[Frontiers in Bioscience 12, 246-264, January 1, 2007]

Vaccines against human papillomavirus

Yen-Yu Lin [1], Hannah Alphs [1], Chien-Fu Hung [1,3], Richard BS Roden [1,2,3] and T-C Wu [1,2,3,4]

Departments of [1] Pathology, [2] Obstetrics and Gynecology, [3] Oncology and [4] Molecular Microbiology and Immunology, Johns Hopkins Medical Institutions, 600 North Wolfe Street, Baltimore, Maryland, 21287 USA

TABLE OF CONTENTS

1. ABSTRACT

Human papillomavirus has been identified as an etiological factor for cervical cancer, anogenital cancers and a subset of head and neck cancers. These important observations suggest that HPV vaccines have potential in the prevention and treatment of cervical cancer and other HPV-associated malignancies. The HPV genome encodes two HPV late genes, L1 and L2, which form the viral capsid. Early viral proteins support viral genome replication, two of which (E6 and E7) are important for HPV associated malignant transformation. Prophylactic HPV vaccines prevent infection by inducing neutralizing antibodies against HPV capsid proteins L1 and L2. However, because HPV-infected basal keratinocytes and HPV-transformed cells generally do not express L1 or L2, therapeutic HPV vaccines aim to treat established HPV infections and HPV-associated malignancies by targeting non-structural early viral antigens of HPV such as E6 and E7. Results from preclinical HPV vaccine studies have led to several HPV vaccine clinical trials. If these prophylactic and therapeutic HPV vaccines prove as successful in patients as they have in animal models, vaccination may provide for control and eventually eradication of oncogenic HPV infection and HPV-related cancers.

2. INTRODUCTION

Human papillomavirus (HPV) has been found to be associated with several important cancers, including cervical cancer (1), vaginal cancer, vulvar cancer, anal cancer (2), and a subset of head and neck cancer (3). Among these cancers, cervical cancer has the most significant morbidity and mortality and is the second most common cancer in women with approximately 493,243 new cases diagnosed each year worldwide (4). Over 100 human papillomavirus (HPV) genotypes have been identified, though only certain types of HPV, including types 16, 18, 31 and 45, have been reported to be associated with higher risk of carcinogenesis, and are thus deemed 'high-risk' types. These four types of HPV have been estimated to be the causal agents of approximately 80% of all cervical cancers (5, 6). Although as many as 22 high risk types have been isolated, HPV type 16 alone can be detected in half of all cervical cancers and is considered to be the most prevalent subtype (5). Persistent infection with a high-risk type HPV is considered a necessary cause of cervical cancer (1, 7, 8). Therefore, prophylactic or therapeutic vaccines targeting these high-risk HPVs may be beneficial for the prevention of HPV infection and the control of existing HPV infection and HPV-related lesions

Studies have shown that the genomic organization and sequence of steps in the progression from HPV infection to HPV-associated cancer is evolutionarily conserved among the numerous subtypes of HPV. HPV is a non-enveloped, double-stranded, circular DNA virus belonging to the family Papovaviridae. High-risk HPV is sexually transmitted and targets the basal cells of the anogenital mucosa. The genes in the HPV genome can be categorized into 'early' and 'late' genes. Protein products of early genes have non-structural, regulatory functions. For example, early genes E1 and E2 can regulate viral DNA replication, E4 can disrupt the cytokeratin matrix, and E5, E6, and E7 can cause cellular transformation. E2 also regulates viral transcription. Protein products of the late genes (L1, L2) form the structural components of the viral capsid. As the infected basal epithelial cells differentiate, the viral expression program changes and the genome copy number is increased from ~10^2/cell to >10^4/cell. Late genes are only turned on when infected epithelial cells reach the superficial epithelium where they encapsidate the viral genome. The assembled virus can be released, a process that may be facilitated by the actions of E4 on cytokeratin networks, and transmitted sexually. While HPV DNA ordinarily replicates in an extra-chromosomal form, malignant progression is associated with integration of viral DNA into the host genomic DNA. Large deletions of the viral genome often occur upon integration. E2 is frequently deleted. E4, E5, L1, L2 are also often deleted, thus leaving E6 and E7 as the principle open reading frames found in high grade squamous intraepithelial lesions (HSIL) and invasive cancers. The E6 and E7 proteins have the capability to interact with p53 and Rb and influence normal cell cycle regulation resulting in cell cycle elongation, apoptosis suppression and genetic instability. These phenomena may all contribute to the development of invasive cancer (for a review, see reference (9)).

