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(54) Title: THERAPEUTIC HPV18 VACCINES

(57) Abstract: The invention provides designer nucleic acid constructs and polypeptides that used as therapeutic vaccines against HPV18 and/or HPV16.

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Title: Therapeutic HPV18 vaccines

The invention relates to the field of medicine and more in particular to nucleic acid constructs and polypeptides that can be used in therapeutic vaccines against human papillomavirus type 18, and/or type 16.

Background of the invention

The family of human papillomaviruses (HPVs) consist of more than 100 types (also referred to as subtypes) that are capable of infecting keratinocytes of the skin or mucosal membranes. Over 40 types of HPV are typically transmitted through sexual contact and HPV infections of the anogenital region are very common in both men and women. Some sexually transmitted HPV types may cause genital warts. Persistent infections with "high-risk" HPV types (e.g. types 16, 18, 31, 45) — different from the ones that cause skin warts — may progress to precancerous lesions and invasive cancer, e.g. of the cervix, vulva, vagina, penis, oropharynx and anus. The majority of HPV infections are spontaneously cleared within one to two years after infection. In healthy individuals circulating Th1- and Th2-type CD4+ T-cells specific for the viral early proteins E2, E6 and E7 of HPV-16 as well as E6- specific CD8+ T-cells, migrate into the skin upon antigenic challenge, indicating that successful defense against HPV-16 infection is commonly associated with a systemic effector T-cell response against these viral early antigens. In a minority (~1%) of infected individuals, HPV infection persists, ultimately resulting in genital neoplastic lesions. Among the high-risk HPVs, HPV16 and HPV18 are the main cause of cervical cancer, together causing about 70% of the cases, and these two types also play a major role in other HPV-induced cancers such as anal and oropharyngeal cancer. Worldwide, HPV is one of the most important infectious agents causing cancer.

Vaccination against HPV is deemed a feasible strategy to reduce the incidence or effects of infection by HPV (van der Burg and Melief, 2011, *Curr Opinion Immunol* 23: 252–257).

Prophylactic HPV vaccines based on virus like particles (VLPs) formed by the (envelope) protein L1 of the HPV types 16 and 18, are very efficient in the prevention of persistent infection and the associated disease by HPV16 and HPV18. These vaccines are believed to provide sterile immunity via the induction of neutralizing

antibodies against the L1 proteins. Addition of L1-based VLPs from additional high-risk HPV types may further increase the breadth of protection conferred by such vaccines.

However, while such vaccines can *prevent* initial infection (i.e., they result in prophylaxis), there is no evidence of a beneficial effect on established genital lesions caused by HPV16 and HPV18, so they are not considered *therapeutic* vaccines against HPV (Hildesheim *et al.,* 2007, *JAMA* 298: 743-53).

Despite the introduction of these prophylactic vaccines, large numbers of people have already obtained or are still at risk of obtaining persistent high-risk HPV infections and, therefore, are at risk of getting cancer. Therapeutic vaccines for the eradication of established HPV infections and associated diseases are an urgent unmet medical need.

Some attempts to address this need have been described. For example, clinical trials have been carried out with a variety of different vaccination strategies, such as a fusion protein consisting of a heat shock protein (Hsp) from *Mycobacterium bovis* and HPV-16 E7 or consisting of a fusion protein of E6, E7 and L2 from HPV-16 and HPV-18, chimeric L1-E7 VLPs, recombinant vaccinia viruses expressing either E6 and E7 of HPV-16 and HPV-18 or bovine papilloma virus E2, DNA vaccines expressing CTL epitopes of E6 and E7 of HPV-16 and HPV-18, a live-attenuated Listeria monocytogenes (Lm) that secretes the HPV-16 E7 antigen, and synthetic long-peptides (SLPs) comprising HPV-16 E6 and E7 peptides. While some of these approaches show some, but limited, clinical efficacy, most have failed, demonstrating that improvement of the current strategies is needed.

Integration of the genes encoding the early HPV proteins E6 and E7 is a necessary step in the process from infection to cancer and continuous expression of E6 and E7 is required for the maintainance of the neoplastic phenotype of cervical cancer cells. E6 and E7 are therefore considered good targets for therapeutic vaccination. As mentioned some studies have shown that therapeutic vaccination of women infected with high-risk HPV can induce regression of existing lesions. Kenter et al showed a durable and complete regression in 47% of patients having Vulvar Intraepithelial Neoplasia (VIN) using SLPs derived from the HPV16 E6 and E7 proteins and an adjuvant as a therapeutic vaccine (Kenter *et al.,* 2009, *N Engl J Med* 361: 1838-47). Similarly, a study in which a protein-based vaccine (TA-CIN, consisting of a fusion protein of HPV16 E6, E7 and L2) was combined with local

immune modulation in VIN 2/3 patients, showed complete regression in 63% of patients (Daayana *et al.,* 2010, *Br J Cancer* 102: 1129-36). Possible drawbacks of the synthetic long peptides as a vaccine include manufacturability at large scale and costs associated therewith, the need for potentially reactogenic adjuvant and the associated adverse effects associated with immunization (especially pain and swelling). Due to the high level of discomfort it is not likely that SLPs will be used in early stage disease when the spontaneous clearance rate is still high. Similarly, due to the need for local imiquimod treatment in the case of TA-CIN treatment, tolerability is a significant issue as the majority of women experience local and systemic side effects lasting for the duration of imiquimod treatment, which may affect daily activities.

A possible alternative is to use nucleic acid based vaccination such as DNA vaccines or viral vectored vaccines encoding the HPV E6 and/or E7 protein for vaccination.

However, the HPV E6 and E7 proteins have oncogenic potential and thus vaccination with vaccines that comprise nucleic acids encoding these proteins poses a risk of inducing cellular transformation due to the possibility of prolonged expression of the antigens.

Therefore, in case of genetic vaccination, non-oncogenic/detoxified versions of E6 and/or E7 can be used in order to exclude any risk of cellular transformation due to the vaccination. Loss of oncogenic potential of wild-type E6 and E7 is commonly achieved by deletion and/or substitution of residues known to be important for the function of these proteins (e.g., Smahel *et al.,* 2001, *Virology* 281:231-38; Yan *et al.,* 2009, *Vaccine* 27: 431–40; Wieking *et al.,* 2012, *Cancer Gene Ther* 19: 667-74). However, a disadvantage of these approaches is that they carry the risk of removing important T-cell epitopes from and/or introducing new undesired T-cell epitopes into the proteins, and may thus not lead to the desired immune response.

In an alternative strategy to remove the oncogenic potential of HPV16 E6 and E7, shuffled versions (i.e. polypeptides wherein fragments of the wild-type protein are re-ordered) of the E6 and E7 proteins have been constructed (e.g. Öhlschläger *et al.,* 2006, *Vaccine* 24: 2880–93; Oosterhuis *et al.,* 2011, *Int J Cancer* 129: 397-406; Oosterhuis *et al.,* 2012, *Hum Gen Ther* 23: 1301-12). However, these approaches would still require manufacturing, formulation and administration of multiple molecules to ensure inclusion of all possible epitopes of both the E6 and E7 proteins, resulting in sub-optimal logistics and relatively high costs, and moreover the

strategies described introduce potentially strong non-natural epitopes that are not present in E6 and E7 and since immune responses could be diverted from relevant E6/E7 epitopes towards such non-natural epitopes, the described constructs may not have the optimal immunological characteristics. A therapeutic DNA vaccine expressing an intracellularly targeted fusion protein with built-in genetic adjuvant and shuffled fragments of E6 and E7 of both HPV16 and HPV18 has also been described, and electroporation-enhanced immunization therewith elicited a significant E6/E7-specific T-cell response in CIN3 patients (Kim *et al.*, 2014).

Another approach that has been described to make immunogenic constructs is making so-called multi-epitope constructs or minigenes (e.g. US 2007/014810; Mishra et al, 2014; Moise et al, 2011; Moss et al, 2010). This has the objective of generating the smallest peptide that encompasses the epitopes of interest. However, in such approaches potential disadvantages are that only a subset of the epitopes of a natural protein are present and further that typically spacer sequences are introduced that are not naturally present in the protein of interest.

There remains a need in the art for therapeutic vaccines against HPV, preferably having less of the drawbacks of the approaches described before.

Summary of the invention

The present invention provides nucleic acid molecules that encode polypeptides that comprise essentially all possible T-cell epitopes of HPV16 or HPV18 oncoproteins E6 and E7, but nevertheless have a strongly reduced (as compared to wt E6 and E7), up to non-detectable, transforming activity, by comprising fragments of the E6 and E7 proteins that have been re-ordered, while at the same time containing a minimized number of undesired strong neo-epitopes. This is in contrast to molecules previously reported by others. The invention provides molecules that can be used in therapeutic vaccines against either HPV16 or HPV18. Such molecules can also be combined in therapeutic vaccines against both HPV16 and HPV18.

The invention for HPV16 provides a nucleic acid molecule encoding a polypeptide comprising a sequence as set forth in SEQ ID NO: 1. For HPV18 the invention provides a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 20.

The encoded polypeptide may further comprise a leader sequence.

In certain embodiments, the encoded polypeptide further comprises at least one epitope of a human papillomavirus (HPV) E2 protein, for example an HPV16 E2 protein or an HPV18 E2 protein. The E2 protein may be inactivated in for instance its transactivation and/or DNA binding domain, e.g. by deletion, mutation or by structural rearrangement of different parts of the protein. In certain embodiments for HPV16, the encoded polypeptide comprises a sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5. In certain embodiments for HPV18, the encoded polypeptide comprises a sequence as set forth in SEQ ID NO: 22.

In certain embodiments, the nucleic acid sequence is codon-optimized, e.g. for expression in human cells.

In certain embodiments for HPV16, the nucleic acid sequence comprises a sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 4 , or SEQ ID NO: 6. In certain embodiments for HPV18, the nucleic acid sequence comprises a sequence as set forth in SEQ ID NO: 21 or SEQ ID NO: 23.

The invention also provides a vector comprising a nucleic acid molecule according to the invention, wherein the sequence encoding the polypeptide is operably linked to a promoter.

In certain embodiments, the vector is a DNA vector such as a plasmid. In other embodiments the vector is a viral vector, such as an MVA vector or a recombinant adenoviral vector. In certain preferred embodiments, the vector is a recombinant adenovirus.

In certain embodiments, the promoter in the vector is operably coupled to a repressor operator sequence, to which a repressor protein can bind in order to repress expression of the promoter in the presence of said repressor protein. In certain embodiments, the repressor operator sequence is a TetO sequence or a CuO sequence.

The invention also provides a vaccine composition comprising a vector according to the invention, and a pharmaceutically acceptable excipient.

The invention also provides a method of inducing an immune response against HPV, in particular HPV16 or HPV18, or HPV16 and HPV18 in a subject, the method comprising administering to the subject a vaccine composition according to the invention. The invention also provides a vaccine according to the invention for use in inducing an immune response against HPV, in particular HPV16 or HPV18, or both HPV16 and HPV18.

In certain embodiments, the vaccine is administered to the subject more than once.

The invention also provides a method for treating any of: persistent HPV infection (in particular persistent HPV16 or HPV18 infection), vulvar intraepithelial neoplasia (VIN), cervical intraepithelial neoplasia (CIN), vaginal intraepithelial neoplasia (VaIN), anal intraepithelial neoplasia (AIN), cervical cancer (such as cervical squamous cell carcinoma (SCC), oropharyngeal cancer, penile cancer, vaginal cancer or anal cancer in a subject, the method comprising administering to the subject a vaccine according to the invention. The invention also provides a vaccine according to the invention for use in treatment of any of: persistent HPV infection (in particular persistent HPV16 or HPV18 infection), vulvar intraepithelial neoplasia (VIN), cervical intraepithelial neoplasia (CIN), vaginal intraepithelial neoplasia (VaIN), anal intraepithelial neoplasia (AIN), cervical cancer (such as cervical squamous cell carcinoma (SCC), oropharyngeal cancer, penile cancer, vaginal cancer or anal cancer in a subject.

The invention for HPV16 also provides a polypeptide comprising a sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 3 or SEQ ID NO: 5. The invention for HPV18 also provides a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 20 or SEQ ID NO: 22.

The invention also provides combinations of molecules as described above for HPV16 and HPV18. Such molecules can be combined as separate molecules in a single composition (e.g. one nucleic acid for HPV16, i.e. encoding a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 1, and one nucleic acid for HPV18, i.e. encoding a polypeptide comprising an amino acid comprising a sequence as set forth in SEQ ID NO: 20, for instance each on a separate vector). Alternatively, such molecules could be used in combination via administration to a single subject of at least two separate compositions (one for HPV16 and one for HPV18). Alternatively, such molecules could also be combined by having the HPV16 and HPV18 molecules present in a single nucleic acid molecule, e.g. a single vector. In certain embodiments therefore, the invention provides a vector according to the invention, comprising both a nucleic acid molecule encoding a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO: 1 and a nucleic acid molecule encoding a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO: 20. In other embodiments, the invention provides a composition

comprising a vector comprising a nucleic acid molecule encoding a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO: 1 and a further vector comprising a nucleic acid molecule encoding a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO: 20. In certain embodiments, the invention provides a method for inducing an immune response against HPV, in particular HPV16 and HPV18, in a subject, the method comprising administering to the subject a nucleic acid molecule encoding a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO: 1 and a nucleic acid molecule encoding a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO: 20. In certain embodiments, the invention provides a method for treating any of: persistent HPV infection (in particular persistent HPV16 or HPV18 infection), vulvar intraepithelial neoplasia (VIN), cervical intraepithelial neoplasia (CIN), vaginal intraepithelial neoplasia (VaIN), anal intraepithelial neoplasia (AIN), cervical cancer (such as cervical squamous cell carcinoma (SCC), oropharyngeal cancer, penile cancer, vaginal cancer or anal cancer in a subject, the method comprising administering to the subject a nucleic acid molecule encoding a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO: 1 and a nucleic acid molecule encoding a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO: 20. In certain aspects of any of these HPV16/18 combination embodiments, the polypeptide that comprises the amino acid sequence set forth in SEQ ID NO: 1, may comprise a sequence as set forth in SEQ ID NO:3 or SEQ ID NO: 5, and optionally the nucleic acid sequence may comprise a sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 6, while the polypeptide that comprises the amino acid sequence set forth in SEQ ID NO: 20, may comprise a sequence as set forth in SEQ ID NO:22, and optionally the nucleic acid sequence may comprise a sequence as set forth in SEQ ID NO: 21 or SEQ ID NO: 23. Any of the features in embodiments described for the individual HPV16 or HPV18 nucleic acids, vectors, polypeptides, vaccine compositions, uses or methods above, can of course also be applied to the combinations of HPV16 and 18 as disclosed herein.

Brief description of the Figures

Fig. 1. Expression of fusion proteins of HPV16 E6 and E7. HEK-293T cells were transiently transfected with DNA vectors expressing the transgenes indicated above

the figure. 24 hr after transfection the cells were harvested and cell extracts were analyzed by SDS-PAGE and western blotting with an antibody against HPV16 E7 (upper panel). A loading control showing NF-kB (lower panel) confirms similar loading of cell lysates in all lanes. A molecular weight marker is indicated on the left. Expected sizes of the fusion proteins: E6E7SH approx. 38kDa; E2E6E7SH and E6E7E2SH approx. 75kDa, LSE2E6E7SH approx. 78kDa.

Fig. 2. Colony formation in soft agar. A) Schematic representation of the setup of the soft-agar assay. B) Representative microscopic images at 40x magnification of the cells in agar six weeks post seeding. The white arrows highlight colonies observed in the E7wt transfected cells. C) Colony quantification six weeks post seeding in agar using the Gelcount™ and associated software. *: $p<0.05$ (Poisson regression model); **: non-inferior (generalized linear model with non-inferiority margin of 5%).

Fig. 3. HPV16 E6E7SH has lost E6 and E7 activities. A) Representative western blot demonstrating absence of p53 degradation by E6E7SH. Human p53 null NCI-H1299 cells were co-transfected with a plasmid expressing p53 in combination with a plasmid expressing HPV16 E6 wild-type, HPV16 E6E7SH or the empty vector. Non-TF indicates non-transfected cells. 24 hours after transfection cell lysates were prepared and 30 µg of total protein was loaded on gel. Upper panel - p53 staining, middle panel - E6 staining, lower panel - NF-kB staining (loading control). (B) Quantification of p53 levels in four independent assays. The p53 signal was normalized to the NF-κB signal. C) Western blot demonstrating lack of pRb degradation by E6E7SH. pRb null Saos-2 cells were transfected with a plasmid expressing pRb in combination with a plasmid expressing HPV16 E7 wild-type, HPV16 E6E7SH or the empty vector. Non-TF indicates non-transfected cells. 24 hours after transfection cell lysates were prepared and 10 µg of total protein was loaded on gel. Upper panel - pRb staining, middle panel – E7 staining, lower panel - NF-κB staining (loading control). D) Quantification of pRb levels in four independent assays. The pRb signal was normalized to the NF-κB signal. *: $p<0.05$ (ANOVA models); **: non-inferior (testing was based on 95% CI's derived from ANOVA models. Non-inferiority margin was set at 75%).

Fig. 4. HPV16 E6E7SH does not immortalize primary human epidermal keratinocytes. Primary human epidermal keratinocytes were transduced with lentiviruses encoding either the wild-type E6- and E7-encoding open reading frame of HPV16 (E6E7wt), the HPV16 E6E7SH sequence or eGFP. Non-transduced donor cells were used as a control. Only expression of E6E7wt induces immortalization of primary keratinocytes as indicated by the extended lifespan and hTERT activation around day 200 (not shown). The cross symbol indicates that the cells died in senescence and could not be further cultured. For details see example 2. Similar results were obtained in two additional donors (not shown).

Fig. 5. Immune response induced by HPV16 E6E7SH after DNA immunization – IFNγ ELISPOT analysis. A. Immunization scheme. CB6F1 mice were immunized with DNA plasmids expressing HPV16 E6E7SH or a plasmid expressing no transgene (control). Two weeks after immunization the mice were sacrificed and isolated splenocytes were stimulated overnight with 15mer peptide pools corresponding to E7. B. HPV16 E7-specific immune responses in individual mice as measured by IFNγ ELISPOT assays are given as spot forming units (SFU) per 10^6 splenocytes.

Fig. 6. Immunogenicity of HPV16 E6E7SH – IFNγ ELISPOT analysis. (A). Immunization scheme. Mice were immunized with adenovectors with inserts as indicated. E7-specific responses at two weeks (B) and at eight weeks (C) were analyzed by IFNγ ELISPOT (represented as spot-forming units (SFU) per 10^6 splenocytes). The closed circles represent mice immunized with a dosage of $1*10^{10}$ vp, and open circles represent mice immunized with $5*10^9$ vp. The black bar represents the geometric mean of the responses. The dotted line indicates the lower detection limit in the ELISPOT assay. ANOVA Post-hoc Bonferroni statistical analysis was performed on log transformed data. *: $p<0.05$. For details see example 3.

Fig. 7. Immunogenicity of HPV16 E2E6E7SH – E7-tetramer staining. (A). Immunization scheme. CB6F1 mice were immunized with $1*10^{10}$ vp of adenovectors expressing the transgenes as indicated. Two weeks after immunization the mice were sacrificed and isolated splenocytes analyzed for the presence of CD8+ cells capable of interacting with $E7_{49-57}$-H2-Db tetramers (B). The percentage of E7-tetramer positive

CD8+ T-cells is indicated on the y-axis. ANOVA Post-hoc Bonferroni statistical analysis was performed on log transformed data, the differences between the different E6E7SH variants were not statistically significant.

Fig. 8. Immunogenicity of HPV16 E2E6E7SH – IFNγ ELISPOT analysis. (A). Immunization scheme. CB6F1 mice were immunized with adenovectors expressing the transgenes indicated below panels B and C. Two weeks after immunization the mice were sacrificed and isolated splenocytes were stimulated overnight with 15mer peptide pools corresponding to E2 (B), E6 (not shown) or E7 (C). Responses are given as SFU per 10^6 splenocytes. ANOVA Post-hoc Bonferroni statistical analysis was performed on log transformed data. The E2 response induced by Adenovectors encoding E2 alone is higher than the response induced by the polypeptides of the invention that include the E6 and E7 fragments. The difference is significant for E2 vs E2E6E7SH and E2 vs E6E7E2SH (*: $p<0.05$). ANOVA Post-hoc Bonferroni statistical analysis was performed on log transformed data.

Fig. 9. Sustained HPV16 immune responses in immunized mice. (A) Immunization scheme. CB6F1 mice were immunized with $1*10^{10}$ vp of Ad35 vectors expressing variants HPV16 LSE2E6E7SH, HPV16 E2E6E7SH, HPV16 E6E7SH, or with an adenovector not expressing a transgene (Emtpy). Blood samples were taken every two weeks to determine the percentage E7-specific CD8+ T-cells by tetramer staining. (B) Immune responses two weeks after immunization. The vector including a leader sequence induced a higher response than vectors without the leader sequence; LSE2E6E7SH vs E2E6E7SH (*: $p<0.05$). (C) Kinetics of the responses. ANOVA Post-hoc Bonferroni statistical analysis was performed on log transformed data of the week 2 data set. The E7 response induced by molecules including E2 tend to be higher compared to the molecule without E2, though the results were not statistically significant.

Fig. 10. Use of different Adenoviral vectors to boost immune responses. (A). Immunization scheme. CB6F1 mice were immunized with an Ad26 vector expressing HPV16 E2E6E7SH (HPV16-Tx) or with an Ad26 vector expressing no transgene (empty). Two weeks later the immunizations were repeated with Ad35-based vectors as indicated below the figure. Four weeks after the second immunization the mice

were sacrificed and blood samples were used to determine the percentage of E7-specific CD8+ T-cells by tetramer staining (B). * indicates the comparison of Ad26.HPV16-Tx/Ad35.HPV16-Tx versus Ad26.HPV16-Tx/Ad35.Empty, $p<0.05$ (student t-test on log transformed data, with alpha = 0.01 for multiple comparisons).

Fig. 11. Cellular immunogenicity of HPV16 E2E6E7SH in Rhesus macaques.. (A) Immunization scheme. Rhesus macaques were immunized at day 0: Eight animals received Ad26.HPV16-E2E6E7SH and two control animals received Ad26.Empty by intramuscular immunization (i.m). A boost immunization was given (Ad26.HPV16-E2E6E7SH or Ad26.Empty) at 8 weeks. At 16 weeks, animals received a second boost immunization with Ad35 vectors expressing the same HPV16 E2E6E7SH, while control animals received Ad35.Empty. The dose of adenovectors was $1*10^{11}$ vp per immunization. Blood drawings were performed at several time points. (B) Cellular immune responses in PBMCs were measured by IFNγ ELISPOT. PBMCs were stimulated with peptide pools corresponding to HPV16 E2, E6 or E7 and the number of spot-forming units (SFU) in $1*10^6$ PBMCs are depicted. The empty control animal (n=2) showed no detectable response. For details see example 4.

Fig. 12. Therapeutic effect of Adenovectors expressing HPV16-E2E6E7SH. (A) TC-1 injection and immunization scheme. CB6F1 mice were injected sub-cutaneously with $1*10^5$ TC-1 cells at day 0. After six days, when tumors were palpable, mice were immunized with two SLPs covering HPV16 E6 and E7 immunodominant epitopes (i.e., HPV16 E6, aa41-65 (KQQLLRREVYDFAFRDLCIVYRDGN; SEQ ID NO: 18) and HPV16 E7 aa 43-77 (GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR; SEQ ID NO: 19)) at 150 µg in a final volume of 200 µl 0.9% saline supplemented with 5 nmol ODN1826-CpG (B) or Ad26.HPV16-E2E6E7SH (C). Control mice received either CpG alone (D) or Ad26.Empty (E). All mice received a boost immunization at day 20. Mice that received Ad26 vectors in the prime immunization were subsequently immunized with the corresponding Ad35 vectors. The other mice received, SLP adjuvanted with CpG or CpG alone as in the prime immunizations. (B-E) Tumor measurement in TC-1 injected mice. Tumor volume was calculated as $(width^2 * length)/2$. Mice were sacrificed when tumor volumes surpassed 1000 mm^3. Two mice had to be sacrificed due to weight loss of more than 20% (indicated with

asterisks). (F-G) Close up of panels B and C for first 35 days. (H) Survival after TC-1 injection. The survival of mice treated with Ad.HPV16-E2E6E7SH was significantly increased compared with mice immunized with SLP and CpG (Log-rank test $p<0.05$). Three mice immunized with the Ad.HPV16-E2E6E7SH were tumor free at the end of the experiment (at day 92).

Fig. 13. Adenoviral vectors carrying transgenes encoding either HPVAg or LSE2E6E7SH show increased viral yields on cells capable of repressing transgene expression. A) Viral yield assay for Ad35 vectors. PER.C6, PER.C6/CymR, and PER.C6/TetR cells were infected by Ad35 vectors carrying GFP-Luc- or HPVAg-encoding transgenes. These transgenes were driven by either CuO- or TetO-containing CMV promoters. Viral yields were determined four days after infection by an Ad35 hexon-specific qPCR-based method. B) Viral yield assay for Ad26 vectors. PER.C6 and PER.C6/TetR cells were infected by Ad26 vectors carrying GFP-Luc, HPVAg, or LSE2E6E7SH-encoding transgenes, which were all driven by a TetO-containing CMV promoter. Viral yields were determined three days after infection by an Ad26 hexon-specific qPCR-based method. For details see Example 6.

Fig. 14. Employment of a repressor system to repress transgene expression during vector production prevents transgene cassette instability in an adenoviral vector carrying an HPVAg-encoding transgene. An Ad35 vector expressing HPVAg under the control of CMVCuO was rescued by DNA transfection on either PER.C6 or PER.C6/CymR cell lines. Resultant viral plaques were picked – five per cell line – and used for consecutive infection rounds on the respective cell lines. A) Analysis of the integrity of the vector transgene cassette region by PCR after 10 viral passages. PCR products obtained from viral isolates passaged on PER.C6 and PER.C6/CymR are shown in the middle and right panels, respectively. The full-length-appearing PCR products obtained for PER.C6-passaged viral isolates 1, 2, 4, and 5, and those seen for PER.C6/CymR-passaged isolates 1 to 5 were analyzed by Sanger DNA sequencing. Analysis of the chromatogram traces (not shown) revealed that all isolates grown on PER.C6, but not those grown on PER.C6/CymR, contained either frameshifting small deletions or premature stop mutations within the coding sequence for HPVAg. B) Analysis of the ability of the vectors to express HPVAg after seven viral passages.

A549 cells were transduced by the PER.C6- and PER.C6/CymR-grown viral isolates and HPVAg expression was analyzed by Western Blot using an HPV16 E7-specific antibody. The predicted size for HPVAg is 83 kDa. For details see Example 6.

Fig. 15. Expression of fusion proteins of HPV18 E6 and E7. HEK-293T cells were transiently transfected with DNA vectors expressing the transgenes indicated above the figure. 24 hr after transfection the cells were harvested and cell extracts were analyzed by SDS-PAGE and western blotting with an antibody against HPV18 E6 (upper panel). A loading control showing NF-kB (lower panel) confirms similar loading of cell lysates in both lanes. A molecular weight marker is indicated on the left and arrows indicate the fusion proteins. Expected sizes: E6E7SH approx. 38kDa; E2E6E7SH approx. 75kDa.

Fig. 16. No colony formation in soft agar by the HPV18 E6E7SH designer construct. A) Representative microscopic images at 40x magnification of the cells in agar six weeks post seeding. Large colonies are observed in the E7wt transfected cells. B) Colony quantification six weeks post seeding in agar using the Gelcount™ and associated software. *: $p<0.05$ (Poisson regression model); **: non-inferior (generalized linear model with non-inferiority margin of 5%).

Fig. 17. HPV18 E6E7SH has lost the ability to degrade p53 and pRb. A) Representative western blot demonstrating absence of p53 degradation by HPV18 E6E7SH. Human p53 null NCI-H1299 cells were co-transfected with a plasmid expressing p53 in combination with a plasmid expressing HPV18 E6 wild-type, E6E7SH or the empty vector. Non-TF indicates non-transfected cells. 24 hours after transfection cell lysates were prepared and 30 µg of total protein was loaded on gel. Upper panel - p53 staining, middle panel - E6 staining, lower panel - NF-kB staining (loading control). (B) Quantification of p53 levels in four independent assays. The p53 signal was normalized to the NF-κB signal. C) Western blot demonstrating lack of pRb degradation by HPV18 E6E7SH. pRb null Saos-2 cells were transfected with a plasmid expressing pRb in combination with a plasmid expressing HPV18 E7 wild-type, E6E7SH or the empty vector. Non-TF indicates non-transfected cells. 24 hours after transfection cell lysates were prepared and 10 µg of total protein was loaded on

gel. Upper panel - pRb staining, middle panel – E7 staining, lower panel - NF-κB staining (loading control). D) Quantification of pRb levels in four independent assays. The pRb signal was normalized to the NF-κB signal. *: $p<0.05$ (ANOVA models); **: non-inferior (testing was based on 95% CI's derived from ANOVA models. Non-inferiority margin was set at 75%).

Fig. 18. HPV18 E6E7SH does not immortalize primary human genital keratinocytes. Primary human genital keratinocytes were transduced with lentiviruses encoding either the wild-type E6- and E7-encoding open reading frame of HPV18 (E6E7wt), the E6E7SH sequence or eGFP. Non-transduced donor cells were used as a control. Only expression of HPV18 E6E7wt induces immortalization of primary keratinocytes as indicated by the extended lifespan (and hTERT activation around day 200, data not shown). The cross symbol indicates that the cells died in senescence and could not be further cultured. For details see example 8. Similar results were obtained in two additional donors (data not shown).

Fig. 19. Immunogenicity of HPV18 E6E7SH variants – Intracellular Cytokine staining. CB6F1 mice were immunized with adenovectors expressing the transgenes indicated below the panels. Two weeks after immunization the mice were sacrificed and isolated splenocytes were stimulated overnight with 15mer peptide pools corresponding to HPV18 E6. Responses are given as percentage of IFNγ-positive CD8+ T-cells.

Fig. 20. Immunogenicity of combined HPV16 and HPV18 vectors - IFNγ ELISPOT analysis. CB6F1 mice were immunized with adenovectors (type 26) expressing the E2E6E7SH transgenes from both HPV16 (encoding SEQ ID NO: 3) and HPV18 (encoding SEQ ID NO: 22). Four weeks after prime immunization the mice received an heterologous boost immunization with adenoviral vectors of type 35 with the same E2E6E7SH transgenes. Two weeks after the boost immunization the mice were sacrificed and isolated splenocytes were stimulated overnight with 15mer peptide pools corresponding to HPV16 E7 (A) or HPV18 E6 (B). Responses are given as SFU per 10^6 splenocytes.

Fig. 21. Cellular immunogenicity of combined HPV16 and HPV18 vaccine in Rhesus macaques. Rhesus macaques were immunized according to the scheme as presented in Fig. 11, with a combination of HPV16 and HPV18 designer constructs. At day 0: Eight animals received amixture of Ad26.HPV16-E2E6E7SH and Ad26.HPV18-E2E6E7SH by intramuscular immunization (i.m). A boost immunization with the same vectors was given at 8 weeks. At 16 weeks, animals received a second boost immunization with a mixture of two Ad35 vectors expressing the same HPV16 and HPV18 E2E6E7SH fusion proteins. The dose of adenovectors was $1*10^{11}$ vp per vector per immunization. Blood drawings were performed at several time points. Cellular immune responses in PBMCs were measured by IFNγ ELISPOT. PBMCs were stimulated with peptide pools corresponding to E2, E6 or E7 of HPV16 and HPV18 and the number of spot-forming units (SFU) in $1*10^6$ PBMCs were determined. The figure shows cumulative responses for all six tested peptide pools at 2 weeks after each immunization. For details see example 11.

Fig. 22. Therapeutic effect of combined adenovectors expressing HPV16 and HPV18 E2E6E7SH. C57BL/6 mice were injected sub-cutaneously with $5*10^4$ TC-1 cells at day 0. After six days, when tumors were palpable, mice were immunized with Ad26.HPV16-E2E6E7SH or a mixture of Ad26.HPV16-E2E6E7SH and Ad26.HPV18-E2E6E7SH. Control mice received Ad26.Empty. All mice received a boost immunization at day 20 with the corresponding Ad35 vectors. Tumor volume was calculated as ($width^2$ * length)/2. Mice were sacrificed when tumor volumes surpassed 1000 mm^3. The graphs show survival after TC-1 injection. Three mice immunized with the combined HPV16 + HPV18 vaccine were tumor free at the end of the experiment. The median survival time of mice treated with Ad.HPV16-E2E6E7SH was not significantly different compared with mice immunized with Ad.HPV16/18-E2E6E7SH.

Detailed description of the invention

The invention provides a nucleic acid molecule encoding a polypeptide comprising SEQ ID NO: 1. The polypeptide is a fusion polypeptide, and is sometimes referred to herein as the polypeptide of the invention, or the fusion polypeptide of the

invention. This polypeptide is useful to generate an immune response against the E6 and E7 proteins of HPV16, and thus the nucleic acid molecule can be used as a therapeutic vaccine to prevent persistent HPV16 infection, and diseases associated therewith.

The polypeptide of the invention is a carefully designed molecule that contains virtually the complete E6 and E7 amino acid sequences of HPV16 (it lacks only one amino acid from the C-terminus of the native HPV16 E6 protein) in the form of fragments that are re-ordered and partly overlapping such that (essentially) all T-cell epitopes of the HPV16 E6 and E7 protein are present. Earlier molecules with some potential as HPV vaccines have been described by others (e.g. Kenter *et al.,* 2009, *N Engl J Med* 361: 1838-47; Daayana *et al.,* 2010, Br J Cancer 102: 1129-36; Smahel *et al.,* 2001, *Virology* 281: 231-38; Yan *et al.,* 2009, *Vaccine* 27: 431–40; Öhlschläger *et al.,* 2006, *Vaccine* 24: 2880–93; Oosterhuis *et al.,* 2011, *Int J Cancer* 129: 397-406; EP1183368, WO 2013/083287), but each of these molecules has one or more drawbacks. The designer polypeptide molecules of the invention are advantageous in at least one and typically several aspects with respect to the approaches described earlier. In particular, advantages of the molecules and/or vectors of the present invention include: (i) they have a desired safety profile, as the nucleic acid has a strongly reduced (as compared to native E6 and E7 proteins), down to non-detectable, transforming activity; (ii) they are single nucleic acid molecules, which are easy to manufacture at industrial scale in an economically feasible manner, and do not pose logistic challenges unlike multiple molecule approaches; (iii) the encoded polypeptides comprise essentially all T-cell epitopes of the native HPV16 E6 and E7 proteins; (iv) the design of the encoded polypeptides has minimized the introduction of undesired potential strong neo-epitopes (i.e. epitopes not present in the native E6 and E7 proteins); and (v) in certain embodiments, they are not dependent on highly reactogenic adjuvants to raise a desired immune response. Thus, the molecules of the invention represent a major step forward by combining various advantageous characteristics in a single design, and are excellent candidates primarily for therapeutic vaccination against HPV16. These molecules could also possibly work as prophylactic vaccines against HPV16, meaning that they are likely to prevent persistent infection with HPV16 of vaccinated subjects.

