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Therapeutic human papillomavirus vaccines: current clinical trials and future directions

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Abstract

Background—Cervical cancer is the second largest cause of cancer deaths in women worldwide. It is now evident that persistent infection with high-risk human papillomavirus (HPV) is necessary for the development and maintenance of cervical cancer. Thus, effective vaccination against HPV represents an opportunity to restrain cervical cancer and other important cancers. The FDA recently approved the HPV vaccine Gardasil for the preventive control of HPV, using HPV virus-like particles (VLP) to generate neutralizing antibodies against major capsid protein, L1. However, prophylactic HPV vaccines do not have therapeutic effects against pre-existing HPV infections and HPV-associated lesions. Furthermore, due to the considerable burden of HPV infections worldwide, it would take decades for preventive vaccines to affect the prevalence of cervical cancer. Thus, in order to speed up the control of cervical cancer and treat current infections, the continued development of therapeutic vaccines against HPV is critical. Therapeutic HPV vaccines can potentially eliminate pre-existing lesions and malignant tumors by generating cellular immunity against HPV-infected cells that express early viral proteins such as E6 and E7.

Objective—This review discusses the future directions of therapeutic HPV vaccine approaches for the treatment of established HPV-associated malignancies, with emphasis on current progress of HPV vaccine clinical trials.

Methods—Relevant literature is discussed.

Results/conclusion—Though their development has been challenging, many therapeutic HPV vaccines have been shown to induce HPV-specific antitumor immune responses in preclinical animal models and several promising strategies have been applied in clinical trials. With continued progress in the field of vaccine development, HPV therapeutic vaccines may provide a potentially promising approach for the control of lethal HPV-associated malignancies.

Keywords

clinical trials; HPV; human papillomavirus; therapeutic; vaccines

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1. Introduction

Human papillomavirus (HPV) has been identified as an etiological factor for several important cancers, including anogenital cancers [1], and a subset of head and neck cancers (for review see [2]). Among these cancers, cervical cancer has the most significant morbidity, being the second most common cancer in women with an estimated half a million new cases diagnosed and ~ 274,000 deaths per year [3]. Of the > 200 HPV genotypes identified, four 'high-risk' types of HPV (16, 18, 31 and 45) are of particular importance because of their high level of association with ~ 80% of all cervical cancers [4]. Of these high-risk types, HPV type 16 (HPV-16) is the most prevalent, being present in more than half of all cervical cancers [5]. Furthermore, high-risk HPV types are associated with high-grade squamous intraepithelial lesions (HSILs), which are precursors of cervical cancer. The identification of HPV as an etiological factor for cervical cancer has created an opportunity to control cervical cancer through vaccination against HPV.

In order to successfully design vaccines against HPV, it is essential to have a clear understanding of HPV biology. The genomic organization of HPV is quite conserved among various types of papillomaviruses. HPVs are non-enveloped, double-stranded, circular DNA viruses of the family Papovaviridae whose genome encodes six 'early' proteins (E1, E2, E4, E5, E6 and E7) and two 'late' proteins (L1 and L2). The early proteins interact with cellular gene products and facilitate viral DNA replication, while the late proteins comprise the structural components of the viral capsid involved in the packaging of new virions. Though HPV DNA usually replicates in episomal form, it may integrate into host DNA, which often results in the deletion of some viral genes, including several early (*E2, E4* and *E5*) and late (*L1* and *L2*) genes, leaving E6 and E7 as the principal proteins expressed within the infected cell. Because E2 is a transcriptional repressor of E6 and E7, loss of E2 leads to upregulation of the *E6* and *E7* genes. The E6 and E7 proteins interact with the p53 and retinoblastoma (Rb) proteins respectively, which are important cell cycle regulatory proteins. The uncontrolled expression of E6 and E7 proteins results in the disruption of cell cycle regulation and leads to genomic instability, thereby contributing to the progression of HPV-associated cervical cancer (for a review, see [6]).

Based on our understanding of HPV biology, we realize that for the prevention of HPV infections it is necessary to develop vaccines that are capable of generating HPV-neutralizing antibodies. The newly licensed HPV preventive vaccine, Gardasil represents a triumph for HPV preventive vaccine development. Gardasil is a quadrivalent HPV L1 virus-like particle (VLP) recombinant vaccine produced by Merck that protects against HPV types 6, 11, 16 and 18. Likewise, the other HPV L1 VLP vaccine, Cervarix developed by Glaxo SmithKline that contains HPV types 16 and 18 is also expected to be available in the market soon. In general, these vaccines provide type-restricted protection, that is they protect against cervical disease relating to the HPV types included in the vaccine but not against other HPV types [7,8]. However, partial cross-protection has been observed for closely related HPV types. For example, vaccination with HPV VLPs against HPV types 16 and 18 also induces protection against HPV types 31 and 45 as well [9,10]. Gardasil and Cervarix have demonstrated excellent safety profiles and are highly effective against the included HPV types. Since HPV-16 and 18 account for ~ 70 – 75% of cervical cancers, Gardasil and Cervarix may protect up to 80% of all cervical cancers, including the partial protection against closely related types (HPV types 31 and 45).

However, Gardasil and Cervarix are unlikely to have an effect on the incidence of cervical cancer. Since cervical cancer has a high prevalence in developing countries, vaccines need to be made available in low-resource areas in order to affect the incidence of cervical cancer worldwide. Gardasil, which costs several hundred US dollars per person and Cervarix,

which is expected to cost a similar amount, may not be ideal for low-resource areas. Furthermore, the current HPV L1 VLP preventive vaccines require refrigeration for storage, which might be problematic in remote and low-resource areas. Thus, in low-resource settings, the relative benefits of these vaccines may be restricted. In order to have an effect on the incidence of cervical cancer, it is therefore necessary to develop cost-effective, stable and effective preventive vaccines that are capable of inducing broader protection against most HPV types and which are suitable for low-resource areas.

Another important obstacle to the elimination of cervical cancer is the prevalence of established HPV infections and HPV-associated disease. The existing HPV L1 VLP vaccines, Gardasil and Cervarix, do not generate therapeutic effects against pre-existing HPV infection. Since infected basal epithelial cells and cervical cancers cells do not express detectable levels of capsid antigen (L1 and/or L2), preventive HPV vaccines targeting L1 and/or L2 are unlikely to be effective in the elimination of pre-existing infection and HPV-related disease. This is a serious concern since there is currently a considerable burden of HPV infections worldwide. It is estimated that it would take ~ 20 years from the implementation of mass vaccination for highly effective preventive vaccines to affect the cervical cancer rates due to the prevalence of a significant population with existing HPV infections and the slow process of carcinogenesis. Thus, in order to accelerate the control of cervical cancer and treat currently infected patients, it is important to develop therapeutic vaccines against HPV.

