce and more unstable,
25 however they can be modified to be very stable in complex biological samples such as blood in order to resist nucleases;
- PNA (*peptide nucleic acids*) aptamers: they have a structure similar to DNA but based on a peptide backbone; therefore it is insensitive to the action of both nucleases and proteases;
- 30 - XNA (*xeno nucleic acids*) aptamers: they contain “unnatural” analogues of the nitrogenous bases that confer a particular resistance to nucleases and proteases on them;
- LNA (*locked nucleic acids*) aptamers: they contain ribonucleotides modified

- 14 -

with an additional bond that connects oxygen at the 2' with carbon at the 4'; they are extremely similar, specific and stable;

- spiegelmers: they are RNA aptamers but with a backbone based on L-ribose instead of D-ribose.

5 In possible embodiments, aptamers can be DNA, RNA, PNA, XNA, LNA, and spiegelmer aptamers. A possible example embodiment are DNA aptamers. Another possible embodiment are RNA aptamers.

10 The Applicant has advantageously used the SELEX technique (*Systematic Evolution of Ligands by Exponential enrichment*) by means of which it is possible to identify and select aptamers that bond targets such as a protein, for example.

In particular, the Applicant has used the SELEX technique to select aptamers that meet the following requirements:

15 a) they bond not only the single proteins of the viral capsid but also bond complex structures of the viral capsid such as conformational epitopes also comprising protein elements with post-translational modifications: in fact SELEX was not performed with viral proteins but with whole capsids;

20 b) the Applicant has found that not everything that bonds the capsid inhibits the viral infection; therefore, in conjunction with the bond, the capacity of the selected aptamers is inhibitory toward the viral infection caused by Papillomavirus;

c) the aptamers have characteristics that are intrinsic and/or due to chemical and/or nucleotide modifications able to confer stability of the molecules in body fluids;

25 d) the possibility of being used successfully for the diagnosis or treatment of the Papillomavirus.

30 The aptamers used according to the present description have been selected in vitro by means of SELEX technique. The aptamers bonding the Papillomavirus capsid have proved to have a high affinity and stability. The selected aptamers have a significant in vitro protective effect, since they protect cells from viral infection preserving their vitality if infected with Papillomavirus.

Since DNA is generally recognized as safe for topical applications, it is an appealing candidate for broad-spectrum topical anti-viral use, to block the

transmission and self-infection of HPV (DNA is classified as skin conditioning - CAS-no 9007-49-2).

Therefore, the aptamers of the embodiments described here have been selected against the Papillomavirus pseudovirions, not against the individual
5 Papillomavirus proteins such as E2, E7 and L1; these pseudovirions are very complex structures, they are whole assembled capsids that have infective power. Advantageously, these pseudovirion structures are the structure most similar to the naturally occurring Papillomaviruses, unlike the VLPs viral particles since the latter contain only the L1 protein, while the pseudovirions are composed of L1
10 and L2 assembled in a capsid, as occurs in wild-type Papillomavirus. In addition, pseudovirions are produced in eukaryotic cells and have a glycosylation pattern which is much more similar to that of natural Papillomavirus virions, as opposed to the individual proteins, which are only parts of viruses, produced in bacteria; the latter, therefore, are very different organisms to eukaryotic cells in that they
15 are not provided with an apparatus able to perform post-translational modifications.

In this way, as better described below, the aptamers obtained by means of this SELEX are further selected for inhibitory capacity of the viral infection and therefore biologically active. Populations of biologically active aptamers are
20 therefore selected that also recognize linkage elements of the capsomeres, protein epitopes with post-translational modifications and conformational epitopes that it would not be possible to obtain if single HPV proteins were used, such as for example E2, E7 and L1. In particular, the target part of the SELEX technique is not an individual protein, but an active biological unit, that is, the entire structure
25 of the pseudovirion, that is, whole assembled Papillomavirus capsids that have infective power.

According to some embodiments, therefore, a method to select aptamers is also provided for the therapy, prevention, diagnosis and detection of the Papillomavirus, said method providing to generate aptamers having specific
30 characteristics not only defined by the bond to the viral capsid but also that simultaneously satisfy the following conditions:

- i) high affinity bond with the capsid (SELEX);
- ii) bond with inhibitory effect on the viral infection (functional assay);

- 16 -

iii) stability in body fluids and/or iv) stability in products for topical use application on the skin or mucous membranes, for example, creams, gels and oils;

The aspects iii) and iv) can both be present or alternative, for example
5 depending on whether the aptamers are provided for diagnostic use in a biological sample (aspect iii), or for use in topical application on the skin and/or mucous membranes (aspect iv).

Here and in the following description, by 'mucous membrane' we mean a membrane that covers the internal face of some organic apparatuses, such as the
10 bladder mucous membrane, the nasal mucous membrane, the vaginal mucous membrane, the rectal mucous membrane, the oral mucous membrane, meaning also the internal region of the mouth including gums, the labial mucous membrane, the oropharyngeal mucous membrane, and which is continuously kept moist by the secretion of muciparous glands or glands of a different nature.

15 In particular, the aptamers usable in accordance with the embodiments described here could be contained, comprised or formulated in products for topical application or use on skin or mucous membranes such as for example:

- creams and soaps for the skin and mucous membranes;
- oils, gels, soaps and body moisturizers;
- 20 - mouthwashes, toothpastes, ointments, sprays and other products for oral health and hygiene;
- mechanical contraceptives;
- non-viral vectors able to transfect the aptamers at the intracellular level.

As described above, to date no active molecule exists that can effectively
25 prevent or directly counter the Papillomavirus infection. In this context, the embodiments described here solve this currently unresolved problem, and in particular can be used:

- 1) for preventive purposes, to avoid or reduce the possibility of contagion both at the cervical, anogenital, oral level and also at the cutaneous level, that is, in all
30 those compartments where the Papillomaviruses can generate an infection that is harmful to tissue;
- 2) for therapeutic purposes, in the case of clear Papillomavirus lesions at the cervical, anogenital, oral or cutaneous level, in order to prevent the self-infection

- 17 -

and propagation of the Papillomavirus by reducing the recurrence rates and guaranteeing the adequate and decisive treatment of the infection itself;

3) for diagnostic purposes, as the stained aptamer can be used to detect the Papillomavirus in biological samples and consequently to diagnose the infection;

5 4) as a scientific tool for molecular investigation to discover and characterize new epitopes responsible for viral infectivity. Not all epitopes related to viral infectivity are known; in fact, by finding, using the technique described here, an aptamer able to strongly inhibit the infection and proceeding to study the epitope, it is possible to characterize new sites of still unknown interactions.

10 Furthermore, embodiments described here can also be used in the field of basic and applied research to show viral particles in cellular models, animal models, or in vitro systems.