The benefits described in the previous two paragraphs for the nucleic acid molecule encoding HPV16 designer molecules (comprising an amino acid sequence

set forth in SEQ ID NO: 1), also apply mutatis mutandis to nucleic acid molecules encoding novel designer molecules for HPV18 (comprising an amino acid sequence set forth in SEQ ID NO: 20), which is an object of the present invention.

We used the IEDB-AR to determine the possible formation of non-natural strong epitopes that could be introduced at the newly created junctions between the different A6 and E7 fragments. In certain embodiments for the HPV16 designer molecule, by careful design the number of neo-epitopes with a length of nine amino acids with a predicted binding affinity <50 nM for the 20 most common HLA-A, 20 most common HLA-B and 20 most common HLA-C alleles in the re-ordered HPV16 E6 and E7 sequences was miminized to only 1. This is a significant improvement over constructs described by others, which for a single shuffled HPV16 E6 protein already contained more than 30 of such neo-epitopes, and which constructs will higly likely comprise even several more neo-epitopes in sequences that were appended to these constructs to prevent loss of epitopes (Öhlschläger *et al.,* 2006, *Vaccine* 24: 2880–93). Hence the constructs of the invention have a significantly improved immunologic profile since chances of an altered immune response as compared to native E6 and E7 have been minimized in the molecules of the invention, as compared to approaches described by others.

Skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides described to reflect the codon usage of any particular host organism in which the polypeptides are to be expressed. Therefore, unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

In a preferred embodiment, the nucleic acid encoding the polypeptide according to the invention is codon optimized for expression in mammalian cells, preferably human cells. Methods of codon-optimization are known and have been described previously (e.g. WO 96/09378). A sequence is considered codon optimized if at least one non-preferred codon as compared to a wild type sequence is replaced by a codon that is more preferred. Herein, a non-preferred codon is a codon that is used less frequently in an organism than another codon coding for the same amino acid, and a codon that is more preferred is a codon that is used more frequently in an

organism than a non-preferred codon. The frequency of codon usage for a specific organism can be found in codon frequency tables, such as in http://www.kazusa.or.jp/codon. Preferably more than one non-preferred codon, e.g. more than 10%, 40%, 60%, 80% of non-preferred codons, preferably most (e.g. at least 90%) or all non-preferred codons, are replaced by codons that are more preferred. Preferably the most frequently used codons in an organism are used in a codon-optimized sequence. Replacement by preferred codons generally leads to higher expression.

Nucleic acid sequences can be cloned using routine molecular biology techniques, or generated de novo by DNA synthesis, which can be performed using routine procedures by service companies having business in the field of DNA synthesis and/or molecular cloning (e.g. GeneArt, GenScripts, Invitrogen, Eurofins).

It will be appreciated by a skilled person that changes can be made to a protein, e.g. by amino acid substitutions, deletions, additions, etc, e.g. using routine molecular biology procedures. Generally, conservative amino acid substitutions may be applied without loss of function or immunogenicity of a polypeptide. This can be checked according to routine procedures well known to the skilled person.

In certain embodiments, the encoded polypeptide according to the invention further comprises a leader sequence, also referred to as signal sequence or signal peptide. This is a short (typically 5-30 amino acids long) peptide present at the N-terminus of the majority of newly synthesized proteins that are destined towards the secretory pathway. The presence of such a sequence may lead to increased expression and immunogenicity. Non-limiting examples that can be used are an IgE leader peptide (see e.g. US 6,733,994; e.g. having sequence MDWTWILFLVAAATRVHS (SEQ ID NO: 7)) or a HAVT20 leader peptide (e.g. having sequence MACPGFLWALVISTCLEFSMA (SEQ ID NO: 9)). One of these can optionally be added to the N-terminus of a polypeptide of the invention. In other embodiments, a polypeptide according to the invention does not comprise a leader sequence.

Diverse types of HPV exist (over 120 types have been identified and are referred to by number), and generally for each type that needs to be covered by a vaccine, type-specific antigens may need to be incorporated in the vaccine, although for certain antigens some cross-reactivity might exist. Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are carcinogenic "high-risk" sexually transmitted HPVs and may lead to the development of cervical intraepithelial neoplasia (CIN),

vulvar intraepithelial neoplasia (VIN), vaginal intraepithelial neoplasia (VaIN), penile intraepithelial neoplasia (PIN), and/or anal intraepithelial neoplasia (AIN). The HPV according to the invention (i.e. the HPV from which the E6 and E7 fragments in the encoded polypeptide are derived) is HPV16 (for SEQ ID NOs: 1-6), or HPV18 (for SEQ ID NOs: 20-23). It can be used for subjects that are infected with HPV16 or HPV18, respectively. It may in certain embodiments also suitably be combined with vaccines against other HPV types. In certain embodiments, this combination is with a vaccine against HPV of a high risk type as identified above, e.g. a vaccine against HPV16 with a vaccine against HPV18. In other embodiments, the vaccine of the invention is combined with a vaccine against one or more of HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, -73, or -82. Such combinations could for instance be used if the exact type of HPV infection is not yet certain, or if an immune response with a prophylactic effect is desired against more than one HPV type. Also combinations of the vaccines of the invention with vaccines against HPV types that cause genital warts, such as HPV6 and/or HPV11, are envisaged. Sequences of these HPV types and the proteins encoded thereby (e.g. E6, E7, E2) are available to the skilled person in public databases, such as the GenBank sequence database provided by the National Center for of technology Information (NCBI).

The polypeptide according to the invention for HPV16 comprises SEQ ID NO: 1, and in one embodiment the nucleic acid molecule according to the invention comprises SEQ ID NO: 2. The polypeptide according to the invention for HPV18 comprises SEQ ID NO: 20, and in one embodiment the nucleic acid molecule according to the invention comprises SEQ ID NO: 21.

Sequences herein are provided from 5' to 3' direction or from N- to C-terminus, as custom in the art.

The polypeptide according to the invention comprises the epitopes of HPV16 E6 and E7 proteins, or alternatively the epitopes of HPV18 E6 and E7 proteins. In certain embodiments, the polypeptide according to the invention further comprises (and hence the nucleic acid encoding the polypeptide further encodes) at least one further antigen or epitope(s) of such further antigen. Such a further antigen preferably is an HPV antigen, preferably of the same HPV type as the E6 and E7 proteins in the polypeptide, i.e. HPV16 or HPV18 respectively. Such a further antigen can thus be an HPV protein or an immunogenic fragment thereof, and in certain embodiments comprises an E2 protein or a fragment thereof comprising at least one epitope of E2 of

HPV, preferably from HPV16 or HPV18. Such further antigens or epitopes could be placed internally between two fragments of E6 and/or E7 in the polypeptide comprising SEQ ID NO: 1 or SEQ ID NO: 20, but preferably are fused N-terminally or C-terminally to the E6/E7 polypeptide comprising SEQ ID NO: 1 or SEQ ID NO: 20. Alternatively or in addition, amino acid sequences can be present that stimulate the immune response. Thus, in certain embodiments the invention provides nucleic acid molecules according to the invention, encoding a polypeptide comprising SEQ ID NO: 1 or SEQ ID NO: 20, and wherein the polypeptide further comprises at least one other antigen, e.g. HPV E2 protein or at least one epitope, but preferably more epitopes, thereof. One advantage of the addition of E2 antigen for the instant invention is that E2 is known to be expressed early during infection/in low grade lesions where E6 and E7 expression is still very low. During the development towards cervical cancer E2 expression is lost and as a result E6 and E7 levels are increased (Yugawa and Kiyono, 2009, *Rev Med Virol* 19: 97–113). Combining epitopes from E2, E6 and E7 in one vaccine allows for treatment in a broad target group of patients, ranging from having persistent infection to invasive cervical cancer (or other HPV16-caused cancers). In certain embodiments, the E2 protein is a wild-type E2 protein. In certain other embodiments, the E2 protein has a deletion or one or more mutations in its DNA binding domain (as compared to a wild type E2 protein). The sequence of the HPV16 and HPV18 E2 proteins can be found in the NCBI protein database (www.ncbi.nlm.nih.gov/protein) under numbers NP_041328.1 and AAP20597.1, respectively. Several single amino acid changes in HPV16 E2 such as G293V, K299M, or C300R in the C-terminal part of this protein are known to abrogate DNA binding. For HPV18 E2, the corresponding amino acid changes are G294V, K300M, C301R. An advantage of using a variant or fragment of E2 that lacks DNA binding capacity is that it could prevent unpredictable transcriptional changes via direct binding to host cell DNA in the cells where it is expressed. In addition to or as an alternative to mutations in the DNA binding domain described above, further approaches to prevent E2 activity are to introduce mutations that abrogate activity of the more N-terminally located E2 transactivation domain, and/or that are reported to affect the structure of the E2 polypeptide. For HPV16 E2, non-limiting examples of amino acid changes at positions that have previously been described (e.g. Brokaw et al, 1996; Sakai et al, 1996) are R37A, I73A, W92A, E39A, W33A, P106A and G156A, and HPV16 E2 according to the invention could optionally comprise one or

more of these mutations in the transactivation domain. For HPV 18 E2, the corresponding amino acid changes are R41A, I77A, W96A, E43A, W37A, P110A and G161A, and HPV18 E2 according to the invention could thus optionally comprise one or more of these mutations in the transactivation domain. In certain embodiments, E2 has mutations in the transactivation domain, in other embodiments E2 has mutations in the DNA binding domain, and in further embodiments E2 has mutations in both the transactivation domain and in the DNA binding domain. In yet another alternative embodiment, the E2 polypeptide according to the invention is divided in fragments which are reordered (shuffled), to abrogate E2 activity while maintaining the E2 epitopes for immunogenicity. Such embodiment could optionally be combined with one or more of the mutations described above, e.g. in the DNA binding domain and/or in the transactivation domain. Besides wild-type HPV E2 polypeptides, all such E2 mutants can be used as the E2 protein or part or variant thereof according to the invention.

The E2 protein or part or variant thereof can be added internally, but preferably is fused to the N-terminus or to the C-terminus of the polypeptide of the invention having SEQ ID NO: 1 or SEQ ID NO: 20. In one embodiment for HPV16, the nucleic acid molecule of the invention encodes a polypeptide comprising SEQ ID NO: 3. In one embodiment thereof, the nucleic acid molecule of the invention comprises SEQ ID NO: 4. In another embodiment for HPV16 , the nucleic acid molecule of the invention encodes a polypeptide comprising SEQ ID NO: 5. In one embodiment thereof, the nucleic acid molecule of the invention comprises SEQ ID NO: 6. In one embodiment for HPV18, the nucleic acid molecule of the invention encodes a polypeptide comprising SEQ ID NO: 22. In one embodiment thereof, the nucleic acid molecule of the invention comprises SEQ ID NO: 23.

It is also possible to make further fusions of the designer polypeptides of the invention with further proteins, e.g. so called carrier proteins, such as Calreticulin, Mycobacterium Tubercelosis heat shock protein-70, IP10, or Tetanus toxin fragment C (see Oosterhuis et al., *Human Gene Ther*, 2012, *supra*, for more examples), which could further enhance the immune respsonse to the HPV E6 and E7 (and optionally E2) epitopes. The invention thus also provides such further fusion proteins, and nucleic acids encoding such.

In certain embodiments, a nucleic acid molecule according to the invention is incorporated into a vector. A “vector” as used herein, is typically a vehicle to

artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed, and according to the invention can be any nucleic acid molecule that incorporates a nucleic acid molecule according to the invention. These can be prepared according to routine molecular biology techniques such as cloning. Typically such vectors can be propagated in at least one type of suitable hosts such as bacteria, yeast, insect cells, mammalian cells, and the like. Four major types of vectors are plasmids, viral vectors, cosmids, and artificial chromosomes. The vector itself is generally a DNA sequence that consists of an insert (transgene; in the present invention the nucleic acid encoding the fusion polypeptide of the invention) and a sequence that serves as the "backbone" of the vector. The purpose of a vector which transfers genetic information to another cell is typically to isolate, multiply, or express the insert in the target cell. Preferably, the sequence encoding the polypeptide is operably linked to a promoter in the vector. The term "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the promoter in a manner that allows for expression of the nucleotide sequence (e.g., in a host cell when the vector is introduced into the host cell). Expression regulatory sequences can be operably linked to a transgene. In certain embodiments, vectors are designed for the expression of the transgene in the target cell, and generally have a promoter sequence that drives expression of the transgene. In certain embodiments, one or more of routinely used vector elements such as transcription terminator sequences, polyadenylation tail sequences, Kozak sequences, UTRs, origin of replication, multiple cloning sites, genetic markers, antibiotic resistance, and further sequences may be present, and the skilled person can design a vector such that it has the desired properties, e.g. for replication in certain cells for propagation and multiplication of the vector, and for expression of the transgene of the vector in target cells into which the vector is introduced. Vectors comprising the nucleic acid encoding the fusion polypeptide according to the invention, preferably designed for expression in mammalian cells, are suitable as vaccines according to the invention. In certain embodiments, a vector according to the invention is a plasmid, a cosmid, a yeast artificial chromosome, a bacterial artificial chromosome, a viral vector, or the like. The person skilled in the art is aware that various promoters can be used to obtain expression of a gene in host cells. Some well-known and much used promoters for expression in eukaryotic cells comprise promoters derived from viruses, such as adenovirus, e.g. the E1A promoter, promoters derived from cytomegalovirus (CMV),

such as the CMV immediate early (IE) promoter (referred to herein as the CMV promoter) (obtainable for instance from pcDNA, Invitrogen), promoters derived from Simian Virus 40 (SV40) (e.g. obtainable from pIRES, cat.no. 631605, BD Sciences), and the like. Suitable promoters can also be derived from eukaryotic cells, such as methallothionein (MT) promoters, elongation factor 1α (EF-1α) promoter, ubiquitin C or UB6 promoter, actin promoter, an immunoglobulin promoter, heat shock promoters, and the like (see e.g. WO 2006/048459). A non-limiting example of a suitable promoter for obtaining expression in eukaryotic cells is a CMV-promoter (US 5,385,839), e.g. the CMV immediate early promoter, for instance comprising nt. –735 to +95 from the CMV immediate early gene enhancer/promoter, e.g. a CMV promoter as provided herein with a sequence as set forth in SEQ ID NO: 13. A polyadenylation signal, for example the bovine growth hormone polyA signal (US 5,122,458), may be present behind the transgene(s).

Further regulatory sequences may also be added. The term "regulatory sequence" is used interchangeably with "regulatory element" herein and refers to a segment of nucleic acid, typically but not limited to DNA, that modulate the transcription of the nucleic acid sequence to which it is operatively linked, and thus acts as a transcriptional modulator. A regulatory sequence often comprises nucleic acid sequences that are transcription binding domains that are recognized by the nucleic acid-binding domains of transcriptional proteins and/or transcription factors, enhancers or repressors etc. For example, it is possible to operably couple a respressor sequence to the promoter, which repressor sequence can be bound by a repressor protein that can decrease or prevent the expression of the transgene in a production cell line that expresses this repressor protein. This may improve genetic stability and/or expression levels of the nucleic acid molecule upon passaging and/or when this is produced at high quantities in the production cell line. Such systems have been described in the art. For example, a regulatory sequence could include one or more tetracycline operon operator sequences (tetO), such that expression is inhibited in the presence of the tetracycline operon repressor protein (tetR). In the absence of tetracycline, the tetR protein is able to bind to the tetO sites and repress transcription of a gene operably linked to the tetO sites. In the presence of tetracycline, however, a conformational change in the tetR protein prevents it from binding to the operator sequences, allowing transcription of operably linked genes to occur. In certain

embodiments, a nucleic acid molecule, e.g. when present in a recombinant adenovirus vector, of the present invention can optionally include tetO operatively linked to a promoter, such that expression of one or more transgenes is inhibited in recombinant adenoviruses that are produced in the producer cell line in which tetR protein is expressed. Subsequently, expression would not be inhibited if the recombinant adenovirus is introduced into a subject or into cells that do not express the tetR protein (e.g., international patent application WO 07/ 073513). In certain other embodiments, a nucleic acid molecule of the present invention, e.g. when present in a recombinant adenovirus, can optionally include a cumate gene-switch system, in which regulation of expression is mediated by the binding of the repressor (CymR) to the operator site (CuO), placed downstream of the promoter (e.g., Mullick et al. *BMC Biotechnol.* 2006 6:43). As used herein, the term "repressor," refers to entities (e.g., proteins or other molecules) having the capacity to inhibit, interfere, retard and/or repress the production of heterologous protein product of a recombinant expression vector. For example, by interfering with a binding site at an appropriate location along the expression vector, such as in an expression cassette. Examples of repressors include tetR, CymR, the lac repressor, the trp repressor, the gal repressor, the lambda repressor, and other appropriate repressors known in the art. Examples of the use of the tetO/tetR operator/repressor system and of the CuO/CymR operator/repressor system are provided herein. Repression of vector transgene expression during vector propagation can prevent transgene instability, and may increase yields of vectors having a transgene of the invention during production. Hence, in some embodiments, the vectors of the invention have a promoter that can be repressed by binding of a repressor protein, e.g. by having a promoter that is operably coupled to a repressor operator sequence (e.g. in non-limiting embodiments, a TetO-containing sequence, e.g. the one set forth in SEQ ID NO: 11, or a CuO-containing sequence, e.g. the one set forth in SEQ ID NO: 12), to which a repressor protein (e.g. the TetR protein, e.g. having an amino acid sequence as set forth in SEQ ID NO: 15, or the CymR protein, e.g. having an amino acid sequence as set forth in SEQ ID NO: 17) can bind.

In certain embodiments, the vector is a plasmid DNA molecule, or a fragment thereof. These can be used for DNA vaccination. Other platforms are also possible for use as vectors, for instance live-attenuated double-deleted *Listeria monocytogenes* strains.

In other embodiments, the vector is a recombinant viral vector, which may be replication competent or replication deficient. In certain embodiments, a viral vector comprises a recombinant DNA genome. In certain embodiments, a vector according to the invention is for instance a recombinant adenovirus, a recombinant retrovirus, a recombinant pox virus such as a vaccinia virus (e.g. Modified Vaccinia Ankara (MVA)), a recombinant alphavirus such as semliki forest virus, a recombinant paramyxovirus, such as a recombinant measles virus, or another recombinant virus. In certain embodiments, a vector according to the invention is an MVA vector.

In preferred embodiments, a vector according to the invention is a recombinant adenovirus. Advantages of adenoviruses for use as vaccines include ease of manipulation, good manufacturability at large scale, and an excellent safety record based on many years of experience in research, development, manufacturing and clinical trials with numerous adenoviral vectors that have been reported. Adenoviral vectors that are used as vaccines generally provide a good immune response to the transgene-encoded protein, including a cellular immune response. An adenoviral vector according to the invention can be based on any type of adenovirus, and in certain embodiments is a human adenovirus, which can be of any serotype. In other embodiments, it is a simian adenovirus, such as chimpanzee or gorilla adenovirus, which can be of any serotype. In certain embodiments, a vector according to the invention is of a human adenovirus serotype 5, 26 or 35. The preparation of recombinant adenoviral vectors is well known in the art. In certain embodiments, an adenoviral vector according to the invention is deficient in at least one essential gene function of the E1 region, e.g. the E1a region and/or the E1b region, of the adenoviral genome that is required for viral replication. In certain embodiments, an adenoviral vector according to the invention is deficient in at least part of the non-essential E3 region. In certain embodiments, the vector is deficient in at least one essential gene function of the E1 region and at least part of the non-essential E3 region.

Adenoviral vectors, methods for construction thereof and methods for propagating thereof, are well known in the art and are described in, for example, U.S. Pat. Nos. 5,559,099, 5,837,511, 5,846,782, 5,851,806, 5,994,106, 5,994,128, 5,965,541, 5,981,225, 6,040,174, 6,020,191, and 6,113,913, and Thomas Shenk, "Adenoviridae and their Replication", M. S. Horwitz, "Adenoviruses", Chapters 67 and 68, respectively, in *Virology*, B. N. Fields *et al.*, *eds.*, 3d ed., Raven Press, Ltd., New York (1996), and other references mentioned herein. Typically, construction of

adenoviral vectors involves the use of standard molecular biological techniques, such as those described in, for example, Sambrook *et al.*, *Molecular Cloning, a Laboratory Manual*, 2d ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), Watson *et al.*, *Recombinant DNA*, 2d ed., Scientific American Books (1992), and Ausubel *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience Publishers, NY (1995), and other references mentioned herein.

Particularly preferred serotypes for the recombinant adenovirus are human serotype 35 or human serotype 26. Preparation of rAd26 vectors is described, for example, in WO 2007/104792 and in Abbink *et al.,* 2007 *Virology* 81: 4654-63. Exemplary genome sequences of Ad26 are found in GenBank Accession EF 153474 and in SEQ ID NO:1 of WO 2007/104792. Preparation of rAd35 vectors is described, for example, in US Patent No. 7,270,811, in WO 00/70071, and in Vogels *et al.,* 2003, *J Virol* 77: 8263-71. Exemplary genome sequences of Ad35 are found in GenBank Accession AC_000019 and in Fig. 6 of WO 00/70071.

In certain embodiments, the adenovirus is replication deficient, e.g. because it contains a deletion in the E1 region of the genome. As known to the skilled person, in case of deletions of essential regions from the adenovirus genome, the functions encoded by these regions have to be provided in trans, preferably by the producer cell, i.e. when parts or whole of E1, E2 and/or E4 regions are deleted from the adenovirus, these have to be present in the producer cell, for instance integrated in the genome thereof, or in the form of so-called helper adenovirus or helper plasmids. The adenovirus may also have a deletion in the E3 region, which is dispensable for replication, and hence such a deletion does not have to be complemented.

A producer cell (sometimes also referred to in the art and herein as 'packaging cell' or 'complementing cell') that can be used can be any producer cell wherein a desired adenovirus can be propagated. For example, the propagation of recombinant adenovirus vectors is done in producer cells that complement deficiencies in the adenovirus. Such producer cells preferably have in their genome at least an adenovirus E1 sequence, and thereby are capable of complementing recombinant adenoviruses with a deletion in the E1 region. Any E1-complementing producer cell can be used, such as human retina cells immortalized by E1, e.g. 911 or PER.C6 cells (see US patent 5,994,128), E1-transformed amniocytes (See EP patent 1230354), E1-transformed A549 cells (see e.g. WO 98/39411, US patent 5,891,690), GH329:HeLa (Gao *et al.,* 2000, *Hum Gene Ther* 11: 213-19), 293, and the like. In certain

embodiments, the producer cells are for instance HEK293 cells, or PER.C6 cells, or 911 cells, or IT293SF cells, and the like. Production of adenoviral vectors in producer cells is reviewed in (Kovesdi *et al.,* 2010, *Viruses* 2: 1681-703).

In certain embodiments, an E1-deficient adenovirus comprises the E4-orf6 coding sequence of an adenovirus of subgroup C such as Ad5. This allows propagation of such adenoviruses in well known complementing cell lines that express the E1 genes of Ad5, such as for example 293 cells or PER.C6 cells (see, e.g. Havenga *et al.,* 2006, *J Gen Virol* 87: 2135-43; WO 03/104467, incorporated in its entirety by reference herein).

"Heterologous nucleic acid" (also referred to herein as 'transgene') in vectors of the invention is nucleic acid that is not naturally present in the vector, and according to the present invention the nucleic acid encoding the fusion polypeptide of the invention is considered heterologous nucleic acid when present in a vector. It is introduced into the vector for instance by standard molecular biology techniques. It can for instance be cloned into a deleted E1 or E3 region of an adenoviral vector, or in the region between the E4 region and the rITR. A transgene is generally operably linked to expression control sequences. In preferred embodiments, the transgene is cloned into the E1-region of an adenoviral vector.

Production of vectors such as DNA vectors, MVA vectors, or recombinant adenovirus vectors, can be performed according to various methods well known to the person skilled in the art. Generally, the production entails propagation in cultured cells to generate a substantial amount of vector material, followed by harvest of the vector from the cell culture, and typically followed by further purification of the vector to remove other substances and obtain purified vectors that can be formulated into pharmaceutical compositions (e.g., Hoganson *et al.,* 2002, *BioProcessing J* 1: 43-8; Evans *et al.,* 2004, *J Pharm Sci* 93:2458-75). For example, methods for harvesting adenovirus from cultures of producer cells have for instance been extensively described in WO 2005/080556. For example WO 2010/060719, and WO 2011/098592, both incorporated by reference herein, describe suitable methods for obtaining and purifying large amounts of recombinant adenoviruses.

In certain aspects, the invention also provides a polypeptide that is encoded by a nucleic acid molecule according to the invention. Such a polypeptide comprises SEQ ID NO: 1 (for HPV16), or SEQ ID NO: 20 (for HPV18). In certain

embodiments, such a polypeptide may comprise SEQ ID NO: 3 or SEQ ID NO: 5 (each for HPV16), or SEQ ID NO: 22 (for HPV18). The characteristics of such a polypeptide are as described above. Such a polypeptide can for instance be used directly as a vaccine against HPV.

The invention further provides vaccines comprising nucleic acid molecules, vectors or polypeptides according to the invention, wherein embodiments for each of these aspects can include those as described above. In preferred embodiments, a vaccine according to the invention comprises a nucleic acid molecule according to the invention. In further preferred embodiments, the vaccine comprises a vector according to the invention, preferably a DNA vector, an MVA vector, or a recombinant adenovirus vector.

In certain embodiments, a vaccine according to the invention that encodes the HPV16 designer polypeptide comprises further active ingredients, e.g. nucleic acid encoding at least one epitope of E6 and/or E7 protein of at least one HPV type different from HPV16, e.g. a high risk HPV type such as HPV18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, -73, or -82. In certain embodiments, a vaccine according to the invention that encodes the HPV18 designer polypeptide comprises further active ingredients, e.g. nucleic acid encoding at least one epitope of E6 and/or E7 protein of at least one HPV type different from HPV18, e.g. a high risk HPV type such as HPV16, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, -73, or -82.

Particularly preferred are vaccines comprising nucleic acids encoding both HPV16 and HPV18 designer polypeptides of the invention, i.e. encoding a polypeptide with SEQ ID NO: 1 as well as a polypeptide with SEQ ID NO: 20. In such vaccines, the HPV16 and HPV18 components may be in the same composition as separate molecules, or they may be in the same molecule, e.g. encoded on the same vector, or they could be provided as a kit of parts with a separate HPV16 component and a separate HPV18 component for combined use in vaccination, e.g. for reconstitution prior to administration, or for separate but essentially simultaneous administration. One advantage of such combinations is that such vaccines can work therapeutically in subjects that are infected with either HPV16 or with HPV18 (the two most prevailing high risk HPV types that together account for the majority of HPV-induced cancers), so that such vaccines have increased applicability over the monotype vaccines that have either HPV16 or HPV18 designer molecules.

The term "vaccine" refers to an agent or composition containing an active component effective to induce a prophylactic and/or therapeutic degree of immunity in a subject against a certain pathogen or disease, in this case therapeutically against HPV. The vaccine typically comprises the nucleic acid molecule, or vector, according to the invention, and a pharmaceutically acceptable excipient. Upon administration to a subject, the polypeptide encoded by the nucleic acid molecule according to the invention will be expressed in the subject, which will lead to an immune response towards E6 and/or E7 antigenic fragments that are present in the polypeptide. The advantage of the instant molecules is that essentially all T-cell epitopes of HPV16 (for SEQ ID NOs: 1-6) or HPV18 (for SEQ ID NOs: 20-23) E6 and E7 are present and thus a T-cell response to any epitope present in wild-type E6 or E7 can be mounted in the vaccinee. Further, the vaccine has all the safety and efficacy advantages as outlined above for the nucleic acid molecules according to the invention.

For administering to humans, the invention may employ pharmaceutical compositions comprising the vector and a pharmaceutically acceptable carrier or excipient. In the present context, the term "Pharmaceutically acceptable" means that the carrier or excipient, at the dosages and concentrations employed, will not cause any unwanted or harmful effects in the subjects to which they are administered. Such pharmaceutically acceptable excipients are well known in the art (see Remington's Pharmaceutical Sciences, 18th edition, A. R. Gennaro, Ed., Mack Publishing Company [1990]; Pharmaceutical Formulation Development of Peptides and Proteins, S. Frokjaer and L. Hovgaard, Eds., Taylor & Francis [2000]; and Handbook of Pharmaceutical Excipients, 3rd edition, A. Kibbe, Ed., Pharmaceutical Press [2000]). An excipient is generally a pharmacologically inactive substance formulated with the active ingredient of a medication. Excipients are commonly used to bulk up formulations that contain potent active ingredients (thus often referred to as "bulking agents," "fillers," or "diluents"), to allow convenient and accurate dispensation of a drug substance when producing a dosage form. They also can serve various therapeutic-enhancing purposes, such as facilitating drug absorption or solubility, or other pharmacokinetic considerations. Excipients can also be useful in the manufacturing process, to aid in the handling of the active substance concerned such as by facilitating powder flowability or non-stick properties, in addition to aiding in vitro stability such as prevention of denaturation over the expected shelf life. The

selection of appropriate excipients also depends upon the route of administration and the dosage form, as well as the active ingredient and other factors.

The purified nucleic acid molecule, vector or polypeptide preferably is formulated and administered as a sterile solution although it is also possible to utilize lyophilized preparations. Sterile solutions are prepared by sterile filtration or by other methods known per se in the art. The solutions are then lyophilized or filled into pharmaceutical dosage containers. The pH of the solution generally is in the range of pH 3.0 to 9.5, e.g pH 5.0 to 7.5. The nucleic acid molecule or vector or polypeptide typically is in a solution having a suitable buffer, and the solution of vector may also contain a salt. Optionally stabilizing agent may be present, such as albumin. In certain embodiments, detergent is added. In certain embodiments, vaccine may be formulated into an injectable preparation. These formulations contain effective amounts of nucleic acid molecule, vector or polypeptide are either sterile liquid solutions, liquid suspensions or lyophilized versions and optionally contain stabilizers or excipients.

For instance recombinant adenovirus vector may be stored in the buffer that is also used for the Adenovirus World Standard (Hoganson *et al.,* 2002, *Bioprocessing J* 1: 43-8): 20 mM Tris pH 8, 25 mM NaCl, 2.5% glycerol. Another useful formulation buffer suitable for administration to humans is 20 mM Tris, 2 mM $MgCl_2$, 25 mM NaCl, sucrose 10% w/v, polysorbate-80 0.02% w/v. Another formulation buffer that is suitable for recombinant adenovirus comprises 10-25 mM citrate buffer pH 5.9-6.2, 4-6% (w/w) hydroxypropyl-beta-cyclodextrin (HBCD), 70-100 mM NaCl, 0.018-0.035% (w/w) polysorbate-80, and optionally 0.3-0.45% (w/w) ethanol. Obviously, many other buffers can be used, and several examples of suitable formulations for the storage and for pharmaceutical administration of purified vectors are known.

In certain embodiments a composition comprising the vector further comprises one or more adjuvants. Adjuvants are known in the art to further increase the immune response to an applied antigenic determinant. The terms “adjuvant” and "immune stimulant" are used interchangeably herein, and are defined as one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to the polypeptides encoded by the nucleic acid molecules in the vectors of the invention. Examples of suitable adjuvants include aluminium salts such as aluminium hydroxide and/or aluminium phosphate and/or aluminium potassium phosphate; oil-emulsion compositions (or oil-in-water

compositions), including squalene-water emulsions, such as MF59 (see e.g. WO 90/14837); saponin formulations, such as for example QS21 and Immunostimulating Complexes (ISCOMS) (see e.g. US 5,057,540; WO 90/03184, WO 96/11711, WO 2004/004762, WO 2005/002620); bacterial or microbial derivatives, examples of which are monophosphoryl lipid A (MPL), 3-O-deacylated MPL (3dMPL), CpG-motif containing oligonucleotides, ADP-ribosylating bacterial toxins or mutants thereof, such as *E. coli* heat labile enterotoxin LT, cholera toxin CT, and the like. It is also possible to use vector-encoded adjuvant, e.g. by using heterologous nucleic acid that encodes a fusion of the oligomerization domain of C4-binding protein (C4bp) to the antigen of interest (e.g. Solabomi *et al.,* 2008, *Infect Immun* 76: 3817-23), or by using a vector encoding both the transgene of interest and a TLR-3 agonist such as heterologous dsRNA (e.g. WO 2007/100908), or the like.

In other embodiments, the compositions of the invention do not comprise adjuvants.

Pharmaceutical compositions may be administered to a subject, e.g. a human subject. The total dose of the vaccine active component provided to a subject during one administration can be varied as is known to the skilled practitioner, and for adenovirus is generally between $1x10^7$ viral particles (vp) and $1x10^{12}$ vp, preferably between $1x10^8$ vp and $1x10^{11}$ vp, for instance between $3x10^8$ and $5x10^{10}$ vp, for instance between 10^9 and $3x10^{10}$ vp. For a DNA vaccine, total amounts of DNA per administration may for instance be between 1 μg and 10 mg. If a gene gun is used for administration , typically low amounts are used, e.g. 10 μg. For intramuscular injection, typically higher amounts are used, e.g. up to 5 mg.

Administration of pharmaceutical compositions can be performed using standard routes of administration. Non-limiting embodiments include parenteral administration, such as by injection, e.g. intradermal, intramuscular, etc, or subcutaneous or transcutaneous, or mucosal administration, e.g. intranasal, oral, intravaginal, rectal, and the like. In one embodiment a composition is administered by intramuscular injection, e.g. into the deltoid muscle of the arm, or vastus lateralis muscle of the thigh. In certain embodiments the vaccine is a DNA vaccine, and this can for instance be administered intradermally, e.g. by DNA tattooing (see, e.g. Oosterhuis *et al.,* 2012, *Curr Top Microbiol Immunol* 351: 221-50). This route is also feasible for adenoviral vectors. In certain embodiments a composition according to the invention comprises an adenoviral vector and is administered by intramuscular injection. The

skilled person knows the various possibilities to administer a composition, such as a vaccine in order to induce an immune response to the antigen(s) in the vaccine.

A subject as used herein preferably is a mammal, for instance a rodent, e.g. a mouse, or a non-human-primate, or a human. Preferably, the subject is a human subject.