2. Therapeutic human papillomavirus vaccines

Therapeutic vaccines can be used to treat established HPV infections and could therefore have an immediate effect on the prevalence of HPV-associated malignancies. Therapeutic vaccine strategies aim to eliminate pre-existing lesions and even malignant tumors by generating cell-mediated immunity against HPV-infected cells. Current approaches include live-vector-based, peptide- and protein-based, nucleic acid-based and cell-based vaccines. Table 1 summarizes the strengths and weaknesses of each strategy and Table 2 summarizes the current strategies to enhance the potency of each of these vaccine approaches. This review discusses the future directions of therapeutic HPV vaccine approaches for the treatment of established HPV-associated malignancies, with emphasis on current progress of HPV vaccine clinical trials. Table 3 lists the various therapeutic HPV vaccine clinical trials.

In order to eliminate existing lesions, a therapeutic vaccine should target HPV antigens that are continuously expressed in the infected cells and cancer cells. Thus, the choice of target antigen is extremely important for therapeutic HPV vaccine design. The HPV early proteins are potential target antigens since they are expressed throughout the life cycle and help regulate progression of the disease. In particular, the HPV-encoded proteins E6 and E7 represent ideal targets for the development of therapeutic HPV vaccines. Firstly, E6 and E7 proteins are constitutively expressed by HPV-associated tumors. Secondly, because E6 and E7 are critical for the induction and maintenance of cellular transformation in HPV-infected cells, it is unlikely that the tumor cells can escape immune attack through antigen loss. Thirdly, as E6 and E7 are foreign proteins, immunization against HPV-associated tumors circumvents some common cancer-vaccine-associated problems such as immune tolerance. Thus, many therapeutic HPV vaccine strategies have focused primarily on stimulating the production and activation of T cells by targeting E6 and/or E7 proteins Figure 1 summarizes various therapeutic HPV vaccine strategies and their effect on HPV progress.

2.1 Live-vector-based vaccines

Live-vector-based vaccines can be classified into: i) bacterial vectors, such as *Salmonella typhimurium* and *Listeria monocytogenes*; and ii) viral vectors, such as adenovirus (AdV)

and vaccinia virus. The use of vector-based vaccines for the delivery of antigen to dendritic cells (DC) is an appealing strategy for HPV vaccination. They can be used to express HPV E6 and/or E7 for the treatment of HPV-associated malignancies. Many live-vector vaccines are highly immunogenic because they can replicate within host cells and facilitate intercellular spread of antigen. In addition, the wide variety of available vectors allows for the use of custom vectors that produce a desired effect.

Though live-vector-based vaccines have strong immunogenicity and are able to spread antigen efficiently from cell to cell, there are several potential limitations that must be addressed. The production of neutralizing antibodies in the host during vaccination could reduce the potency of repeat immunizations. In addition, there is the potential risk of toxicity associated with the use of live vectors in human patients. Vaccination with live vectors may also elicit immunosuppressive factors in the host; eliminating these factors may improve the efficacy of these vaccines.

2.1.1 Bacterial vectors—Attenuated bacteria can be used to deliver genes or proteins of interest to DCs. Examples include *Listeria monocytogenes*, *Salmonella*, *Lactococcus lactis* [11,12], *Lactobacillus plantarum* [13], and bacillus Calmette-Guérin. Bacterial vector-based vaccines have been shown to be capable of eliciting strong E7-specific T-cell-mediated immune responses. In particular, *L. monocytogenes* has emerged as an exciting prospect as a live-vector-based vaccine due to its ability to generate both $CD8^+$ and $CD4^+$ immune responses and induce regression of established tumors expressing a model antigen. *L. monocytogenes* is a Gram-positive intracellular bacterium that usually infects macrophages. Unlike other intracellular pathogens, however, it evades phagocytosis by macrophages by secreting a factor, listeriolysin O, to escape into the cytoplasm of the macrophage. Its presence in both the endosomal compartments and the cytoplasm allows it to deliver antigens or carry foreign antigens into both MHC-I and MHC-II pathways, inducing strong cellular immune responses. Recently, it has been shown that *Listeria*-based vaccines against E7 can induce the regression of solid implanted tumors in transgenic mice with tissue-specific expression of HPV 16 E6 and E7 oncoproteins and overcome central tolerance by expanding low avidity $CD8^+$ T cells specific for E7 [14]. Several strategies employing fusion proteins have been used to enhance the Listeria-based vaccine potency [15,16]. Recently, a Phase I/II clinical trial is currently ongoing using the Listeria-based therapeutic HPV vaccine targeting the HPV E7 antigen in patients with cervical cancer (Y Paterson, pers. commun.).

2.1.2 Viral vectors—The high immunogenecity of viral vectors has also been harnessed in a variety of recombinant DNA and RNA vectors. These live-vector-based vaccines, which include vaccinia virus, adenovirus, adeno-associated virus and alphavirus RNA replicon particles such as Sindbis virus and Venezuelan equine encelphalitis (VEE) virus, have mostly been tested in preclinical models (for a review, see [17]). Among viral vectors, the vaccinia virus is considered to be particularly promising for antigen-specific immunotherapy due to its high efficiency of infection. Vaccinia virus constructs encoding E7 linked to proteins that enhance antigen presentation in DCs have been shown to generate E7-specific immune responses that can cause regression of E7-expressing tumors in mice [18–20].

Preclinical studies have also shown that adenoviruses can serve as effective vectors. In one adenoviral vaccine model testing a papillomavirus protein, E2, a therapeutic effect of reduced papilloma-forming sites has been observed in rabbits [21]. Recent studies employed the CRT/E7 fusion delivered by a replication-deficient adenovirus vector (Ad-CRT/E7) and it was observed that vaccination with Ad-CRT/E7 protected mice against E7-expressing tumor challenge and induced a therapeutic effect against established tumors. Rechallenge with tumor cells showed that these mice generated long-term immunological memory

against E7-expressing TC-1 cells [22]. Additionally, several adeno-associated viruses have been engineered to express E7 linked to either *Mycobacterium tuberculosis* heat-shock protein 70 (hsp70) [23] or the cytokine interleukin (IL)-12 [24], and have been shown to induce E7-specific immune responses *in vivo*.

Another viral vector of interest is the novel Semliki Forest virus (SFV), which has been shown to be capable of inducing immune responses strong enough to break host tolerance to HPV antigens. This has significant implications in established infections and cancers where the observed lack of clinical response may be due to immune tolerance of HPV-infected cells [25,26].

A recombinant vaccinia vector encoding an HPV-16/18 E6/E7 fusion protein, termed TA-HPV, has been evaluated in Phase I/II clinical trials. The vaccine was well tolerated and induced T-cell-mediated immune responses in patients with cervical intraepithelial neoplasia (CIN) [27–29]. TA-HPV has also been tested with some success in patients with other HPV-associated malignancies including vulvar intraepithelial neoplasia (VIN) [30,31] and vaginal intraepithelial neoplasia (VAIN) [32]. In a clinical study in VIN and VAIN patients vaccinated with TA-HPV, 5 out of 12 patients showed at least a 50% reduction in lesion diameter over a 24-week period, and 1 patient showed complete regression of the lesion [32].

More recently, a recombinant vaccinia vector encoding the E2 viral protein, termed MVA-E2, has been tested in patients with CIN [33,34] and flat condyloma lesions [35]. These studies demonstrated that patients developed antibodies against the MVA-E2 vaccine and generated a specific cytotoxic T lymphocyte (CTL) response against papilloma-transformed cells. However, no definitive conclusion can yet be drawn as to whether the vaccines can generate an E2-specific immune response. One possibility is that the E2-mediated cell death leads to the uptake of the antigen by the immune cells via cross-priming. However, no data are available to support this notion.