Specifically, for example, the Applicant has conducted experiments to investigate the effect of Papillomavirus-specific aptamers selected by SELEX
15 according to the present description, on HaCaT cells, measuring the protective effect of aptamers in the presence of Papillomavirus through analysis of cellular vitality (see fig. 1). From fig. 1 it can be observed that the administration of the full library of aptamers (*full library*, second column) does not preserve the vitality of the cells, which is comparable to the value found in the case of
20 Papillomavirus infection in the absence of aptamers (first column). However, the addition of Papillomavirus-specific aptamers allows to increase cell vitality (third column), demonstrating a protective effect.

Aptamers

The aptamers or mixture of aptamers according to the present description can
25 comprise DNA aptamers with a length from 15 to 100 nucleotides stable in body fluids since chemically modified and/or subjected to nucleotide modification, having both the ability to bond the Papillomavirus capsid and also at the same time to inhibit its entry into the target cells, with the result of limiting viral infectivity.

30 In possible embodiments, consideration is given to both variants in which a specific aptamer recognizes only one genotype, and therefore specific for a single strain (for example an aptamer for HPV16), and also variants in which aptamers are provided in multiplex for a group of genotypes.

In some embodiments, a mixture of aptamers can be provided which is able to bond and inhibit the infection specifically of one or more human Papillomaviruses (HPV) of the type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73.

5 In a preferred embodiment, the mixture of aptamers is able to bond and inhibit the infection specifically of one or more human Papillomaviruses (HPV) of the type 6, 11, 40, 42, 43, and 44.

In a preferred embodiment, the mixture of aptamers is able to bond and inhibit the infection specifically of one or more human Papillomaviruses (HPV) of the
10 type 27, 57, 2, 4, 5, 1, 65.

In a preferred embodiment, the mixture of aptamers is able to bond and inhibit the infection specifically of at least one of the non-human Papillomaviruses such as: AaPV1 (EEPV), BgPV1, BPV1, BPV2, BPV3, BPV4, BPV5, BPV6, BPV7, BPV8, BPV9, BPV10, BPV11, BPV12, BPV13, CdPV1, CdPV2, ChPV1,
15 CcaPV1, (RdPV1, CcPV1), OvPV1 (DPV), OaPV1 (OvPV1), OaPV2, OvPV1 (RPV), SsPV1, Canis familiaris oral Papillomavirus, CPV2 (CfPV2), CPV3, CPV4, CPV5, CPV6, CPV7, CPV8, CPV9, CPV10, CPV11, CPV12, CPV13, CPV14, CPV15, CcrPV1, FcaPV2 (FdPV2), LrPV1, PlpPV1, PlPV1, PcPV1, UuPV1, UmPV1, ZcPV1, Delphinus delphis Papillomavirus 1, PphPV1,
20 PphPV2, PphPV4, PsPV1, TtPV1, TtPV2, TtPV3, TtPV4, TtPV5, TtPV6, TtPV7, Mini-pus V1, TT20F, MrPV1, RaPV1, Bettongia penicillata Papillomavirus 1, Erinaceus europaeus Papillomavirus 1, Francolinus leucoscepus Papillomavirus 1, Oryctolagus cuniculus Papillomavirus 1, SfPV1 (CRPV, SPV), Fringilla coelebs Papillomavirus 1, Equus caballus Papillomavirus
25 1, EcPV2 (EqPV2), EcPV3, EcPV4, EcPV5, EcPV6, EcPV7, Colobus guereza Papillomavirus 1, CgPV2, MfPV1, MfPV2, MfPV3, MfPV5 (MfPV-a, MfPV6, MfPV7, MfPV8 (RhPV-a), MfPV9, MfPV10, MfPV11 (RhPV-b), MmPV1 (RhPV1), PpPV1 (PCPV), PtPV1 (CCPV), PhPV1, Psittacus erithacus Papillomavirus 1, EdPV1, MnPV1 (MrPV, MmPV), McPV2, MaPV1 (HaOPV),
30 MmiPV1 (MmPV1), MmuPV1, PmPV1, RnPV1, RnPV2, Trichechus manatus latirostris Papillomavirus 1, TmPV2, Morelia spilota Papillomavirus 1, Caretta caretta Papillomavirus 1, CmPV1.

In a preferred embodiment, the mixture of aptamers bonds the HPV2 capsid

and simultaneously inhibits its cellular internalization decreasing its infective power.

For example, in some embodiments, combinable with all the embodiments described here, the aptamers or mixture of aptamers comprise at least one of the following sequences of aptamers:

5 SEQ ID No.: 1, SEQ ID No.: 2, SEQ ID No.: 3, SEQ ID No.: 4, SEQ ID No.: 5, SEQ ID No.: 6, SEQ ID No.: 7, SEQ ID No.: 8, SEQ ID No.: 9, SEQ ID No.: 10, SEQ ID No.: 11, SEQ ID No.: 12, SEQ ID No.: 13, SEQ ID No.: 14, SEQ ID No.: 15, SEQ ID No.: 16, SEQ ID No.: 17, SEQ ID No.: 18, SEQ ID No.: 19, 10 SEQ ID No.: 20, SEQ ID No.: 21, SEQ ID No.: 22, SEQ ID No.: 23, SEQ ID No.: 24, SEQ ID No.: 25, SEQ ID No.: 26, SEQ ID No.: 27, SEQ ID No.: 28, SEQ ID No.: 29, SEQ ID No.: 30, SEQ ID No.: 31, SEQ ID No.: 32, SEQ ID No.: 33, SEQ ID No.: 34, SEQ ID No.: 35, SEQ ID No.: 36, SEQ ID No.: 37, SEQ ID No.: 38, SEQ ID No.: 39, SEQ ID No.: 40, SEQ ID No.: 41, SEQ ID 15 No.: 42, SEQ ID No.: 43, SEQ ID No.: 44, SEQ ID No.: 45, SEQ ID No.: 46, SEQ ID No.: 47, SEQ ID No.: 48, SEQ ID No.: 49, SEQ ID No.: 50, SEQ ID No.: 51, SEQ ID No.: 52, SEQ ID No.: 53, SEQ ID No.: 54, SEQ ID No.: 55, SEQ ID No.: 56, SEQ ID No.: 57, SEQ ID No.: 58, SEQ ID No.: 59, SEQ ID No.: 60, SEQ ID No.: 61, SEQ ID No.: 62, SEQ ID No.: 63, SEQ ID No.: 64, 20 SEQ ID No.: 65, SEQ ID No.: 66, SEQ ID No.: 67, SEQ ID No.: 68, SEQ ID No.: 69, SEQ ID No.: 70, SEQ ID No.: 71, SEQ ID No.: 72, SEQ ID No.: 73, SEQ ID No.: 74, SEQ ID No.: 75, SEQ ID No.: 76, SEQ ID No.: 77, SEQ ID No.: 78, SEQ ID No.: 79, SEQ ID No.: 80, SEQ ID No.: 81, SEQ ID No.: 82, SEQ ID No.: 83, SEQ ID No.: 84, SEQ ID No.: 85, SEQ ID No.: 86, SEQ ID 25 No.: 87.

The 87 sequences indicated above were identified by the Applicant as effective in the therapy, prevention and detection, as well as for diagnostic use, at least of human Papillomavirus HPV2.