The vaccines of the invention can be used to treat patients having one of various stages of diseases caused by HPV (in particular type 16 for vaccines comprising or encoding any of SEQ ID NOs: 1-6, or type 18 for vaccines comprising or encoding any of SEQ ID NOs: 20-23, or both types for vaccines that comprise or encode both HPV16 and HPV18 designer molecules described herein), from incident and persistent HPV infection as such (e.g. as detected by HPV DNA testing), thus before (pre-)cancerous lesions are formed, as well as cervical intraepithelial neoplasia (CIN; also known as cervical dysplasia and cervical interstitial neoplasia, which is the potentially premalignant transformation and abnormal growth (dysplasia) of squamous cells on the surface of the cervix) up to and including cervical cancer (such as cervical squamous cell carcinoma (SCC). In addition, other HPV-induced neoplasias, such as vulvar intraepithelial neoplasia (VIN), vaginal intraepithelial neoplasia (VaIN), penile intraepithelial neoplasia (PIN), anal intraepithelial neoplasia (AIN) can be targeted as well as more advanced stages of oropharyngeal cancer (also known as head- and neck cancer), penile cancer, vaginal cancer, vulvar cancer and anal cancer. The vaccines of the invention thus can target a wide range of HPV induced lesions, and are likely most effective at the precancerous stages of HPV-induced disease, e.g. at the (persistent) infection and/or the neoplasia stages, where expression of E2, E6 and/or E7 is highest. It is also possible to combine the treatment using a vaccine of the invention with compounds that counteract or can overcome immune escape mechanisms in advanced cancer cells e.g. anti-PD1/PD-L1 antibodies, anti CTLA-4 antibodies such as Ipilimumab, anti-LAG-3 antibodies, anti-CD25 antibodies, IDO-inhibitors, CD40 agonistic antibodies, CD137 agonistic antibodies, etc (see, e.g. Hamid and Carvajal, 2013, *Expert Opinion Biol Ther* 13: 847-861; Mellman *et al.*, 2011, *Nature Rev* 480: 480-89). The therapeutic vaccination method could in principle also be used for treating external genital warts or precursors thereof in case the vaccine comprises further (sequences encoding) E6 and/or E7 of an HPV type causing external genital warts and is administered to a subject infected by such an HPV type.

As used herein, 'treating' means administration of the vaccine to induce a therapeutic immune response against cells that express (epitopes of) HPV16 or 18 E6 and/or E7 in the patient, which leads to at least reduction of the level of and preferably complete removal of HPV16 or 18 infection, which results in at least slowing and preferably stopping the progress of HPV16- or HPV18-caused disease such as neoplasias and/or symptoms thereof. Preferably treatment with the vaccine results also in remission of more advanced stages of HPV-induced cancers. It is preferred to administer the vaccine to patients that have an established HPV infection that has been typed, so that the vaccine that encodes the polypeptide of the corresponding HPV type can be administered. In the absence of screening the vaccine can also be administered in the part of the population that is likely to be HPV infected, i.e. sexually active people. It is also possible to administer a vaccine of the invention to subjects that have not been infected by HPV16 or 18, e.g. for prophylactic use, possibly in combination with a vaccine against another HPV type by which the patient has been infected, or alternatively in non-infected subjects. A vaccine of the invention can also be administered to a subject that is subject to further treatment by other means, e.g. surgery (removal of a lesion caused by HPV16 or 18 infection), or treatment with imiquimod (comprising a TLR-7/8 agonist, see e.g. Dayaana *et al.,* 2010, *Br J Cancer* 102: 1129 – 36). The effect of the treatment can be measured either by cytology or by HPV testing.

The vaccination comprises administering the vaccine of the invention to a subject or patient at least once. It is also possible to provide one or more booster administrations of one or more further vaccines. If a boosting vaccination is performed, typically, such a boosting vaccination will be administered to the same subject at a moment between one week and one year, preferably between two weeks and four months, after administering an immunogenic composition with the same antigen to the subject for the first time (which is in such cases referred to as 'priming vaccination'). In alternative boosting regimens, it is also possible to administer different vectors, e.g. one or more adenoviruses of different serotype, or other vectors such as MVA, or DNA, or protein, to the subject as a priming or boosting vaccination. In certain embodiments, the same form of a vaccine of the invention is administered at least twice to the same patient in a prime-boost regimen, e.g. with the same recombinant adenovirus (such as Ad26) according to the invention. In certain embodiments, a vaccine of the invention is administered at least twice in a prime-boost regimen, but

the vector of the vaccine is different, e.g. two different serotypes of adenoviral vectors are used, e.g. priming with recombinant Ad26 and boosting with recombinant Ad35, or vice versa; or priming with DNA and boosting with an adenoviral vector, or vice versa; or priming with an adenoviral vector and boosting with an MVA vector, or vice versa. Exemplary embodiments include priming with Ad26 vector and boosting with Ad35 vector, priming with Ad26 vector and boosting with MVA vector, priming with Ad35 vector and boosting with MVA vector, priming with Ad35 vector and boosting with Ad26 vector, etc, wherein in each case the priming and boosting vector comprise nucleic acid encoding a designer polypeptide of the invention, preferably the priming and boosting vector each encoding the same designer polypeptide of the invention. In certain embodiments, a vaccine according to the invention is administered at least three times, in a prime-boost-boost regimen. Further booster administrations might be added to the regimen. It is also possible to simultaneously or substantially simultaneously (e.g. not more than 10 minutes apart) administer an adenoviral vector and an MVA vector (which can either be in the same composition or in different compositions), to induce an immune response (see e.g. WO 2010/073043).

It is also an aspect of the invention to induce a CTL response against HPV16 or HPV18 in a subject, comprising administering a vaccine according to the invention to the subject. The skilled person will understand that the vaccines that include HPV16 sequences (encoding or comprising any of SEQ ID NOs: 1-6) work best against and are intended for use against HPV16 infection, while the vaccines that include HPV18 sequences (encoding or comprising any of SEQ ID NOs: 20-23) work best against and are intended for use against HPV18 infection.

The invention provides also the following non-limiting embodiments:

1) a nucleic acid encoding a polypeptide comprising SEQ ID NO: 1;

2) a nucleic acid according to embodiment 1, wherein the polypeptide further comprises at least part of HPV E2 protein;

3) a nucleic acid according to embodiment 2, wherein the at least part of the HPV E2 protein is from the E2 protein of HPV16;

4) a nucleic acid according to embodiment 2, wherein the polypeptide comprises at least part of the E2 protein fused to the N-terminal side of the polypeptide with SEQ ID NO: 1;

5) a nucleic acid according to embodiment 2, wherein the polypeptide comprises at

least part of the E2 protein fused to the C-terminal side of the polypeptide with SEQ ID NO: 1;

6) a nucleic acid according to embodiment 3, wherein the polypeptide comprises at least part of the E2 protein fused to the N-terminal side of the polypeptide with SEQ ID NO: 1;

7) a nucleic acid according to embodiment 3, wherein the polypeptide comprises at least part of the E2 protein fused to the C-terminal side of the polypeptide with SEQ ID NO: 1;

8) a nucleic acid according to embodiment 2, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

9) a nucleic acid according to embodiment 3, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

10) a nucleic acid according to embodiment 4, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

11) a nucleic acid according to embodiment 5, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

12) a nucleic acid according to embodiment 6, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

13) a nucleic acid according to embodiment 7, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

14) a vector comprising a nucleic acid according to embodiment 1, wherein a sequence encoding the polypeptide is operably linked to a promoter;

15) a vector comprising a nucleic acid according to embodiment 2, wherein a sequence encoding the polypeptide is operably linked to a promoter;

16) a vector comprising a nucleic acid according to embodiment 3, wherein a sequence encoding the polypeptide is operably linked to a promoter;

17) a vector comprising a nucleic acid according to embodiment 4, wherein a sequence encoding the polypeptide is operably linked to a promoter;

18) a vector comprising a nucleic acid according to embodiment 5, wherein a sequence encoding the polypeptide is operably linked to a promoter;

19) a vector comprising a nucleic acid according to embodiment 6, wherein a sequence encoding the polypeptide is operably linked to a promoter;

20) a vector comprising a nucleic acid according to embodiment 7, wherein a sequence encoding the polypeptide is operably linked to a promoter;

21) a vector comprising a nucleic acid according to embodiment 8, wherein a sequence encoding the polypeptide is operably linked to a promoter;

22) a vector comprising a nucleic acid according to embodiment 9, wherein a sequence encoding the polypeptide is operably linked to a promoter;

23) a vector comprising a nucleic acid according to embodiment 10, wherein a sequence encoding the polypeptide is operably linked to a promoter;

24) a vector comprising a nucleic acid according to embodiment 11, wherein a sequence encoding the polypeptide is operably linked to a promoter;

25) a vector comprising a nucleic acid according to embodiment 12, wherein a sequence encoding the polypeptide is operably linked to a promoter;

26) a vector comprising a nucleic acid according to embodiment 13, wherein a sequence encoding the polypeptide is operably linked to a promoter;

27) a vector according to embodiment 14, wherein the vector is an adenovirus;

28) a vector according to embodiment 15, wherein the vector is an adenovirus;

29) a vector according to embodiment 16, wherein the vector is an adenovirus;

30) a vector according to embodiment 17, wherein the vector is an adenovirus;

31) a vector according to embodiment 18, wherein the vector is an adenovirus;

32) a vector according to embodiment 19, wherein the vector is an adenovirus;

33) a vector according to embodiment 20, wherein the vector is an adenovirus;

34) a vector according to embodiment 21, wherein the vector is an adenovirus;

35) a vector according to embodiment 22, wherein the vector is an adenovirus;

36) a vector according to embodiment 23, wherein the vector is an adenovirus;

37) a vector according to embodiment 24, wherein the vector is an adenovirus;

38) a vector according to embodiment 25, wherein the vector is an adenovirus;

39) a vector according to embodiment 26, wherein the vector is an adenovirus;

40) a vector according to embodiment 27, wherein the adenovirus is a human adenovirus of serotype 26;

41) a vector according to embodiment 28, wherein the adenovirus is a human

adenovirus of serotype 26;

42) a vector according to embodiment 29, wherein the adenovirus is a human adenovirus of serotype 26;

43) a vector according to embodiment 30, wherein the adenovirus is a human adenovirus of serotype 26;

44) a vector according to embodiment 31, wherein the adenovirus is a human adenovirus of serotype 26;

45) a vector according to embodiment 32, wherein the adenovirus is a human adenovirus of serotype 26;

46) a vector according to embodiment 33, wherein the adenovirus is a human adenovirus of serotype 26;

47) a vector according to embodiment 34, wherein the adenovirus is a human adenovirus of serotype 26;

48) a vector according to embodiment 35, wherein the adenovirus is a human adenovirus of serotype 26;

49) a vector according to embodiment 36, wherein the adenovirus is a human adenovirus of serotype 26;

50) a vector according to embodiment 37, wherein the adenovirus is a human adenovirus of serotype 26;

51) a vector according to embodiment 38, wherein the adenovirus is a human adenovirus of serotype 26;

52) a vector according to embodiment 39, wherein the adenovirus is a human adenovirus of serotype 26;

53) a vector according to embodiment 28, wherein the adenovirus is a human adenovirus of serotype 35;

54) a vector according to embodiment 29, wherein the adenovirus is a human adenovirus of serotype 35;

55) a vector according to embodiment 30, wherein the adenovirus is a human adenovirus of serotype 35;

56) a vector according to embodiment 31, wherein the adenovirus is a human adenovirus of serotype 35;

57) a vector according to embodiment 32, wherein the adenovirus is a human adenovirus of serotype 35;

58) a vector according to embodiment 33, wherein the adenovirus is a human

adenovirus of serotype 35;

59) a vector according to embodiment 34, wherein the adenovirus is a human adenovirus of serotype 35;

60) a vector according to embodiment 35, wherein the adenovirus is a human adenovirus of serotype 35;

61) a vector according to embodiment 36, wherein the adenovirus is a human adenovirus of serotype 35;

62) a vector according to embodiment 37, wherein the adenovirus is a human adenovirus of serotype 35;

63) a vector according to embodiment 38, wherein the adenovirus is a human adenovirus of serotype 35;

64) a vector according to embodiment 39, wherein the adenovirus is a human adenovirus of serotype 35;

65) a vaccine composition comprising a vector according to embodiment 14, and a pharmaceutically acceptable excipient;

66) a vaccine composition comprising a vector according to embodiment 15, and a pharmaceutically acceptable excipient;

67) a vaccine composition comprising a vector according to embodiment 16, and a pharmaceutically acceptable excipient;

68) a vaccine composition comprising a vector according to embodiment 17, and a pharmaceutically acceptable excipient;

69) a vaccine composition comprising a vector according to embodiment 18, and a pharmaceutically acceptable excipient;

70) a vaccine composition comprising a vector according to embodiment 19, and a pharmaceutically acceptable excipient;

71) a vaccine composition comprising a vector according to embodiment 20, and a pharmaceutically acceptable excipient;

72) a vaccine composition comprising a vector according to embodiment 21, and a pharmaceutically acceptable excipient;

73) a vaccine composition comprising a vector according to embodiment 22, and a pharmaceutically acceptable excipient;

74) a vaccine composition comprising a vector according to embodiment 23, and a pharmaceutically acceptable excipient;

75) a vaccine composition comprising a vector according to embodiment 24, and a pharmaceutically acceptable excipient;

76) a vaccine composition comprising a vector according to embodiment 25, and a pharmaceutically acceptable excipient;

77) a vaccine composition comprising a vector according to embodiment 26, and a pharmaceutically acceptable excipient;

78) a vaccine composition comprising a vector according to embodiment 27, and a pharmaceutically acceptable excipient;

79) a vaccine composition comprising a vector according to embodiment 28, and a pharmaceutically acceptable excipient;

80) a vaccine composition comprising a vector according to embodiment 29, and a pharmaceutically acceptable excipient;

81) a vaccine composition comprising a vector according to embodiment 30, and a pharmaceutically acceptable excipient;

82) a vaccine composition comprising a vector according to embodiment 31, and a pharmaceutically acceptable excipient;

83) a vaccine composition comprising a vector according to embodiment 32, and a pharmaceutically acceptable excipient;

84) a vaccine composition comprising a vector according to embodiment 33, and a pharmaceutically acceptable excipient;

85) a vaccine composition comprising a vector according to embodiment 34, and a pharmaceutically acceptable excipient;

86) a vaccine composition comprising a vector according to embodiment 35, and a pharmaceutically acceptable excipient;

87) a vaccine composition comprising a vector according to embodiment 36, and a pharmaceutically acceptable excipient;

88) a vaccine composition comprising a vector according to embodiment 37, and a pharmaceutically acceptable excipient;

89) a vaccine composition comprising a vector according to embodiment 38, and a pharmaceutically acceptable excipient;

90) a vaccine composition comprising a vector according to embodiment 39, and a pharmaceutically acceptable excipient;

91) a vaccine composition comprising a vector according to embodiment 40, and a pharmaceutically acceptable excipient;

92) a vaccine composition comprising a vector according to embodiment 41, and a pharmaceutically acceptable excipient;

93) a vaccine composition comprising a vector according to embodiment 42, and a pharmaceutically acceptable excipient;

94) a vaccine composition comprising a vector according to embodiment 43, and a pharmaceutically acceptable excipient;

95) a vaccine composition comprising a vector according to embodiment 44, and a pharmaceutically acceptable excipient;

96) a vaccine composition comprising a vector according to embodiment 45, and a pharmaceutically acceptable excipient;

97) a vaccine composition comprising a vector according to embodiment 46, and a pharmaceutically acceptable excipient;

98) a vaccine composition comprising a vector according to embodiment 47, and a pharmaceutically acceptable excipient;

99) a vaccine composition comprising a vector according to embodiment 48, and a pharmaceutically acceptable excipient;

100) a vaccine composition comprising a vector according to embodiment 49, and a pharmaceutically acceptable excipient;

101) a vaccine composition comprising a vector according to embodiment 50, and a pharmaceutically acceptable excipient;

102) a vaccine composition comprising a vector according to embodiment 51, and a pharmaceutically acceptable excipient;

103) a vaccine composition comprising a vector according to embodiment 52, and a pharmaceutically acceptable excipient;

104) a vaccine composition comprising a vector according to embodiment 53, and a pharmaceutically acceptable excipient;

105) a vaccine composition comprising a vector according to embodiment 54, and a pharmaceutically acceptable excipient;

106) a vaccine composition comprising a vector according to embodiment 55, and a pharmaceutically acceptable excipient;

107) a vaccine composition comprising a vector according to embodiment 56, and a pharmaceutically acceptable excipient;

108) a vaccine composition comprising a vector according to embodiment 57, and a pharmaceutically acceptable excipient;

109) a vaccine composition comprising a vector according to embodiment 58, and a pharmaceutically acceptable excipient;

110) a vaccine composition comprising a vector according to embodiment 59, and a pharmaceutically acceptable excipient;

111) a vaccine composition comprising a vector according to embodiment 60, and a pharmaceutically acceptable excipient;

112) a vaccine composition comprising a vector according to embodiment 61, and a pharmaceutically acceptable excipient;

113) a vaccine composition comprising a vector according to embodiment 62, and a pharmaceutically acceptable excipient;

114) a vaccine composition comprising a vector according to embodiment 63, and a pharmaceutically acceptable excipient;

115) a vaccine composition comprising a vector according to embodiment 64, and a pharmaceutically acceptable excipient;

116) a method for inducing an immune response against HPV in a subject, comprising administering to the subject a vaccine composition according to any one of embodiments 65-115;

117) a method for treating persistent HPV (type 16) infection, comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from persistent HPV infection;

118) a method for treating vulvar intraepithelial neoplasia (VIN) (with underlying HPV type 16 infection), comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from VIN;

119) a method for treating vulvar cancer (with underlying HPV type 16 infection), comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from vulvar cancer;

120) a method for treating cervical intraepithelial neoplasia (CIN) (with underlying HPV type 16 infection), comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from CIN;

121) a method for treating cervical cancer (with underlying HPV type 16 infection), comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from cervical cancer;

122) a method for treating opopharyngeal cancer (with underlying HPV type 16 infection), comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from oropharyngeal cancer;

123) a method for treating penile intraepithelial neoplasia (PIN) (with underlying HPV type 16 infection), comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from PIN;

124) a method for treating penile cancer (with underlying HPV type 16 infection), comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from penile cancer;

125) a method for treating vaginal intraepithelial neoplasia (VaIN) (with underlying HPV type 16 infection), comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from VaIN;

126) a method for treating vaginal cancer (with underlying HPV type 16 infection), comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from vaginal cancer;

127) a method for treating anal intraepithelial neoplasia (AIN) (with underlying HPV type 16 infection), comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from AIN;

128) a method for treating anal cancer (with underlying HPV type 16 infection), comprising administering a vaccine according to any one of embodiments 65-115 to a subject that suffers from anal cancer;

129) a polypeptide comprising SEQ ID NO: 1;

130) a polypeptide according to embodiment 129, wherein the polypeptide further comprises at least part of HPV E2 protein;

131) a polypeptide according to embodiment 130, wherein the at least part of the HPV E2 protein is from the E2 protein of HPV16;

132) a polypeptide according to embodiment 130, wherein at least part of the E2 protein is fused to the N-terminal side of the polypeptide with SEQ ID NO: 1;

133) a polypeptide according to embodiment 130, wherein at least part of the E2 protein is fused to the C-terminal side of the polypeptide with SEQ ID NO: 1;

134) a polypeptide according to embodiment 131, wherein at least part of the E2 protein is fused to the N-terminal side of the polypeptide with SEQ ID NO: 1;

135) a polypeptide according to embodiment 131, wherein at least part of the E2 protein is fused to the C-terminal side of the polypeptide with SEQ ID NO: 1;

136) a polypeptide according to embodiment 130, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

137) a polypeptide according to embodiment 131, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

138) a polypeptide according to embodiment 132, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

139) a polypeptide according to embodiment 133, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

140) a polypeptide according to embodiment 134, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

141) a polypeptide according to embodiment 135, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

142) a nucleic acid according to embodiment 3, encoding a polypeptide according to SEQ ID NO: 3;

143) a nucleic acid according to embodiment 3, encoding a polypeptide according to SEQ ID NO: 5;

144) a vector encoding a nucleic acid according to embodiment 142, wherein a sequence encoding the polypeptide is operably linked to a promoter;

145) a vector encoding a nucleic acid according to embodiment 143, wherein a sequence encoding the polypeptide is operably linked to a promoter;

146) a vector according to embodiment 144, wherein the vector is an adenovirus;

147) a vector according to embodiment 145, wherein the vector is an adenovirus;

148) a vector according to embodiment 146, wherein the adenovirus is a human adenovirus of serotype 26;

149) a vector according to embodiment 147, wherein the adenovirus is a human adenovirus of serotype 26;

150) a vector according to embodiment 146, wherein the adenovirus is a human adenovirus of serotype 35;

151) a vector according to embodiment 147, wherein the adenovirus is a human adenovirus of serotype 35;

152) a vaccine composition comprising a vector according to embodiment 144, and a pharmaceutically acceptable excipient;

153) a vaccine composition comprising a vector according to embodiment 145, and a pharmaceutically acceptable excipient;

154) a vaccine composition comprising a vector according to embodiment 146, and a pharmaceutically acceptable excipient;

155) a vaccine composition comprising a vector according to embodiment 147, and a pharmaceutically acceptable excipient;

156) a vaccine composition comprising a vector according to embodiment 148, and a pharmaceutically acceptable excipient;

157) a vaccine composition comprising a vector according to embodiment 149, and a pharmaceutically acceptable excipient;

158) a vaccine composition comprising a vector according to embodiment 150, and a pharmaceutically acceptable excipient;

159) a vaccine composition comprising a vector according to embodiment 151, and a pharmaceutically acceptable excipient;

160) a method for inducing an immune response against HPV in a subject, comprising administering to the subject a vaccine composition according to any one of embodiments 152-159;

161) a method for treating vulvar intraepithelial neoplasia (VIN), comprising administering a vaccine according to any one of embodiments 152-159 to a subject that suffers from VIN;

162) a method for treating vulvar cancer, comprising administering a vaccine according to any one of embodiments 152-159 to a subject that suffers from vulvar cancer;

163) a method for treating cervical intraepithelial neoplasia (CIN), comprising administering a vaccine according to any one of embodiments 152-159 to a subject that suffers from CIN;

164) a method for treating cervical cancer, comprising administering a vaccine according to any one of embodiments 152-159 to a subject that suffers from cervical cancer;

165) a method for treating opopharyngeal cancer, comprising administering a vaccine according to any one of embodiments 152-159 to a subject that suffers from oropharyngeal cancer;

166) a method for treating penile intraepithelial neoplasia (PIN), comprising administering a vaccine according to any one of embodiments 152-159 to a subject that suffers from PIN;

167) a method for treating penile cancer, comprising administering a vaccine according to any one of embodiments 152-159 to a subject that suffers from penile cancer;

168) a method for treating vaginal intraepithelial neoplasia (VaIN), comprising administering a vaccine according to any one of embodiments 152-159 to a subject that suffers from VaIN;

169) a method for treating vaginal cancer, comprising administering a vaccine according to any one of embodiments 152-159 to a subject that suffers from vaginal cancer;

170) a method for treating anal intraepithelial neoplasia (AIN), comprising administering a vaccine according to any one of embodiments 152-159 to a subject that suffers from AIN;

171) a method for treating anal cancer, comprising administering a vaccine according to any one of embodiments 152-159 to a subject that suffers from anal cancer;

172) a nucleic acid encoding a polypeptide comprising SEQ ID NO: 20;

173) a nucleic acid according to embodiment 172, wherein the polypeptide further comprises at least part of HPV E2 protein;

174) a nucleic acid according to embodiment 173, wherein the at least part of the HPV E2 protein is from the E2 protein of HPV18;

175) a nucleic acid according to embodiment 173, wherein the polypeptide comprises at least part of the E2 protein fused to the N-terminal side of the polypeptide with SEQ ID NO: 20;

176) a nucleic acid according to embodiment 173, wherein the polypeptide comprises at least part of the E2 protein fused to the C-terminal side of the polypeptide with SEQ ID NO: 20;

177) a nucleic acid according to embodiment 174, wherein the polypeptide comprises at least part of the E2 protein fused to the N-terminal side of the polypeptide with SEQ ID NO: 20;

178) a nucleic acid according to embodiment 174, wherein the polypeptide comprises at least part of the E2 protein fused to the C-terminal side of the polypeptide with SEQ ID NO: 20;

179) a nucleic acid according to embodiment 173, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

180) a nucleic acid according to embodiment 174, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

181) a nucleic acid according to embodiment 175, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

182) a nucleic acid according to embodiment 176, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

183) a nucleic acid according to embodiment 177, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

184) a nucleic acid according to embodiment 178, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

185) a vector comprising a nucleic acid according to embodiment 172, wherein a sequence encoding the polypeptide is operably linked to a promoter;

186) a vector comprising a nucleic acid according to embodiment 173, wherein a sequence encoding the polypeptide is operably linked to a promoter;

187) a vector comprising a nucleic acid according to embodiment 174, wherein a sequence encoding the polypeptide is operably linked to a promoter;

188) a vector comprising a nucleic acid according to embodiment 175, wherein a sequence encoding the polypeptide is operably linked to a promoter;

189) a vector comprising a nucleic acid according to embodiment 176, wherein a sequence encoding the polypeptide is operably linked to a promoter;

190) a vector comprising a nucleic acid according to embodiment 177, wherein a sequence encoding the polypeptide is operably linked to a promoter;

191) a vector comprising a nucleic acid according to embodiment 178, wherein a

sequence encoding the polypeptide is operably linked to a promoter;

192) a vector comprising a nucleic acid according to embodiment 179, wherein a sequence encoding the polypeptide is operably linked to a promoter;

193) a vector comprising a nucleic acid according to embodiment 180, wherein a sequence encoding the polypeptide is operably linked to a promoter;

194) a vector comprising a nucleic acid according to embodiment 181, wherein a sequence encoding the polypeptide is operably linked to a promoter;

195) a vector comprising a nucleic acid according to embodiment 182, wherein a sequence encoding the polypeptide is operably linked to a promoter;

196) a vector comprising a nucleic acid according to embodiment 183, wherein a sequence encoding the polypeptide is operably linked to a promoter;

197) a vector comprising a nucleic acid according to embodiment 184, wherein a sequence encoding the polypeptide is operably linked to a promoter;

198) a vector according to embodiment 185, wherein the vector is an adenovirus;

199) a vector according to embodiment 186, wherein the vector is an adenovirus;

200) a vector according to embodiment 187, wherein the vector is an adenovirus;

201) a vector according to embodiment 188, wherein the vector is an adenovirus;

202) a vector according to embodiment 189, wherein the vector is an adenovirus;

203) a vector according to embodiment 190, wherein the vector is an adenovirus;

204) a vector according to embodiment 191, wherein the vector is an adenovirus;

205) a vector according to embodiment 192, wherein the vector is an adenovirus;

206) a vector according to embodiment 193, wherein the vector is an adenovirus;

207) a vector according to embodiment 194, wherein the vector is an adenovirus;

208) a vector according to embodiment 195, wherein the vector is an adenovirus;

209) a vector according to embodiment 196, wherein the vector is an adenovirus;

210) a vector according to embodiment 197, wherein the vector is an adenovirus;

211) a vector according to embodiment 198, wherein the adenovirus is a human adenovirus of serotype 26;

212) a vector according to embodiment 199, wherein the adenovirus is a human adenovirus of serotype 26;

213) a vector according to embodiment 200, wherein the adenovirus is a human adenovirus of serotype 26;

214) a vector according to embodiment 201, wherein the adenovirus is a human adenovirus of serotype 26;

215) a vector according to embodiment 202, wherein the adenovirus is a human adenovirus of serotype 26;

216) a vector according to embodiment 203, wherein the adenovirus is a human adenovirus of serotype 26;

217) a vector according to embodiment 204, wherein the adenovirus is a human adenovirus of serotype 26;

218) a vector according to embodiment 205, wherein the adenovirus is a human adenovirus of serotype 26;

219) a vector according to embodiment 206, wherein the adenovirus is a human adenovirus of serotype 26;

220) a vector according to embodiment 207, wherein the adenovirus is a human adenovirus of serotype 26;

221) a vector according to embodiment 208, wherein the adenovirus is a human adenovirus of serotype 26;

222) a vector according to embodiment 209, wherein the adenovirus is a human adenovirus of serotype 26;

223) a vector according to embodiment 210, wherein the adenovirus is a human adenovirus of serotype 26;

224) a vector according to embodiment 198, wherein the adenovirus is a human adenovirus of serotype 35;

225) a vector according to embodiment 199, wherein the adenovirus is a human adenovirus of serotype 35;

226) a vector according to embodiment 200, wherein the adenovirus is a human adenovirus of serotype 35;

227) a vector according to embodiment 201, wherein the adenovirus is a human adenovirus of serotype 35;

228) a vector according to embodiment 202, wherein the adenovirus is a human adenovirus of serotype 35;

229) a vector according to embodiment 203, wherein the adenovirus is a human adenovirus of serotype 35;

230) a vector according to embodiment 204, wherein the adenovirus is a human adenovirus of serotype 35;

231) a vector according to embodiment 205, wherein the adenovirus is a human adenovirus of serotype 35;

232) a vector according to embodiment 206, wherein the adenovirus is a human adenovirus of serotype 35;

233) a vector according to embodiment 207, wherein the adenovirus is a human adenovirus of serotype 35;

234) a vector according to embodiment 208, wherein the adenovirus is a human adenovirus of serotype 35;

235) a vector according to embodiment 209, wherein the adenovirus is a human adenovirus of serotype 35;

236) a vector according to embodiment 210, wherein the adenovirus is a human adenovirus of serotype 35;

237) a vaccine composition comprising a vector according to embodiment 185, and a pharmaceutically acceptable excipient;

238) a vaccine composition comprising a vector according to embodiment 186, and a pharmaceutically acceptable excipient;

239) a vaccine composition comprising a vector according to embodiment 187, and a pharmaceutically acceptable excipient;

240) a vaccine composition comprising a vector according to embodiment 188, and a pharmaceutically acceptable excipient;

241) a vaccine composition comprising a vector according to embodiment 189, and a pharmaceutically acceptable excipient;

242) a vaccine composition comprising a vector according to embodiment 190, and a pharmaceutically acceptable excipient;

243) a vaccine composition comprising a vector according to embodiment 191, and a pharmaceutically acceptable excipient;

244) a vaccine composition comprising a vector according to embodiment 192, and a pharmaceutically acceptable excipient;

245) a vaccine composition comprising a vector according to embodiment 193, and a pharmaceutically acceptable excipient;

246) a vaccine composition comprising a vector according to embodiment 194, and a pharmaceutically acceptable excipient;

247) a vaccine composition comprising a vector according to embodiment 195, and a pharmaceutically acceptable excipient;

248) a vaccine composition comprising a vector according to embodiment 196, and a pharmaceutically acceptable excipient;

249) a vaccine composition comprising a vector according to embodiment 197, and a pharmaceutically acceptable excipient;

250) a vaccine composition comprising a vector according to embodiment 198, and a pharmaceutically acceptable excipient;

251) a vaccine composition comprising a vector according to embodiment 199, and a pharmaceutically acceptable excipient;

252) a vaccine composition comprising a vector according to embodiment 200, and a pharmaceutically acceptable excipient;

253) a vaccine composition comprising a vector according to embodiment 201, and a pharmaceutically acceptable excipient;

254) a vaccine composition comprising a vector according to embodiment 202, and a pharmaceutically acceptable excipient;

255) a vaccine composition comprising a vector according to embodiment 203, and a pharmaceutically acceptable excipient;

256) a vaccine composition comprising a vector according to embodiment 204, and a pharmaceutically acceptable excipient;

257) a vaccine composition comprising a vector according to embodiment 205, and a pharmaceutically acceptable excipient;

258) a vaccine composition comprising a vector according to embodiment 206, and a pharmaceutically acceptable excipient;

259) a vaccine composition comprising a vector according to embodiment 207, and a pharmaceutically acceptable excipient;

260) a vaccine composition comprising a vector according to embodiment 208, and a pharmaceutically acceptable excipient;

261) a vaccine composition comprising a vector according to embodiment 209, and a pharmaceutically acceptable excipient;

262) a vaccine composition comprising a vector according to embodiment 210, and a pharmaceutically acceptable excipient;

263) a vaccine composition comprising a vector according to embodiment 211, and a pharmaceutically acceptable excipient;

264) a vaccine composition comprising a vector according to embodiment 212, and a pharmaceutically acceptable excipient;

265) a vaccine composition comprising a vector according to embodiment 213, and a pharmaceutically acceptable excipient;

266) a vaccine composition comprising a vector according to embodiment 214, and a pharmaceutically acceptable excipient;

267) a vaccine composition comprising a vector according to embodiment 215, and a pharmaceutically acceptable excipient;

268) a vaccine composition comprising a vector according to embodiment 216, and a pharmaceutically acceptable excipient;

269) a vaccine composition comprising a vector according to embodiment 217, and a pharmaceutically acceptable excipient;

270) a vaccine composition comprising a vector according to embodiment 218, and a pharmaceutically acceptable excipient;

271) a vaccine composition comprising a vector according to embodiment 219, and a pharmaceutically acceptable excipient;

272) a vaccine composition comprising a vector according to embodiment 220, and a pharmaceutically acceptable excipient;

273) a vaccine composition comprising a vector according to embodiment 221, and a pharmaceutically acceptable excipient;

274) a vaccine composition comprising a vector according to embodiment 222, and a pharmaceutically acceptable excipient;

275) a vaccine composition comprising a vector according to embodiment 223, and a pharmaceutically acceptable excipient;

276) a vaccine composition comprising a vector according to embodiment 224, and a pharmaceutically acceptable excipient;

277) a vaccine composition comprising a vector according to embodiment 225, and a pharmaceutically acceptable excipient;

278) a vaccine composition comprising a vector according to embodiment 226, and a pharmaceutically acceptable excipient;

279) a vaccine composition comprising a vector according to embodiment 227, and a pharmaceutically acceptable excipient;

280) a vaccine composition comprising a vector according to embodiment 228, and a pharmaceutically acceptable excipient;

281) a vaccine composition comprising a vector according to embodiment 229, and a pharmaceutically acceptable excipient;

282) a vaccine composition comprising a vector according to embodiment 230, and a pharmaceutically acceptable excipient;

283) a vaccine composition comprising a vector according to embodiment 231, and a pharmaceutically acceptable excipient;

284) a vaccine composition comprising a vector according to embodiment 232, and a pharmaceutically acceptable excipient;

285) a vaccine composition comprising a vector according to embodiment 233, and a pharmaceutically acceptable excipient;

286) a vaccine composition comprising a vector according to embodiment 234, and a pharmaceutically acceptable excipient;

287) a vaccine composition comprising a vector according to embodiment 235, and a pharmaceutically acceptable excipient;

288) a vaccine composition comprising a vector according to embodiment 236, and a pharmaceutically acceptable excipient;

289) a method for inducing an immune response against HPV in a subject, comprising administering to the subject a vaccine composition according to any one of embodiments 237-288;

290) a method for treating persistent HPV (type 18) infection, comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from persistent HPV infection;

291) a method for treating vulvar intraepithelial neoplasia (VIN) (with underlying HPV type 18 infection), comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from VIN;