2.2 Peptide-/protein-based vaccines

2.2.1 Peptide-based vaccines—Direct administration of peptides derived from HPV antigens provides a means of vaccination against HPV. HPV antigenic proteins are taken up by dendritic cells (DCs) and presented in association with the MHC class I and/or class II pathways on human leukocyte antigen (HLA) molecules, to mount an immune response against the pathogen. In general, peptide-based vaccines are safe, easy to produce, and stable. One limitation, however, is that peptide-based vaccines are MHC-specific. Due to the highly polymorphic nature of these HLA molecules, however, it is necessary to identify specific immunogenic epitopes of HPV antigens before a vaccine can be developed, and it may be difficult to produce a peptide-based vaccine that is effective in a variety of patients with different HLA haplotypes, making it impractical for large scale vaccination treatments. Another drawback is that peptide vaccines tend to be poorly immunogenic. Consequently, most of the research in this area has focused on the use of adjuvants such as chemokines, cytokines and costimulatory molecules to enhance vaccine potency (for a review, see [36]).

In preclinical studies, progress has been achieved in augmenting peptide vaccine potency by employing the intranasal route of administration [37], linking peptides to immunostimulatory molecules to generate protective immunity and specific CTL responses [38] and using DC-activating agents such as 4′-monophosphoryl lipid A (MPL) and GM-CSF to increase and sustain levels of CTL responses [39]. Combining peptides with CpG oligodeoxynucleotide (CpG ODN), which provides a ‘danger signal’ for Toll-like receptor 9 by mimicking bacterial DNA, has also been shown to enhance the immunogenicity of peptide vaccines [40,41]. Other methods for potentiating peptide-based vaccines include the

linkage of peptides to lipids and the enhancement of epitopes to prevent peptide degradation. In particular, therapeutic vaccination with E6 and E7 long peptides has been shown to result in the control of both established virus-induced lesions and latently infected sites in a preclinical cottontail rabbit papillomavirus model [42].

There have been several clinical trials employing peptide-based vaccines. In recent Phase I/II human clinical trials, several peptide vaccines have been found to be safe and well tolerated [43,44]. In one study, 18 women with high grade cervical or vulvar intraepithelial neoplasia were vaccinated with lipidated HPV-16 E7 peptides. Of the 18 patients, 3 showed cleared dysplasia and 6 demonstrated measurable lesion regression [45]. In another non-randomized Phase I clinical trial using lipidated E7 peptide, it was found that the vaccine did not elicit regression of tumor burden [46]. A recent study has demonstrated that VIN 3 patients vaccinated with 30–35-mer overlapping peptides of HPV-16 E6 and E7 sequences in Montamide ISA 51 adjuvant generated HPV-16-specific $T_H 1/T_H 2$ cells infiltrating both the vaccination site and the VIN lesions in 6 out of 9 patients analyzed [47]. Several such strategies employing synthetic peptide vaccines are currently being developed (for review see [48]).

2.2.2 Protein-based vaccines—Like peptide vaccines, protein-based vaccines are safe and easy to produce. Moreover, protein-based vaccination can circumvent the limited specificity of MHC responses associated with peptide-based vaccines. Protein antigens can be processed and presented on the surface of DCs, and they contain all of the possible human leukocyte antigen (HLA) epitopes of an antigen. Thus, protein-based vaccination circumvents the need to determine the HLA types of prospective patients. However, like peptide-based vaccines, protein-based vaccines also suffer from low immunogenicity, and as a result, adjuvant and fusion protein strategies are often used to enhance vaccine potency. Another limitation of protein-based vaccines is that since proteins used for vaccination are administered exogenously, antigen-presenting cells (APCs) may only occasionally encounter and engulf an injected protein for MHC class I presentation. Additionally, a potential disadvantage of using proteins for therapeutic vaccination is that proteins may elicit better antibody responses than CTL responses.

Preclinical studies have focused on addressing these drawbacks in a number of different ways. To enhance immunogenecity, adjuvant and fusion protein strategies have been used, such as adding the liposome-polycation-DNA (LPD) adjuvant [49] or the saponin-based adjuvant ISCOMATRIX [50]. Because exogenously administered protein vaccines are not usually efficiently engulfed and presented by APCs, there have also been strategies aimed at promoting protein uptake by APCs. For example, fusing the antigen of interest with special proteins, such as *Bordetella pertussis* adenylyl cyclase (CyaA), a protein that targets APCs through specific interaction with $\alpha_M \beta_2$ integrin [51], or with the translocation domain of bacterial exotoxin *Pseudomonas aeruginosa* exotoxin A (EXA) [52], has been shown to enhance MHC class I and II presentation. Heat-shock proteins (hsp) have also been shown to help enhance protein-based vaccines by targeting antigen to APCs [53]. The unusually immunogenic nature of hsp derived from bacteria makes them good adjuvant-free carriers for protein-based vaccines. For example, we have shown that a fusion protein comprised of HPV-16 E7 and *Mycobacterium bovis* bacille Calmette–Guérin hsp65 could induce CTL responses in mice leading to tumor regression [54].

Various protein vaccines have moved to clinical trials. Fusion proteins containing HPV capsid proteins and HPV early proteins can potentially induce prophylactic and therapeutic immune responses. One example of this experimental fusion vaccine is TA-GW, a fusion of HPV-6 L2 and E7 absorbed onto Alhydrogel. It has been well tolerated by patients in two clinical trials and was effective in clearing HPV genital warts in a subset of patients [55,56].

A vaccine containing an HPV-16 E6/E7 fusion protein mixed with the ISCOMATRIX adjuvant has also recently been tested in a Phase I study. Immunization with this protein-based vaccine was shown to be safe and immunogenic and resulted in significantly enhanced $CD8^+$ T cell responses to both E6 and E7 in vaccinated patients compared with those observed in placebo recipients [57]. Another protein vaccine, TA-CIN, a fusion of HPV-16 L2, E6 and E7, induced antibodies in all the women tested and induced T cell immunity in a subset of them, proving to be safe [58]. A vaccine termed PD-E7, comprised of mutated HPV-16 E7 fused with a fragment of *Haemophilus influenzae* protein D and formulated in the GlaxoSmithKline Biologicals adjuvant AS02B, has been evaluated in Phase I/II clinical trials and was shown to induce significant E7-specific CTL responses in patients with CIN-1 and CIN-3 lesions [59]. Recently, a fusion of HPV-16 E7 and *M. bovis* hsp65 has been shown to be well tolerated in patients with high-grade anal intraepithelial neoplasia (AIN) [60,61] ; however, further tests are needed to determine the clinical efficacy of the vaccine. A recent trial employing the same vaccine was conducted in women with CIN III lesions. However, there was no significant difference in regression in women infected with HPV 16 compared with those without HPV 16 infection [62].