The aptamers used here have a series of characteristics which make their use advantageous in association with the embodiments described here:

- high affinity, similar to an antibody;
- high stability, even in aggressive environments;
- excellent storage properties;

- 20 -

- low production cost;
- high specificity due to the selection methods;
- almost total absence of immunogenicity;
- reproducibility of specifications (whereas antibodies vary from batch to batch);
- 5 - they are chemically synthesized based on the sequence;
- very quick synthesis;
- they are completely engineered in a test tube.

The aptamers can also be modified, these modifications increase their diversity and make the affinity even higher (sub-nanomolar) expanding the range of possible interactions (for example hydrophobic) and therefore of further bonds.

In some embodiments, the aptamers or mixture of aptamers can therefore be modified. Specifically, the following modifications to the aptamers can be included:

- terminal internucleotide linkage (3'-3' and 5'-5'). These modifications can increase the stability and resistance of the aptamers to the 3'-exonuclease present in human serum;
- biotinylation at the 3' (3'-biotin). In some cases, this modification could decrease the efficacy of the 3'-exonuclease and therefore inhibit the degradation of the modified aptamer;
- 20 - modifications to the ribose group. The modifications to sugars present in the DNA structure such as the addition of 2'-fluorine (2'-F), or 2'-amino (2'-NH₂) on the pyrimidine residues, or 2'-O-methyl (2'-OMe) increase resistance to nucleases;
- LNA, UNA, 2'-F. The locked nucleic acids constitute an analogue of ribonucleic acid that has a methylene bond between 2'-O and 4'-C of the sugar ring. This modification confers great resistance to nucleases and greater thermostability. Unlocked nucleic acid (UNA) in which there is a further bond between the C2 and C3 atoms of the sugar ring is more flexible with respect to the LNA. As a consequence, the modification of 2'-deoxy-2'-fluoro-d-
- 25 arabinonucleic acid (2'-F ANA) is very suitable to improve the biological and physical-chemical properties of the G quartets of DNA;
- changes to phosphodiester links (Methylphosphonate and phosphorothioate). The linkage of the phosphodiester substituted with the methylphosphonate or
- 30

- 21 -

phosphorothioate analogue is commonly used to modify the aptamers. Thermodynamic studies revealed that the loss of the negative charge of the phosphate, as in the case of the methylphosphonate analogue, destabilized the G-quadruplex structure. The complete substitution with thiophosphorylated oligonucleotides has to be limited at the systemic level (if these molecules are used in therapeutic applications) because they have high toxicity (partial substitutions are preferred). However, this modification allows high resistance of the DNA to degradation by nucleases present in the blood serum or other body fluids. Phosphor toxic bonds (PS2) have been used to stabilize the DNA structure. Furthermore, it has been reported that the substitutions of PS2 have significantly improved the affinity of the target bond by approximately 1000 times;

- triazole. The substitution of the bond of the oligonucleotide phosphodiester with triazole-based compounds is particularly promising. Oligonucleotide analogues linked to internucleotide triazole have a higher resistance to DNase. This modification can also strongly influence the bonding properties of the aptamer;

- I-DNA. The transition from the form of natural DNA (D-DNA) to the mirror image leads to a chiral form of DNA that can show high resistance to degradation by nucleases and maintain affinity with the targets;

- 5'-Addition of cholesterol. The aptamers are subject to rapid excretion through renal clearance primarily through glomerular filtration. The cholesterol is able to prolong the half-life of the aptamers in the blood stream;

- Polyethylene glycol (PEG) linkage. This modification increases the stability in the serum from one day up to a few weeks;

In preferred embodiments, the aptamers or mixture of aptamers can be modified with one or more of the following modifications: 3'-3' and 5'-5' internucleotide inversions of the DNA, biotinylation at the 3' of DNA, modifications to the ribose group, 2'-fluoro (2'-F) and/or 2'-amino (2'-NH₂) on pyrimidine residues, 2'-O-methyl (2'-OMe), Locked Nucleic Acid, Unlocked Nucleic Acid, 2'-deoxy-2'-fluoro-d-arabinonucleic (2'-F ANA) acid, modifications to phosphodiester linkages (methylphosphonate, phosphorothioate, thiophosphorylated oligonucleotides), modification with triazole, chiral specular DNA (I-DNA), addition of cholesterol at the 5' of DNA,

Polyethylene glycol (PEG) linkage.

Aptamer selection technique

The Applicant has developed a method to select aptamers according to the present description, described below in detail using fig. 2. The following
5 description refers by way of example to DNA aptamers, however it is also applicable in a suitable manner to other types of aptamers as described here. Fig. 2 is used to describe embodiments used to select aptamers with the following characteristics:

- high affinity for Papillomavirus pseudovirions;
- 10 - able to limit viral infection;
- stable in conditions similar to physiological ones;
- stable even if contained in products for topical application on skin or mucous membranes, for example creams, gels, and lotions based on mineral, synthetic and vegetable oils.

15 Initially, the SELEX technique applied allowed, after 9 cycles, to select aptamers, for example DNA aptamers, having a high affinity for the Papillomavirus pseudovirions. Using pseudovirions in the selection process was an important step in obtaining a mixture of aptamers that could effectively inhibit the viral infection. The use of pseudovirions implies an enormous advantage with
20 respect to a selection made by means of single denatured viral proteins produced in prokaryotic systems.

The selection of aptamers against the entire viral capsid (pseudovirions) allows to enrich the selection with aptamers that recognize the viral capsid in its conformational elements, and/or with post-translational modifications, and also at
25 the linkage points between capsomeres. For example, this would not have been possible if the capsid viral proteins L1 and L2 had been used individually as a selection parameter in the SELEX technique.

Pseudovirions, although difficult to produce, mimic the behavior of the Papillomavirus capsid in vivo more faithfully. For this reason, pseudovirions
30 have been used as a target of SELEX, since the selection of aptamers against pseudovirions allows to obtain more effective aptamers with greater protective action in vivo.

Once the aptamers are obtained, they are cloned and sequenced. Experiments

- 23 -

conducted by the Applicant both with mixtures thereof and also individually are performed in order to test their protective action against the Papillomavirus infection: the aptamers are dissolved in a culture medium and administered to the cells, and the cells are then infected with the Papillomavirus.

- 5 The medium used for the cell culture partly mimics the physiological conditions, the action of the aptamers is tested at 37°C, physiological temperature.

- 24 hours after the viral infection, cell vitality tests are performed to identify the aptamers or aptamer mixtures that have had a protective effect on the cells in
10 terms of cell vitality via the ATPlite assay.

- The selected aptamers can, advantageously, undergo various chemical and/or nucleotide modifications in order to be even more stable and effective if contained in products for topical application on the skin or mucous membranes, for example creams, oils and gels; these modifications are subject to further
15 functional assays and therefore subsequent selections performed directly on cells in vitro (fig. 2).