292) a method for treating vulvar cancer (with underlying HPV type 18 infection), comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from vulvar cancer;

293) a method for treating cervical intraepithelial neoplasia (CIN) (with underlying HPV type 18 infection), comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from CIN;

294) a method for treating cervical cancer (with underlying HPV type 18 infection), comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from cervical cancer;

295) a method for treating opopharyngeal cancer (with underlying HPV type 18 infection), comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from oropharyngeal cancer;

296) a method for treating penile intraepithelial neoplasia (PIN) (with underlying HPV type 18 infection), comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from PIN;

297) a method for treating penile cancer (with underlying HPV type 18 infection), comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from penile cancer;

298) a method for treating vaginal intraepithelial neoplasia (VaIN) (with underlying HPV type 18 infection), comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from VaIN;

299) a method for treating vaginal cancer (with underlying HPV type 18 infection), comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from vaginal cancer;

300) a method for treating anal intraepithelial neoplasia (AIN) (with underlying HPV type 18 infection), comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from AIN;

301) a method for treating anal cancer (with underlying HPV type 18 infection), comprising administering a vaccine according to any one of embodiments 237-288 to a subject that suffers from anal cancer;

302) a polypeptide comprising SEQ ID NO: 20;

303) a polypeptide according to embodiment 302, wherein the polypeptide further comprises at least part of HPV E2 protein;

304) a polypeptide according to embodiment 303, wherein the at least part of the HPV E2 protein is from the E2 protein of HPV18;

305) a polypeptide according to embodiment 303, wherein at least part of the E2 protein is fused to the N-terminal side of the polypeptide with SEQ ID NO: 20;

306) a polypeptide according to embodiment 303, wherein at least part of the E2 protein is fused to the C-terminal side of the polypeptide with SEQ ID NO: 20;

307) a polypeptide according to embodiment 304, wherein at least part of the E2 protein is fused to the N-terminal side of the polypeptide with SEQ ID NO: 20;

308) a polypeptide according to embodiment 304, wherein at least part of the E2 protein is fused to the C-terminal side of the polypeptide with SEQ ID NO: 20;

309) a polypeptide according to embodiment 303, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

310) a polypeptide according to embodiment 304, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

311) a polypeptide according to embodiment 305, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

312) a polypeptide according to embodiment 306, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

313) a polypeptide according to embodiment 307, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

314) a polypeptide according to embodiment 308, wherein the at least part of the E2 protein comprises a variant of the E2 protein with a mutation that abrogates DNA binding of E2;

315) a nucleic acid according to embodiment 174, encoding a polypeptide according to SEQ ID NO: 22;

316) a vector encoding a nucleic acid according to embodiment 315, wherein a sequence encoding the polypeptide is operably linked to a promoter;

317) a vector according to embodiment 316, wherein the vector is an adenovirus;

318) a vector according to embodiment 317, wherein the adenovirus is a human adenovirus of serotype 26;

319) a vector according to embodiment 317, wherein the adenovirus is a human adenovirus of serotype 35;

320) a vaccine composition comprising a vector according to embodiment 316, and a pharmaceutically acceptable excipient;

321) a vaccine composition comprising a vector according to embodiment 317, and a pharmaceutically acceptable excipient;

322) a vaccine composition comprising a vector according to embodiment 318, and a pharmaceutically acceptable excipient;

323) a vaccine composition comprising a vector according to embodiment 319, and a pharmaceutically acceptable excipient;

324) a method for inducing an immune response against HPV in a subject, comprising administering to the subject a vaccine composition according to any one of embodiments 320-324;

325) a method for treating vulvar intraepithelial neoplasia (VIN), comprising administering a vaccine according to any one of embodiments 320-324 to a subject that suffers from VIN;

326) a method for treating vulvar cancer, comprising administering a vaccine according to any one of embodiments 320-324 to a subject that suffers from vulvar cancer;

327) a method for treating cervical intraepithelial neoplasia (CIN), comprising administering a vaccine according to any one of embodiments 320-324 to a subject that suffers from CIN;

328) a method for treating cervical cancer, comprising administering a vaccine according to any one of embodiments 320-324 to a subject that suffers from cervical cancer;

329) a method for treating opopharyngeal cancer, comprising administering a vaccine according to any one of embodiments 320-324 to a subject that suffers from oropharyngeal cancer;

330) a method for treating penile intraepithelial neoplasia (PIN), comprising administering a vaccine according to any one of embodiments 320-324 to a subject that suffers from PIN;

331) a method for treating penile cancer, comprising administering a vaccine according to any one of embodiments 320-324 to a subject that suffers from penile cancer;

332) a method for treating vaginal intraepithelial neoplasia (VaIN), comprising administering a vaccine according to any one of embodiments 320-324 to a subject that suffers from VaIN;

333) a method for treating vaginal cancer, comprising administering a vaccine according to any one of embodiments 320-324 to a subject that suffers from vaginal cancer;

334) a method for treating anal intraepithelial neoplasia (AIN), comprising administering a vaccine according to any one of embodiments 320-324 to a subject that suffers from AIN;

335) a method for treating anal cancer, comprising administering a vaccine according to any one of embodiments 320-324 to a subject that suffers from anal cancer;

336) a composition comprising nucleic acid encoding SEQ ID NO: 1 and nucleic acid encoding SEQ ID NO: 20;

337) a composition according to embodiment 336, comprising nucleic acid encoding SEQ ID NO: 3 and nucleic acid encoding SEQ ID NO: 22;

338) a method for inducing an immune response in a subject against HPV, comprising administering to the subject nucleic acid encoding SEQ ID NO: 1 and nucleic acid encoding SEQ ID NO: 20;

339) a method according to embodiment 338, comprising administering to the subject nucleic acid encoding SEQ ID NO: 3 and nucleic acid encoding SEQ ID NO: 22;

340) a kit of parts comprising (i) nucleic acid encoding SEQ ID NO: 1 and (ii) nucleic acid encoding SEQ ID NO: 20;

341) a vaccine composition comprising nucleic acid encoding SEQ ID NO: 1 and nucleic acid encoding SEQ ID NO: 20, and a pharmaceutically acceptable excipient;

342) a method for treating persistent HPV infection, vulvar intraepithelial neoplasia (VIN), cervical intraepithelial neoplasia (CIN), vaginal intraepithelial neoplasia (VaIN), anal intraepithelial neoplasia (AIN), cervical cancer (such as cervical squamous cell carcinoma (SCC), oropharyngeal cancer, penile cancer, vaginal cancer or anal cancer in a subject, the method comprising administering to the subject nucleic acid encoding SEQ ID NO: 1 and nucleic acid encoding SEQ ID NO: 20.

The practice of this invention will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology, microbiology, cell biology, and recombinant DNA, which are within the skill of the art. See e.g. Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual, 2nd edition, 1989; Current Protocols in Molecular Biology, Ausubel FM, et al., eds, 1987; the series Methods in Enzymology (Academic Press, Inc.); PCR2: A Practical Approach, MacPherson MJ, Hams BD, Taylor GR, eds, 1995; Antibodies: A Laboratory Manual, Harlow and Lane, eds, 1988.

The invention is further explained in the following examples. The examples do not limit the invention in any way. They merely serve to clarify the invention.

EXAMPLES

Example 1: construction of a designer polypeptide comprising essentially all HPV16 E6 and E7 CTL epitopes

We designed a novel, non-tumorigenic polypeptide (and nucleic acid encoding such) that contains essentially all CTL epitopes of HPV16 E6 and E7 proteins, and has a minimum number of anticipated/predicted strong neo-epitopes (neo-epitopes meaning epitopes not present in the wild type HPV16 E6 and E7 proteins). The polypeptide of the invention (also sometimes referred to as 'E6E7SH' herein) for HPV16 comprises a sequence as provided in SEQ ID NO: 1. A codon-optimized nucleic acid encoding this polypeptide is provided in SEQ ID NO: 2.

The molecules of the invention are single molecules, which provides manufacturing advantages over strategies where multiple molecules are used. In addition, the polypeptide of the invention comprises essentially all putative CTL epitopes that are present in wild-type E6 and E7 of HPV16, and at the same time have a minimum number of anticipated/predicted strong neo-epitopes that could potentially be immunodominant and thus divert the immune response from relevant wild-type CTL epitopes. Thus the constructs of the present invention are immunologically more favourable than molecules described by others that either lack possible CTL epitopes and/or that contain more or stronger neo-epitopes.

For instance, the construct of SEQ ID NO: 1 contains only one neo-epitope with a length of nine amino acids with a predicted binding affinity <50 nM for the 20 most common HLA-A, 20 most common HLA-B and 20 most common HLA-C alleles (HLA-A*01:01, HLA-A*02:01, HLA-A*02:03, HLA-A*02:06, HLA-A*02:07, HLA-A*03:01, HLA-A*11:01, HLA-A*23:01, HLA-A*24:02, HLA-A*26:01, HLA-A*29:02, HLA-A*30:01, HLA-A*30:02, HLA-A*31:01, HLA-A*32:01, HLA-A*33:01, HLA-A*33:03, HLA-A*34:01, HLA-A*68:01, HLA-A*68:02, HLA-B*07:02, HLA-B*07:04, HLA-B*08:01, HLA-B*13:01, HLA-B*15:01, HLA-B*18:01, HLA-B*35:01, HLA-B*37:01, HLA-B*39:01, HLA-B*40:01, HLA-B*40:02, HLA-B*40:06, HLA-B*44:02, HLA-B*44:03, HL-B*46:01, HLA-B*48:01, HLA-B*51:01, HLA-B*52:01, HLA-B*53:01, HLA-

B*58:01, HLA-C*07:02, HLA-C*04:01, HLA-C*03:04, HLA-C*01:02, HLA-C*07:01, HLA-C*06:02, HLA-C*03:03, HLA-C*08:01, HLA-C*15:02, HLA-C*12:02, HLA-C*02:02, HLA-C*05:01, HLA-C*14:02, HLA-C*03:02, HLA-C*16:01, HLA-C*08:02, HLA-C*12:03, HLA-C*04:03, HLA-C*17:01, HLA-C*14:03), as determined using the ANN (Lundegaard *et al.,* 2008, *Nucl Acids Res* 36: W509-12) and SMM method (Peters *et al.,* 2003, *Bioinformatics* 19: 1765-72) for HLA-A and HLA-B and the NetMHCpan method (Hoof *et al.,* 2009, I*mmunogenetics* 61: 1-13) for HLA-C of the prediction tool for 'Peptide binding to MHC class I molecules' at the IEDB website (http://tools.immuneepitope.org/analyze/html/mhc_binding.html, version 2009-09-01B). Zhang et al (2008) describe the IEDB analysis resource.

As a non-limiting example, using the SMM prediction tool at the IEDB website, the shuffled E6 and E7 sequences as described by Oosterhuis *et al.,* 2011, *Int J Cancer* 129: 397-406 and Öhlschläger *et al.,* 2006, *Vaccine* 24: 2880–93 contain each nine potential strong unique neo-epitopes (ANN or SMM IC50<50 nM) for the 20 most HLA-A and -B, in the core part. This even excludes the appendices used in that approach (in which appendices will further contribute to additional neo-epitopes, and may miss out on more native MHC II epitopes due to the limited length of the 'overlap'). Indeed, a reportedly improved molecule containing a variant with shuffled E6 and E7 proteins that was described in WO 2013/083287, contains 22 unique neo-epitopes with a length of nine amino acids with a predicted IC50 <50 nM (ANN, SMM or NetMHCPan) for the 20 most common HLA-A, 20 most common HLA-B and 20 most common HLA-C alleles.

Hence, the designer molecules of the invention clearly are favourable in having much lower number of predicted neo-epitopes compared to other published approaches where E6 and E7 where shuffled to remove functionality.

Nucleic acid encoding our thus designed HPV16 E6E7SH molecule (i.e. a polypeptide having amino acid sequence as provided in SEQ ID NO:1) was synthesized, the nucleic acid sequence comprising SEQ ID NO: 2, and flanked by a HindIII site and a Kozak sequence on the 5'end and an XbaI site on the 3'site (custom synthesis and standard molecular cloning at Invitrogen Life technologies, Germany).

The synthezised fragments were cloned using HindIII and XbaI into a standard expression vector, pCDNA2004.Neo, harbouring both a bacterial resistance marker

(Ampiciline) and a mammalian resistance marker (Neomycine), to obtain plasmid vectors encoding a molecule of the invention, e.g. for (transient) transfection based experiments.

These molecules could be used as such, but also as the basis for further molecules that contain additional features. As non-limiting examples, some further variants were prepared as described below.

The HPV16 E6E7SH fusion protein sequence can be combined with sequences of other HPV16 early proteins to target individuals with persistent infection and to broaden the immune repertoire in an immunized individual. Immune responses against E2 have been suggested to play an important role in the clearance of HPV16 infections (de Jong *et al.,* 2002, *Cancer Res* 62: 472-479). Fusion of E2 to E6E7SH will give a vaccine component that harbours antigens against the stages of HPV-related cancer from persistent infection to invasive cancer or recurrent/refractory disease after LEEP surgery. Therefore, as a non-limiting example of such embodiments, we prepared a sequence coding for a fusion protein of E6E7SH with E2 at its N-terminus. In the E2 sequence modifications can be made to abrogate DNA binding activity that might affect gene expression in cells expressing the fusion protein. We mutated Glycine at position 293, Lysine at position 299 and Cysteine at position 300 of the wt HPV16 E2 protein into respectively Valine, Methionine and Arginine. Each of these mutations on its own already completely abrogates the binding of E2 to DNA sequences that harbour E2 binding domains (Prakash et al., 1992, *Genes Dev* 6: 105-16).

The resulting polypeptide is referred to as HPV16 E2E6E7SH and comprises SEQ ID NO: 3. A codon-optimized sequence encoding this polypeptide was prepared and is provided in SEQ ID NO: 4.

We also constructed a variant wherein the same E2 mutant protein was fused to the C-terminus of the HPV16 E6E7SH fusion polypeptide, giving rise to a polypeptide referred to as HPV16 E6E7E2SH, which comprises SEQ ID NO: 5. The sequence encoding this construct is provided as SEQ ID NO: 6.

For control purposes, we also constructed sequences encoding a polypeptide that contains the wild-type sequences for full-length HPV16 E6 and E7 as a fusion

protein (E6 from aa 1 to 158 directly fused to E7 from aa 1 to 98, named herein E6E7wt).

We also tested the effect of adding leader sequences to the polypeptide. As a non-limiting example, a sequence encoding an IgE leader sequence (see e.g. US 6,733,994) [the sequence of the leader peptide is provided in SEQ ID NO: 7] was fused at the N-terminus of some of the constructs, e.g. in the E6E7wt construct, which rendered LSE6E7wt, and in the E2E6E7SH construct, which rendered LSE2E6E7SH. The effect thereof was significantly ($p< 0.05$) enhanced immunogenicity in comparison to the same antigen without the LS sequence as measured by E7-tetramer analysis in immunized mice (as can for instance be seen in Fig. 9).

The sequences that encode the E6E7SH polypeptides of the invention, with or without E2, can for instance be expressed from DNA constructs, from RNA or from viral vectors. Fig.1 demonstrates expression in HEK-293T cells upon transient transfection with DNA vectors expressing the transgenes as described above. After transfection, cells were harvested and cell extracts were analyzed by SDS-PAGE and western blotting with an antibody against HPV16 E7. This experiment demonstrates expression of the expected fusion proteins of appropriate size upon transfection of the expression vectors.

Adenoviral vectors can be used to express the E6E7, either with or without E2, and with or without additional sequences to augment the immunogenicity of the encoded fusion protein.

The genes, coding for HPV16 E6E7 wt control or HPV16 designer sequences described above were gene optimized for human expression and synthesized, at Geneart. A Kozak sequence (5' GCCACC 3') was included directly in front of the ATG start codon, and two stop codons (5' TGA TAA 3') were added at the end of the respective coding sequence. The genes were inserted in the pAdApt35BSU plasmid and in the pAdApt26 plasmid (Havenga *et al.*, 2006, *J Gen Virol* 87, 2135-43) via HindIII and XbaI sites.

All adenoviruses were generated in PER.C6 cells by single homologous recombination and produced as previously described (for rAd35: Havenga et al., 2006, *J Gen Virol* 87: 2135–43; for rAd26: Abbink et al., 2007, *J Virol* 81: 4654-63).

PER.C6 cells (Fallaux *et al.*, 1998, *Hum Gene Ther* 9: 1909-17) were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), supplemented with 10mM MgCl2.

Briefly, PER.C6 cells were transfected with Ad vector plasmids, using Lipofectamine according to the instructions provided by the manufacturer (Life Technologies). Cells were harvested one day after full cytopathic effect (CPE) was reached, freeze-thawed, centrifuged for 5 min at 3,000 rpm, and stored at -20°C. The viruses were plaque purified and amplified in PER.C6 cells cultured in a single well of a multiwell 24 tissue culture plate. Further amplification was carried out in PER.C6 cells cultured in a T25 tissue culture flask and subsequently in a T175 tissue culture flask. Of the crude lysate prepared from the cells obtained after the T175 flask, 3 to 5 ml was used to inoculate 24×T1000 five-layer tissue culture flasks containing 70% confluent layers of PER.C6 cells. The virus was purified using a two-step CsCl purification method. Finally, the virus was stored in aliquots at -85°C.

Ad35.HPV16-E6E7wt, and Ad35.HPV16-E6E7SH are recombinant adenovirus serotype 35 (Ad35) vectors comprising the codon-optimized nucleotide sequences for the expression of, respectively, a fusion protein of the wild type HPV16 E6 and E7 proteins (E6E7wt), and a designer fusion protein variant as described above (E6E7SH, having the amino acid sequence provided in SEQ ID NO: 1). The combined E6 and E7 sequences were placed under the control of a CMV promoter in the E1 region of the E1,E3 deleted adenovirus genome. Ad26.HPV16-E6E7wt, and Ad26.HPV16-E6E7SH are the equivalent vectors based on recombinant adenovirus serotype 26.

Similarly, Ad26 and Ad35-based recombinant adenoviral vectors were produced that encode the HPV16 E2E6E7SH (SEQ ID NO: 3) variant. Likewise, Ad26 and Ad35 encoding the HPV16 E6E7E2SH (SEQ ID NO: 5) variant were produced. Also, an Ad35 vector encoding the E2E6E7SH fusion protein with an IgE leader sequence at the N-terminus was produced, named Ad35.HPV16-LSE2E6E7SH. Also a control adenovirus with the E6E7wt fused to the IgE leader sequence at the N-terminus was produced.

The recombinant adenoviruses were produced on PER.C6 cells and purified by centrifugation on cesium chloride gradients.

Further examples of constructs that were coupled to repressor systems are provided in a later example below.

Example 2. Lack of transforming activity of the HPV16 designer constructs

Wild-type HPV16 E6 and E7 proteins have tumorigenic potential, which is apparent as transforming activity in certain assays, such as colony formation in a soft-agar assay (Massimi and Banks, 2005, *Methods Mol Med* 119: 381-395). The E6E7SH polypeptide as described in example 1 comprises the fragments of the E6 and E7 proteins in a re-ordered fashion. This is expected to remove the tumorigenic potential, as can be measured for instance by a significantly reduced transforming activity as compared to either of wt E6 and E7 proteins in such assays.

Others reported that gene-shuffled variants of HPV16 E6 and E7 have indeed lost their oncogenic potential (Öhlschläger *et al.,* 2006, *Vaccine* 24: 2880–93; Henken *et al.,* 2012, *Vaccine* 30: 4259-66), demonstrating that gene shuffling destroys the wild-type functions of E6 and E7 proteins.

To assess the loss of tumorigenic properties, we assessed the ability of our E6E7SH constructs to confer the ability to grow in soft agar upon NIH 3T3 cells (as described by e.g. Massimi and Banks, 2005, *Methods Mol Med* 119: 381-395). Transfection of NIH3T3 cells with a plasmid expressing the wild type HPV16 E7 resulted consistently in colony formation. In these assays, expression of wild type HPV16 E6 alone did not cause colony formation above background. This is in line with published observations that E7wt is much more efficient than E6wt in this assay (Sedman *et al.,* 1991, *J Virol* 65: 4860-66). Transfection with our E6E7SH construct did not lead to growth of colonies of cells in soft agar (Fig. 2) in four independent experiments, demonstrating that nucleic acids encoding a polypeptide of the invention, E6E7SH, have lost the transforming capacity that is associated with E7.

The tumorigenic potential of E6 and E7 is associated with their ability to reduce the levels of the cellular proteins p53 and pRb respectively. p53 and pRb degradation assays were performed todemonstrate that nucleic acid encoding a polypeptide of the invention, E6E7SH, construct does not have the biological activity associated with the wild-type E6 and E7 at the molecular level. In short, HPV16 E6wt

and our E6E7SH construct were expressed in NCI-H1299 cells that lack endogenous p53 for the p53 degradation assay. For the pRb degradation assay HPV16 E7wt and the E6E7SH construct were expressed in pRb null Saos-2 cells. As can be seen in Fig. 3, co-expression of p53 with E6wt, but not with E6E7SH, leads to reduced p53 levels (panels A and B). Likewise, panels 3C and 3D show that co-expression of pRb with E7wt, but not with E6E7SH, leads to reduced pRB levels. These data demonstrate that nucleic acid encoding a polypeptide of the invention has no ability to form colonies in soft agar and does not containmain biological activities of the wild-type E6 and E7 polypeptides, namely the inactivation of p53 and pRb respectively.

To further demonstrate the safety of nucleic acid constructs encoding polypeptide of the invention, we made use of primary human foreskin keratinocytes that are the natural target cells for HPV mediated transformation. Immortalization of primary human keratinocytes requires the action of both E6 and E7 wild-type (Munger *et al.,* 1989, *J Virol* 63: 4417-21). This assay is probably the physiologically most relevant *in vitro* assay to demonstrate the safety of our constructs (Massimi and Banks, 2005, *Methods Mol Med* 119: 381-395). Cells transduced with lentiviruses expressing wild type E6 and E7 from HPV16 (E6E7wt) induce immortalization in primary keratinocytes as indicated by the extension of their lifespan as compared to non-transduced control cells (Fig. 4) and activation of hTERT, the catalytic subunit of telomerase (data not shown). Expression of the polypeptide of the invention (E6E7SH) is not able to to extend the lifespan compared to GFP-transduced or non-transduced keratinocytes. A similar result was obtained in two additional independent donors (data not shown). Taken together these data demonstrate that our constructs have lost the ability to induce immortalization in primary human keratinocytes, that are considered a highly physiological model.

Another construct wherein comparable fragments of HPV16 E6 and E7 were recombined in a different order was also incapable of immortalization of primary human foreskin keratinocytes. However, an expanded life span up to approximately 120-150 days was observed for that construct. This indicates some unpredictability in this field, and demonstrates the superiority of the selected designer molecules according to the invention in this safety-related aspect.

All together the experiments in this example provide strong evidence of the lack of transforming activity of nucleic acids encoding HPV16 designer polypeptides according to the invention, and thus a strongly improved safety over HPV16 E6 and E7 wt constructs.

Example 3. Immune responses to the HPV16 E6E7SH designer constructs

We have prepared DNA vectors and adenoviral vectors, as described in example 1.

We used the CB6F1 mouse strain for measuring immune responses, based on initial experiments where mice where immunized with DNA plasmids encoding wild type E2, or E6 or E7, and immunization with HPV16 E2, E6 and E7 antigens induced a broader cellular immune response in CB6F1 than in C57BL/6 mice or Balb/c mice. In a separate experiment mice were immunized with DNA vectors encoding molecules of the invention and cellular immune responses were measured. HPV16 E7-specific immune responses could be measured in mice immunized with DNA plasmids expressing E6E7SH (Fig. 5).

The following data shown in this example are from mouse experiments that were carried out with adenoviral vectors.

To evaluate the vaccine induced immunogenicity, CB6F1 mice were immunized with adenovectors (Ad35) expressing HPV16 E6E7wt, LSE6E7wt, E6E7SH or adenovectors not encoding a transgene (Empty). Two doses were tested for administration to the mice: $5*10^9$ viral particles (vp) and $1*10^{10}$ vp. Two and eight weeks after immunization the mice were sacrificed and isolated splenocytes were stimulated overnight with an HPV16 E7 15mer peptide pool. E7-specific responses at two weeks and at eight weeks were analyzed by IFNγ ELISPOT. The data are presented in Fig. 6.

This shows that immunization of mice with Ad35.HPV16-E6E7SH induces E7-specific immune responses as measured by ELISPOT analysis. In addition, the results in Fig. 6 demonstrates the possibility to enhance the immune response against

an adenoviral expressed transgene by adding an N-terminal leader sequence to the transgene.

Next the effect of adding HPV16 E2 to the HPV16 E6E7SH polypeptide with respect to immunogenicity was tested. The Ad35 vectors encoded polypeptides that had E2 either fused to the N-terminus (E2E6E7SH) or to the C-terminus (E6E7E2SH). CB6F1 mice were immunized with a dose of $1x10^{10}$ vp. Fig. 7 (E7-tetramer staining) and Fig. 8 (Panel C, IFNγ ELISPOT) show the immune responses against E7, which for the designer constructs including E2 tends to be higher in comparison to the construct without E2, although the differences were not statistically significant. The response against E2 was higher for adenoviral vectors encoding only E2 compared to the response for adenoviral vectors that had E2 fused to the E6E7SH designer polypeptide (Fig. 8B), with differences being significant for both E2 vs E2E6E7SH and E2 vs E6E7E2SH (p-value: <0.05).

It is concluded that the designer constructs that further include E2 can still provide an immune response against E7, and in addition also provide an immune response against E2, thus increasing the breadth of the immune response over the constructs that do not include E2.

Addition of a leader sequence was shown to result in higher E7-specific responses when fused to the N-terminus of the fusion protein of wild type E6 and E7 (Fig. 6C). Similarly, the effect of the leader sequence on immunogenicity of the E2E6E7SH fusion protein was determined. Therefore, Ad35 vectors encoding the HPV16 designer polypeptide, with or without N-terminal E2 and an Ad35 vector encoding LSE2E6E7SH were used for immunization of mice and blood samples were taken at two-week intervals to measure E7-specific immune responses (Fig. 9). As shown in Figs. 7 and 8 the presence of E2 at either N- or C-terminally fused to E6E7SH tended to increase the immune responses. Addition of the IgE leader sequence further increased the E7-specific response (Fig. 9B). Over time sustained immune responses were observed for all three adenoviral vectors that encoded designer molecules according to the invention, and the highest response after the immunization corresponded with the highest responses over the duration of the experiment.

It is concluded that the responses that are induced by the designer construct that further includes N-terminal E2 can be increased by addition of specific sequences, e.g., the IgE leader sequence, that target the encoded protein to specific cellular compartments.

The cellular immune response against the peptide of the invention can be induced with different types of adenoviral vectors. In the previous experiment we used Ad35 vectors, while in the experiment of Fig. 10, mice were immunized with an Ad26 adenoviral vector expressing HPV16 E2E6E7SH. The data show that also immunization with an Ad26-based vaccine induced E7-specific T-cells. In addition, the results demonstrate that a second immunization with an Ad35 adenoviral vector expressing HPV16 E2E6E7SH further boosted the cellular immune responses (Fig. 10).

Example 4. Immunogenicity of HPV16 designer constructs in rhesus macaques.

To evaluate the ability of the adenoviral vectors expressing the designer sequence of the invention to induce immune responses in non-human primates, rhesus macaques were immunized by intramuscular injection with adenovectors (Ad26) expressing HPV16 E2E6E7SH or adenovectors not encoding a transgene (Empty), with a dose of $1*10^{11}$ vp. Eight weeks after the immunization the immune responses were boosted by immunization with Ad26 vectors expressing the same antigen. At week 16 the animals received one more injection with the Ad35 vectors expressing the same antigen. Blood samples were taken at several time points and isolated white blood cells were stimulated overnight with a peptide pools corresponding to HPV16 E2, E6 or E7. Specific responses were measured by IFNγ ELISPOT. The data are presented in Fig. 11. In addition at week 10 and week 18 post prime immunization, the celluar immune response specific to peptides spanning the novel junctions in the invention was evaluated. The induction of IFNγ response was in all animals below the limit of detection of < 50 SFU per $1*10^6$ PBMC (data not shown).

The data show that immunization of non-human primates with Ad26.HPV16-E2E6E7SH resulted in cellular immune responses against all three HPV16 proteins that are present in the encoded transgene, but not against the novel junctions.

Responses could be boosted by the additional immunization with Ad26.HPV16-E2E6E7SH and additional boost at week 16 with the corresponsing Ad35 vector further increased the HPV16 E2, E6 and E7-specific immune responses.

In a separate experiment (not shown), Rhesus macaques were immunized by intravaginal administration with a combination of two adenoviral vectors, one expressing HPV16 E6E7SH and the other the HPV16 L1 protein. Low but measurable cellular responses were measured in peripheral mononuclear blood cells against both E6 and E7. In these experiments, strong cellular immune responses against L1 were detected.

Example 5. Therapeutic efficacy in a mouse tumor model.

The polypeptide of the invention for HPV16 (comprising SEQ ID NO: 1) is capable of inducing HPV16-specific cellular immune response in animals, which can exert a therapeutic effect on cells expressing HPV16 E6 and/or E7. Therapeutic immunization, i.e. immunization after tumor growth has started, can be used to demonstrate efficacy of a therapeutic HPV vaccine candidate. The therapeutic effect of Ad26 and Ad35 vectors was tested in mice that were injected with TC-1 cells (mouse cells expressing HPV16 E6 and E7) (Lin *et al.,* 1996, *Cancer Res* 56: 21-6). TC-1 cells will form solid tumor within a few days to weeks after sub-cutaneous injection in mice. Without vaccine the tumors grew rapidly and reach a pre-determined size of 1000 mm^3 within 30 days (panels D and E). Upon reaching this size the mice are sacrificed for ethical reasons.

With a prime-boost immunization scheme with SLPs (used as a positive control; Kenter *et al.,* 2009, *N Engl J Med* 361:1838-47; Zwaveling *et al.,* 2002, *J Immunol* 169:350-8) or adenoviral vectors expressing HPV16-E2E6E7SH, a marked decrease of the growth of TC-1 induced tumors was observed (Fig. 12, panels B and C). Closer inspection of the first 30 days after the prime immunizations (Panels F and G) shows that the immunization with the adenovectors expressing E2E6E7SH have a substantially larger impact on tumor growth than immunization with the SLPs. The initial growth rate is much lower and in most cases the tumors shrunk. In 3 out of 11 mice immunized with the adenoviral vectors, the tumors were completely eradicated, which is reflected in the survival plot (panel H).

In conclusion, immunization with adenoviral vectors expressing an HPV16 designer polypeptide of the invention significantly reduced tumor growth or completely eradicated established tumors in a well-established challenge model for HPV16-induced cancer.

Example 6: Employment of repressor systems to improve the productivity and genetic stability of adenoviral vectors expressing HPV-derived antigens

It has previously been reported that transgenes inserted into adenovirus vectors under the control of powerful constitutively active promoters can, depending on the properties of the transgene product, negatively impact vector production (Yoshida & Yamada, 1997, *Biochem Biophys Res Commun* 230:426-30; Rubinchik *et al.,* 2000, *Gene Ther* 7:875-85; Matthews *et al.,* 1999, *J Gen Virol* 80:345-53; Edholm *et al.,* 2001, *J Virol* 75:9579-84; Gall *et al.,* 2007, *Mol Biotechnol* 35:263-73). Examples of transgene-dependent vector productivity issues include inefficient vector rescue and growth, low final vector yields, and, in severe cases, rapid outgrowth of viral mutants with defective transgene cassettes. To solve these issues, multiple studies explored the possibility to silence vector transgene expression during vector replication in producer cells (Matthews *et al.,* 1999, *J Gen Virol* 80:345-53; Edholm *et al.,* 2001, *J Virol* 75:9579-84; Gall *et al.,* 2007, *Mol Biotechnol* 35:263-73; Cottingham *et al.,* 2012, *Biotechnol Bioeng* 109:719-28; Gilbert *et al.,* 2014, *J Virol Methods* 208:177-88). In this regard, different repression systems have previously been implemented in the context of Ad vectors and have indeed shown to improve vector productivity and genetic stability for vectors encoding different types of (inhibitory) transgenes.

It was observed that some of the adenovirus vectors described herein, as well as some other adenoviral vectors encoding certain HPV antigen variants, displayed some of the transgene-dependent vector productivity issues described above, and therefore could possibly be further improved in that respect. We therefore sought to investigate whether usage of systems to repress vector transgene expression can improve production characteristics of Ad vectors expressing HPV-derived antigens as those described herein. For this purpose, we implemented two existing repressor-operator systems, i.e. TetR/TetO (Yao & Eriksson, 1999, *Hum Gene Ther* 10:419-22, EP0990041B1) and CymR/CuO (Mullick *et al.,* 2006, *BMC Biotechnol* 6:43), into our adenovirus vector platform. Both the TetR/TetO and the CymR/CuO system have

previously been used by others to improve adenovirus vector productivity through vector transgene silencing during vector replication (Gall *et al.,* 2007, *Mol Biotechnol* 35:263-73; Cottingham *et al.,* 2012, *Biotechnol Bioeng* 109:719-28; Gilbert *et al.,* 2014, *J Virol Methods* 208:177-88). Implementation of these two systems involved the generation of adenoviral vectors expressing genes of interest under the control of either a TetO or a CuO sequence-containing CMV promoter. Furthermore, the implementation entailed the generation of cell lines stably expressing the respective cognate repressors proteins (i.e. TetR or CymR).

Several E1-deleted, Ad26- and Ad35-based vectors were generated in which sequences encoding heterologous polypeptides were operably linked to a CMV promoter containing either TetO or CuO operator sequences. First, certain TetO- or CuO-containing sequences (SEQ ID NO: 11 and SEQ ID NO: 12, respectively) were inserted near the transcription start site (TSS) of the CMV promoter (SEQ ID NO: 13) of pAdapt26 and pAdapt35.Bsu plasmids (Abbink *et al.,* 2007, *J Virol* 81:4654-63; Havenga *et al.,* 2006, *J Gen Virol* 87:2135-43). The operator-containing sequences were inserted at precisely the same positions of the CMV promoter as previously described for the two systems (Yao & Eriksson, 1999, *Human Gene Ther* 10:419-22; EP0990041B1; Mullick *et al.,* 2006, *BMC Biotechnol* 6:43; EP1385946B1). Specifically, relative to the TSS (as originally assigned; Stenberg *et al.* 1984, J Virol 49:190-9), the TetO- and CuO-containing sequences were inserted directly downstream of positions -20 and +7, respectively. In SEQ ID NO: 13, these two positions correspond to positions 716 and 742, respectively. The resulting operator-containing CMV promoters are termed, respectively, CMVTetO and CMVCuO. Next, different transgenes were inserted downstream of the (modified) CMV promoters of the resulting constructs using HindIII and XbaI restriction sites. These transgenes included genes encoding a fusion protein of green fluorescent protein and luciferase (GFP-Luc), HPV16 LSE2E6E7SH as described above in example 1, and another polypeptide with some similarity to HPV16 LSE2E6E7SH (a construct referred to in this example as 'HPVAg'). HPVAg comprises the same leader sequence as present in LSE2E6E7SH, as well as E2, E6, and E7 sequences of HPV16. Using methods as described herein, the resulting modified pAdapt26 and pAdapt35.Bsu plasmids were used for the generation of adenoviral vectors expressing the above mentioned reporter and HPV transgenes under the control of either the CMVTetO or the CMVCuO promoter.