2.3 Nucleic acid-based vaccines

2.3.1 DNA-based vaccines—DNA vaccines have emerged as an attractive and potentially effective strategy for antigen-specific immunotherapy. Naked DNA is safe, stable, relatively easy to manufacture and can be used to sustain the expression of antigen in cells for longer periods of time than RNA or protein vaccines. Furthermore, unlike live-vector vaccines, DNA vaccines do not elicit neutralizing antibody production in the patient, and thus can be repeatedly administered to the same patient effectively.

Although HPV DNA vaccines are considered to be relatively safe compared with other forms of vaccines such as viral-vector-based vaccines, some concerns have been raised. For example, DNA may potentially integrate into the host genome, causing genomic instability, though there is no evidence that shows that integration of DNA occurs in numerous organs or tissues. Vaccination with E6 and/or E7 DNA also has the potential hazard of cellular trans formation, as E6 and E7 are virus-encoded oncoproteins, which could be addressed through modification of E6 or E7, which abolishes the transformative capacity of these proteins. Generally, however, DNA vaccines are poorly immunogenic because DNA lacks the intrinsic ability to amplify or spread from transfected cells to surrounding cells *in vivo*. Several strategies have been developed to enhance the potency of DNA vaccines by: i) increasing the number of antigen-expressing DCs; ii) enhancing antigen processing and presentation in DCs; and iii) improving DC interaction with T cells to augment T-cell-mediated immune responses (for reviews, see [63,64]).

2.3.1.1 Strategies for increasing antigen-expressing dendritic cell populations: One approach for boosting antigen-expressing DC populations is to find the most effective routes for delivery of DNA vaccines. Among the different routes of DNA administration, vaccination via gene gun has been to shown to be one of the most potent methods for the delivery of genes of interest into DCs [65]. The gene gun is used to fire DNA-coated gold particles into the epidermis and efficiently transfect intradermal DCs that can mature and migrate to the lymphoid organs for T cell priming. This represents a convenient and effective method for the *in vivo* introduction of naked DNA into DCs. Another method for increasing the number of antigen-expressing DCs by DNA vaccines is to promote the spread of encoded antigen between DCs by linking antigen with proteins capable of intercellular transport. The authors have investigated the use of DNA encoding HPV-16 E7 fused to herpes simplex virus type 1 VP22 (HSV-1 VP22), a viral protein with intercellular trafficking properties, in a DNA vaccine. *In vivo* experiments showed that the vaccine

dramatically enhanced E7-specific $CD8^+$ T-cell responses and generated greater antitumor effects than DNA vaccines encoding E7 alone [66]. We then generated a vaccine encoding E7 linked to Marek's disease virus type 1 VP22 (MDV-1 VP22), a protein with some homology to HSV-1 VP22, and observed powerful antitumor immunity as well [67]. Other strategies for improving antigen-expressing DC populations include the linkage of antigen to molecules that can target the antigen to the surface of DCs, such as FMS-like tyrosine kinase 3 (flt3) ligands, which bind with flt3 receptors on DCs, and hsp, which bind with scavenger receptors on DCs such as CD91.

2.3.1.2 Strategies for enhancing antigen expression, processing and presentation in dendritic cells: One method for improving antigen expression of DNA vaccines in DCs is codon optimization. This technique is used to modify antigen gene sequences by replacing rarely used codons with more commonly recognized codons, and can enhance translation of a DNA vaccine in DCs. It has also been shown to be effective in increasing the CTL response induced by DNA vaccines [68–70].

DCs must present antigens through the MHC class I pathway to generate populations of antigen-specific $CD8^+$ T cells. Linkage of antigen to proteins that target the antigen to the endoplasmic reticulum or facilitate proteasome degradation, for example, can lead to enhanced MHC class I antigen presentation and greater ensuing CTL responses. The authors have demonstrated the potent effects against E7-expressing tumors of vaccines encoding E7 linked to various MHC class I-targeting proteins and protein domains, including *M. tuberculosis* hsp70 [71], γ-tubulin [72], the extracellular domain of Flt3-ligand [73], and the translocation domain of *Pseudomonas aeruginosa* exotoxin A [74]. Among these strategies, it was found that DNA vaccines containing E7 linked to calreticulin (CRT), a protein shown to significantly enhance MHC class I antigen presentation, elicited the greatest E7-specific $CD8^+$ T-cell responses among all the DNA vaccines tested [75]. These findings suggest that the use of CRT for the generation of potent antigen-specific immune responses may be applicable to other types of immunotherapeutic vaccines.

Another important strategy for circumventing antigen processing and eliciting stable MHC class I presentation of a peptide encoded by a DNA vaccine is the employment of MHC class I single-chain trimer (SCT) technology [76]. This technique involves the linkage of an antigenic peptide to β2-microglobulin and MHC class I heavy chain, producing a single-chain construct encoding an MHC class I molecule fused to the peptide antigen. It has been shown that immunization of mice with a DNA vaccine encoding a SCT composed of an immunodominant CTL epitope of HPV-16 E6, β2-microglobulin and $H\text{-}2K^b$ MHC class I heavy chain (termed pIRES-E6- $\beta 2m\text{-}K^b$) could generate increased E6 peptide-specific $CD8^+$ T-cell responses compared with mice vaccinated with DNA encoding wild type E6 [76].

On the other hand, fusion of antigen to MHC class II-targeting molecules can redirect an antigen to the class II pathway and generate greater $CD4^+$ T cell responses that augment CTL responses (for a review, see [77]). We have previously shown that linkage of antigen to a signal peptide for the endoplasmic reticulum (Sig) and the lysosomal targeting domains of lysosome-associated membrane protein 1 (LAMP-1) can enhance MHC class II antigen presentation and generate significant $CD4^+$ T cell responses [78]. This construct, termed Sig/E7/LAMP-1, was tested in the context of a DNA vaccine and produced greater numbers of E7-specific $CD4^+$ T cells and also higher E7-specific CTL activity in mice than vaccines composed of Sig/E7 or wild-type E7 DNA alone [79].

The MHC class II invariant chain has recently been employed in the context of a DNA vaccine to effectively enhance class II presentation of antigens. In the endoplasmic

reticulum (ER), Ii binds to MHC class II molecules and the class II-associated invariant -chain peptide (CLIP) region of the invariant chain occupies the class II peptide-binding groove, preventing premature binding of antigenic peptides into the groove. In the lysosomal compartment, CLIP is replaced by an antigenic peptide and the MHC class II/peptide complex is presented on the surface of the DC. By substituting the CLIP region of the invariant chain with a desired T helper (T_H) epitope such as pan-DR helper T-lymphocyte epitope (PADRE) (Ii-PADRE), the epitope can be efficiently presented via the class II pathway. Mice vaccinated with a DNA vaccine encoding Ii-PADRE generated significant PADRE-specific $CD4^+$ T cell immune responses. Furthermore, coadministration of DNA encoding E7 and DNA encoding Ii-PADRE in mice was shown to elicit potent $CD8^+$ T-cell responses compared with coadministration of DNA encoding E7 and DNA encoding unmodified invariant chain [80].