Materials and methods

Chemical reagents used

- The chemical reagents used to perform the entire selection and construction
20 process of anti-Papillomavirus aptamers were purchased from Thermo Fisher Scientific, Sigma-Aldrich, Euroclone, Invitrogen, and Qiagen.

Base solutions:

- 1x PBS: 137mM NaCl, 2.7mM KCl, 10mM Na₂HPO₄, 1.8 mM KH₂PO₄ in dH₂O, pH 7.4;
- 25 - TE: 0.1 mM EDTA, 10 mM Tris-HCl pH 8.0 in dH₂O;
- 10x TBE (tris-borate EDTA) buffer: 89 mM Tris, 89 mM boric acid, 2 mM EDTA in dH₂O, pH 7.5.

Preparation of Pseudovirions

- All operations relating to the handling of Papillomavirus pseudovirions have
30 to be performed using a biological hood (class II).

DNA transfection to produce Pseudovirions

The DNA transfection to produce pseudovirions is performed in HEK 293TT (Human Embryonic Kidney) cells. The HEK 293TT cells are plated in 10 cm x

- 24 -

10 cm Petri dishes containing DMEM medium with the addition of fetal bovine serum at 10% and 1% penicillin/streptomycin. The dishes are then deposited in a humidified incubator that maintains a controlled temperature of 37° C (5% CO₂). When the cells reach about 70% confluence, the transfection of the plasmid DNA is performed by means of calcium phosphate. For each dish of cells, 2.5 µg of plasmid encoding the L1 and L2 proteins and 12.5 µg of plasmid encoding a reporter gene (luciferase), are mixed in a test tube containing 22 µL of 2.5 M CaCl₂ and 200 µL of TE. The solution is transferred drop by drop into a test tube containing 200 µL of HBS 2x (50 mM Hepes pH 7.0, 28 mM NaCl, 1.5 mM Na₂HPO₄, pH 7.12). After 20 minutes, 420 µL of the transfection solution is added to each dish containing medium and cells. The dishes are then placed once again in an incubator at 37° C for 48 hours.

Maturation of the pseudovirions

The culture medium is removed from the cell growth dishes and the cells are treated with 1 mL of trypsin/EDTA. The trypsin washing is then removed and replaced with 2 mL of fresh trypsin/EDTA. The dishes are then incubated with trypsin for at least 5 minutes and the cells are then collected in a 50 mL test tube containing 20 mL of PBS. At this point, the test tube has to be gently centrifuged at 850xg for 5 minutes and this creates a cell sedimentation pellet; the pellet has to then be resuspended in 1 mL of PBS. After the removal of the supernatant, the addition of PBS + 9.5 mM MgCl₂, and a solution of 10% Triton-X, 0.3% and 0.4%, respectively, benzonase and endonuclease V (RecBCD) occurs. Both benzonase and also endonuclease were useful to eliminate any residual genomic DNA, improving the purity of the preparation. The solution has to then be mixed and incubated in a thermoblock at 37° C for 24 hours, with occasional mixing.

Purification of the Pseudovirions from HEK293TT cells

After the maturation step of the pseudovirions, the cell lysate has to be cooled on ice for 10 minutes. 0.17 volumes of PBS + 5 M NaCl are then mixed with the lysate, followed by 3 freezing and thawing cycles using dry ice and a thermal block heated to 37° C. The cell lysate is then centrifuged at 54000xg at 4° C for 5 minutes and the supernatant has to be preserved. The pellet is washed twice with 800 µL of HSB (25 mM HEPES pH 7.5, 500 mM NaCl, 1 mM MgCl₂, 100 µM EDTA, 0.5% ethanol). All supernatants have to be recovered and preserved.

- 25 -

Meanwhile, 4.5 mL of “A” CsCl mixture (prepared by dissolving 54 g of CsCl in 200 mL of PBS) have to be dispensed into an ultracentrifuge test tube. In addition, 4.5 mL of “B” CsCl mixture (prepared dissolving 77.7 g of CsCl in 200 mL of PBS) have to be dispensed. HSB has to be added at the end to fill the
5 ultracentrifuge test tube. The test tube is then centrifuged at 20000xg at 4° C for 16 hours.

The pseudovirions will be collected using an 18G needle and a 2 ml syringe, through one side of the test tube. Subsequently, they have to be distributed in a filtering unit and centrifuged at maximum speed for 10 minutes. After two
10 washes with HSB, the pseudovirions are resuspended in 50 µL of HSB and aliquoted.

Functional assay of viral infectivity:

Cell cultures

The HaCaT (CLS Cell Lines Service, 300493) is a spontaneously transformed
15 aneuploid immortal keratinocyte cell line, these cells represent one of the best models in the world to study the infectivity of the human Papillomavirus.

Cells have to be stored at -80° C. The cell culture medium is DMEM (Dulbecco's Modified Eagle's Medium) with 4.5 g/L glucose and 3.7 g/L NaHCO₃, without L-glutamine and sodium pyruvate (PAN-Biotech). The
20 medium is completed with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin.

Cell Vitality Test

The ATPlite 1step (PerkinElmer) test kit was used for the quantitative assessment of the protective effect of the aptamers on the viral infection. The
25 assay is performed according to the manufacturer's instructions. Briefly, different concentrations of cells per well are seeded in a 96-well plate. Cells have to be incubated with mixtures of aptamers. At different times after the treatment with aptamers the cells have to be infected with the pseudovirions for 48 hours. After the cell lysis, following the manufacturer's instructions, the assay ends with a
30 luminescence signal representative of the cell vitality, which is detected by means of a multiple-detection microplate reader. The Applicant used the EnVision 2104 (PerkinElmer) model.

SELEX

- 26 -

The starting point for SELEX is a library of secondary structures of nucleic acids, which in this case is a DNA library composed of sequences containing an aptamer region of 60 nucleotides. Two identical single-stranded DNA (ssDNA) libraries were used. The identification sequences for the amplification of the two
5 libraries are perfectly identical (SEQ ID No.: 88: 5'-AGACCACAACGGTTTCCC- (N50) -TAGCATAACCCCTTG-3'). The main objective of SELEX is to identify a small subset of aptamers that bond with high affinity the particles of Papillomavirus pseudovirions.

Positive and negative selection in vitro

10 For positive selection, the Heparin-Agarose (Sigma Aldrich) spheres are used, because the cell surface heparan-sulfate is necessary for the Papillomavirus infection since it represents a fundamental receptor for the cellular adhesion of the virus. 60 µL of heparin-agarose have to be centrifuged at 500xg for 1 minute. The supernatant is then removed and 50 µL of HSB + 2 µg of Papillomavirus
15 pseudovirions have to be added. The test tube has to be incubated on a Mini LabRoller™ (Labnet) at 4° C overnight.

12 hours later, 2 nmol of aptamer library (20 µL of the 100 µM parent solution) have to be added to 1 mL of selection buffer (5 mM MgCl₂, 10 mM KCl, 0.01% (v/v) Tween- 20, 1x PBS).