Cell lines expressing either TetR or CymR were generated by stable transfection of PER.C6® cells using, respectively, plasmid pcDNA^{TM}6/TR (LifeTechnologies, V1025-20) and a derivative of pcDNA^{TM}6/TR in which the TetR-coding sequence (SEQ ID NO: 14, which encodes polypeptide SEQ ID NO: 15) is replaced by a codon-optimized CymR-coding sequence (SEQ ID NO: 16, which encodes polypeptide SEQ ID NO: 17). Stable cell line generation was performed largely as described by the supplier of pcDNA^{TM}6/TR using a transient transfection-based assay to screen for cell clones capable of repressing expression of CMVTetO- or CMVCuO-driven genes. The resulting PER.C6/TetR and PER.C6/CymR cell lines were analyzed for their ability to repress transgene expression during vector replication in these cells. Experiments conducted with vectors expressing GFP-Luc under the control of operator-containing CMV-promoters showed at least a 10-fold reduction of luciferase gene expression throughout the complete virus replication cycle in the cell lines expressing the repressor corresponding to the respective operator sequences (data not shown). This confirmed that the PER.C6/TetR and PER.C6/CymR cell lines were capable of repressing vector transgene expression in the context of replicating adenovirus vectors.

The effect of TetR- and CymR-mediated repression of adenovector transgene expression on vector yields was investigated for Ad35-based vectors expressing HPVAg (Fig. 13A). To this end, PER.C6, PER.C6/TetR, and PER.C6/CymR cell lines, seeded at $3*10^5$ cells per well in 24-well plate wells, were subjected to quadruplicate infections – at 1000 virus particles per cell and for a duration of three hours – by vectors expressing HPVAg from either CMVTetO or CMVCuO promoters. As controls, parallel infections were performed with corresponding vectors expressing GFP-Luc instead of HPVAg. Four days after infection, crude viral lysates were prepared by subjecting the contents of the wells (i.e. infected cells and medium) to two freeze-thaw cycles. Adenovector titers were subsequently determined by an Ad35 hexon sequence-specific quantitative PCR-based protocol that uses a purified Ad35 vector with known virus particle titer as a standard. The results show that both the TetO- and the CuO-containing HPVAg-encoding Ad35 vectors, compared to the control vectors expressing GFP-Luc, display decreased vector yields on normal PER.C6 cells. By contrast, when produced on cells expressing their cognate repressors (i.e. TetR and CymR, respectively), these same vectors gave yields as high as those obtained with the control vectors. These data indicate that repression of

transgene expression during vector production in producer cells can be beneficial for the productivity of Ad35 vectors carrying HPVAg as a transgene.

The effect that repression of adenovector transgene expression may have on vector yields was also investigated for vectors derived from adenovirus serotype 26 (Ad26) (Fig. 13B). In an assay performed essentially as described above for the Ad35 vectors, Ad26 vectors carrying CMVTetO promoter-controlled transgenes encoding either GFP-Luc, HPVAg, or LSE2E6E7SH were used to infect PER.C6 and PER.C6/TetR cells at 1500 virus particles per cell. Three days later the infections were harvested and virus particle titers determined by an Ad26 hexon sequence-specific quantitative PCR-based method. The results show that on PER.C6 cells the yields for the vectors encoding HPVAg and LSE2E6E7SH are lower than obtained with the control vector encoding GFP-Luc. In contrast, on PER.C6/TetR cells, both these vectors showed titers that are as high as that obtained for the control vector. Together with the results above (for Ad35 vectors), these data indicate that repression of transgene expression during adenovector production increases the yields of vectors expressing HPVAg and LSE2E6E7SH.

We have observed major issues regarding the genetic stability of an adenovirus vector that carried a CMV promoter-driven transgene for HPVAg. For example, it was observed that after several passaging rounds of this vector on PER.C6 the majority of the vector population consisted of a mutant vector that carried a large deletion in the HPVAg coding sequence (data not shown).

We reasoned that employment of a transgene expression repression system, such as one of the two described above, could prevent genetic stability issues associated with transgenes, such as HPVAg that are inhibitory to vector growth. To test this, an Ad35-based vector with CMVCuO promoter-driven HPVAg expression was assessed for transgene cassette stability upon growth of the vector on either PER.C6 or PER.C6/CymR cells (Fig. 14). In brief, vector DNA was transfected into the two different cell lines and resultant viral plaques were allowed to grow under an agarose layer. From each of the two transfections, five viral plaques were isolated and separately passaged further on the same cell line (i.e. as used for the transfection), for ten consecutive viral passages. Transgene integrity was assessed by PCR amplification of the transgene cassette at viral passage number ten (VPN10), and the subsequent analysis of resultant PCR products by gel electrophoresis and Sanger sequencing. In addition, at VPN7, the passaged viral clones were assessed for their

ability to express HPVAg. This was done by using the passaged viral isolates to infect A549 cells at 1000 virus particles per cell, lysing the cells at 48 hours post infection, and subsequently analyzing the expression of HPVAg by western blotting using a monoclonal antibody directed against HPV16 E7 (Santa-Cruz Biotechnology). The results of the gel electrophoresis and sequencing analyses showed that all five viral isolates that had been passaged on PER.C6 each carried either small frameshifting deletions or premature stop mutations within the transgene cassette. By contrast, such deletions or mutations could not be detected in any of the vector isolates that had been passaged on the cell line expressing CymR (PER.C6/CymR). In agreement with these data, all PER.C6/CymR-propagated vector isolates were able to express HPVAg, while all PER.C6-grown vectors completely lost this ability, suggesting defective transgene cassettes for these vectors. In conclusion, our data demonstrate that employment of a repressor system, as for instance the CymR/CuO system, to repress vector transgene expression during vector propagation is an effective means to prevent severe transgene cassette instability, such as that seen for vectors carrying a transgene expressing HPVAg.

Example 7: construction of a designer polypeptide comprising essentially all HPV18 E6 and E7 CTL epitopes

Similar to our design for HPV16 E6 and E7, we designed a novel, non-tumorigenic polypeptide (and nucleic acid encoding such) that contains essentially all CTL epitopes of HPV18 E6 and E7 proteins, and has a minimum number of anticipated/predicted strong neo-epitopes (neo-epitopes meaning epitopes not present in the wild type HPV18 E6 and E7 proteins). The polypeptide of the invention for HPV18 (also sometimes referred to as HPV18 'E6E7SH' herein) comprises the amino acid sequence as provided in SEQ ID NO: 20. A codon-optimized nucleic acid encoding this polypeptide is provided in SEQ ID NO: 21.

The molecules of the invention for HPV18 have the same advantages as described under example 1 for HPV16. They are single molecules, which provides manufacturing advantages over strategies where multiple molecules are used. In addition, the polypeptide of the invention comprises essentially all putative CTL epitopes that are present in wild-type E6 and E7 of HPV18, and at the same time have

a minimum number of anticipated/predicted strong neo-epitopes that could potentially be immunodominant and thus divert the immune response from relevant wild-type CTL epitopes. Thus the constructs of the present invention are immunologically more favourable than molecules described by others that either lack possible CTL epitopes and/or that contain more or stronger neo-epitopes.

For instance, the HPV18 designer construct of SEQ ID NO: 20 contains only five neo-epitopes with a length of nine amino acids with a predicted binding affinity <50 nM for the 20 most common HLA-A, 20 most common HLA-B and 20 most common HLA-C alleles, as described in example 1 for the HPV16 designer construct (having SEQ ID NO: 1).

Nucleic acid encoding our thus designed HPV18 E6E7SH molecule (i.e. a polypeptide having amino acid sequence as provided in SEQ ID NO:20) was synthesized, the nucleic acid sequence comprising SEQ ID NO: 21, and flanked by a HindIII site and a Kozak sequence on the 5'end and an XbaI site on the 3'site (custom synthesis and standard molecular cloning at Invitrogen Life technologies, Germany).

The synthezised fragments were cloned using HindIII and XbaI into a standard expression vector, pCDNA2004.Neo, harbouring both a bacterial resistance marker (Ampiciline) and a mammalian resistance marker (Neomycine), to obtain plasmid vectors encoding an HPV18 designer molecule of the invention, e.g. for (transient) transfection based experiments.

These molecules could be used as such, but also as the basis for further molecules that contain additional features. As non-limiting examples, some further variants were prepared as described below.

The HPV18 E6E7SH fusion protein sequence can be combined with sequences of other HPV18 early proteins to target individuals with persistent infection and to broaden the immune repertoire in an immunized individual. As a non-limiting example of such embodiments, we prepared a sequence coding for a fusion protein of E6E7SH with E2 at its N-terminus. We mutated Glycine at position 294, Lysine at position 300 and Cysteine at position 301 of the wt HPV18 E2 protein (Genbank: AAP20597.1) into respectively Valine, Methionine and Arginine to abrogate DNA

binding activity. Each of these mutations on its own already completely abrogates the binding of E2 to DNA sequences that harbour E2 binding domains (Prakash et al., 1992, *Genes Dev* 6: 105-16).

The resulting polypeptide is referred to as HPV18 E2E6E7SH and comprises SEQ ID NO: 22. A codon-optimized sequence encoding this polypeptide was prepared and is provided in SEQ ID NO: 23.

The sequences that encode the HPV18 E6E7SH polypeptides of the invention, with or without E2, can for instance be expressed from DNA constructs, from RNA or from viral vectors. Fig. 15 demonstrates expression in HEK-293T cells upon transient transfection with DNA vectors expressing transgenes as described above. After transfection, cells were harvested and cell extracts were analyzed by SDS-PAGE and western blotting with an antibody that recognizs E6 of HPV18. This experiment demonstrates expression of the expected fusion proteins of appropriate size upon transfection of the expression vectors.

Adenoviral vectors can be used to express the E6E7, either with or without E2, and with or without additional sequences to augment the immunogenicity of the encoded fusion protein.

The genes, coding for HPV18 designer sequences described above were gene optimized for human expression and synthesized, at Geneart. A Kozak sequence (5' GCCACC 3') was included directly in front of the ATG start codon, and two stop codons (5' TGA TAA 3') were added at the end of the respective coding sequence. The genes were inserted in the pAdApt35BSU plasmid and in the pAdApt26 plasmid (Havenga *et al.*, 2006, *J Gen Virol* 87, 2135-43) via HindIII and XbaI sites.

Ad35.HPV18- E6E7SH ia a recombinant adenovirus serotype 35 (Ad35) vector comprising the codon-optimized nucleotide sequences for the expression of the HPV18 designer fusion protein variant as described above (HPV18 E6E7SH, having the amino acid sequence provided in SEQ ID NO: 20). The combined E6 and E7 sequences were placed under the control of a CMV promoter in the E1 region of the E1,E3 deleted adenovirus genome. Ad26.HPV18-E6E7SH is the equivalent vector based on recombinant adenovirus serotype 26.

Similarly, Ad26 and Ad35-based recombinant adenoviral vectors were produced that encode the HPV18 E2E6E7SH (SEQ ID NO: 22) variant.

All adenoviruses were generated, prepared, purified and stored as described in example 1 above.

Example 8. Lack of transforming activity of the HPV18 designer constructs

The E6 and E7 proteins of HPV18 have tumorigenic potential, which is apparent as transforming activity in certain assays, such as colony formation in a soft-agar assay (Massimi and Banks, 2005, *Methods Mol Med* 119: 381-395). The E6E7SH polypeptide as described in example 7 comprises the fragments of the E6 and E7 proteins in a re-ordered fashion. This is expected to remove the tumorigenic potential, as can be measured for instance by lack of transforming activity as compared to either of wt E6 and E7 proteins in such assays.

Others reported that gene-shuffled variants of HPV16 E6 and E7 have indeed lost their oncogenic potential (Öhlschläger *et al.,* 2006, *Vaccine* 24: 2880–93; Henken *et al.,* 2012, *Vaccine* 30: 4259-66), demonstrating that gene shuffling destroys the wild-type functions of HPV16 E6 and E7 proteins. In example 2, we have shown that our designer construct for HPV16 has lost its E6 and E7 activities.

To assess the loss of tumorigenic properties, we assessed the ability of our HPV18 E6E7SH construct to confer the ability to grow in soft agar upon NIH 3T3 cells (as described by e.g. Massimi and Banks, 2005, *Methods Mol Med* 119: 381-395). Transfection of NIH3T3 cells with a plasmid expressing the wild type HPV18 E7 resulted consistently in colony formation. Similar to the results obtained with HPV16 E6, expression of wild type HPV18 E6 alone did not cause colony formation above background. Transfection with our HPV18 E6E7SH construct did not lead to growth of colonies of cells in soft agar (Fig. 16) in four independent experiments, demonstrating that nucleic acids encoding a polypeptide of the invention, HPV18 E6E7SH, have lost the transforming capacity that is associated with E7.

The tumorigenic potential of E6 and E7 is associated with their ability to reduce the levels of the cellular proteins p53 and pRb respectively. p53 and pRb degradation assays were performed to demonstrate that nucleic acid encoding a polypeptide of the invention, HPV18 E6E7SH, does not have the biological activity associated with the wild-type E6 and E7 at the molecular level. In short, HPV18 E6wt

and our HPV18 E6E7SH construct were expressed in NCI-H1299 cells that lack endogenous p53 for the p53 degradation assay. For the pRb degradation assay HPV18 E7wt and the HPV18 E6E7SH construct were expressed in pRb null Saos-2 cells. As can be seen in Fig. 17, co-expression of p53 with HPV18 E6wt, but not with HPV18 E6E7SH, leads to reduced p53 levels (panels A and B). Likewise, panels 17C,D show that co-expression of pRb with HPV18 E7wt, but not with HPV18 E6E7SH, leads to reduced pRB levels. These data demonstrate that nucleic acid encoding an HPV18 designer polypeptide of the invention has no ability to form colonies in soft agar and does not contain main biological activities of the wild-type HPV18 E6 and E7 polypeptides, namely the inactivation of p53 and pRb respectively.

To further demonstrate the safety of nucleic acid constructs encoding polypeptide of the invention, we made use of primary human genital keratinocytes derived from neonatal foreskin (HEKn cells) that closely resemble the natural target cells for HPV mediated transformation. Immortalization of primary human keratinocytes requires the action of both E6 and E7 wild-type (Munger *et al.,* 1989, *J Virol* 63: 4417-21). This assay is probably the physiologically most relevant *in vitro* assay to demonstrate the safety of our constructs (Massimi and Banks, 2005, *Methods Mol Med* 119: 381-395). Cells transduced with lentiviruses expressing wild type E6 and E7 from HPV18 (E6E7wt) induce immortalization in primary keratinocytes as indicated by the extension of their lifespan as compared to non-transduced control cells (Fig. 18) and activation of hTERT, the catalytic subunit of telomerase (data not shown). Expression of the HPV18 designer polypeptide of the invention (HPV18 E6E7SH) is not able to to extend the lifespan compared to GFP-transduced or non-transduced keratinocytes. A similar result was obtained in two additional independent donors (data not shown). Taken together these data demonstrate that our constructs have lost the ability to induce immortalization in primary human keratinocytes, that are considered a highly physiological model.

All together the experiments in this example provide strong evidence of the lack of transforming activity of nucleic acids encoding polypeptides according to the invention, and thus a strongly improved safety over HPV18 E6 and E7 wt constructs.

Comparative example 8A. The constructs of the invention have unique properties

A further HPV18 designer construct had been prepared (referred to herein as 'HPV18DC2'; the amino acid sequence of this construct is provided as SEQ ID NO: 24). HPV18DC2 has the following features in common with the HPV18 E6E7SH (SEQ ID NO: 20) construct of the invention: (a) it also contains virtually the complete E6 and E7 amino acid sequences of HPV18, (b) in the form of the same number of re-ordered fragments, (c) which fragments are partly overlapping such that essentially all T-cell epitopes of HPV18 E6 and E7 are present, and (d) it was designed to minimize the introduction of undesired strong neo-epitopes. The designer construct of the invention and HPV18DC2 therefore structurally only differ in the exact amino acid sequence.

However, this does translate into at least one biologic difference, which demonstrates that such molecules cannot be considered mere alternatives that could be substituted for each other.

In particular, the molecule of the invention was entirely devoid of measurable functional activity in extending lifespan of primary foreskin keratinocytes, as shown above (example 8). In contrast, HPV18DC2 did induce an extended life span in primary keratinocytes. For example, according to the experiment described in example 8, cells of a donor expressing the HPV18 E6E7SH construct of the invention had a life span of 81 days in which they had 7 passage doublings, whereas in comparison cells with HPV18DC2 had a longer life span of 120 days in which they had 67 passage doublings. Similar differences were found in independent assays in keratinocytes from different donors (in an average from 3 donors, cells with the construct of the invention had a lifespan of 62 days with 9 passage doublings, whereas cells with HPV18DC2 had a much longer lifespan of 156 days with 62 passage doublings), showing the differences resulted from the difference between the constructs. In line with this observation that life span was extended by HPV18DC2, cells transduced with HPV18DC2 displayed some residual E7 activity (i.e. pRb degradation/p16 upregulation), in contrast to cells transduced with the HPV18 E6E7SH molecule of the invention that lacked detectable activity in these assays (example 8).

Similar observations were made with an alternative HPV16 designer construct when compared to the HPV16 designer construct of the invention (as referred to in example 2).

The observed differences between seemingly highly similar molecules in a biologic model system demonstrate that such molecules cannot be considered mere alternatives that could be substituted for each other. This underscores the uniqueness of the designer molecules of the invention, and the unpredictability in this field.

Importantly, it can be concluded from the experiments in examples 2 and 8 that the designer molecules of the invention have lost the oncogenic activities of wild-type HPV16/18 E6 and E7 proteins in the used model systems.

Example 9. Immune responses to the HPV18 E6E7SH designer constructs

We have prepared DNA vectors and adenoviral vectors, as described in example 7. To evaluate the vaccine induced immunogenicity, CB6F1 mice were immunized with adenovectors (Ad35) expressing HPV18 E6E7SH or E2E6E7SH, or with adenovectors not encoding a transgene (Empty) as controls. Two weeks after the prime immunization the mice were sacrificed and isolated splenocytes were stimulated overnight with an HPV18 E6 15mer peptide pool. E6-specific immune responses were analyzed by intracellular cytokine staining. In a separate experiment, CB6F1 mice were immunized with adenovectors (Ad35 or Ad26) expressing HPV18 E2E6E7SH or with adenovectors not encoding a transgene (Empty) as control.

Fig. 19A shows that immunization of mice with Ad35.HPV18-E6E7SH induces E6-specific immune responses as measured by ICS analysis. In addition, the results in Fig. 19A demonstrate that fusion of E2 to the N-terminus of the designer construct does not decrease the immunogenicity, despite the lower expression of this E2E6E7 variant that was observed upon transfection (Fig. 15). Fig.19B shows that immunization of mice with Ad35.HPV18-E6E7SH or Ad26.HPV18-E2E6E7SH induces comparable percentage of IFNγ-producing HPV18-E6 specific CD8 T-cells.

The cellular immune response against the peptide of the invention can be induced with different types of adenoviral vectors. In the experiment presented in Fig. 19B, mice were immunized with either Ad26 or Ad35 adenoviral vectors expressing HPV18 E2E6E7SH. The data show that these adenoviral vectors induced HPV18 E6-specific T-cells to similar levels.

Example 10. Combining adenoviral vectors expressing HPV16 and HPV18 designer constructs.

Combining designer constructs for different HPV types offers the possibility to make a treatment vaccine for different HPV types. To evaluate the ability of the adenoviral vectors expressing different designer sequences to induce immune responses, mice were immunized by intramuscular injection with the adenovectors (Ad26) expressing HPV16 E2E6E7SH (encoding protein comprising amino acid sequence set forth in SEQ ID NO: 3) and with Ad26 expressing HPV18 E2E6E7SH (encoding protein comprising amino acid sequence set forth in SEQ ID NO: 22) with a dose of $1*10^{10}$ vp for each vector or adenovectors not encoding a transgene (Empty). Four weeks after the immunization the immune responses were boosted by immunization with Ad35 vectors expressing the same antigens. Immune responses were measured two weeks after the boost immunization. Cells were stimulated overnight with peptide pools corresponding to E6 of HPV18 or E7 of HPV16 and responses were measured by IFNγ ELISPOT. The data are presented in Fig. 20.

The data show that immunization of mice with Ad26/35 vectors expressing HPV16 E2E6E7SH and HPV18 E2E6E7SH resulted in cellular immune responses against both (i.e. HPV16 and HPV18) designer proteins.

In an independent experiment with a similar immunization schedule (Ad26 prime and Ad35 boost) we compared the immune response induced by Ad expressing HPV16 E2E6E7SH and Ad expressing HPV18 E2E6E7SH together to that induced in mice immunized with Ad expressing HPV16 E2E6E7SH alone or Ad expressing HPV18 E2E6E7SH alone. Immune responses were measured two weeks after boost immunization, and cells were stimulated overnight with peptide pools corresponding to E2, E6 or E7 of HPV16 and HPV18 and the responses were measured by IFNγ ELISPOT as well as intracellular cytokine staining. Although co-administration in a single composition of Ad expressing HPV16 E2E6E7SH and Ad expressing HPV18 E2E6E7SH did result in an overall lower magnitude of CD4 and CD8 responses as compared to animals that were only immunized with the individual vaccine components, the co-administration induced a similar breadth of the immune responses (data not shown).

Co-administration of HPV16 E2E6E7SH and HPV18 E2E6E7SH expressing constructs according to the invention is thus possible to induce cellular immune responses to both HPV16 and HPV18.

Example 11. Immunogenicity of combined designer constructs in rhesus macaques.

To evaluate the ability of the adenoviral vectors expressing the designer sequences of the invention to induce immune responses in non-human primates, rhesus macaques were immunized by intramuscular injection with the mix of two separate adenovectors as in the previous example, i.e. Ad26 vectors together expressing HPV16 and HPV18 E2E6E7SH, at a dose of $1*10^{10}$ vp for each vector, or adenovectors not encoding a transgene (Empty). Eight weeks after the immunization, animals received a boost immunization with Ad26 vectors expressing the same antigens. At week 16 the animals received one more injection with the Ad35 vectors expressing the same antigens. Blood samples were taken at several time points and isolated white blood cells were stimulated overnight with peptide pools corresponding to E2, E6 or E7 for both HPV16 and HPV18. Specific responses were measured by IFNγ ELISPOT. The data are presented in Fig. 21. In addition at week 10 and week 18 post prime immunization, the cellular immune response specific to peptides spanning the novel junctions in the HPV18 designer molecules of the invention was evaluated. The induction of IFNγ response against these junctional peptides was in all animals below the limit of detection of < 50 SFU per $1*10^6$ PBMC (data not shown).

The data show that immunization of non-human primates with a combination of Ad26 vectors together expressing HPV16 E2E6E7SH and HPV18 E2E6E7SH resulted in cellular immune responses against several of the HPV proteins that are present in the encoded transgenes. Responses could be boosted by the additional immunization with Ad26 vectors. The additional boost immunization at week 16 with the corresponsing Ad35 vector further increased the immune responses.

Example 12. Therapeutic efficacy of combined constructs in a mouse tumor model.

The polypeptide of the invention corresponding to HPV16 E6 and E7 is capable of inducing cellular immune responses in mice that will lead to a therapeutic effect in the TC-1 model (as shown in example 5). The therapeutic effect of a combination of adenoviral vectors together expressing both HPV16 and HPV18 designer proteins was tested in this same model. Without vaccine the tumors grew rapidly and reach a pre-determined size of 1000 mm^3 within 30 days at which point the mice were sacrificed for ethical reasons.

In this experiment, prime-boost immunizations with adenoviral vectors expressing HPV16 E2E6E7SH prolonged the survival of the mice significantly (Fig 22). With a combination of adenoviral vectors together expressing both HPV16 E2E6E7SH and HPV18 E2E6E7SH, a similar mean survival time was observed. In the group of mice that received the combination vaccine, three animals were tumor free at the end of the monitoring period of 90 days. In conclusion, immunization with a combination of adenoviral vectors together expressing HPV16- and HPV18-specific designer polypeptides of the invention significantly reduced tumor growth or completely eradicated established tumors in a well-established challenge model for HPV16-induced cancer.

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Table I. sequences

SEQ ID NO: 1 (HPV16-E6E7SH, amino acid sequence of HPV16 E6/E7 designer polypeptide)

MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLEDEIDG
PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV
CPICSQKPGT TLEQQYNKPL CDLLIRCINC QKPLCPEEKQ RHLDKKQRFH
NIRGRWTGRC MSCCRSSRTR RETQMHGDTP TLHEYMLDLQ PETTDLYCYE
QLNDSSEEED EIDGPAGQAE PDRAHYNIVT FCCQLCTELQ TTIHDIILEC
VYCKQQLLRR EVYDFAFRDL CIVYRDGNPY AVCDKCLKFY SKISEYRHYC
YSLYGTTLEQ QYNKPLCDLL IRCINCQK

SEQ ID NO: 2 (HPV16-E6E7SH, nucleotide sequence encoding amino acid sequence of HPV16 E6/E7 designer polypeptide)

ATGCACCAGA AACGGACCGC CATGTTCCAG GACCCCCAGG AACGGCCCAG AAAGCTGCCC
CAGCTGTGCA CCGAGCTGCA GACCACCATC CACGACATCA TCCTGGAATG CGTGTACTGC
AAGCAGCAGC TGGAAGATGA GATCGACGGC CCTGCTGGCC AGGCCGAACC CGACAGAGCC
CACTACAATA TCGTGACCTT CTGCTGCAAG TGCGACAGCA CCCTGCGGCT GTGCGTGCAG
AGCACCCACG TGGACATCCG GACCCTGGAA GATCTGCTGA TGGGCACCCT GGGCATCGTG
TGCCCCATCT GCAGCCAGAA GCCCGGCACC ACCCTGGAAC AGCAGTACAA CAAGCCCCTG
TGCGACCTGC TGATCCGGTG CATCAACTGC CAGAAACCCC TGTGCCCCGA GGAAAAGCAG
CGGCACCTGG ACAAGAAGCA GCGGTTCCAC AACATCCGGG GCAGATGGAC AGGCAGATGC
ATGAGCTGCT GCAGAAGCAG CCGGACCAGA CGGGAAACCC AGATGCACGG CGACACCCCC
ACCCTGCACG AGTACATGCT GGACCTGCAG CCCGAGACAA CCGACCTGTA CTGCTACGAG
CAGCTGAACG ACAGCAGCGA GGAAGAGGAC GAGATTGACG GACCCGCTGG ACAGGCCGAG
CCTGACCGGG CTCACTATAA CATCGTGACA TTTTGCTGTC AGCTCTGTAC TGAACTCCAG
ACAACAATTC ACGATATTAT TCTCGAATGT GTGTATTGTA AACAGCAGCT CCTGCGGAGA
GAGGTGTACG ACTTCGCCTT CCGGGACCTC TGCATCGTGT ATCGGGACGG CAACCCCTAC
GCCGTGTGCG ACAAGTGCCT GAAGTTCTAC AGCAAGATCA GCGAGTACCG GCACTACTGC
TACAGCCTGT ACGGAACAAC ACTCGAACAG CAGTATAACA AACCACTCTG TGATCTGCTG
ATTCGCTGTA TCAATTGTCA GAAGTGATAA

SEQ ID NO: 3 (HPV16 E2E6E7SH, amino acid sequence of HPV16 E2/E6/E7 designer polypeptide)

METLCQRLNVCQDKILTHYENDSTDLRDHIDYWKHMRLECAIYYKAREMGFKHINHQVVPTL
AVSKNKALQAIELQLTLETIYNSQYSNEKWTLQDVSLEVYLTAPTGCIKKHGYTVEVQFDGD
ICNTMHYTNWTHIYICEEASVTVVEGQVDYYGLYYVHEGIRTYFVQFKDDAEKYSKNKVWEV
HAGGQVILCPTSVFSSNEVSSPEIIRQHLANHPAATHTKAVALGTEETQTTIQRPRSEPDTG
NPCHTTKLLHRDSVDSAPILTAFNSSHKGRINCNSNTTPIVHLKVDANTLMRLRYRFKKHCT
LYTAVSSTWHWTGHNVKHKSAIVTLTYDSEWQRDQFLSQVKIPKTITVSTGFMSIMHQKRTA
MFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQQLEDEIDGPAGQAEPDRAHYNIVTFCC
KCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKPGTTLEQQYNKPLCDLLIRCINCQ
KPLCPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRETQMHGDTPTLHEYMLDLQPET
TDLYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCQLCTELQTTIHDIILECVYCKQ
QLLRREVYDFAFRDLCIVYRDGNPYAVCDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLC
DLLIRCINCQK

SEQ ID NO: 4 (HPV16 E2E6E7SH, nucleotide sequence encoding HPV16 E2/E6/E7 designer polypeptide)

ATGGAAACCCTGTGCCAGCGGCTGAACGTGTGCCAGGACAAGATCCTGACCCACTACGAGAA
CGACAGCACCGACCTGCGGGACCACATCGACTACTGGAAGCACATGCGGCTGGAATGCGCCA
TCTACTACAAGGCCAGAGAGATGGGCTTCAAGCACATCAACCACCAGGTGGTGCCCACCCTG
GCCGTGTCCAAGAACAAGGCCCTGCAGGCCATCGAGCTGCAGCTGACCCTGGAAACCATCTA
CAACAGCCAGTACAGCAACGAGAAGTGGACCCTGCAGGACGTGTCCCTGGAAGTGTACCTGA
CCGCTCCCACCGGCTGCATCAAGAAACACGGCTACACCGTGGAAGTGCAGTTCGACGGCGAC
ATCTGCAACACCATGCACTACACCAACTGGACCCACATCTACATCTGCGAAGAGGCCAGCGT
GACCGTGGTGGAAGGCCAGGTGGACTACTACGGCCTGTACTACGTGCACGAGGGCATCCGGA
CCTACTTCGTGCAGTTCAAGGACGACGCCGAGAAGTACAGCAAGAACAAAGTGTGGGAGGTG
CACGCTGGCGGCCAGGTCATCCTGTGCCCCACCAGCGTGTTCAGCAGCAACGAGGTGTCCAG
CCCCGAGATCATCCGGCAGCACCTGGCCAATCACCCTGCCGCCACCCACACAAAGGCCGTGG
CCCTGGGCACCGAGGAAACCCAGACCACCATCCAGCGGCCCAGAAGCGAGCCCGACACCGGC
AATCCCTGCCACACCACCAAGCTGCTGCACCGGGACAGCGTGGACAGCGCCCCTATCCTGAC
CGCCTTCAACAGCAGCCACAAGGGCCGGATCAACTGCAACAGCAACACCACCCCCATCGTGC
ACCTGAAGGTGGACGCCAACACCCTGATGCGGCTGCGGTACAGATTCAAGAAGCACTGCACC
CTGTACACCGCCGTGTCCTCCACCTGGCACTGGACCGGCCACAACGTGAAGCACAAGAGCGC
CATCGTGACCCTGACCTACGACAGCGAGTGGCAGCGGGACCAGTTCCTGAGCCAGGTCAAAA
TCCCCAAGACCATCACCGTGTCCACCGGCTTCATGAGCATCATGCACCAGAAACGGACCGCC
ATGTTCCAGGACCCCCAGGAACGGCCCAGAAAGCTGCCCCAGCTGTGCACCGAGCTGCAGAC

CACCATCCACGACATCATCCTGGAATGCGTGTACTGCAAGCAGCAGCTGGAAGATGAGATCG
ACGGCCCTGCTGGCCAGGCCGAACCCGACAGAGCCCACTACAATATCGTGACCTTCTGCTGC
AAGTGCGACAGCACCCTGCGGCTGTGCGTGCAGAGCACCCACGTGGACATCCGGACCCTGGA
AGATCTGCTGATGGGCACCCTGGGCATCGTGTGCCCCATCTGCAGCCAGAAGCCCGGCACCA
CCCTGGAACAGCAGTACAACAAGCCCCTGTGCGACCTGCTGATCCGGTGCATCAACTGCCAG
AAACCCCTGTGCCCCGAGGAAAAGCAGCGGCACCTGGACAAGAAGCAGCGGTTCCACAACAT
CCGGGGCAGATGGACAGGCAGATGCATGAGCTGCTGCAGAAGCAGCCGGACCAGACGGGAAA
CCCAGATGCACGGCGACACCCCCACCCTGCACGAGTACATGCTGGACCTGCAGCCCGAGACA
ACCGACCTGTACTGCTACGAGCAGCTGAACGACAGCAGCGAGGAAGAGGACGAGATTGACGG
ACCCGCTGGACAGGCCGAGCCTGACCGGGCTCACTATAACATCGTGACATTTTGCTGTCAGC
TCTGTACTGAACTCCAGACAACAATTCACGATATTATTCTCGAATGTGTGTATTGTAAACAG
CAGCTCCTGCGGAGAGAGGTGTACGACTTCGCCTTCCGGGACCTCTGCATCGTGTATCGGGA
CGGCAACCCCTACGCCGTGTGCGACAAGTGCCTGAAGTTCTACAGCAAGATCAGCGAGTACC
GGCACTACTGCTACAGCCTGTACGGAACAACACTCGAACAGCAGTATAACAAACCACTCTGT
GATCTGCTGATTCGCTGTATCAATTGTCAGAAGTGATAA

SEQ ID NO: 5 (HPV16 E6E7E2SH, amino acid sequence encoding HPV16 E6/E7/E2 designer polypeptide

MHQKRTAMFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQQLEDEIDGPAGQAEPDRAHY
NIVTFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKPGTTLEQQYNKPLCDLL
IRCINCQKPLCPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRETQMHGDTPTLHEYM
LDLQPETTDLYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCQLCTELQTTIHDIIL
ECVYCKQQLLRREVYDFAFRDLCIVYRDGNPYAVCDKCLKFYSKISEYRHYCYSLYGTTLEQ
QYNKPLCDLLIRCINCQKMETLCQRLNVCQDKILTHYENDSTDLRDHIDYWKHMRLECAIYY
KAREMGFKHINHQVVPTLAVSKNKALQAIELQLTLETIYNSQYSNEKWTLQDVSLEVYLTAP
TGCIKKHGYTVEVQFDGDICNTMHYTNWTHIYICEEASVTVVEGQVDYYGLYYVHEGIRTYF
VQFKDDAEKYSKNKVWEVHAGGQVILCPTSVFSSNEVSSPEIIRQHLANHPAATHTKAVALG
TEETQTTIQRPRSEPDTGNPCHTTKLLHRDSVDSAPILTAFNSSHKGRINCNSNTTPIVHLK
VDANTLMRLRYRFKKHCTLYTAVSSTWHWTGHNVKHKSAIVTLTYDSEWQRDQFLSQVKIPK
TITVSTGFMSI