2.3.1.3 Strategies for enhancing dendritic cell and T-cell interaction: Once an antigen has been processed and presented, the interactions between DCs and T cells become critical for T-cell activation. After priming, DCs can become targets of activated armed effector T cells. To prevent T cell-mediated apoptosis in DCs, DNA encoding antiapoptotic proteins can be used to prolong DCs survival and enhance the long-term ability of DCs to prime T cells. In our studies, co-delivery of DNA encoding E7 with DNA encoding inhibitors of apoptosis such as B-cell leukemia/lymphoma × (BCL-xL), B-cell lymphoma protein 2 (BCL-2), X-linked inhibitor of apoptosis protein (XIAP) and dominant-negative caspases was shown to enhance E7-specific $CD8^+$ T cell responses in mice [81]. Approaches involving the linkage of the *E7* gene with *Escherichia coli* β-glucuronidase have also been employed to enhance vaccine potency [82]. In addition, DNA vaccines employing strategies to enhance antigen presentation with strategies to prolong DC life were shown to further improve antigen-specific CTL responses [83–85]. The introduction of DNA-encoding antiapoptotic proteins into cells, however, raises concerns of oncogenicity. Inhibition of proapoptotic proteins using RNA interference (RNAi) may potentially alleviate these problems. We have recently demonstrated that coadministration of DNA vaccines encoding E7 with short interfering RNA (siRNA) targeting the key proapoptotic proteins BCL-2 homologous antagonist/killer (Bak) and BCL2-associated X protein (Bax) was able to effectively improve DC resistance to apoptotic cell death and enhance antitumor $CD8^+$ T cell responses in mice [86].

The activation of naive antigen-specific T cells is dependent on signals delivered by DCs to T cells, such as co-stimulatory factors and cytokines. Numerous studies using this strategy have shown enhanced antigen-specific immune responses. Examples include coadministration of HPV DNA vaccines with DNA encoding GM-CSF [87], IL-2 [88] or IL-12 [89].

Several DNA vaccines have translated into clinical trials. A microencapsulated DNA vaccine encoding multiple HLA-A2-restricted E7-derived epitopes, termed ZYC-101 (ZYCOS, Inc., now acquired by MGI Pharma), has been tested in patients with CIN-2/3 lesions [90] and in patients with high-grade anal intra-epithelial lesions [91]. The vaccine was well tolerated in both trials. Out of the 12 individuals with anal displasia, 10 had increased immune responses to the peptide epitopes encoded within the DNA vaccine. Out of 15 women with CIN-2/3 5 had complete histological responses and 11 induced HPV-specific T-cell responses. A new version of the vaccine, termed ZYC-101a, encodes HPV-16 and HPV-18 E6- and E7-derived epitopes and was shown to resolve CIN-2/3 lesions in a subset of young women enrolled in the trial [92]. At the Johns Hopkins Hospital, a Phase I trial using a DNA vaccine encoding modified HPV-16 E7 DNA (which abolished the Rb binding site) linked to *M. tuberculosis* hsp70 (pNGVL4a-Sig/E7(detox)/hsp70) was performed in patients with CIN-2/3 lesions. The vaccine was well tolerated by all patients,

and some of the patients who received the maximum dose of DNA vaccine (4 mg/vaccination) showed detectable E7-specific $CD8^+$ T-cell immune responses (C Trimble, pers. commun.). The same DNA vaccine has also been tested in a subset of HPV-16-positive patients with head and neck squamous cell carcinoma. The same group of investigators have also planned to initiate a Phase I trial with a DNA vaccine encoding modified HPV-16 E7 DNA linked to CRT in patients with stage 1B1 cervical cancer using a good manufacturing practice-grade gene gun device (C Trimble, pers. commun.).

2.3.2 RNA replicon vaccines

The use of RNA replicons is a relatively new and potentially interesting strategy for HPV vaccination. RNA replicons are naked RNA molecules that replicate within transfected cells. They may be derived from alphaviruses, such as Sindbis virus [93,94], Semliki Forest virus [95,96], and VEE [97]. These subgenomic alphavirus RNA vaccines are self-replicating and self-limiting, and may be administered as either RNA or DNA, which is then transcribed into RNA replicons. RNA replicon-based vectors have several potential advantages for cancer vaccine development. For example, RNA replicons can replicate in a wide range of cell types and can be used to produce sustained levels of antigen expression in cells, making them more immunogenic than conventional DNA vaccines. Many replicon vectors do not contain viral structural genes, and thus no infectious particles are produced and the host immune response to these vectors is likely to be limited. Thus, RNA-replicon-based vaccines can be used in patients repeatedly. In addition, RNA replicons alleviate the risk of potential chromosomal integration and cellular transformation associated with naked DNA vaccines. This is particularly important in the development of vaccines targeting potentially oncogenic proteins such as E6 and E7. However, RNA replicons are less stable than DNA. One alternative has been to combine the benefits of RNA replicon and DNA vaccines into a DNA-launched RNA replicon, termed ‘suicidal’ DNA.

DNA-launched RNA replicons have been used for HPV vaccine development in preclinical models [98,99]. This ‘suicidal DNA’ is transcribed into RNA within the transfected cell and provides a stable and efficient way to express tumor antigen. However, transfected cells eventually undergo apoptosis. When DNA-launched RNA replicons are delivered to DCs via gene gun, this apoptotic change mediated by suicidal DNA may lead to diminished immune responses. To prevent this, suicidal DNA encoding the E7 antigen linked to antiapoptotic proteins such as BCL-xL was administered to mice and was shown to delay cell death in DCs and generate significantly higher E7-specific $CD8^+$ T-cell immune responses and better antitumor effects than DNA encoding wild type E7 alone [99]. Another alternative is to generate replication-defective, self-limiting replicon particles using a safe packaging cell line. These replicon particles offer advantages over naked nucleic acid vaccines such as efficient gene delivery and large-scale production, and may prove useful in the development of effective RNA-based HPV vaccines.

The potency of HPV RNA replicon vaccines can also be enhanced by employment of the intracellular targeting and intercellular spreading strategies used in DNA-based immunization [100–102]. Another replicon system uses a flavivirus termed Kunjin (KUN) to deliver replicons. Vaccination of mice with KUN replicons expressing an HPV-16 E7 epitope induced specific T-cell responses and protected mice from tumor challenge [103]. A new generation of KUN replicon vectors has been developed which allows for the synthesis of replicon RNA from plasmid DNA. The primary advantage of this DNA-based KUN replicon system is that the replicon does not induce cellular apoptosis, and thus antigen presentation by DCs to T cells is prolonged, enhancing the elicited immune response [104]. However, despite the general success of RNA replicons in preclinical models, RNA replicon-based vaccines have had limited clinical testing.

2.4 Whole cell vaccines

2.4.1 Dendritic cell-based vaccines—The use of DC-based vaccines represents a potentially plausible strategy for therapeutic HPV vaccines against HPV-associated malignancies. To create a dendritic cell-based HPV vaccine, it is necessary to load DCs with viral antigens and deliver them into patients. DCs can be prepared *ex vivo* by the physical loading of MHC class I and class II molecules, and antigen loading can be accomplished by pulsing the DCs with antigenic peptides or proteins or by transfecting DCs with DNA or RNA encoding HPV antigen. Significant advances in our knowledge of DC differentiation, migration and maturation, as well as antigen processing and presentation have provided a rationale for the usage of DCs as natural adjuvants in antigen-specific cancer immunotherapy (for a review, see [105]).