20 Agarose spheres for negative selection: 60 µL of heparin-agarose have to be centrifuged at 500xg for 1 minute. The supernatant has to then be removed and the spheres washed three times with 500 µL of selection buffer followed by centrifugation at 500xg for 1 minute. Finally, 150 µL of bonding buffer (selection buffer integrated with 2 µg/mL of yeast tRNA (Invitrogen) and 125 µg/mL of
25 bovine serum albumin (BSA; Millipore)) and the library has to be added to the agarose spheres. The test tube has to be incubated on a Mini LabRoller™ at 37° C for 15 minutes.

The agarose spheres have to then be centrifuged at 500xg for 1 minute. The supernatant is recovered and transferred to the test tube containing the agarose
30 bound with the pseudovirions, which has to be prepared by centrifuging the test tube at 500xg for 1 minute, removing the supernatant and adding 600 µL of bonding buffer. The sample is then incubated on a Mini LabRoller™ at 37° C for 15 minutes. The spheres used for the selection are centrifuged at 500xg for 1

- 27 -

minute and the supernatant, which contained unbound DNA, has to be removed. The pellet is transferred to a new test tube to eliminate the sequences that may have been bound to the walls of the test tube. A wash with 1 ml of selection buffer and a centrifugation at 500xg for 1 minute are then performed. This step
5 has to be repeated three times. 200 μ L of elution buffer (selection buffer integrated with 500 mM of imidazole, pH 7.0) have to be added to the pellet and the library is eluted together with the pseudovirions incubating the test tube on a Mini LabRoller™ at ambient temperature for 10 minutes. The agarose spheres are then pelletized by centrifugation, the supernatant is then recovered and
10 transferred to another test tube.

DNA has to be precipitated with 750 μ L of absolute ethanol and centrifuged at 21000xg at 4° C for 40 minutes. The DNA pellet is then dried and resuspended in 20 μ L of water.

Use of probes ribosylated in PCR

15 PCR is performed using the Phire Hot Start II (Thermo Fisher Scientific) DNA polymerase, following the manufacturer's instructions. The final reaction volume of the PCR is 20 μ L. Initially, a mixture has to be prepared, which comprises 4 μ L of 5x reaction buffer, 0.4 μ L of dnTP 10 mM (Thermo Fisher Scientific), 0.4 μ L of Hot Start II DNA polymer of phosphorus, 0.25 μ L of SYTO™ 9
20 fluorescent nucleic acid from 160 μ M Stain (Thermo Fisher Scientific), 1 μ L of 10 μ M FW probe (SEQ ID No.: 89: 5'-AGACCACAACGGTTTCCC-3'), 1 μ L of 10 μ M REV probe (SEQ ID No.: 90: 5'-CAAGGGGTTATGCTrA-3') and 10.95 μ L of water. The aptamer library is then diluted in TE buffer to obtain samples containing 100 ng/ μ L, 50 ng/ μ L and 25 ng/ μ L of DNA. 2 μ L of each duplicate
25 sample is dispensed into each well. The PCR protocol used is: 98° C for 30 seconds (initial denaturation), 98° C for 5 seconds (denaturation), a temperature range between 50° C and 55° C for 30 seconds and 72° C for 5 seconds for 40 cycles and 72° C at the end for 1 minute (final extension).

In the SELEX process, the Platinum™ Green Hot Start PCR 2x Master Mix
30 (Invitrogen) is used for the PCR amplification, following the manufacturer's instructions. The PCR is performed in a final volume of 100 μ L, which comprises 50 μ L of Master Mix Start Hot Green PCR 2x Platinum™, 2 μ L of 10 μ M forward primer (SEQ ID No.: 91: 5'-AGACCACAACGGTTTCCC-3'), 2 μ L of

- 28 -

10 μ M reverse primer (SEQ ID No.: 92: 5'-CAAGGGGTTATGCTrA-3'), 13 μ L of water and 20 μ L of the sample obtained after the positive and negative selection in vitro (subtitle 3.7.1 positive and negative selection in vitro). The thermal cycling protocol is: 94° C for 2 minutes (initial denaturation), 94° C for 5 30 seconds (denaturation), 52° C for 30 seconds (probe matching) and 72° C for 5 seconds (extension), all for 30 cycles.

Use of biotinylated probes

The biotinylated probes allow to attach the complementary strand of the aptamer to the spheres of Streptavidin-Agarose and to elute only the aptameric 10 one using NaOH.

Separation of double-stranded DNA and recovery of the single-stranded DNA

At the end of each SELEX cycle, double-stranded DNA (dsDNA) is treated in such a way as to recover the single-stranded aptamer. Initially, the dsDNA is treated with NaOH, loaded in a denaturing gel (PAGE) containing 10% urea. The 15 band corresponding to the ssDNA of interest has to be recovered. Due to the low yield of ssDNA obtained with this procedure, the Streptavidin-Agarose reagent is used and the ssDNA has to be eluted using NaOH.

Denaturing gel and extraction of ssDNA

The PCR products obtained using the ribosylated primer are precipitated with 20 250 μ L of absolute cold ethanol by centrifugation of the solution at 21000xg at 4° C for 30 minutes. The supernatant is removed and the pellet obtained is dried and resuspended in 90 μ L of NaOH 0.25 N. The sample is heated in a heating block at 92° C for 10 minutes (this allows the detachment of the ribosylated base). After heating, 10 μ L of 3 M sodium acetate is added to the test tube and 25 the DNA is precipitated with 250 μ L of absolute cold ethanol by centrifugation at 21000xg at 4° C for 30 minutes. The supernatant is removed and the DNA pellet is then dried.

The DNA pellet has to be re-suspended in 20 μ L of colored loading solution (for 40 ml of solution: 4 g of sucrose, 5 mg of blue bromophenol, 5 mg of 30 xlenocianol FF, 200 μ L of sodium dodecyl sulfate at 10%, 5 ml of 10x TBE, 22 g of urea, H₂O) and is heated for 2 minutes at 92° C.

Meanwhile, a 10% denatured PAGE gel (8 M urea) has to be prepared. For 1 liter of solution, 420.43 g of urea, 100 mL of 10x TBE and 250 mL of 40% 29:

- 29 -

1 acrylamide/bis-acrylamide are mixed. 35 mL of this mixture are combined with 350 μ L of APS (ammonium persulfate) and 35 μ L of TEMED (tetramethylethylenediamine). The gel is initially run at 40 mA for 20 minutes by means of an electrophoretic cell. The volume of the gel band is estimated, and 2
5 volumes of elution buffer have to be added. The sample is incubated on a Mini LabRoller™ at 37° C for 12 hours. The solution has to be centrifuged at the maximum speed at 4° C for 1 minute in order to precipitate the acrylamide and the supernatant is recovered in a new test tube. The centrifugation and recovery have to be repeated after the addition of 0.5 volumes of elution buffer to the
10 precipitated acrylamide. 2.5 volumes of cold absolute ethanol and 0.3 M of sodium acetate are added to the sample. The sample is then placed at -20° C for 20 minutes and centrifuged at the maximum speed at 4° C for 20 minutes. The absolute ethanol is substituted with 100 μ L of ethanol at 70%, the test tube is then centrifuged at the maximum speed at 4° C for 10 minutes. The supernatant
15 is removed, the pellet is dried and resuspended with 20 μ L of H₂O.