SEQ ID NO: 6 (HPV16 E6E7E2SH, nucleotide sequence encoding HPV16 E6/E7/E2 designer polypeptide

ATGCACCAGAAACGGACCGCCATGTTCCAGGACCCCCAGGAACGGCCCAGAAAGCTGCCCCA
GCTGTGCACCGAGCTGCAGACCACCATCCACGACATCATCCTGGAATGCGTGTACTGCAAGC
AGCAGCTGGAAGATGAGATCGACGGCCCTGCTGGCCAGGCCGAACCCGACAGAGCCCACTAC

AATATCGTGACCTTCTGCTGCAAGTGCGACAGCACCCTGCGGCTGTGCGTGCAGAGCACCCA
CGTGGACATCCGGACCCTGGAAGATCTGCTGATGGGCACCCTGGGCATCGTGTGCCCCATCT
GCAGCCAGAAGCCCGGCACCACCCTGGAACAGCAGTACAACAAGCCCCTGTGCGACCTGCTG
ATCCGGTGCATCAACTGCCAGAAACCCCTGTGCCCCGAGGAAAAGCAGCGGCACCTGGACAA
GAAGCAGCGGTTCCACAACATCCGGGGCAGATGGACAGGCAGATGCATGAGCTGCTGCAGAA
GCAGCCGGACCAGACGGGAAACCCAGATGCACGGCGACACCCCCACCCTGCACGAGTACATG
CTGGACCTGCAGCCCGAGACAACCGACCTGTACTGCTACGAGCAGCTGAACGACAGCAGCGA
GGAAGAGGACGAGATTGACGGACCCGCTGGACAGGCCGAGCCTGACCGGGCTCACTATAACA
TCGTGACATTTTGCTGTCAGCTCTGTACTGAACTCCAGACAACAATTCACGATATTATTCTC
GAATGTGTGTATTGTAAACAGCAGCTCCTGCGGAGAGAGGTGTACGACTTCGCCTTCCGGGA
CCTCTGCATCGTGTATCGGGACGGCAACCCCTACGCCGTGTGCGACAAGTGCCTGAAGTTCT
ACAGCAAGATCAGCGAGTACCGGCACTACTGCTACAGCCTGTACGGAACAACACTCGAACAG
CAGTATAACAAACCACTCTGTGATCTGCTGATTCGCTGTATCAATTGTCAGAAGATGGAAAC
CCTGTGCCAGCGGCTGAACGTGTGCCAGGACAAGATCCTGACCCACTACGAGAACGACAGCA
CCGACCTGCGGGACCACATCGACTACTGGAAGCACATGCGGCTGGAATGCGCCATCTACTAC
AAGGCCAGAGAGATGGGCTTCAAGCACATCAACCACCAGGTGGTGCCCACCCTGGCCGTGTC
CAAGAACAAGGCCCTGCAGGCCATCGAGCTGCAGCTGACCCTGGAAACCATCTACAACAGCC
AGTACAGCAACGAGAAGTGGACCCTGCAGGACGTGTCCCTGGAAGTGTACCTGACCGCTCCC
ACCGGCTGCATCAAGAAACACGGCTACACCGTGGAAGTGCAGTTCGACGGCGACATCTGCAA
CACCATGCACTACACCAACTGGACCCACATCTACATCTGCGAAGAGGCCAGCGTGACCGTGG
TGGAAGGCCAGGTGGACTACTACGGCCTGTACTACGTGCACGAGGGCATCCGGACCTACTTC
GTGCAGTTCAAGGACGACGCCGAGAAGTACAGCAAGAACAAAGTGTGGGAGGTGCACGCTGG
CGGCCAGGTCATCCTGTGCCCCACCAGCGTGTTCAGCAGCAACGAGGTGTCCAGCCCCGAGA
TCATCCGGCAGCACCTGGCCAATCACCCTGCCGCCACCCACACAAAGGCCGTGGCCCTGGGC
ACCGAGGAAACCCAGACCACCATCCAGCGGCCCAGAAGCGAGCCCGACACCGGCAATCCCTG
CCACACCACCAAGCTGCTGCACCGGGACAGCGTGGACAGCGCCCCTATCCTGACCGCCTTCA
ACAGCAGCCACAAGGGCCGGATCAACTGCAACAGCAACACCACCCCCATCGTGCACCTGAAG
GTGGACGCCAACACCCTGATGCGGCTGCGGTACAGATTCAAGAAGCACTGCACCCTGTACAC
CGCCGTGTCCTCCACCTGGCACTGGACCGGCCACAACGTGAAGCACAAGAGCGCCATCGTGA
CCCTGACCTACGACAGCGAGTGGCAGCGGGACCAGTTCCTGAGCCAGGTCAAAATCCCCAAG
ACCATCACCGTGTCCACCGGCTTCATGAGCATCTGATAA

SEQ ID NO: 7 (IgE leader peptide amino acid sequence)

MDWTWILFLVAAATRVHS

SEQ ID NO: 8 (nucleotide sequence encoding IgE leader peptide)

ATGGACTGGACCTGGATCCTGTTCCTGGTGGCTGCCGCAACCCGGGTGCACAGC

SEQ ID NO: 9 (aa HAVT20 leader peptide amino acid sequence)
MACPGFLWALVISTCLEFSMA

SEQ ID NO: 10 (nucleotide sequence encoding HAVT20 leader peptide)
ATGGCCTGCCCCGGCTTTCTGTGGGCCCTGGTCATCAGCACCTGTCTGGAATTCAGCATGGC
C

SEQ ID NO: 11 (2xTetO-containing sequence)
GAGCTCTCCCTATCAGTGATAGAGATCTCCCTATCAGTGATAGAGATCGTCGAC

SEQ ID NO: 12 (CuO-containing sequence)
AACAAACAGACAATCTGGTCTGTTTGTA

SEQ ID NO: 13 (CMV promoter present in pAdApt26 and pAdApt35 plasmids)
TCAATATTGGCCATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATT
GGCCATTGCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACA
TTACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATT
AGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCT
GACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA
ATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTA
TTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCG
GTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGC
ACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGC
GGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGC
CTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCC
GCGGCCGGGAACGGTGCATTGGA

SEQ ID NO: 14 (TetR, nucleotide sequence encoding amino acid sequence of TetR polypeptide expressed by pcDNA™6/TR)
ATGTCTAGATTAGATAAAAGTAAAGTGATTAACAGCGCATTAGAGCTGCTTAATGAGGTCGG
AATCGAAGGTTTAACAACCCGTAAACTCGCCCAGAAGCTAGGTGTAGAGCAGCCTACATTGT
ATTGGCATGTAAAAAATAAGCGGGCTTTGCTCGACGCCTTAGCCATTGAGATGTTAGATAGG
CACCATACTCACTTTTGCCCTTTAGAAGGGGAAAGCTGGCAAGATTTTTTACGTAATAACGC
TAAAAGTTTTAGATGTGCTTTACTAAGTCATCGCGATGGAGCAAAAGTACATTTAGGTACAC
GGCCTACAGAAAAACAGTATGAAACTCTCGAAAATCAATTAGCCTTTTTATGCCAACAAGGT

TTTTCACTAGAGAATGCATTATATGCACTCAGCGCTGTGGGGCATTTTACTTTAGGTTGCGT
ATTGGAAGATCAAGAGCATCAAGTCGCTAAAGAAGAAAGGGAAACACCTACTACTGATAGTA
TGCCGCCATTATTACGACAAGCTATCGAATTATTTGATCACCAAGGTGCAGAGCCAGCCTTC
TTATTCGGCCTTGAATTGATCATATGCGGATTAGAAAAACAACTTAAATGTGAAAGTGGGTC
CGCGTACAGCGGATCCCGGGAATTCAGATCTTATTAA

SEQ ID NO: 15 (TetR, amino acid sequence of TetR polypeptide expressed by pcDNA™6/TR)

MSRLDKSKVINSALELLNEVGIEGLTTRKLAQKLGVEQPTLYWHVKNKRALLDALAIEMLDR
HHTHFCPLEGESWQDFLRNNAKSFRCALLSHRDGAKVHLGTRPTEKQYETLENQLAFLCQQG
FSLENALYALSAVGHFTLGCVLEDQEHQVAKEERETPTTDSMPPLLRQAIELFDHQGAEPAF
LFGLELIICGLEKQLKCESGSAYSGSREFRSY

SEQ ID NO: 16 (CymR, nucleotide sequence encoding amino acid sequence of CymR polypeptide)

ATGTCTCCCAAACGACGGACTCAAGCGGAAAGGGCAATGGAAACTCAGGGTAAGCTGATTGC
CGCGGCTCTGGGAGTGCTGCGAGAGAAAGGGTATGCCGGGTTTCGCATAGCCGACGTTCCTG
GAGCTGCAGGCGTAAGCAGAGGAGCCCAATCTCATCACTTTCCGACCAAGCTGGAGCTTTTG
CTGGCTACCTTCGAATGGCTGTACGAGCAGATCACGGAAAGGAGTCGTGCTAGGCTGGCCAA
GCTGAAACCCGAGGATGATGTCATTCAGCAGATGCTGGACGATGCAGCCGAGTTCTTCCTGG
ACGACGACTTCAGCATCAGTCTCGACCTCATCGTAGCCGCAGATCGCGATCCAGCTTTGCGC
GAGGGCATACAGAGAACAGTCGAGCGGAATCGGTTTGTGGTGGAGGACATGTGGCTTGGTGT
TCTGGTGAGCAGAGGCCTCTCACGGGATGATGCCGAGGACATCCTGTGGCTGATCTTTAACT
CCGTCAGAGGGTTGGCAGTGAGGTCCCTTTGGCAGAAGGACAAAGAACGGTTTGAACGTGTG
CGAAACTCAACACTCGAGATTGCTAGGGAACGCTACGCCAAGTTCAAGAGATGA

SEQ ID NO: 17 (CymR, amino acid sequence of CymR polypeptide)

MSPKRRTQAERAMETQGKLIAAALGVLREKGYAGFRIADVPGAAGVSRGAQSHHFPTKLELL
LATFEWLYEQITERSRARLAKLKPEDDVIQQMLDDAAEFFLDDDFSISLDLIVAADRDPALR
EGIQRTVERNRFVVEDMWLGVLVSRGLSRDDAEDILWLIFNSVRGLAVRSLWQKDKERFERV
RNSTLEIARERYAKFKR

SEQ ID NO: 18 (HPV16 E6, aa41-65)

KQQLLRREVYDFAFRDLCIVYRDGN

SEQ ID NO: 19 (HPV16 E7 aa 43-77)

GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR

SEQ ID NO: 20 (amino acid sequences of HPV18-E6E7SH designer sequence)

MARFEDPTRRPYKLPDLCTELNTSLQDIEITCVYCKTVLDLLCHEQLSDSEEENDEIDGVNH
QHLPARRAEPQRHTMLCMCCKCEARIELVVESSADDLRAFQQLFLNTLSFVCPWCASQHYSD
SVYGDTLEKLTNTGLYNLLIRCLRCQKPLNPAEKLRHLNEKRRFHNIAGHYRGQCHSCCNRA
RQERLQRRRETMHGPKATLQDIVLHLEPQNEIPVDLLCHEQLSDSEEENDEIDGVNPDLCTE
LNTSLQDIEITCVYCKTVLELTEVFEFAFKDLFVVYRDSIPHAACHKCIDFYSRIRELRHYS
DSVYGDTLEKLTNTGLYNLLI

SEQ ID NO: 21 (nucleotide sequences of HPV18-E6E7SH designer sequence)

ATGGCCAGATTCGAGGACCCCACCAGACGGCCCTACAAGCTGCCCGACCTGTGCACCGAGCT
GAACACATCTCTGCAGGACATCGAGATCACATGCGTGTACTGCAAGACCGTGCTGGACCTGC
TGTGCCACGAGCAGCTGTCCGACTCCGAGGAAGAAAACGACGAGATCGACGGCGTGAACCAT
CAGCATCTGCCCGCCAGACGGGCCGAGCCCCAGAGACACACCATGCTGTGCATGTGCTGCAA
GTGCGAGGCCCGGATTGAGCTGGTGGTGGAAAGCAGCGCCGACGACCTGCGGGCCTTCCAGC
AGCTCTTTCTGAATACCCTGAGCTTCGTGTGCCCTTGGTGCGCCAGCCAGCACTACAGCGAC
TCCGTGTACGGCGATACCCTGGAAAAGCTGACCAATACCGGCCTGTATAACCTGCTGATCCG
GTGCCTGCGGTGCCAGAAGCCCCTGAATCCCGCCGAGAAACTGAGACACCTGAACGAGAAGC
GGCGGTTCCACAATATCGCCGGCCACTACAGAGGCCAGTGCCACAGCTGCTGCAACCGGGCC
AGACAGGAACGGCTGCAGCGGAGGCGGGAAACCATGCACGGACCCAAGGCCACCCTCCAGGA
CATTGTCCTGCACCTGGAACCCCAGAACGAGATCCCCGTCGATCTGCTGTGTCATGAACAGC
TCAGCGACAGCGAAGAGGAAAATGACGAAATTGACGGGGTCAACCCTGACCTCTGTACCGAA
CTCAATACCAGTCTCCAGGATATCGAAATTACCTGTGTCTACTGTAAAACCGTCCTCGAGCT
GACCGAGGTGTTCGAGTTCGCCTTCAAGGACCTGTTTGTGGTGTACAGAGACAGCATCCCCC
ACGCCGCCTGCCACAAGTGCATCGACTTCTACAGCCGGATCAGAGAGCTGCGGCACTACTCC
GATTCTGTGTATGGCGACACACTCGAGAAGCTCACAAACACAGGACTGTACAATCTGCTCAT
CTGATAA

SEQ ID NO: 22 (amino acid sequences of HPV18-E2E6E7SH designer sequence)

MQTPKETLSERLSALQDKIIDHYENDSKDIDSQIQYWQLIRWENAIFFAAREHGIQTLNHQV
VPAYNISKSKAHKAIELQMALQGLAQSAYKTEDWTLQDTCEELWNTEPTHCFKKGGQTVQVY
FDGNKDNCMTYVAWDSVYYMTDAGTWDKTATCVSHRGLYYVKEGYNTFYIEFKSECEKYGNT
GTWEVHFGNNVIDCNDSMCSTSDDTVSATQLVKQLQHTPSPYSSTVSVGTAKTYGQTSAATR
PGHCGLAEKQHCGPVNPLLGAATPTGNNKRRKLCSGNTTPIIHLKVDRNSLMRLRYRLRKHS
DHYRDISSTWHWTGAGNEKTGILTVTYHSETQRTKFLNTVAIPDSVQILVGYMTMMARFEDP

TRRPYKLPDLCTELNTSLQDIEITCVYCKTVLDLLCHEQLSDSEEENDEIDGVNHQHLPARR
AEPQRHTMLCMCCKCEARIELVVESSADDLRAFQQLFLNTLSFVCPWCASQHYSDSVYGDTL
EKLTNTGLYNLLIRCLRCQKPLNPAEKLRHLNEKRRFHNIAGHYRGQCHSCCNRARQERLQR
RRETMHGPKATLQDIVLHLEPQNEIPVDLLCHEQLSDSEEENDEIDGVNPDLCTELNTSLQD
IEITCVYCKTVLELTEVFEFAFKDLFVVYRDSIPHAACHKCIDFYSRIRELRHYSDSVYGDT
LEKLTNTGLYNLLI*

SEQ ID NO: 23 (nucleotide sequences of HPV18-E2E6E7SH designer sequence)

ATGCAGACCCCCAAAGAGACACTGAGCGAGCGGCTGAGCGCCCTGCAGGACAAGATCATCGA
CCACTACGAGAACGACAGCAAGGACATCGACAGCCAGATCCAGTACTGGCAGCTGATCAGAT
GGGAGAACGCCATCTTCTTCGCCGCCAGAGAGCACGGCATCCAGACCCTGAACCACCAGGTG
GTGCCCGCCTACAACATCAGCAAGAGCAAGGCCCACAAGGCTATCGAGCTGCAGATGGCCCT
GCAGGGACTGGCCCAGAGCGCCTACAAGACCGAGGACTGGACCCTGCAGGATACCTGCGAGG
AACTGTGGAACACCGAGCCCACCCACTGCTTCAAGAAAGGCGGCCAGACCGTGCAGGTGTAC
TTCGACGGCAACAAGGACAACTGCATGACCTACGTGGCCTGGGACAGCGTGTACTACATGAC
CGACGCCGGCACCTGGGACAAGACCGCCACCTGTGTGTCCCACCGGGGCCTGTACTACGTGA
AAGAGGGCTACAACACCTTCTACATCGAGTTCAAGAGCGAGTGCGAGAAGTACGGCAACACC
GGCACATGGGAGGTGCACTTCGGCAACAACGTGATCGACTGCAACGACAGCATGTGCAGCAC
CAGCGACGACACCGTGTCCGCCACCCAGCTGGTGAAACAGCTGCAGCACACCCCCAGCCCCT
ACAGCAGCACCGTGTCTGTGGGCACCGCCAAGACCTACGGCCAGACCAGCGCCGCCACCAGA
CCTGGACACTGTGGCCTGGCCGAGAAGCAGCACTGCGGCCCTGTGAACCCTCTGCTGGGAGC
CGCCACCCCCACCGGCAACAACAAGCGGAGAAAGCTGTGCAGCGGCAACACCACCCCCATCA
TCCACCTGAAGGTGGACCGGAACAGCCTGATGCGGCTGCGGTACAGACTGCGGAAGCACAGC
GACCACTACCGGGACATCAGCAGCACCTGGCACTGGACCGGCGCTGGCAACGAGAAAACCGG
CATCCTGACCGTGACCTACCACAGCGAAACCCAGCGGACCAAGTTCCTGAACACCGTGGCCA
TCCCCGACAGCGTGCAGATCCTGGTGGGATATATGACCATGATGGCCAGATTCGAGGACCCC
ACCAGACGGCCCTACAAGCTGCCCGACCTGTGCACCGAGCTGAACACATCTCTGCAGGACAT
CGAGATCACATGCGTGTACTGCAAGACCGTGCTGGACCTGCTGTGCCACGAGCAGCTGTCCG
ACTCCGAGGAAGAAAACGACGAGATCGACGGCGTGAACCATCAGCATCTGCCCGCCAGACGG
GCCGAGCCCCAGAGACACACCATGCTGTGCATGTGCTGCAAGTGCGAGGCCCGGATTGAGCT
GGTGGTGGAAAGCAGCGCCGACGACCTGCGGGCCTTCCAGCAGCTCTTTCTGAATACCCTGA
GCTTCGTGTGCCCTTGGTGCGCCAGCCAGCACTACAGCGACTCCGTGTACGGCGATACCCTG
GAAAAGCTGACCAATACCGGCCTGTATAACCTGCTGATCCGGTGCCTGCGGTGCCAGAAGCC
CCTGAATCCCGCCGAGAAACTGAGACACCTGAACGAGAAGCGGCGGTTCCACAATATCGCCG
GCCACTACAGAGGCCAGTGCCACAGCTGCTGCAACCGGGCCAGACAGGAACGGCTGCAGCGG
AGGCGGGAAACCATGCACGGACCCAAGGCCACCCTCCAGGACATTGTCCTGCACCTGGAACC

CCAGAACGAGATCCCCGTCGATCTGCTGTGTCATGAACAGCTCAGCGACAGCGAAGAGGAAA
ATGACGAAATTGACGGGGTCAACCCTGACCTCTGTACCGAACTCAATACCAGTCTCCAGGAT
ATCGAAATTACCTGTGTCTACTGTAAAACCGTCCTCGAGCTGACCGAGGTGTTCGAGTTCGC
CTTCAAGGACCTGTTTGTGGTGTACAGAGACAGCATCCCCCACGCCGCCTGCCACAAGTGCA
TCGACTTCTACAGCCGGATCAGAGAGCTGCGGCACTACTCCGATTCTGTGTATGGCGACACA
CTCGAGAAGCTCACAAACACAGGACTGTACAATCTGCTCATCTGATAA

SEQ ID NO: 24 (amino acid sequence of 'HPV18DC2')

MHGPKATLQDIVLHLEPQNEIPVDLLCHEQLSDSEEENDEIDGVNHQHLPARRAEPQRHTSL
QDIEITCVYCKTVLELTEVFEFAFKDLFVVYRDSIPHAACHKCIDFYSRIRELRHYSDSVYG
DTLEKLTNTGLYNLLIRCLQRFEDPTRRPYKLPDLCTELNTSLQDIEITCVYCKTVLELTEV
FEFADSEEENDEIDGVNHQHLPARRAEPQRHTMLCMCCKCEARIELVVESSADDLRAFQQLF
LNTLSFVCPWCASQSDSVYGDTLEKLTNTGLYNLLIRCLRCQKPLNPAEKLRHLNEKRRFHN
IAGHYRGQCHSCCNRARQERLQRRRETQ

Claims

1. A nucleic acid molecule encoding a first polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 20.

2. A nucleic acid molecule according to claim 1, wherein the first polypeptide further comprises at least one epitope of a human papillomavirus (HPV) E2 protein.

3. A nucleic acid molecule according to claim 2, wherein the first polypeptide comprises HPV18 E2 protein that has a deletion or mutation in its DNA binding domain and/or a mutation in its transactivation domain.

4. A nucleic acid molecule according to claim 3, wherein the first polypeptide comprises the amino acid sequence of SEQ ID NO: 22.

5. A nucleic acid molecule according to any one of the preceding claims, comprising SEQ ID NO: 21.

6. A nucleic acid molecule according to any one of the preceding claims, comprising SEQ ID NO: 23.

7. A vector comprising a nucleic acid molecule according to any one of the preceding claims, wherein a sequence encoding the first polypeptide is operably linked to a promoter.

8. A vector according to claim 7, wherein the vector is a recombinant adenovirus.

9. A vector according to claim 7 or 8, wherein the promoter is operably coupled to a repressor operator sequence, to which a repressor protein can bind in order to repress expression of the promoter in the presence of said repressor protein.

10. A vector according to any one of claims 7-9, further comprising nucleic acid encoding a second polypeptide operably linked to a promoter, wherein the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 1.

11. A vector according to claim 10, wherein the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

12. A vector according to claim 11, wherein the first polypeptide comprises the amino acid sequence of SEQ ID NO: 22, and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 3.

13. A vaccine composition comprising a vector according to any one of claims 7-12, and a pharmaceutically acceptable excipient.

14. A vaccine composition comprising:
(a) a first vector according to any one of claims 7-9;
(b) a second vector comprising nucleic acid encoding a second polypeptide operably linked to a promoter, wherein the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 1; and
(c) a pharmaceutically acceptable excipient.

15. A vaccine composition according to claim 14, wherein the second polypeptide comprises an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

16. A vaccine composition according to claim 15, wherein the first polypeptide as encoded on the first vector comprises the amino acid sequence of SEQ ID NO: 22, and the second polypeptide as encoded on the second vector comprises the amino acid sequence of SEQ ID NO: 3.

17. A method of inducing an immune response against HPV in a subject, comprising administering to the subject a vaccine composition according to any one of claims 13-16.

18. A method of inducing an immune response against HPV in a subject, comprising administering to the subject:

(a) a first vaccine composition comprising a vector according to any one of claims 7-9, and a pharmaceutically acceptable excipient; and

(b) a second vaccine composition comprising a vector comprising nucleic acid encoding a second polypeptide operably linked to a promoter, wherein the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 1.

19. A method according to claim 18, wherein the second polypeptide comprises an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

20. A method according to claim 19, wherein the first polypeptide as encoded in the first vaccine composition comprises the amino acid sequence of SEQ ID NO: 22, and the second polypeptide as encoded in the second vaccine composition comprises the amino acid sequence of SEQ ID NO: 3.

21. A method according to any one of claims 17-20, comprising
(i) administering a vaccine composition according to any one of claims 13-16 to the subject more than once, or
(ii) administering first and second vaccine compositions according to any one of claims 17-20 to the subject more than once.

22. A method for treating persistent HPV infection, vulvar intraepithelial neoplasia (VIN), cervical intraepithelial neoplasia (CIN), vaginal intraepithelial neoplasia (VaIN), anal intraepithelial neoplasia (AIN), cervical cancer (such as cervical squamous cell carcinoma (SCC), oropharyngeal cancer, penile cancer, vaginal cancer or anal cancer in a subject, the method comprising administering to the subject a vaccine composition according to any one of claims 13-16.

23. A kit of parts comprising:
(a) a first vaccine composition comprising a vector according to any one of claims 7-9, and a pharmaceutically acceptable excipient; and
(b) a second vaccine composition comprising a vector comprising nucleic acid encoding a second polypeptide operably linked to a promoter, wherein the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 1.

24. The kit of parts of claim 23, wherein the second polypeptide comprises an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

25. A kit of parts according to claim 24, wherein the first polypeptide as encoded in the first vaccine composition comprises the amino acid sequence of SEQ ID NO: 22, and the second polypeptide as encoded in the second vaccine composition comprises the amino acid sequence of SEQ ID NO: 3.

26. A polypeptide comprising SEQ ID NO: 20.

27. A polypeptide according to claim 26, comprising SEQ ID NO: 22.

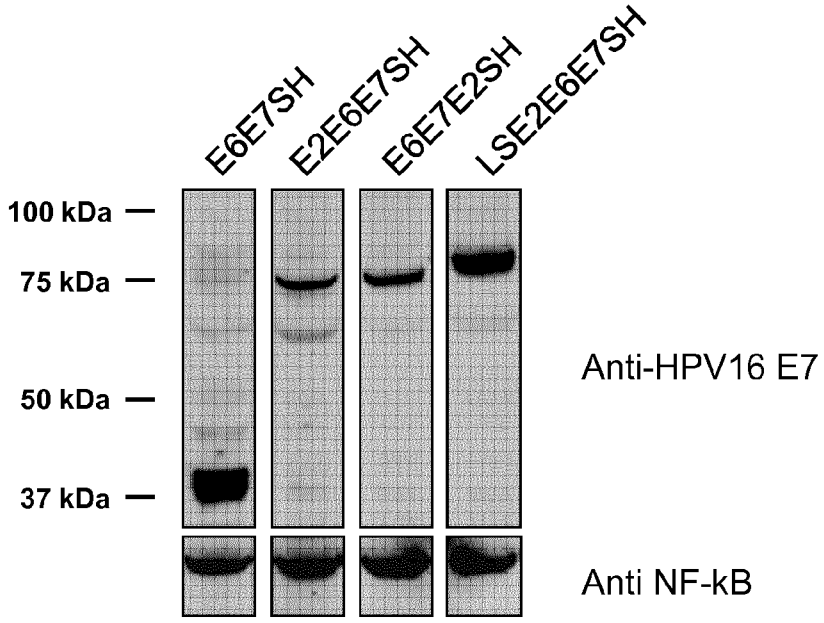

E6E7SH
E2E6E7SH
E6E7E2SH
LSE2E6E7SH
100 kDa
75 kDa
50 kDa
37 kDa
Anti-HPV16 E7
Anti NF-kB

Fig. 1

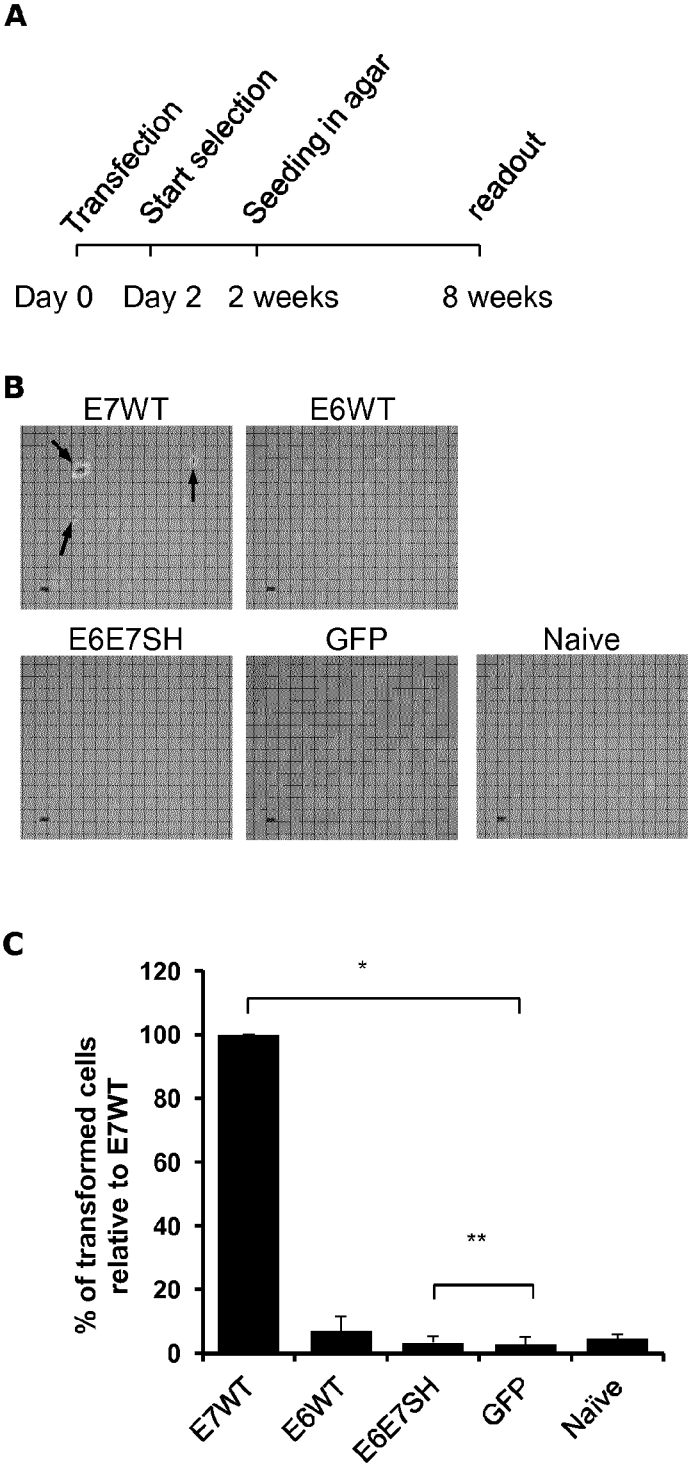

A
Transfection
Start selection
Seeding in agar
readout
Day 0
Day 2
2 weeks
8 weeks
B
E7WT
E6WT
E6E7SH
GFP
Naive
C
% of transformed cells relative to E7WT
120
100
80
60
40
20
0
*
**
E7WT
E6WT
E6E7SH
GFP
Naïve

Fig. 2

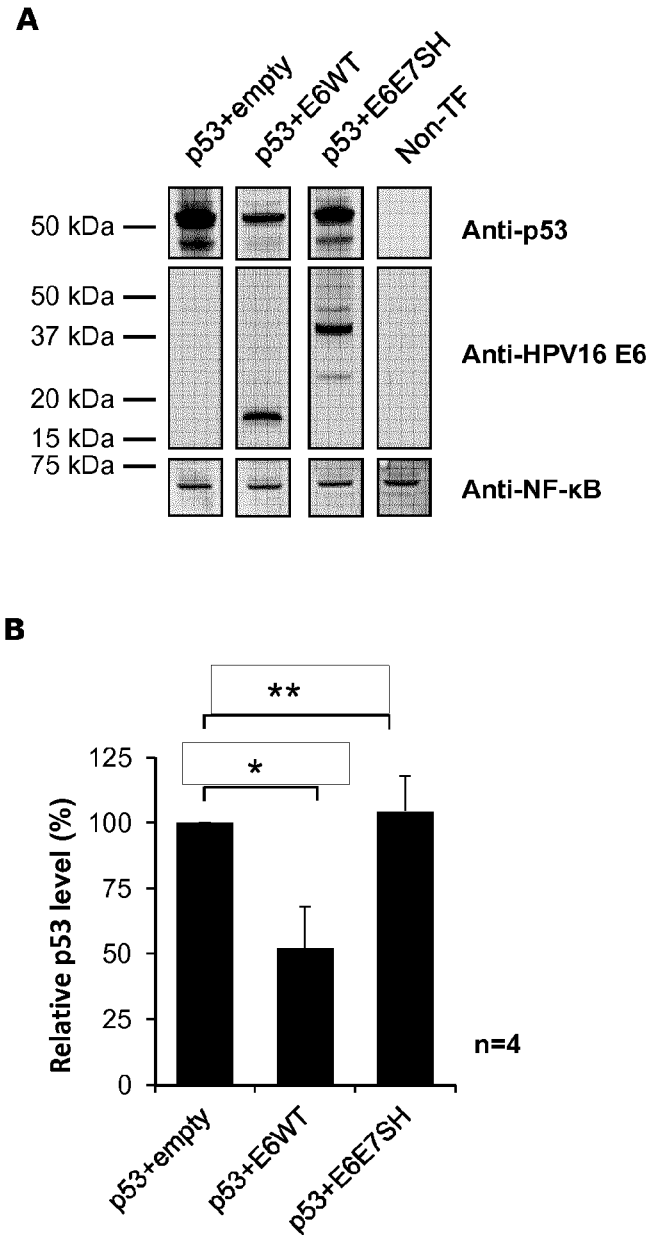
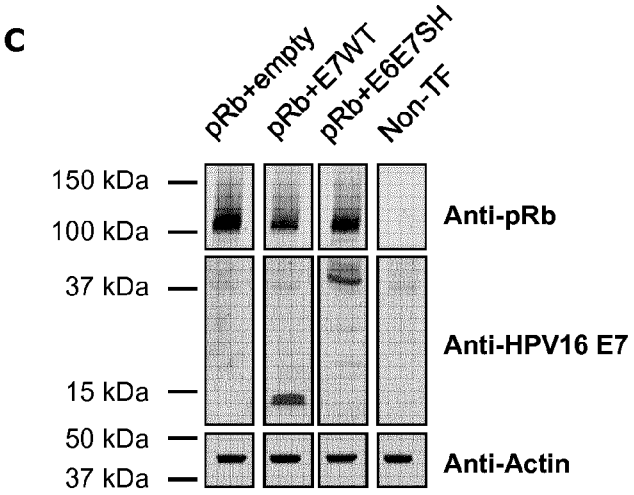

A
p53+empty
p53+E6WT
p53+E6E7SH
Non-TF
50 kDa
Anti-p53
50 kDa
37 kDa
Anti-HPV16 E6
20 kDa
15 kDa
75 kDa
Anti-NF-κB
B
**
*
125
100
75
50
25
0
Relative p53 level (%)
n=4
p53+empty
p53+E6WT
p53+E6E7SH