However, while effective DC-based vaccines can be produced using autologous DCs, individualized vaccines are cumbersome to generate and therefore large-scale production is quite challenging. Additionally, the culturing technique used to prepare DCs may affect the quality of the vaccine, leading to heterogeneity in vaccine quality and a lack of standard criteria for evaluating the vaccines. Furthermore because it is critical for DCs to home to the lymphoid organs where the majority of T cells are located, the route of administration is an important issue for DC-based vaccines for maximizing the effects of the vaccine.

Preclinical models have tried to address these concerns. For effective loading of tumor antigen into DCs, one strategy is to deliver genes to DCs using adenoviral vectors targeted to CD40 via bispecific antibodies. Recombinant adenoviral vectors carrying HPV E7 have been shown to elicit strong antitumor immunity in mice after challenge with E7-expressing tumors [106]. Once DCs are loaded with tumor antigen, the vaccine can be administered via intramuscular, subcutaneous, or intravenous delivery. Among these, it was shown that intramuscular delivery is the most effective method for generating large numbers of E7-specific $CD8^+$ T cell precursors [107]. It is also possible to infect DCs with tumor antigen-expressing vaccinia virus [108] or fuse DCs with tumor cells.

Several methods have been used to enhance the potency of DC-based HPV vaccines. As mentioned previously, prolongation of DC survival improves T cell priming by DCs. It has been shown that transfecting E7-loaded DCs with small interfering RNA (siRNA) targeting the Bak and Bax proapoptotic proteins leads to downregulation of Bak and Bax protein expression in the DCs, which resulted in increased resistance of the DCs to T-cell-mediated apoptosis. Vaccination of mice with E7-loaded DCs transfected with siRNA targeting Bak and Bax led to enhanced E7-specific immune responses and antitumor effects [109]. Furthermore, strategies to prolong DC survival can be used in conjunction with a strategy to improve MHC class I/II presentation of antigen to further enhance DC-based vaccine potency [110]. Thus, some of the strategies used to modify of the properties of DCs described in previous sections may potentially be used to enhance DC-based vaccines.

Several clinical trials of DC-based HPV vaccines in cancer patients have been reported. Although most of the studies were Phase I/II trials focused on assessing feasibility and safety of DC-based vaccines, successful induction of antigen-specific immune responses have been observed. In a case report, subcutaneous injection of HPV-18 E7-pulsed DCs in a patient with metastatic cervical cancer led to inhibition of tumor progression. Although the vaccine did not cause complete remission in this patient, the individual's health status improved and no significant side effects were observed [111]. In a clinical pilot study, autologous DCs pulsed with E7 protein were shown to induce T-cell responses in some late-stage cervical cancer patients [112]. In more recent trials, administration of DCs pulsed with recombinant HPV-16 or HPV-18 E7 was shown to induce E7-specific $CD4^+$ T-cell immune responses in two out of four patients and E7-specific $CD8^+$ T-cell responses in all four

patients with cervical cancers refractory to standard treatments [113]. However, no objective clinical responses were observed.

2.4.2 Tumor-cell-based vaccines—The use of tumor cells in HPV vaccines represents a second approach to whole-cell immunization. Tumor cells can be manipulated *ex vivo* to express immunomodulatory proteins, such as cytokines, in order to enhance their immunogenicity. Cytokine genes used in HPV-transformed tumor vaccines include IL-2 [114], IL-12 [115,116], and GM-CSF [116,117]. One advantage of tumor-cell-based vaccines is the convenience that tumor antigens need not be clearly identified, though cervical cancer has a clear viral etiology, as opposed to other types of cancers without well-defined tumor-specific antigens.

Tumor-cell-based HPV vaccines have been tested in preclinical models. For example, vaccination of mice with GM-CSF-expressing E7-positive tumor cells led to an E7-specific CTL response and potent antitumor effects against E7-expressing tumors *in vivo* [117]. Although tumor-cell-based vaccines, such as GM-CSF-transduced autologous or allogeneic tumor cells have been used in clinical trials for other cancers such as pancreatic cancer, renal cell carcinoma, melanoma and prostate carcinoma, tumor-cell-based vaccines have not been tested against HPV-associated malignancies in the clinical arena. However, using tumor-cell-based vaccines carries the risk of introducing new cancers, which is not acceptable for relatively healthy HPV carriers or patients with mild cervical neoplasia. In addition, although effective tumor vaccines can be derived from autologous tumor cells, individualized vaccine production is cumbersome and large-scale vaccine production is impractical. Furthermore, inconsistencies in the potency and purity of these vaccines restrict their efficacy. Thus, tumor cell-based vaccines have limited scope for HPV vaccine development.

3. Combined approaches: the future of human papillomavirus vaccination

Prime-boost regimens are perhaps the most effective treatment strategy for vaccination against HPV. Because nucleic acid vaccines often generate relatively weak CTL responses, combinatorial vaccination approaches are used to circumvent this limitation. Priming with a DNA or RNA vaccine and then boosting with a viral-vector vaccine has been shown to result in enhanced immune responses relative to single modality vaccinations. For example, in preclinical models, priming the immune system with a DNA vaccine and then boosting with either a live-viral-vector vaccine [118,119] or a tumor vaccine [120] has been shown to elicit stronger antitumor effects than either vaccine alone. In another study, mice first primed with a Sindbis virus RNA replicon containing E7 linked to *M. tuberculosis* hsp70 (E7/hsp70) and then boosted with a vaccinia vector encoding E7/hsp70 showed E7-specific CTL responses as well as strong antitumor effects [121]. The efficacy of other treatment strategies, such as protein-based vaccination, can also be enhanced by prime-boost approaches [122]. On the other hand, combining therapeutic vaccines with antiviral or anticancer agents may also be effective for the treatment of HPV-associated disease [123,124].

Some prime-boost regimens have proven effective in clinical trials. In prime-boost vaccine trials using heterologous HPV vaccines (TA-CIN followed by TA-HPV) in the management of anogenital intraepithelial neoplasia, patients experienced HPV-16-specific T cell responses and showed symptomatic improvement without serious adverse effects [125,126]. In another study, patients were initially primed with TA-HPV and subsequently vaccinated with a booster containing HPV-16 L2/E6/E7. Nine out of ten patients developed HPV 16-specific immune and/or serological responses, and three showed a reduction in lesion size or experienced symptomatic relief [127]. A clinical trial using pNGVL4a/Sig/E7(detox)/Hsp70

DNA prime followed by TA-HPV vaccinia boost is currently being planned at Johns Hopkins University in patients with CIN2/3 lesions (C Trimble, pers. commun.). In general, clinical outcomes have suggested that prime-boost regimens may be more effective than single-modality vaccinations, and thus this strategy merits further study as one of the most promising approaches for the treatment of HPV-associated malignancies.