Use of Streptavidin-Agarose and elution of ssDNA by means of NaOH

The PCR products obtained using the biotinylated primer are purified by means of the QIAquick nucleotide removal kit of the QIAquick gel extraction kit (QIAGEN). Briefly, 1 ml of PN buffer (40% (v/v) 5 M of guanidinium chloride,
20 60% (v/v) of isopropanol) are added to 100 μ L of PCR mixture. The sample is loaded into the QIAquick column and centrifuged at 8950xg for 1 minute. The centrifuged volume is discarded, and centrifugation is repeated after the addition of 750 μ L of PE buffer (10 mM Tris-HCl pH 7.5, 80% ethanol). After discarding the centrifuged volume, the test tube is centrifuged at 17900xg for 1 minute in
25 order to eliminate any ethanol residue. The QIAquick column is then positioned in a clean 1.5 ml tube. To elute the DNA, 35 μ L of H₂O have to be preheated to 50° C and then added to the QIAquick column. The column is then centrifuged at 17900xg for 1 minute and 2 μ L of DNA is eluted. 70 μ L of Streptavidin-Agarose (Sigma Aldrich) have to be centrifuged at 500xg for 1 minute. The supernatant is
30 removed, and two washes are performed with 70 μ L of 1x PBS. The purified PCR products are dispensed to agarose spheres and incubated on a Mini LabRoller™ at ambient temperature for 1 hour. The tube has to be centrifuged at 500xg for 1 minute, the supernatant is then removed, and the pellet is washed

- 30 -

twice with 70 μ L of 1x PBS. The agarose spheres are incubated with 50 μ L of NaOH 20 mM on a Mini LabRoller™ for 10 minutes in order to denature the DNA. The test tube is then centrifuged at 500xg for 1 minute and the supernatant is subsequently recovered. The pH of the solution was raised to about 7.5. The
5 quality and quantity of eluted ssDNA were evaluated by NanoDrop™ 1000.

Cloning of the single-stranded DNA

For sequencing, TOPO® TA Cloning® Kit (Invitrogen) is used, following the instructions provided by the manufacturer. Cloning was performed using LB Amp plates and competent XL1-Blue bacteria.

10 *Production of PCR products*

The PCR is performed on 50 ng of ssDNA obtained in the 9th cycle of the SELEX selection process. The reaction occurs in a volume of 50 μ L. The PCR reaction contains: 1 μ L of GoTaq® DNA Polymerase, 10 μ L of 5x ColorTable GoTaq® Buffer, 1 μ L of 10 μ M forward primer (SEQ ID No.: 93: 5'-
15 AGACCACAACGGTTCCC-3'), 1 μ L of 10 μ M reverse primer (SEQ ID No.: 94: 5'-CAAGGGGTTATGCTA-3'), 0.5 μ L of 50 mM dNTPs, 19.5 μ L of water and 22 μ L of sample. PCR protocol: 95° C for 2 minutes (initial denaturation), 95° C for 30 seconds (denaturation), 52° C for 40 seconds (probe matching) and 72° C for 5 seconds (extension) for 35 cycles and 72° C for 3 minutes (final
20 extension). The products obtained from the PCR are then purified using the QIAquick PCR Purification kit of the QIAquick Gel Extraction Kit (QIAGEN) following the manufacturer's instructions. The success of the reaction has to be verified by means of electrophoresis on a 2% agarose gel.

Selection by chemical modification

25 The aptamers and mixtures of aptamers that have shown in cell vitality assays to be effective against the Papillomavirus infection are further subjected to chemical modifications to improve both their stability and also the antiviral protective effect. For biotinylation, the Thermo Scientific™ Pierce™
biotinylation kit is used.

30 The selected aptamers are synthesized and modified by external companies such as IDT *Integrated DNA Technologies*, and IBA *Lifesciences*. These aptamers are incubated in the cellular medium containing HaCaT cells according to the previously indicated protocols in order to test whether the chemical

modification made produced an improvement in the protective effect against the viral infection. The modified aptamers are tested individually and coupled with increasing concentrations of substances normally present in creams and oils, such as: parabens, silicones, petrolatum, mineral oils, natural oils, detergents, surfactants, preservatives.

Therapeutic treatment and/or prevention

According to some embodiments, the aptamers or mixture of aptamers according to the present description can be contained, comprised or formulated in products for application or topical use on skin or mucous membranes.

10 In one embodiment, the mixture of aptamers described can be an essential component of an oil for the skin.

In one embodiment, the mixture of aptamers described can be an essential component of a lotion to be applied to the mucous membranes of the body.

15 In one embodiment, the mixture of aptamers described can be an essential component of a vaginal cream.

In one embodiment, the mixture of aptamers described can be an essential component of a cream for the penis.

In one embodiment, the mixture of aptamers described can be an essential component of an anal cream.

20 In one embodiment, the mixture of aptamers described can be an essential component of a preparation applicable to the oral cavity.

In one embodiment, the mixture of aptamers described can be an essential component of a mechanical contraceptive.

25 In one embodiment, the mixture of aptamers described can be an essential component of a viral and non-viral vector for transfection and transduction of nucleic acids.

Diagnostics

30 In one embodiment, the mixture of aptamers can bond the capsid of the human Papillomavirus and bond a fluorophore or an enzyme in order to be able to monitor the presence of the human Papillomavirus with diagnostic techniques.

In one embodiment, the mixture of aptamers can bond the capsid of the human Papillomavirus and act as a primary antibody in immunochemical techniques such as for example ELISA, or Western Blot, or immunofluorescence.

- 32 -

In one embodiment, the mixture of aptamers can bond the capsid of the human Papillomavirus and bond to a nano-switch to detect protein analytes. Possible embodiments usable in association with the diagnostic method according to the present description are described in the international applications
5 PCT/EP2017/067904 and PCT/IT2017/000233 in the name of the Applicant and entirely incorporated here as reference.

In some embodiments, the mixture of aptamers can bond the capsid of the human Papillomavirus and be used in biosensor techniques which comprise measurements in amperometry, impedance, voltammetry, capacitance,
10 electrochemiluminescence, chemiluminescence. Possible embodiments usable in association with the diagnostic method according to the present description are described in the international application WO2015/114506 in the name of the Applicant and entirely incorporated here as a reference.

The embodiments described here concern the use of aptamers or a mixture of
15 DNA aptamers selected exclusively to specifically bond the entire Papillomavirus capsid, a bond that occurs between aptamer and linear and conformational protein epitopes, epitopes with post-translational modifications, and epitopes derived from linkage points between viral capsomeres.

Embodiments described here also concern the use of these specific aptamers as
20 agents able to bond to the Papillomavirus capsid limiting its infectivity by means of a mechanism that inhibits virus entry into cells and/or inhibits intracellular trafficking of the virus.