Fig. 3



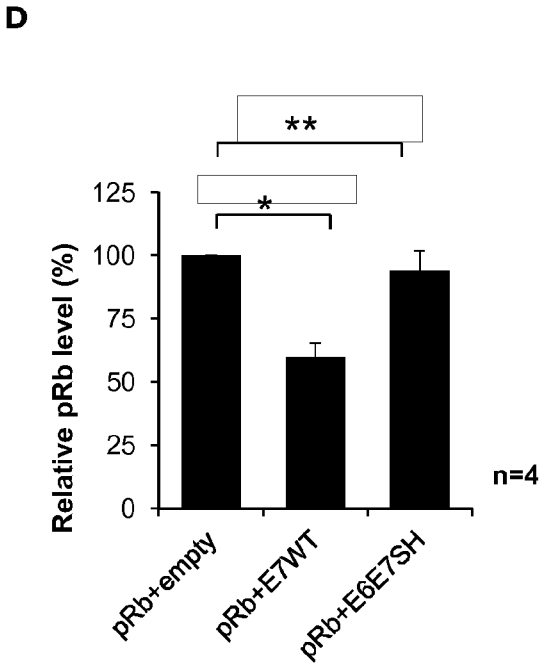


Fig. 3 - continued

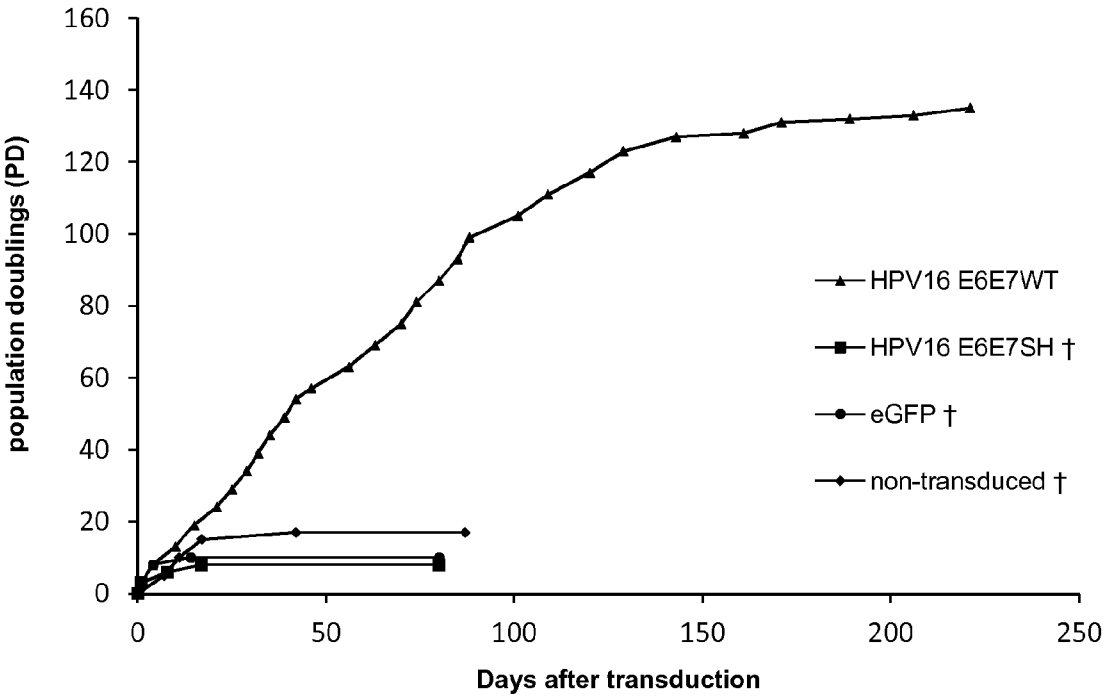
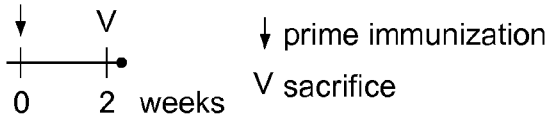

160
140
120
100
80
60
40
20
0
population doublings (PD)
0
50
100
150
200
250
Days after transduction
HPV16 E6E7WT
HPV16 E6E7SH †
eGFP †
non-transduced †

Fig. 4

A


V
prime immunization
V sacrifice
0
2 weeks

B

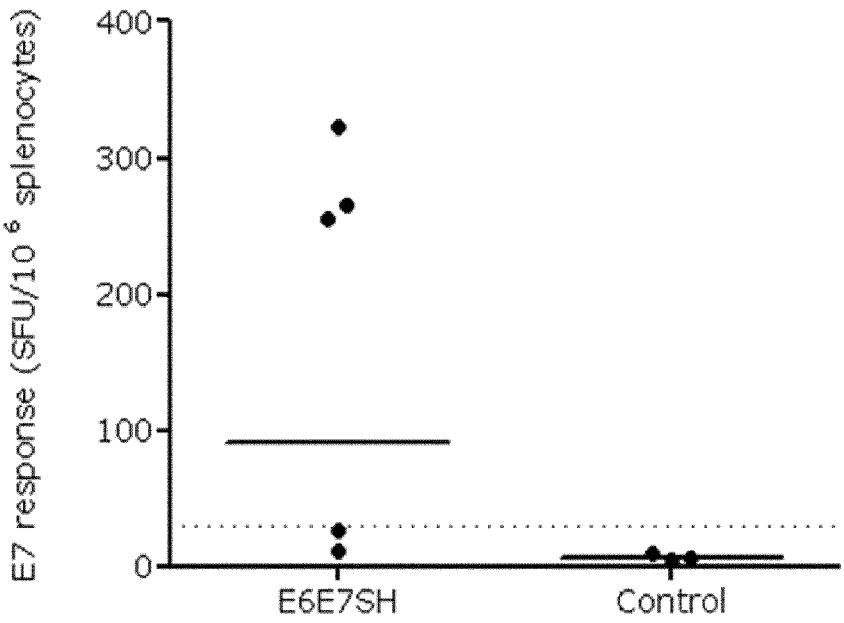
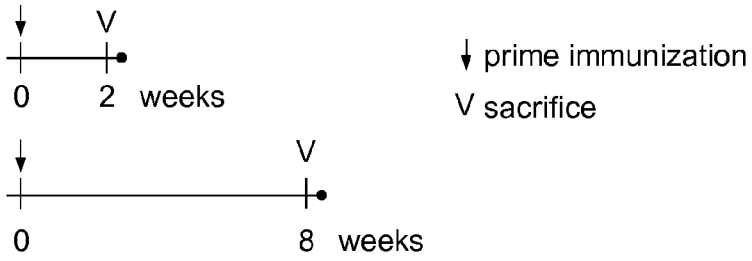
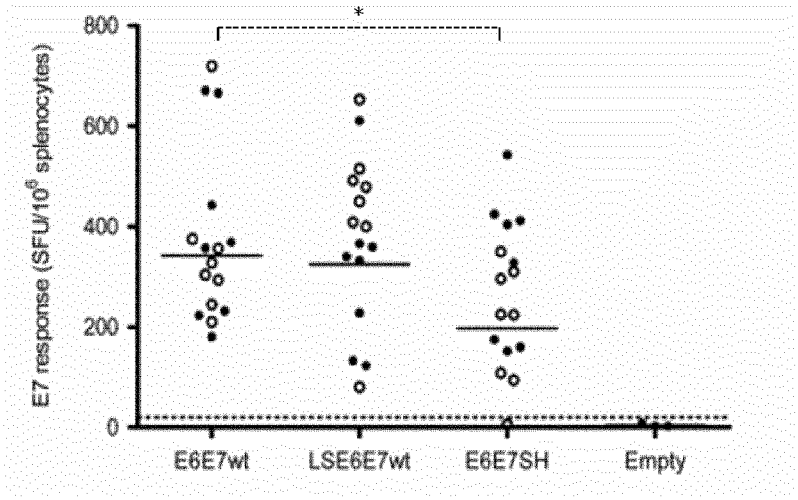

E7 response (SFU/10 6 splenocytes)
400
300
200
100
0
E6E7SH
Control

Fig. 5

A


V
0
2 weeks
prime immunization
V sacrifice
V
0
8 weeks

B


*
800
600
400
200
0
E7 response (SFU/10^6 splenocytes)
E6E7wt
LSE6E7wt
E6E7SH
Empty

C

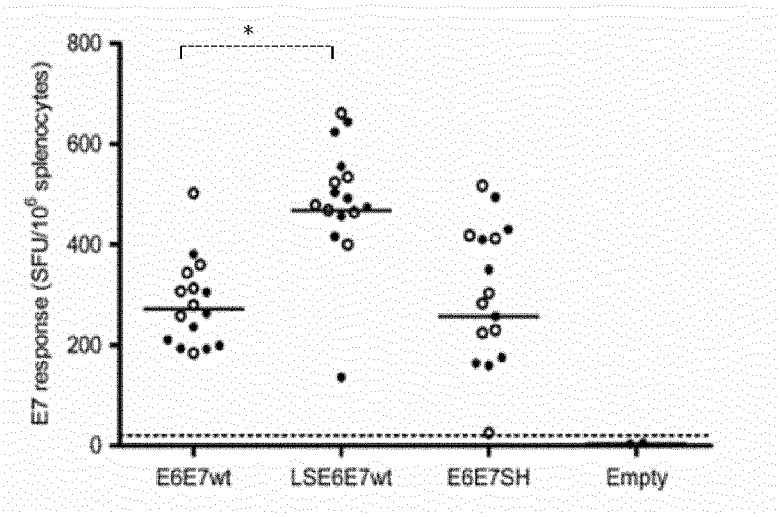

*
800
600
400
200
0
E7 response (SFU/10^6 splenocytes)
E6E7wt
LSE6E7wt
E6E7SH
Empty

Fig. 6

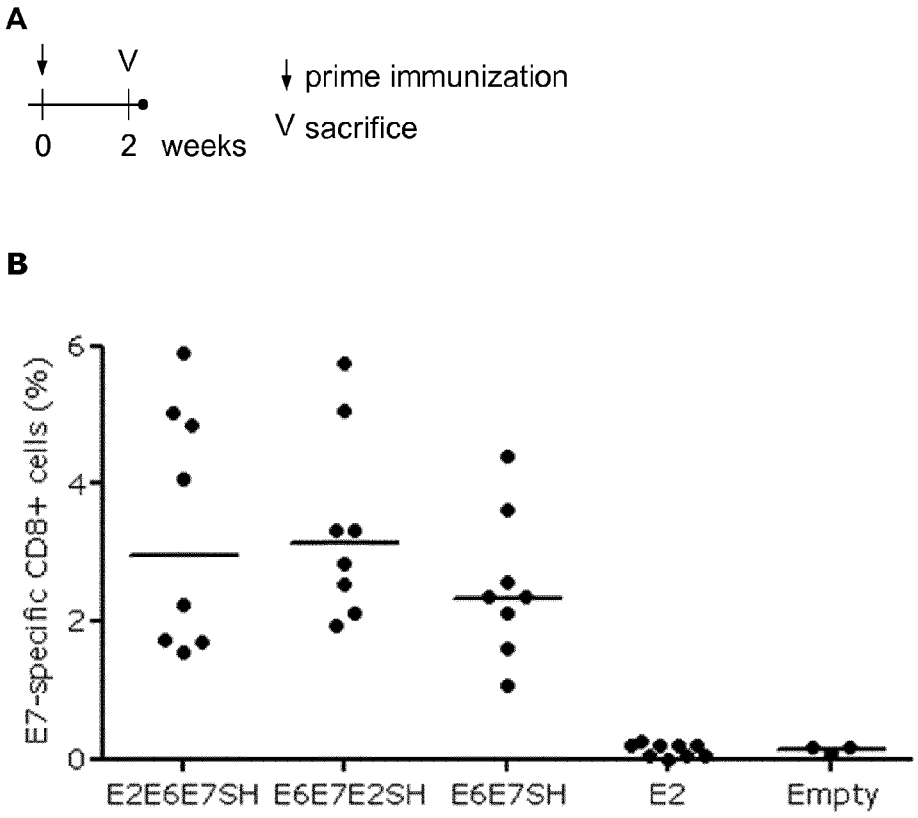

A
V
0
2
weeks
prime immunization
V sacrifice
B
E7-specific CD8+ cells (%)
6
4
2
0
E2E6E7SH
E6E7E2SH
E6E7SH
E2
Empty

Fig . 7

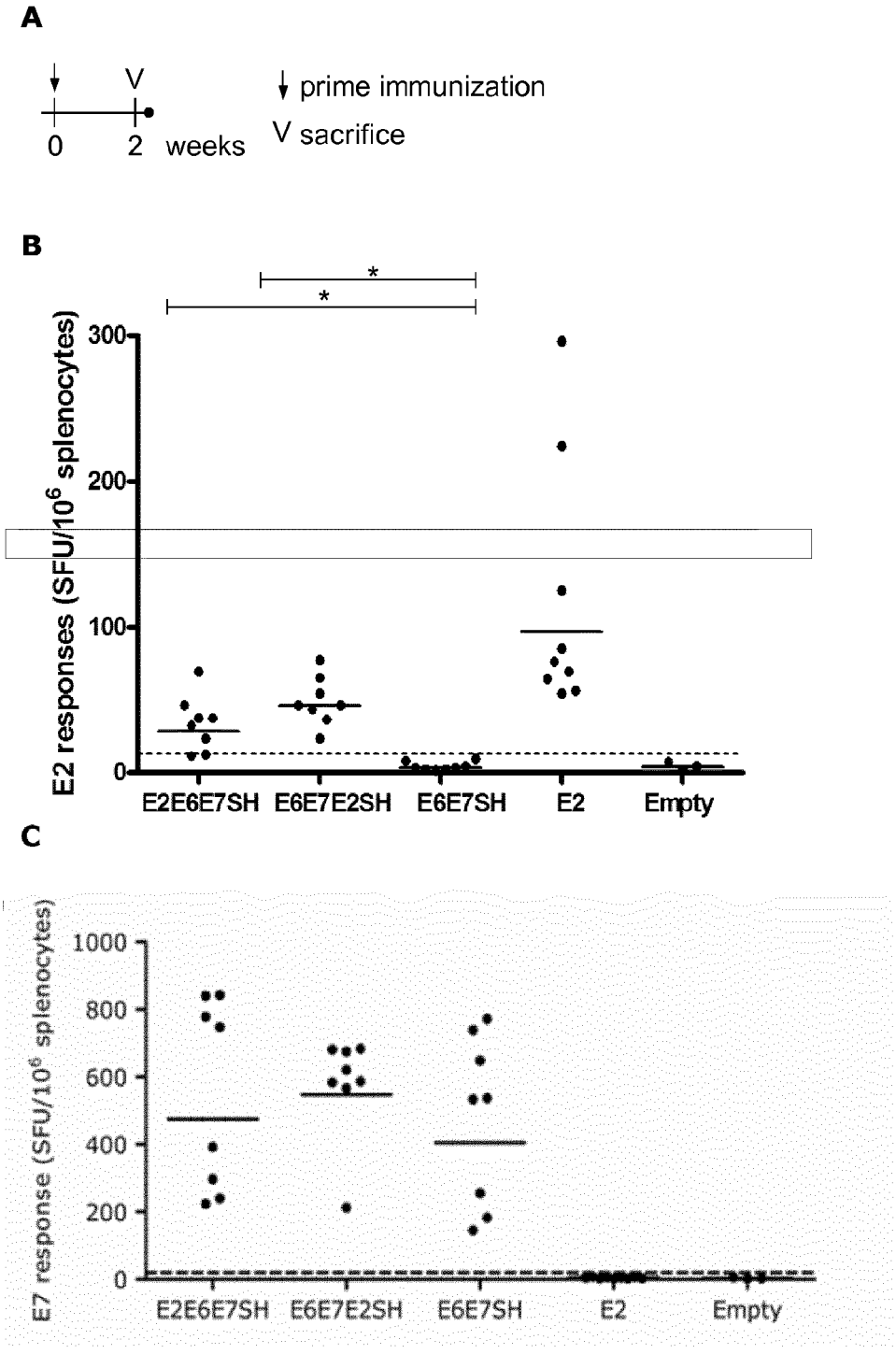

A
V
0
2
weeks
prime immunization
V sacrifice
B
*
*
E2 responses (SFU/10^6 splenocytes)
300
200
100
0
E2E6E7SH
E6E7E2SH
E6E7SH
E2
Empty
C
E7 response (SFU/10^6 splenocytes)
1000
800
600
400
200
0
E2E6E7SH
E6E7E2SH
E6E7SH
E2
Empty

Fig. 8

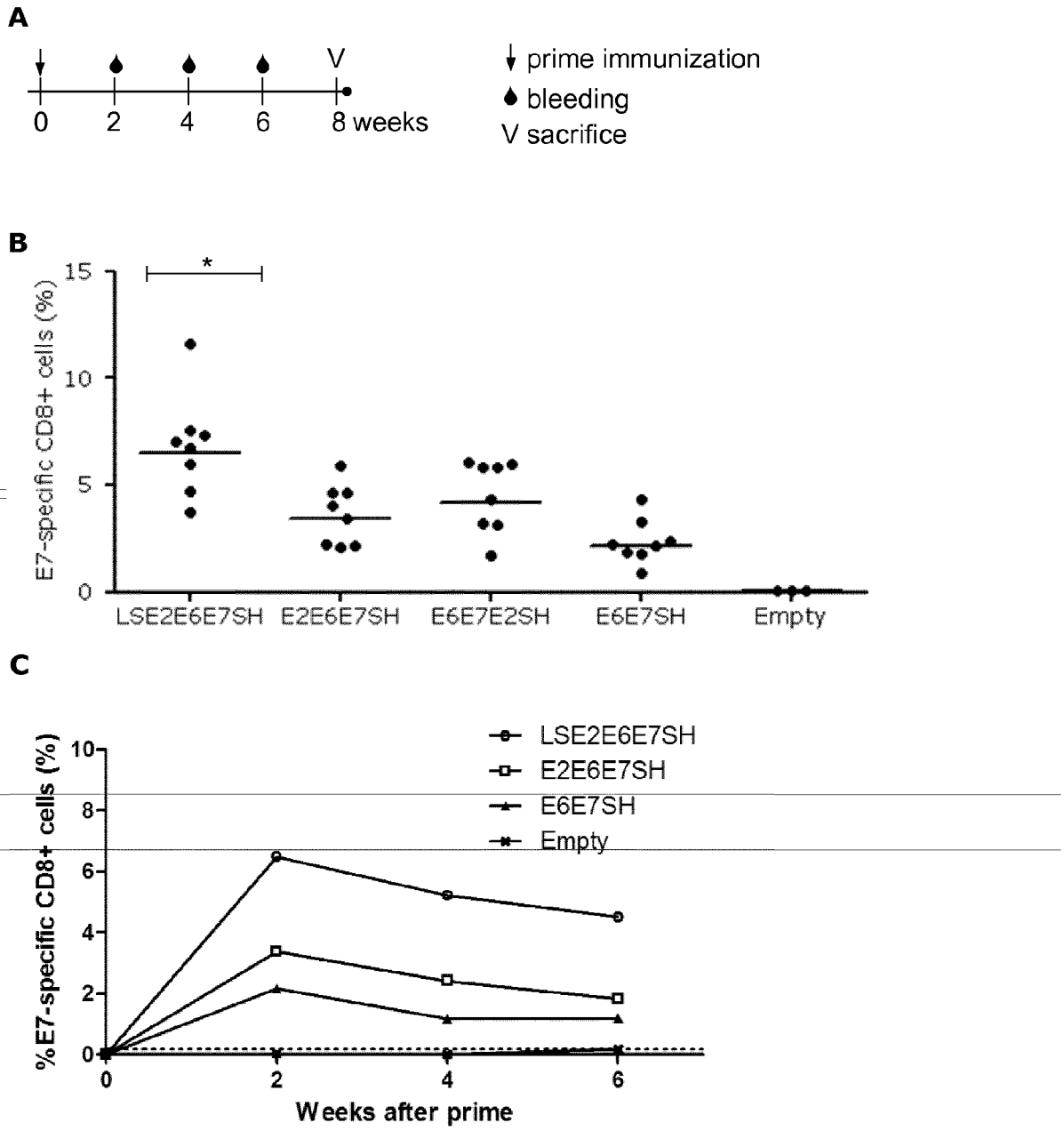

A
V
0
2
4
6
8 weeks
prime immunization
bleeding
V sacrifice
B
*
E7-specific CD8+ cells (%)
15
10
5
0
LSE2E6E7SH
E2E6E7SH
E6E7E2SH
E6E7SH
Empty
C
LSE2E6E7SH
E2E6E7SH
E6E7SH
Empty
%E7-specific CD8+ cells (%)
10
8
6
4
2
0
0
2
4
6
Weeks after prime

Fig. 9

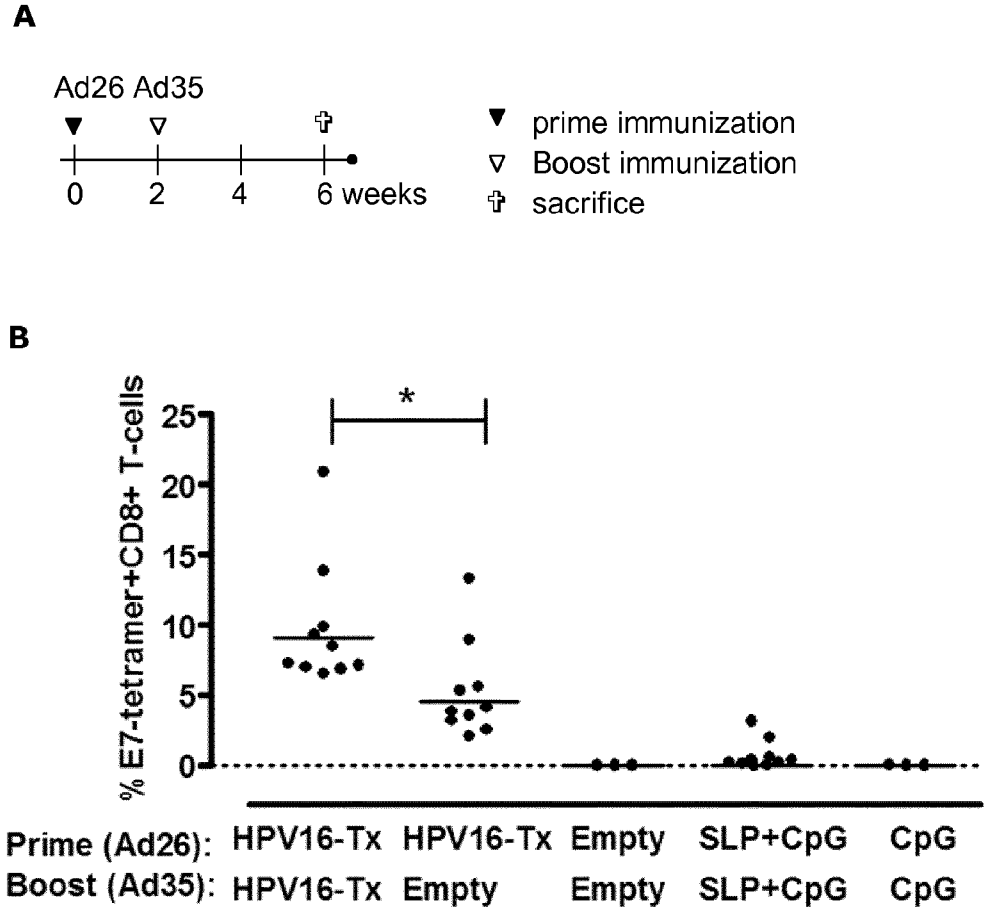

A
Ad26 Ad35
0
2
4
6 weeks
prime immunization
Boost immunization
sacrifice
B
% E7-tetramer+CD8+ T-cells
25
20
15
10
5
0
*
Prime (Ad26): HPV16-Tx HPV16-Tx Empty SLP+CpG CpG
Boost (Ad35): HPV16-Tx Empty Empty SLP+CpG CpG

Fig. 10

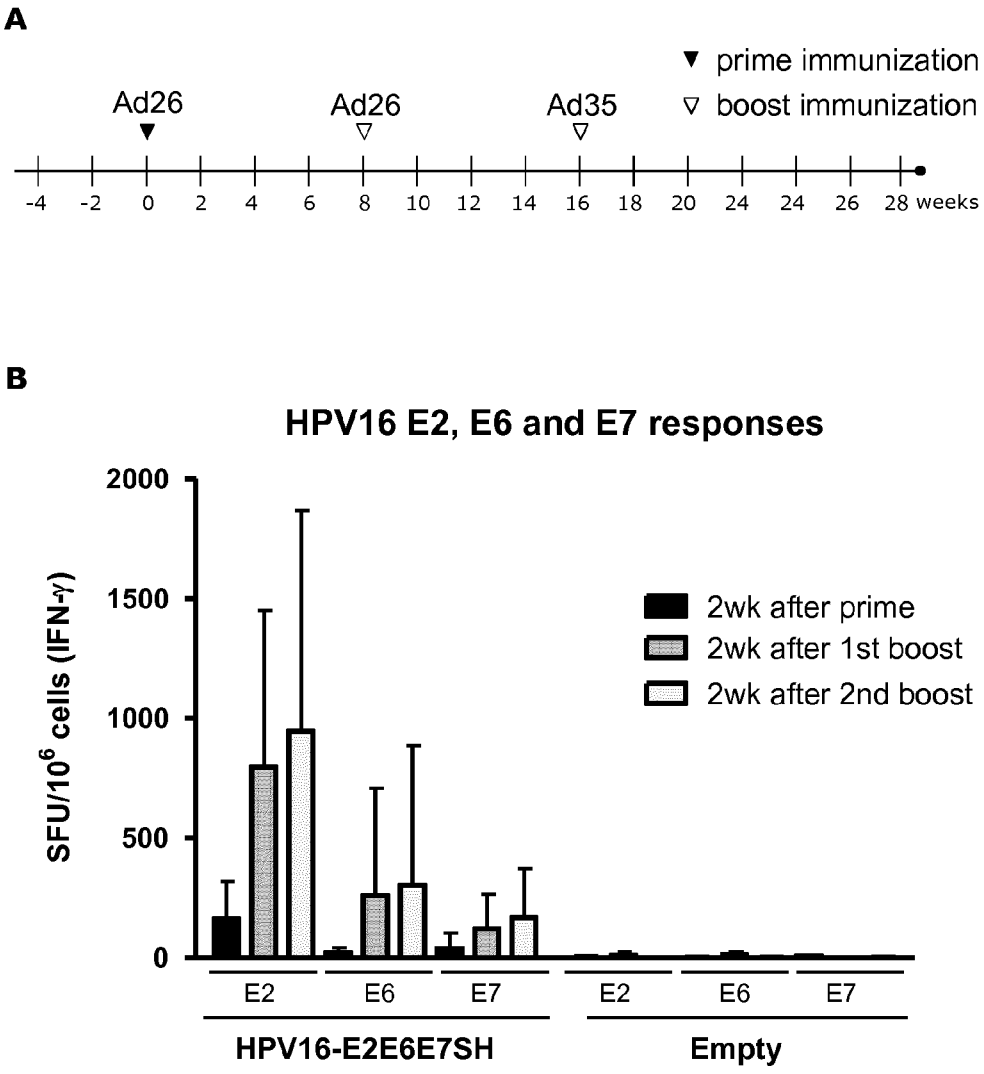

A
prime immunization
boost immunization
Ad26
Ad26
Ad35
-4 -2 0 2 4 6 8 10 12 14 16 18 20 24 24 26 28 weeks
B
HPV16 E2, E6 and E7 responses
SFU/10^6 cells (IFN-γ)
2000
1500
1000
500
0
2wk after prime
2wk after 1st boost
2wk after 2nd boost
E2 E6 E7 E2 E6 E7
HPV16-E2E6E7SH
Empty

Fig. 11

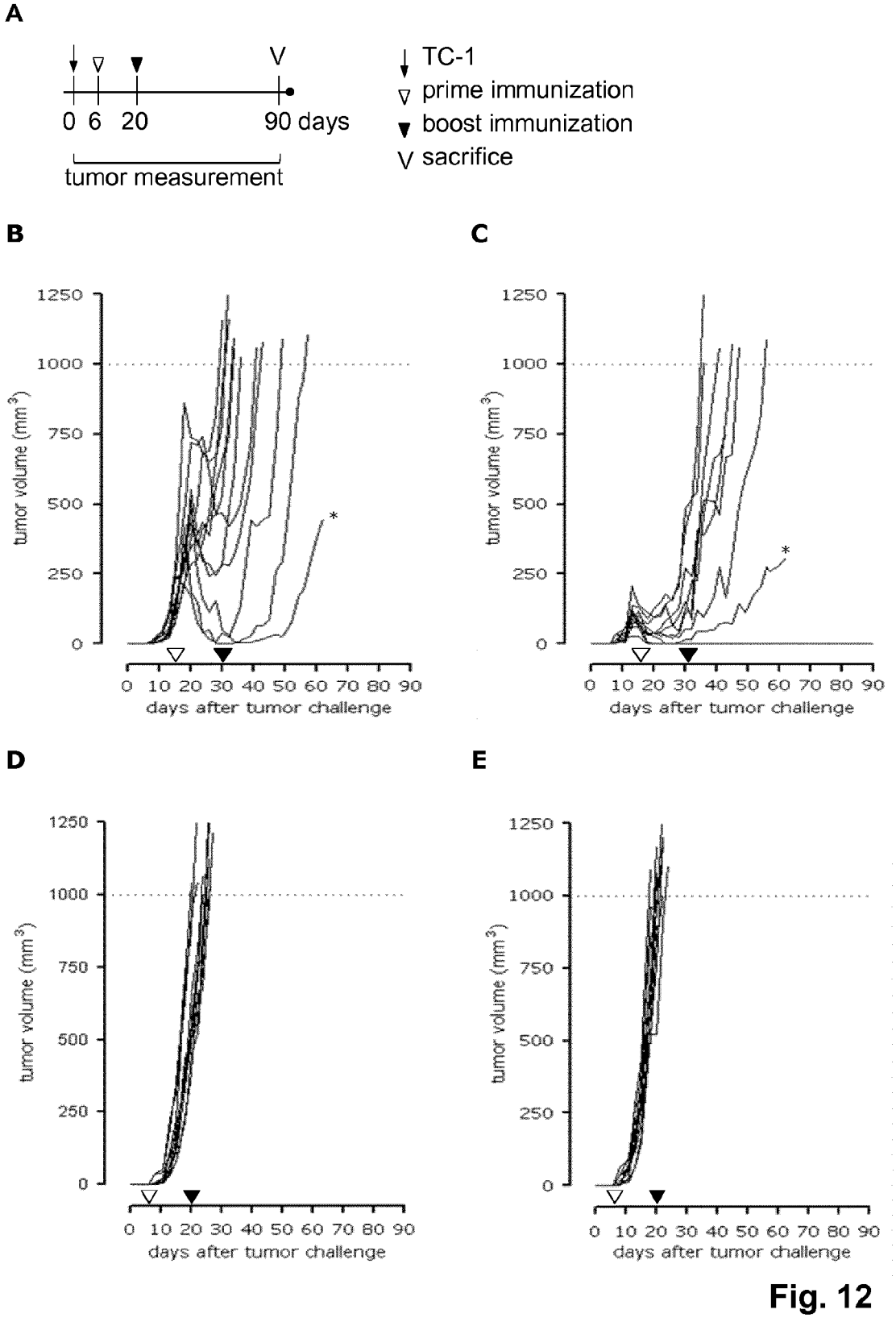
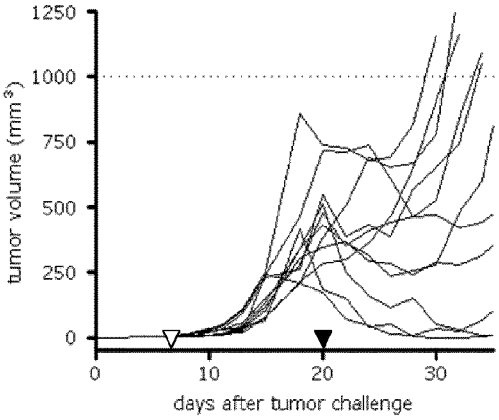
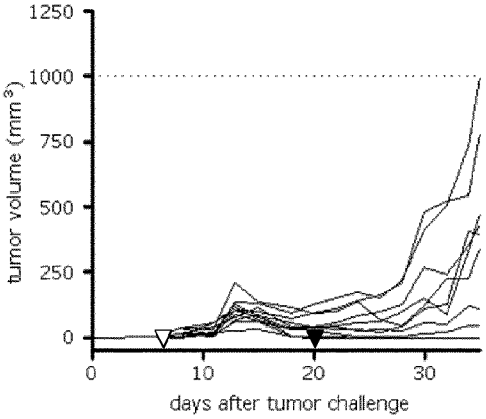

A
V
0 6 20
90 days
tumor measurement
TC-1
prime immunization
boost immunization
sacrifice
B
C
D
E
tumor volume (mm3)
1250
1000
750
500
250
0
0 10 20 30 40 50 60 70 80 90
days after tumor challenge
*