Combination approaches including chemotherapy, radiation or other biotherapeutic agents combined with HPV therapeutic vaccination may also serve to enhance the therapeutic HPV vaccine potency. For example, a recent study has shown that the chemotherapeutic agent epigallocatechin-3-Gallate (EGCG), a chemical derived from green tea, could induce tumor cellular apoptosis and enhance the tumor-antigen-specific T-cell immune responses elicited by DNA vaccination [31]. In addition, peptide/protein-based vaccines have been combined with chemotherapy and/or radiation to improve therapeutic antitumor effects [128,129]. A particular study has combined the employment of recombinant E7 protein-based subunit vaccines in the presence of CpG oligonucleotide adjuvant with chemotherapy using cisplatin and demonstrated therapeutic antitumor synergy against established E7-expressing tumors [128]. These successful results have led to the planning of a Phase I clinical trial at Johns Hopkins involving the combination of oral EGCG administration with intradermal administration of CRT/E7 DNA vaccination via gene gun in patients with advanced HPV-associated head and neck squamous cell carcinomas (HPV-HNSCC) (S Pai, pers. commun.).

The therapeutic effects of HPV vaccines may be further enhanced by combination with blocking of the factors that inhibit T-cell activation, such as cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death protein 1 (PD-1). These molecules are negative coregulators in the T cell costimulatory pathway. Thus, antibody-mediated blockade of CTLA-4 and PD-1 can potentially be used to prolong antitumoral T cell responses (for review, see [130,131]). The combination of HPV therapeutic vaccines with agents that influence the tumor microenvironment may also potentially be used to generate enhanced therapeutic effects against HPV-associated malignancies. It is now clear that several factors present in the tumor microenvironment may potentially hinder immunotherapy. These factors include the expression of B7 homolog-1 B7-H1 [132], signal transducer and activator of transcription 3 (STAT3) [133] and MHC class I polypeptide-related sequence (MIC)-A and B [134], indoleamine 2,3-dioxygenase (IDO) enzyme [135], and galectin-1 [136] on tumor cells, immunosuppressive cytokines such as IL-10 [137] and TGF-β[138], T regulatory cells [139], myeloid-derived suppressor cells [140]. It is conceivable that agents capable of blocking these molecules may potentially be used to enhance the therapeutic effects of the HPV vaccines.

4. Expert opinion

The identification and characterization of high-risk human papillomavirus as a necessary causal agent for cervical cancer provides a promising possibility for the eradication of HPV-related malignancies. In the development of therapeutic HPV vaccines, we have focused on identifying and targeting the most relevant antigens associated with cervical cancer, the E6 and E7 HPV oncoproteins, which represent tumor-specific antigens and potentially ideal targets for therapeutic HPV vaccines. Based on our studies and those of others in the field, we conclude that the various current approaches, including peptide- and protein-based, live-vector-based, nucleic acid-based, and cell-based immunization, are each associated with strengths and weaknesses (Table 1). It is important to consider using strategies such as prime-boost regimens and/or combinations strategies using molecules that are capable of blocking the negative regulators on T cells to further enhance the T cell immune responses. Furthermore, increasing understanding of the molecular mechanisms that hinder immune attack in the tumor microenvironment will lead to the identification of novel molecular

targets that can be blocked in order to enhance the therapeutic effect of HPV vaccines. It is conceivable that effective therapy against HPV-associated malignancies will probably require a combination of therapeutic HPV vaccines with the employment of innovative agents that are capable of eliminating the suppressive factors present in the tumor microenvironment. With continued endeavor in the development of HPV therapeutic vaccines, we can foresee that HPV therapeutic vaccines will become an important approach that can be combined with existing forms of therapy such as chemotherapy and radiation therapy to generate better control of HPV-associated malignancies.

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Declaration of interest

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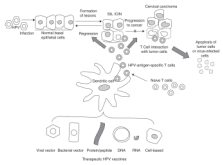

Figure 1. Therapeutic HPV vaccines and HPV progression

Microtrauma is believed to allow HPV to access basal epithelial cells. HPV infection promotes epithelial cell proliferation leading to SIL or CIN, and in some cases, progressing to invasive cervical carcinoma. This diagram provides an overview of the immunologic effects of therapeutic vaccination with live vector-based (viral/bacterial), protein or peptide-based, DNA or RNA-based or cell-based vaccines (DCs or tumor cells). DCs are the most potent professional APCs that prime T cells *in vivo*. DCs also migrate to secondary lymphoid organs to select and stimulate antigen-specific T cells. Thus, many therapeutic vaccine strategies have focused on targeting antigen to professional APCs, such as DCs, and enhancing antigen processing and presentation in DCs in order to augment T-cell-mediated immune responses. DCs activate the HPV-antigen-specific CTLs. These CTLs mediate immune clearance by apoptosis of virus-infected cells, thus blocking progression to cervical cancer or inducing regression of CIN lesions.

APC: Antigen-presenting cell; CIN: Cervical intraepithelial neoplasia; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; HPV: Human papillomaviurs; SIL: Squamous intraepithelial lesions.

Table 1

Characteristics of therapeutic HPV vaccine approaches.

Vaccine approach	Advantages	Disadvantages
Viral-vector-based (i.e., vaccinia, adenovirus, alphavirus)	High immunogenicity Different immunological properties of viruses Wide variety of vectors available Can potentially be engineered to express cytokines or other stimulatory molecules	Risk of toxicity in using live viruses Potential pre-existing immunity Inhibited repeat immunization Immunodominance of viral vector antigens over HPV tumor antigens
Bacterial-vector-based (i.e., *Listeria*, *Salmonella*, *Lactococcus*)	High immunogenicity Can deliver either engineered plasmids or HPV tumor proteins to antigen-presenting cells Wide variety of vectors available	Risk of toxicity in using live bacteria Potential pre-existing immunity Inhibited repeat immunization
Peptide based	Stable, easy to produce, safe Can combine multiple epitopes Can enhance peptides for MHC binding	Low immunogenicity Must determine epitopes Must match patient's HLA
Protein based	Stable, easy to produce, safe Multiple known adjuvants No HLA restriction	Usually better induction of antibody response than CTL response
DNA	Easy to produce, store and transport Versatility in ability to add targeting and/or co-stimulatory genes Capable of multiple immunizations Variety of delivery methods (i.e., direct injection, gene gun, intranasal, biojector) Sustained expression of antigen on MHC-peptide complex (compared with peptide/protein vaccines)	Intrinsically weak immunogenicity Concern of integration into genome or cellular transformation
RNA	Non-infectious, transient; no risk of chromosomal integration or cellular transformation Capable of multiple immunizations RNA replicons replicate within the cell to enhance antigen expression Multiple vectors are available	Difficult to store and handle Labor-intensive preparation Difficult to prepare large amounts
DC based	High immunogenicity; uses the most potent antigen-presenting cells Methods are available to generate large numbers of DCs Multiple methods of antigen loading available Potency can be enhanced by gene transduction or cytokine treatment	Labor intensive, costly, *ex vivo*, individualized cell processing Variable quality control and a lack of standard criteria for quality of vaccines Do not necessarily home to draining lymph nodes Possibility of tolerization by immature DCs
Tumor cell based	Useful if tumor antigen unknown Potency can be enhanced by gene transduction or cytokine treatment Likely to express relevant tumor antigens	Safety concerns about injecting tumor cells into patients Labor-intensive procedure Weak antigen presentation by tumor cells Requires availability of tumor cell lines or autologous tumor cells

DC: Dendritic cell; HPV: Human papillomavirus.