These aptamers, according to the embodiments described here, can be used in creams, ointments, gels, mechanical contraceptives and body lotions applicable
25 on skin and mucous membranes, or lotions and solutions for the oral cavity, in order to limit, contrast and treat the Papillomavirus infection.

At the same time, in the present description, embodiments are also provided concerning the diagnostic use of these aptamers since, in addition to the bond with the viral capsid, they can be directly or indirectly bonded to markers, for
30 example fluorescent reporters or enzymes, whose activity can be monitored in order to detect the presence of the virus in body fluids.

Further embodiments concern a method to select aptamers for the therapy, prevention and diagnosis of the Papillomavirus having specific characteristics

- 33 -

that simultaneously satisfy, in addition to the requirement of the bond to the entire viral capsid, also the following conditions:

- i) high-affinity bond with the capsid defined by means of SELEX technique;
- ii) bond with defined inhibitory power (functional assay);
- 5 iii) stability in body fluids and/or iv) stability in products for topical application or use on skin or mucous membranes, for example, creams, gels and oils;

Aspects iii) and iv) can both be present or alternative, for example according to whether the aptamers are provided for diagnostic use in a biological sample (aspect iii), or for use in topical application on skin and/or mucous membranes
10 (aspect iv).

The selection method developed by the Applicant provides the selection of aptamers able to bond with high affinity the pseudovirions of Papillomavirus which:

- 1) contain both L1 and also L2 and are the active biological entities most similar
15 to Papillomaviruses; on the contrary, Virus-like Particles (VLPs) contain only L1: the method according to the present description allows the selection of aptamers that also recognize L2;
- 2) the pseudovirions were produced in eukaryotic cells, therefore they contain patterns of post-translational protein modifications similar to those of naturally
20 occurring Papillomaviruses: this allows the selection of aptamers that recognize epitopes with previously listed post-translational modifications;
- 3) the pseudovirions are not single inert proteins, but they are biologically active entities with regards to infectivity: this allows the selection of aptamers that recognize important sites for viral infectivity;
- 25 4) the pseudovirions have a conformation that, compared to denatured proteins, allows to select aptamers having affinity for conformational epitopes, which are present in the natural virus: this allows the selection of aptamers that recognize conformational sites of the viral capsid.

Furthermore, it should be noted that the selection method according to the
30 present description is not based only on aptamer-antigen affinity, but also on the ability of the aptamer to inhibit viral entry into the cell and thus limit viral infectivity. This is advantageous because it allows to use aptamers even in a functional essay.

- 34 -

As described above, this method of functional selection is operated in a physiological pH medium, a medium with characteristics similar to biological fluids (cell culture medium), at a controlled temperature of 37° C, therefore a physiological temperature.

5 Furthermore, it is possible to refine the selection, since the best candidate aptamers are further selected based on the chemical modification and/or a possible more suitable nucleotide modification that allows the aptamer to be stable and effective in limiting the Papillomavirus infection even in products for topical application on the skin or mucous membranes, such as creams, oils and
10 gels.

It is clear that modifications and/or additions of parts and/or steps may be made to the use of aptamers for the therapy, prevention, diagnosis and detection of the Papillomavirus as described heretofore, without departing from the field and scope of the present invention as defined in the claims.

15

- 35 -

CLAIMS

1. Aptamers for use in the therapy and/or prevention of the Papillomavirus, said aptamers being configured to specifically bond with high affinity conformational epitopes with post-translational modifications belonging to the entire capsid of the Papillomavirus.
2. Aptamers as in claim 1, wherein said aptamers are also configured to bond said epitopes to limit the infectivity of the Papillomavirus through a mechanism of inhibiting the entry of the Papillomavirus into the cells and inhibiting the endocellular traffic of the Papillomavirus.
3. Aptamers as in claim 1 or 2, wherein said aptamers are configured with a length from 15 to 100 nucleotides.
4. Aptamers as in claim 3, wherein said aptamers are also configured to be stable in body fluids and/or in products for topical application on the skin or mucous membranes.
5. Aptamers as in claim 4, wherein said aptamers are configured to have specific characteristics that satisfy not only the requirement of recognizing epitopes belonging only to the entire viral capsid, but also at the same time the following conditions:
 - i) high affinity bond with said epitopes defined using the SELEX technique;
 - ii) bond with said epitopes, having a defined inhibitory function (functional assay);
 - iii) stability in body fluids and/or iv) stability in products for topical application or use on the skin or mucous membranes, for example, creams, gels and oils; wherein the conditions iii) and iv) can be both satisfied or alternative, depending on whether said aptamers are provided respectively for diagnostic use in a biological sample or for use in topical application on the skin and/or mucous membranes.
6. Aptamers as in any claim from 1 to 5, wherein said aptamers are modified with one or more of the following modifications: 3'-3 and 5'-5 internucleotide inversions of the DNA, 3' biotinylation of the DNA, modifications to the ribose group, 2'-fluoro (2'-F) and/or 2'-amino (2'-NH₂) on the pyrimidine residues, 2'-O-methyl (2'-OMe), Locked Nucleic Acid, Unlocked Nucleic Acid, 2'-deoxy-2'-fluoro-d-arabinonucleic acid (2'-F ANA), modifications to the phosphodiesteric

- 36 -

links (Methylphosphonate, phosphorothioate, thiophosphorylated oligonucleotides), modification with triazole, chiral specular DNA (I-DNA), addition of cholesterol to 5' of the DNA, Polyethylene glycol (PEG) linkage.

7. Aptamers as in any claim from 1 to 6, wherein said aptamers comprise a mixture of aptamers for the therapy and/or prevention of human Papillomaviruses, in particular specifically one or more of:

- Human Papillomaviruses (HPV) type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73;

- Human Papillomaviruses (HPV) type 6, 11, 40, 42, 43 and 44;

10 - Human Papillomaviruses (HPV) type 27, 57, 2, 4, 5, 1 and 65.