Fig. 12

F



G



H

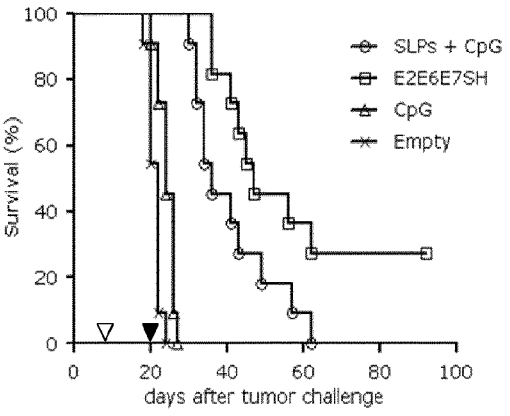


Fig. 12 - continued

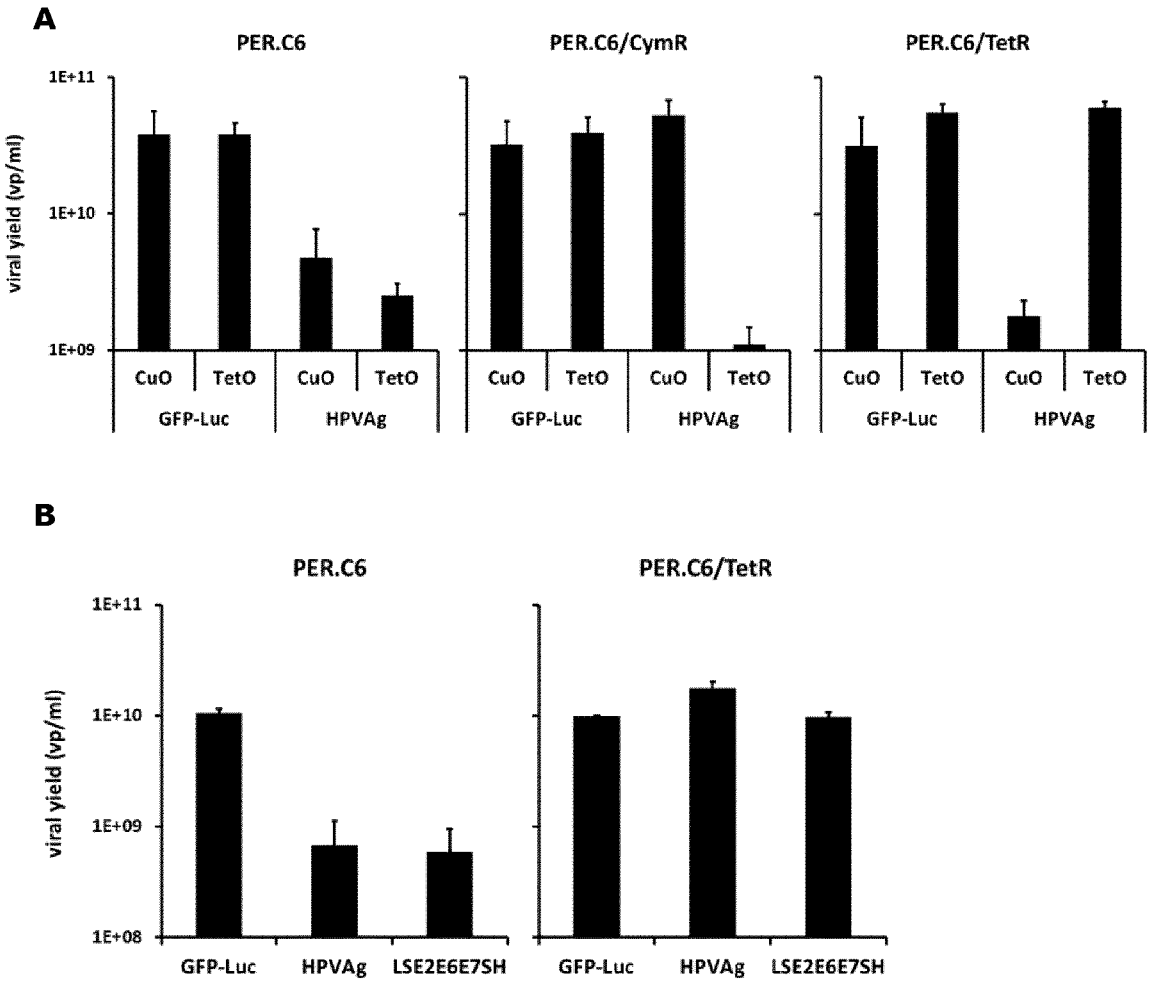

A
PER.C6
PER.C6/CymR
PER.C6/TetR
viral yield (vp/ml)
1E+11
1E+10
1E+09
CuO
TetO
CuO
TetO
GFP-Luc
HPVAg
B
PER.C6
PER.C6/TetR
viral yield (vp/ml)
1E+11
1E+10
1E+09
1E+08
GFP-Luc
HPVAg
LSE2E6E7SH

Fig. 13

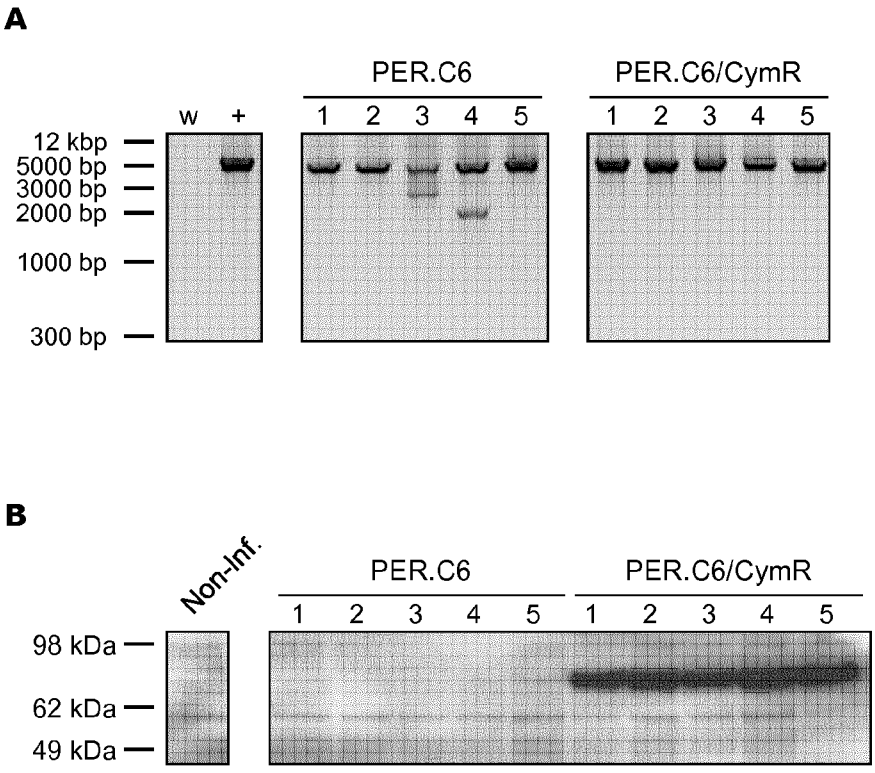

A
PER.C6
PER.C6/CymR
w
+
1 2 3 4 5
1 2 3 4 5
12 kbp
5000 bp
3000 bp
2000 bp
1000 bp
300 bp
B
Non-Inf.
PER.C6
PER.C6/CymR
1 2 3 4 5
1 2 3 4 5
98 kDa
62 kDa
49 kDa

Fig. 14

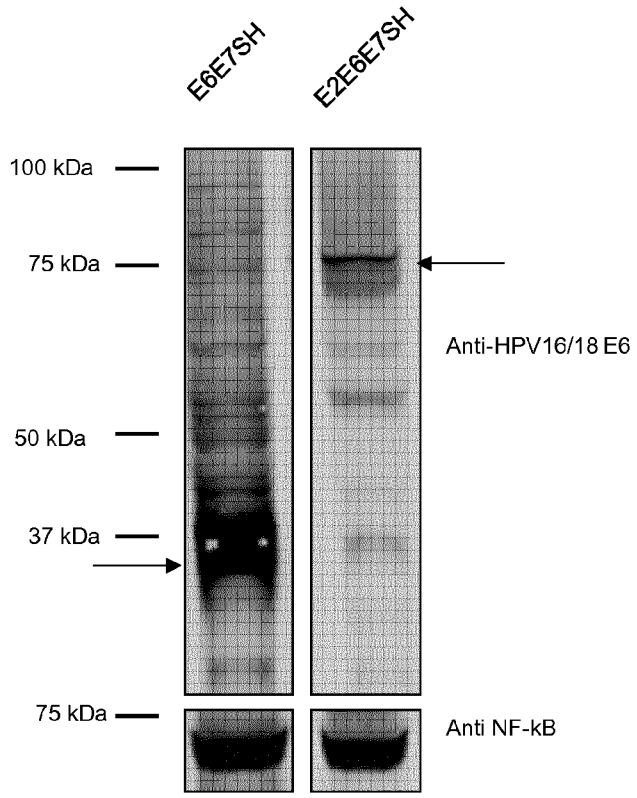

E6E7SH
E2E6E7SH
100 kDa
75 kDa
50 kDa
37 kDa
75 kDa
Anti-HPV16/18 E6
Anti NF-kB

Fig. 15

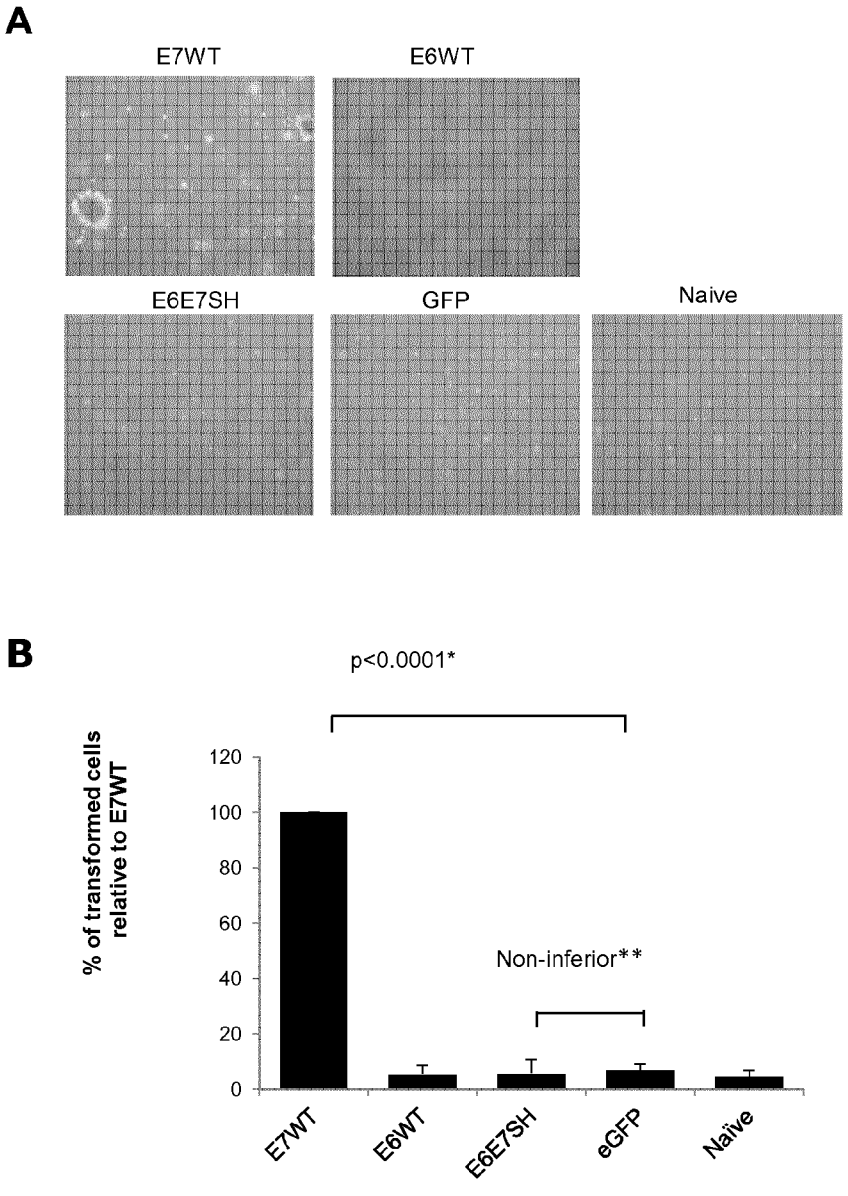

A
E7WT
E6WT
E6E7SH
GFP
Naive
B
p<0.0001*
% of transformed cells relative to E7WT
120
100
80
60
40
20
0
Non-inferior**
E7WT
E6WT
E6E7SH
eGFP
Naive

Fig. 16

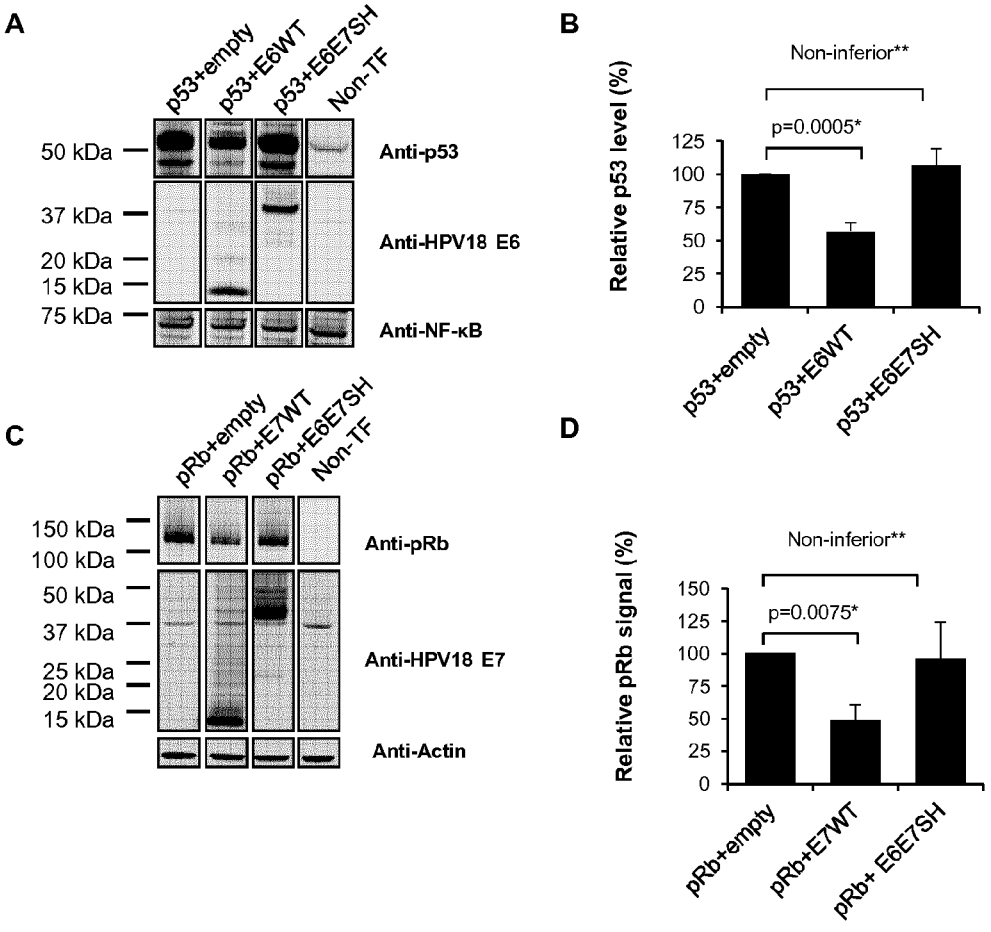

A
p53+empty
p53+E6WT
p53+E6E7SH
Non-TF
50 kDa
37 kDa
20 kDa
15 kDa
75 kDa
Anti-p53
Anti-HPV18 E6
Anti-NF-κB
B
Relative p53 level (%)
Non-inferior**
p=0.0005*
125
100
75
50
25
0
p53+empty
p53+E6WT
p53+E6E7SH
C
pRb+empty
pRb+E7WT
pRb+E6E7SH
Non-TF
150 kDa
100 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
Anti-pRb
Anti-HPV18 E7
Anti-Actin
D
Relative pRb signal (%)
Non-inferior**
p=0.0075*
150
125
100
75
50
25
0
pRb+empty
pRb+E7WT
pRb+ E6E7SH

Fig. 17

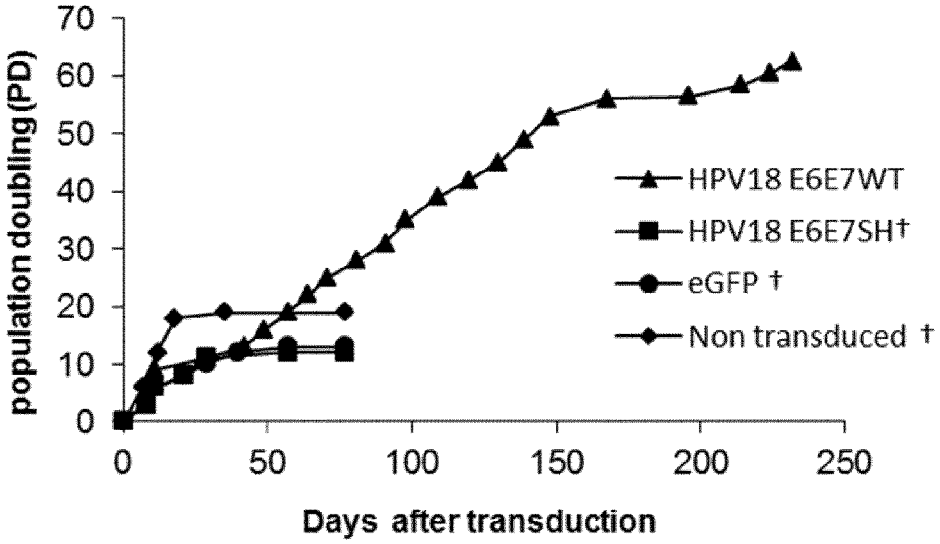

population doubling (PD)
70
60
50
40
30
20
10
0
0
50
100
150
200
250
Days after transduction
HPV18 E6E7WT
HPV18 E6E7SH†
eGFP †
Non transduced †

Fig. 18

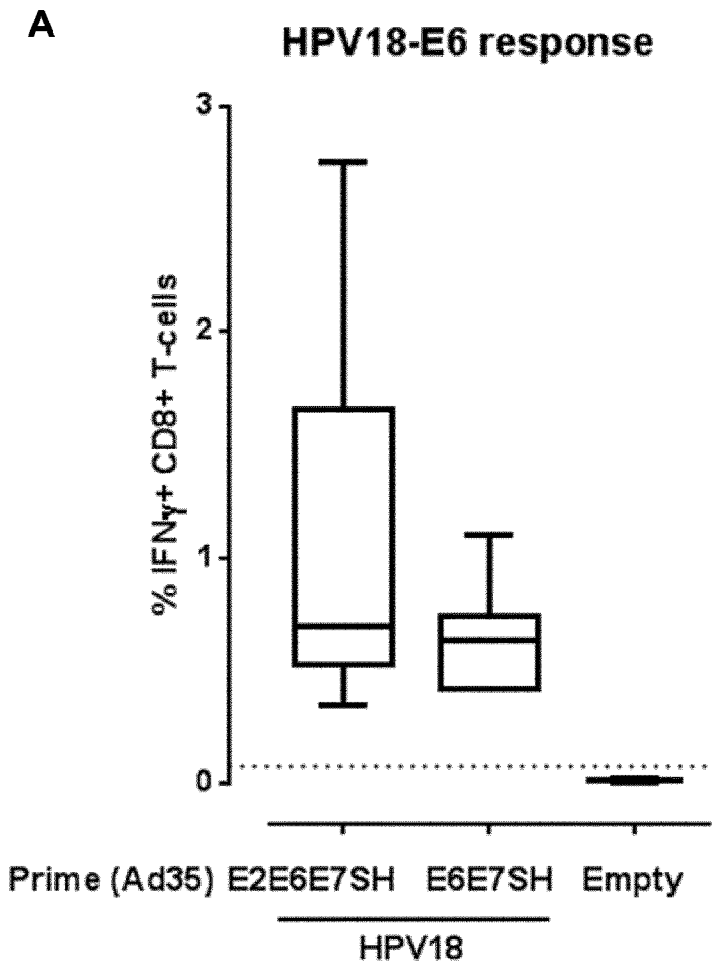

A
HPV18-E6 response
% IFNγ+ CD8+ T-cells
3
2
1
0
Prime (Ad35) E2E6E7SH E6E7SH Empty
HPV18

Fig. 19

B

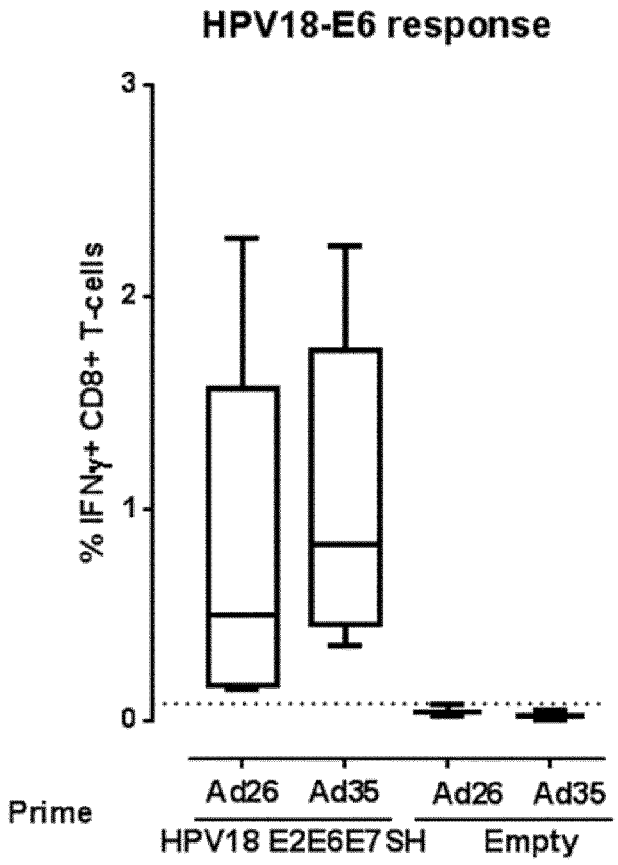


Fig. 19 - continued

A

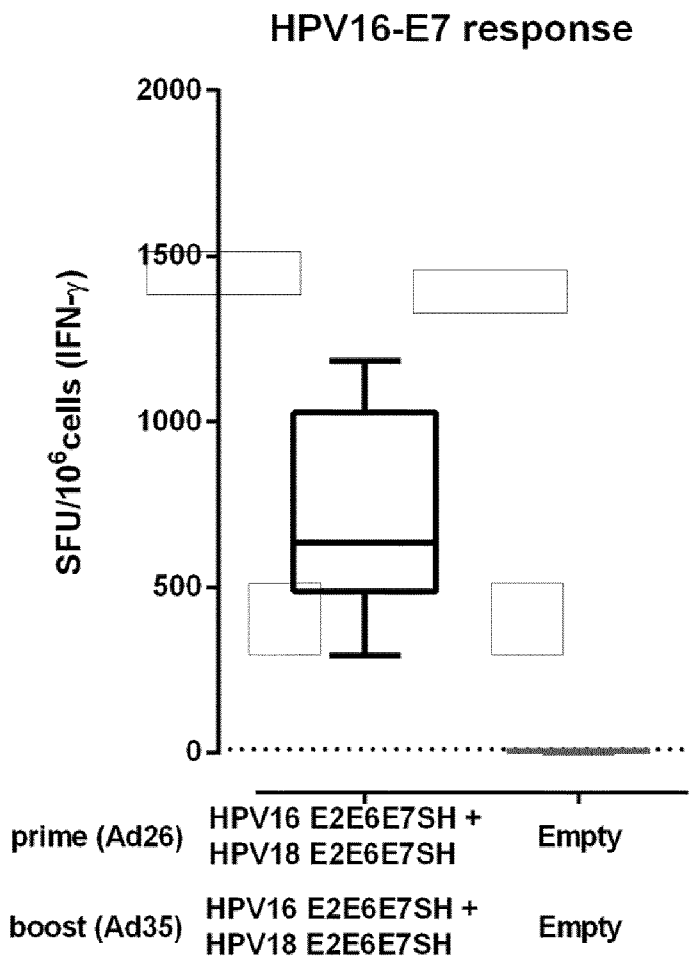

HPV16-E7 response
2000
1500
1000
500
0
SFU/10^6cells (IFN-γ)
prime (Ad26)
HPV16 E2E6E7SH + HPV18 E2E6E7SH
Empty
boost (Ad35)
HPV16 E2E6E7SH + HPV18 E2E6E7SH
Empty

Fig. 20

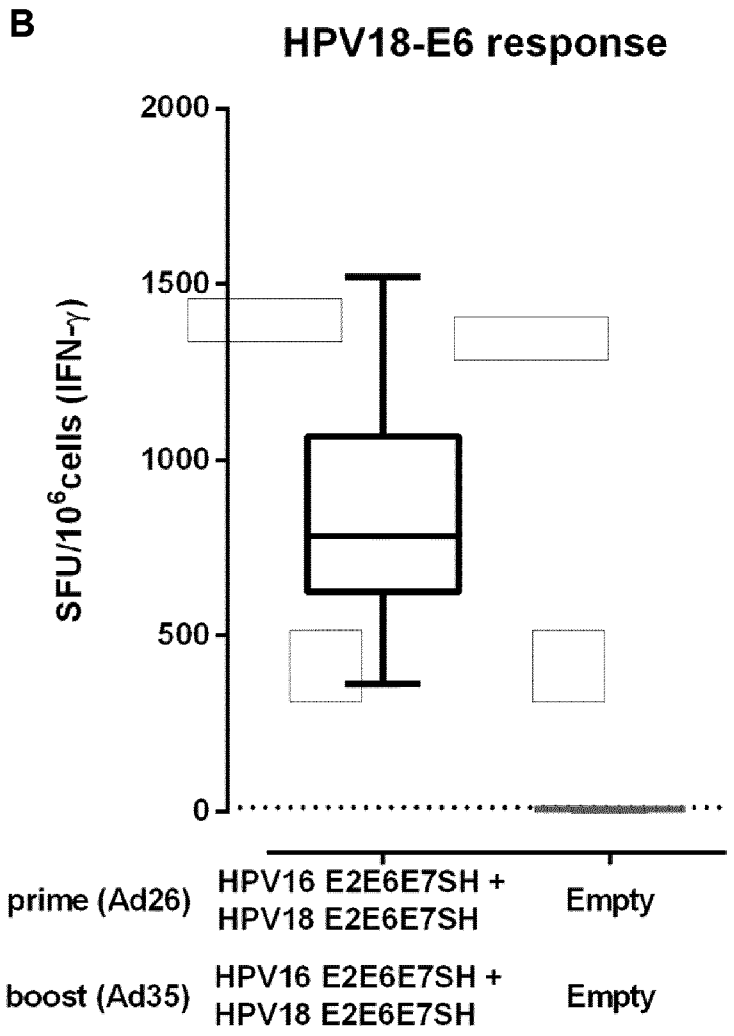


Fig. 20 – continued

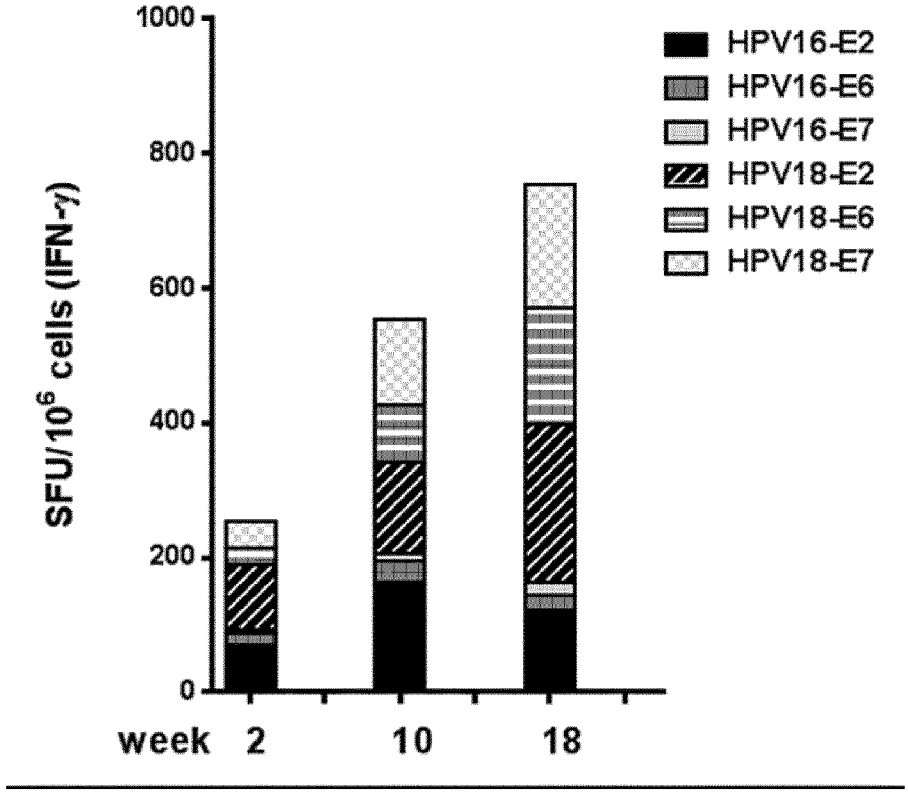

1000
800
600
400
200
0
SFU/10^6 cells (IFN-γ)
week 2
10
18
HPV16-E2
HPV16-E6
HPV16-E7
HPV18-E2
HPV18-E6
HPV18-E7

Fig. 21

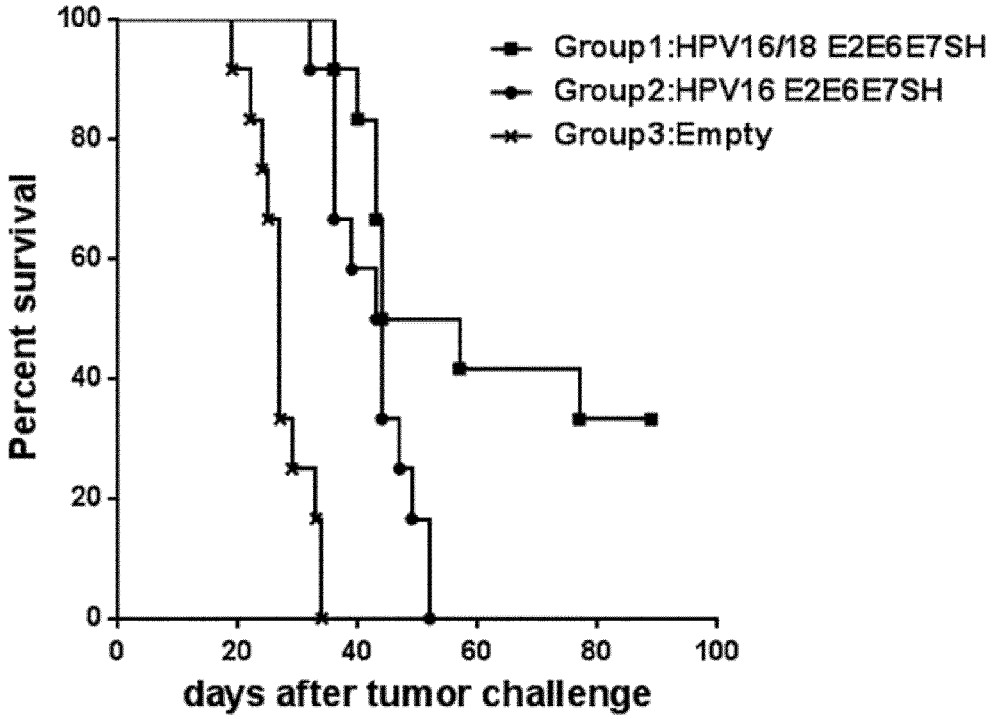

Group1:HPV16/18 E2E6E7SH
Group2:HPV16 E2E6E7SH
Group3:Empty
Percent survival
days after tumor challenge
0
20
40
60
80
100

Fig. 22

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/069618

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 A61K39/12 C07K14/005 C07K14/025
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K 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 practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FAHAD N. ALMAJHDI ET AL: "Design of a Highly Effective Therapeutic HPV16 E6/E7-Specific DNA Vaccine: Optimization by Different Ways of Sequence Rearrangements (Shuffling)", PLOS ONE, vol. 9, no. 11, 25 November 2014 (2014-11-25), page e113461, XP055252178, DOI: 10.1371/journal.pone.0113461 abstract Introduction; figure 1 ----- -/--	1-27

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 2016	27/10/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Turri, Matteo

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	SASMITA MISHRA ET AL: "Dendritic Cell-Mediated, DNA-Based Vaccination against Hepatitis C Induces the Multi-Epitope-Specific Response of Humanized, HLA Transgenic Mice", PLOS ONE, vol. 9, no. 8, 11 August 2014 (2014-08-11) , page e104606, XP055247057, DOI: 10.1371/journal.pone.0104606 page 2 - right-hand column	1-27
Y	MOISE L ET AL: "VennVax, a DNA-prime, peptide-boost multi-T-cell epitope poxvirus vaccine, induces protective immunity against vaccinia infection by T cell response alone", VACCINE, ELSEVIER LTD, GB, vol. 29, no. 3, 10 January 2011 (2011-01-10), pages 501-511, XP027575654, ISSN: 0264-410X [retrieved on 2010-11-04] First full paragraph.; page 505, left-hand column	1-27
Y	STEVEN F MOSS ET AL: "HelicoVax: Epitope-based therapeuticvaccination in a mouse model", VACCINE, ELSEVIER LTD, GB, vol. 29, no. 11, 27 December 2010 (2010-12-27), pages 2085-2091, XP028152754, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2010.12.130 [retrieved on 2011-01-07] page 2087, paragraph 3.1	1-27

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	C. LUNDEGAARD ET AL: "NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11", NUCLEIC ACIDS RESEARCH, vol. 36, no. Web Server, 19 May 2008 (2008-05-19), pages W509-W512, XP055252573, GB ISSN: 0305-1048, DOI: 10.1093/nar/gkn202 cited in the application abstract	1-27
A	WO 2009/106362 A1 (CICHON GUENTER [DE]) 3 September 2009 (2009-09-03) page 14, line 7 - line 10	1-27
A	S S Prakash ET AL: "Amino acids necessary for DNA contact and dimerization imply novel motifs in the papillomavirus E2 trans-activator", , 1 January 1992 (1992-01-01), XP055252213, Retrieved from the Internet: URL:http://genesdev.cshlp.org/content/6/1/105.full.pdf [retrieved on 2016-02-22] cited in the application table 1	4
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2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HENKEN F E ET AL: "Preclinical safety evaluation of DNA vaccines encoding modified HPV16 E6 and E7", VACCINE, ELSEVIER LTD, GB, vol. 30, no. 28, 5 April 2012 (2012-04-05) , pages 4259-4266, XP028511506, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2012.04.013 [retrieved on 2012-04-21] abstract Introduction	1-27
A	WO 2013/083287 A1 (DEUTSCHES KREBSFORSCH [DE]; UNIV KING SAUD [SA]) 13 June 2013 (2013-06-13) cited in the application page 2, line 13 - line 33; claims 1-13	1-27
A	OHLSCHLAGER P ET AL: "An improved rearranged Human Papillomavirus Type 16 E7 DNA vaccine candidate (HPV-16 E7SH) induces an E7 wildtype-specific T cell response", VACCINE, ELSEVIER LTD, GB, vol. 24, no. 15, 5 April 2006 (2006-04-05) , pages 2880-2893, XP028011167, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2005.12.061 [retrieved on 2006-04-05] abstract	1-27
A	US 2007/014810 A1 (BAKER DENISE [US] ET AL) 18 January 2007 (2007-01-18) abstract paragraph [0020]	1-27
A	DE GROOT A S ET AL: "HIV vaccine development by computer assisted design: the GAIA vaccine", VACCINE, ELSEVIER LTD, GB, vol. 23, no. 17-18, 18 March 2005 (2005-03-18), pages 2136-2148, XP027652211, ISSN: 0264-410X [retrieved on 2005-03-18]	1-27

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/069618

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 1732598 A2	20-12-2006
		US 2007014810 A1	18-01-2007
		WO 2005089164 A2	29-09-2005

摘要

本發明提供了被用作針對 HPV18 和/或 HPV16 的治療性疫苗的設計核酸構建體和多肽。