Table 2

Strategies to enhance therapeutic HPV vaccine potency.

Vaccine approach	Improvement strategies
Viral-vector-based (i.e., vaccinia, adenovirus, alphavirus)	Adjuvant and fusion protein strategies to further augment immunogenicity Circumvent neutralizing antibodies
Bacterial-vector-based (i.e., *Listeria*, *Salmonella*, *Lactococcus*)	Adjuvant and fusion protein strategies to further augment immunogenicity Circumvent neutralizing antibodies
Peptide-based	Epitope enhancement Lipopeptide formulations Adjuvant and fusion protein strategies to enhance immunogenicity
Protein-based	Adjuvant and fusion protein strategies to enhance immunogenicity and CTL response
DNA	Increase number of antigen-expressing DCs Enhance antigen processing and presentation in DCs Improve DC interaction with T cells to augment T-cell-mediated immune responses Enhance DC activation
RNA	Combine with DNA for use as DNA-launched RNA replicons
DC based	Improve efficient loading of antigen to DCs Prolong DC survival
Tumor cell based	Address safety concerns Manipulate *ex vivo* to express cytokines

CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; HPV: Human papillomavirus.

Table 3

Therapeutic HPV vaccine clinical trials.

Type	Vaccine construct	Target subtype(s)	Antigen	Sponsor	Study subjects	Ref.
Live vector based	Recombinant Vaccinia Virus (TA-HPV)	HPV-16, HPV-18	E6 and E7 mutated to inactivate Rb binding	Xenova/Cantab	Phase I/II trial in patients with advanced cervical cancer	[27]
					Phase I with early-stage cervical cancer patients	[29]
					Phase II in patients with high-grade VIN or VAIN	[32]
					Phase II with high grade VIN or VAIN patients	[30]
					Phase I/II in patients with high grade VIN	[28]
	Recombinant Vaccinia Virus (MVA-E2)	HPV-16, HPV-18	E2	Instituto Mexicano del Seguro Social (IMSS)	Phase I/II trial in patients with CIN1–3	[33]
					Phase II with high grade CIN	[34]
					Phase I/II in patients with flat con dyloma lesions	[35]
Peptide based	Peptide	HPV-16	E7 epitopes (aa 11 – 22 and 86 – 93) and PADRE	Netherlands National Institute of Public Health and Environmental Protection	Phase I/II trial in patients with cervical cancer	[43]
					Phase I/II trial in patients with advanced cervical cancer	[44]
	Peptide	HPV-16	E7 epitopes (aa 12 – 20 alone or aa 12 – 20 mixed with aa 86 – 93 linked to a T cell epitope peptide)	NCI	Phase I trial in patients with CIN 2/3 or VIN 2/3	[45]
	Lipopeptide	HPV-16	Lipidated E7 (HLA-A * 0201-restricted epitope, aa 86 – 93 lipopeptide)	NCI	Phase I trial in patients with cervical or vaginal cancer	[46]
Protein based	Fusion protein	HPV-16	Recombinant E6/E7 protein with ISCOMATRIX adjuvant	CSL Limited	Phase I trial in patients with CIN 1–3	[57]
	Fusion protein (PD-E7/D16E7)	HPV-16	E7 linked to the first 108 aa of *Haemophilus influenzae* protein D	GlaxoSmithKline	Phase I/II trial in patients with CIN1 or CIN3	[59]
	Fusion protein (TA-GW)	HPV-6	L2/E7	Xenova/Cantab	Phase I trial in healthy volunteers	[56]
					Phase IIa trial in patients with genital warts	[55]
	Fusion protein (TA-CIN)	HPV-6	L2/E6/E7	Xenova/Cantab	Phase I trial in healthy volunteers	[58]

Type	Vaccine construct	Target subtype(s)	Antigen	Sponsor	Study subjects	Ref.
	Fusion protein (SGN-00101 or HSPE7)	HPV-16	E7 linked to HSP65 from *Mycobacterium bovis*	StressGen	Phase I/II trial in HIV patients with AIN	[61]
					Phase III trial in patients with AIN and Phase II trial in patients with AGW and RRP	[60]
DNA based	DNA (ZYC101)	HPV-16	E7 epitope (aa 83 – 95)	Zycos	Phase I trial in patients with anal HSIL	[91]
					Phase I trial in patients with CIN2/3	[90]
	DNA (ZYC101a)	HPV-16, HPV-18	E6 and E7 epitopes	Zycos	Phase II trial in patients with CIN2/3	[92]
	DNA	HPV-16 HPV-16	E7 contained in the pNGVL4a-Sig/E7(detox)/HSP70 plasmid	NCI	Clinical trials have begun for patients with CIN2/3	*
					Clinical trials to begin soon for patients with advanced HNSCC	§
DC based	DC	HPV-18	E7	NA	Case report of a single patient with cervical cancer	[111]
	DC	HPV-16, HPV-18	Autologous DCs pulsed with rHPV16E7 or HPV18E7	NA	Trial in cervical cancer patients with recurrent disease refractory to standard treatment modalities	[113]
	DC	HPV-16, HPV-18	Autologous DCs pulsed with rHPV16E7 or HPV18E7	NA	Clinical pilot study in patients with late stage cervical cancer	[112]
Prime boost	Prime with fusion protein (TA-CIN), boost with recombinant vaccinia virus (TA-HPV)	HPV-16, HPV-18	L2/E6/E7 (TA-CIN) + E6 and E7 mutated to inactivate Rb binding (TA-HPV)	Xenova	Phase II trial in patients with AGIN 3	[125]
					Phase II trial in patients with high-grade AGIN	[126]
	Prime with recombinant vaccinia virus (TA-HPV), boost with fusion protein (TA-CIN)	HPV-16, HPV-18	E6 and E7 mutated to inactivate Rb binding (TA-HPV) + L2/E6/E7 (TA-CIN)	Xenova	Phase I/II trial in patients with high grade AGIN	[127]

*C Trimble, pers. commun.

‡M Gillison, pers. commun.

AA: Amino acids; AGIN: Anogenital intraepithelial neoplasia; AGW: Anogenital warts; AIN: Anal intraepithelial neoplasia; CIN: Cervical intraepithelial neoplasia; CRT: Calreticulin; DC: Dendritic cell; HLA: Human leukocyte antigen; HNSCC: Head and neck squamous cell carcinoma; HPV: Human papillomavirus; HSIL: High-grade squamous intraepithelial lesion; HSP: Heat-shock protein; LGIN: Lower genital tract intraepithelial neoplasia; NCI: National Cancer Institute; PADRE: Pan-DR binding T helper peptide; RRP: Recurrent respiratory papillomatosis (warts of the upper airways); VAIN: Vaginal intraepithelial neoplasia; VIN: Vulvar intraepithelial neoplasia.