8. Aptamers as in any claim from 1 to 5, wherein said aptamers comprise a mixture of aptamers for the therapy and/or prevention of non-human Papillomaviruses, in particular specifically of one or more of: AaPV1 (EEPV), BgPV1, BPV1, BPV2, BPV3, BPV4, BPV5, BPV6, BPV7, BPV8, BPV9, BPV10, BPV11, BPV12, BPV13, CdPV1, CdPV2, ChPV1, CcaPV1, (RdPV1,CcPV1), OvPV1 (DPV), OaPV1 (OvPV1), OaPV2 (OvPV2), OaPV3,RtPV1 (RPV),SsPV1, Canis familiaris oral Papillomavirus, CPV2 (CfPV2), CPV3, CPV4, CPV5, CPV6, CPV7, CPV8, CPV9, CPV10, CPV11, CPV12, CPV13, CPV14, CPV15, CcrPV1, FcaPV1 (FdPV1), FcaPV2 (FdPV2), LrPV1, PlpPV1, PIPV1, PcPV1, UuPV1, UmPV1, ZcPV1, Delphinus delphis Papillomavirus 1, PphPV1, PphPV2, PphPV4, PsPV1, TtPV1, TtPV2, TtPV3, TtPV4, TtPV5, TtPV6, TtPV7, *Miniopterus schreibersii* Papillomavirus 1, MscPV1, TT20F, MrPV1, RaPV1, *Bettongia penicillata* Papillomavirus 1, *Erinaceus europaeus* Papillomavirus 1, *Francolinus leucoscepus* Papillomavirus 1, *Oryctolagus cuniculus* Papillomavirus 1, SfPV1 (CRPV, SPV), *Fringilla coelebs* Papillomavirus 1, *Equus caballus* Papillomavirus 1, EcPV2 (EqPV2), EcPV3, EcPV4, EcPV5, EcPV6, EcPV7, *Colobus guereza* Papillomavirus 1, CgPV2, MfPV1, MfPV2, MfPV3 (RhPV-d), MfPV4, MfPV5 (MfPV-a, MfPV6, MfPV7, MfPV8 (RhPV-a), MfPV9, MfPV10, MfPV11 (RhPV-b), MmPV1 (RhPV1), PpPV1 (PCPV), PtPV1 (CCPV), PhPV1, *Psittacus erithacus* Papillomavirus 1, *Apodemus sylvaticus* Papillomavirus 1, EdPV1, MnPV1 (MrPV, MmPV), McPV2, MaPV1 (HaOPV), MmiPV1 (MmPV1), MmuPV1, PmPV1, RnPV1, RnPV2, *Trichechus manatus latirostris* Papillomavirus 1, TmPV2,

- 37 -

Morelia spilota Papillomavirus 1, Caretta caretta Papillomavirus 1, CmPV1.

9. Aptamers as in any claim from 1 to 5, wherein said aptamers are for the therapy and/or prevention of human Papillomavirus and comprise at least one of the following sequences of aptamers:

5 SEQ ID No.: 1, SEQ ID No.: 2, SEQ ID No.: 3, SEQ ID No.: 4, SEQ ID No.: 5, SEQ ID No.: 6, SEQ ID No.: 7, SEQ ID No.: 8, SEQ ID No.: 9, SEQ ID No.: 10, SEQ ID No.: 11, SEQ ID No.: 12, SEQ ID No.: 13, SEQ ID No.: 14, SEQ ID No.: 15, SEQ ID No.: 16, SEQ ID No.: 17, SEQ ID No.: 18, SEQ ID No.: 19, SEQ ID No.: 20, SEQ ID No.: 21, SEQ ID No.: 22, SEQ ID No.: 23, SEQ ID
10 No.: 24, SEQ ID No.: 25, SEQ ID No.: 26, SEQ ID No.: 27, SEQ ID No.: 28, SEQ ID No.: 29, SEQ ID No.: 30, SEQ ID No.: 31, SEQ ID No.: 32, SEQ ID No.: 33, SEQ ID No.: 34, SEQ ID No.: 35, SEQ ID No.: 36, SEQ ID No.: 37, SEQ ID No.: 38, SEQ ID No.: 39, SEQ ID No.: 40, SEQ ID No.: 41, SEQ ID No.: 42, SEQ ID No.: 43, SEQ ID No.: 44, SEQ ID No.: 45, SEQ ID No.: 46,
15 SEQ ID No.: 47, SEQ ID No.: 48, SEQ ID No.: 49, SEQ ID No.: 50, SEQ ID No.: 51, SEQ ID No.: 52, SEQ ID No.: 53, SEQ ID No.: 54, SEQ ID No.: 55, SEQ ID No.: 56, SEQ ID No.: 57, SEQ ID No.: 58, SEQ ID No.: 59, SEQ ID No.: 60, SEQ ID No.: 61, SEQ ID No.: 62, SEQ ID No.: 63, SEQ ID No.: 64, SEQ ID No.: 65, SEQ ID No.: 66, SEQ ID No.: 67, SEQ ID No.: 68, SEQ ID
20 No.: 69, SEQ ID No.: 70, SEQ ID No.: 71, SEQ ID No.: 72, SEQ ID No.: 73, SEQ ID No.: 74, SEQ ID No.: 75, SEQ ID No.: 76, SEQ ID No.: 77, SEQ ID No.: 78, SEQ ID No.: 79, SEQ ID No.: 80, SEQ ID No.: 81, SEQ ID No.: 82, SEQ ID No.: 83, SEQ ID No.: 84, SEQ ID No.: 85, SEQ ID No.: 86, SEQ ID No.: 87.

25 10. Aptamers as in any claim from 1 to 9, wherein said aptamers are chosen from: DNA aptamers, RNA aptamers, PNA aptamers, XNA aptamers, LNA aptamers, somamers and spiegelmers.

11. Product for topical application on the skin and/or mucous membranes comprising aptamers as in any claim from 1 to 10.

30 12. Product as in claim 11, wherein said product can be selected from creams, ointments, gels, mechanical contraceptives and body lotions applicable on the skin and mucous membranes, or lotions and solutions for the oral cavity.

13. Drug or medical device comprising aptamers as in any claim from 1 to 10.

- 38 -

14. Method for the diagnosis of the Papillomavirus, said method providing to use, in a biological sample of body fluid, aptamers as in any claim from 1 to 10, wherein said aptamers alternatively:

- are bonded with a suitable marker whose activity is monitored in order to detect the presence of the Papillomavirus in body fluids, wherein said marker is a fluorescence reporter, or an enzyme, whose activity can be detectable electrochemically, or via absorbance or luminescence;
- act as primary antibody in immunochemical techniques;
- bond with a nano-switch for the detection of protein analytes;
- are used in biosensor techniques that comprise measurements in amperometry, impedance, voltammetry, capacitance, electrochemiluminescence, chemiluminescence.

15. Kit for the diagnosis of the Papillomavirus, said kit comprising aptamers as in any claim from 1 to 10.

16. Method to select aptamers for the therapy, prevention, diagnosis and detection of the Papillomavirus, said method providing to generate aptamers which are not only configured to specifically bond with high affinity conformational epitopes with post-translational modifications belonging to the entire capsid of the Papillomavirus but also have specific characteristics that also at the same time satisfy the following conditions:

- i) high affinity bond with said epitopes defined using the SELEX technique;
 - ii) bond with said epitopes having a defined inhibitory function;
 - iii) stability in body fluids and/or iv) stability in products for topical application or use on the skin or mucous membranes, for example, creams, gels and oils;
- wherein the conditions iii) and iv) can be both satisfied or alternative, depending on whether said aptamers are provided respectively for diagnostic use in a biological sample or for use in topical application on the skin and/or mucous membranes.

17. Method for the identification of novel epitopes responsible for infectivity of the Papillomaviruses, said method providing to use aptamers selected by a method according to claim 16 to obtain an initial demonstration of a site relevant for infectivity that, once identified, can be used as a target of drugs or monoclonal antibodies.

fig. 1