The design of both prophylactic and therapeutic vaccines requires an understanding of the virology detailed above. Prophylactic vaccines should stimulate the production of antibodies against L1 and L2, the structural components of infectious viral capsids, in order to achieve virus neutralization. On the other hand, therapeutic HPV vaccines generally target the virally encoded early proteins, such as E6 and E7, because while L1 and L2 are not expressed in the more basal cells of established HPV infections and HPV-associated malignancies, E6 and E7 are expressed at all levels of the infected epithelium. Several recent reviews discuss the different strategies of prophylactic vaccines or therapeutic vaccines (10-13). This review discusses HPV vaccines that aim both to prevent HPV infection and to control established HPV infections and HPV-associated malignancies, with special emphasis on current progress of HPV vaccine clinical trials.

3. PROPHYLACTIC HPV VACCINES

Preventing HPV infection would be an effective way to reduce and eventually eradicate the morbidity and mortality of cervical cancer and other HPV-associated disease worldwide. Stimulating the production of neutralizing antibodies could sterilize the virus prior to infection of epithelial cells. Although the use of a live attenuated virus vaccine has been shown to be effective in the prevention of diseases such as measles, mumps and rubella, it has proven difficult to generate a live attenuated HPV vaccine for two reasons. Firstly, *in vitro* culture of HPV is hampered by their dependency on epithelial differentiation during the replication process, thus limiting the number of viral particles necessary for conventional live vaccine development. Secondly, vaccination with live attenuated HPV also carries considerable risk given the necessity of viral oncoproteins for the replication process which would inevitably be introduced into human body. An alternative solution, vaccination with HPV capsid proteins L1 and/or L2 in the absence of the oncogenic viral genome, has emerged as a safe and effective strategy for a prophylactic vaccine.

When the major capsid protein L1 is overexpressed in various cell types, these molecules spontaneously assemble into virus-like particles (VLPs) (14-18) which resemble native HPV virions. Although the viral genome and the minor capsid protein L2 are absent, the VLPs still possess similar morphology and antigenicity to natural virions. Parenteral vaccination with papillomavirus L1 VLPs has been shown to induce high titers of serum neutralizing antibodies in animal models. Passive transfer of immune serum could also protect naive animals from cutaneous or mucosal challenge with infectious papillomaviruses of homologous type (19-21). Importantly, in phase I, II and III clinical trials, intramuscular vaccination with HPV L1 VLPs in women was not only immunogenic but also safe (22-25). Table 1 lists these and other prophylactic vaccines in clinical trials.

None of the animal models of papillomavirus infection mimic sexual transmission. A significant concern was that, because HPV can be transmitted through sexual intercourse, a prophylactic HPV vaccine might require mucosal secretion of neutralizing antibodies at the site of infection (i.e. at the cervical mucosa) for effective protection. Parenteral VLP vaccination is only be capable of generating systemic and not local antibody response; however, human studies confirm that high titers of specific antibodies are present in cervical secretions of women receiving intramuscular HPV-16 VLP immunization (24). The transfer of systemic antibodies to the mucosa occurs via a process of transudation, which results in low levels of antibodies (as compared to the serum concentration) and the efficiency of which is known to vary across the menstrual cycle. It was thus a concern that these titers in the cervical mucosa might not be protective or that protection might occur at only particular phases of the menstrual cycle. An answer to these questions came from recent efficacy trials. A double-blind, randomized, controlled trial in 2392 women demonstrated that HPV-16 L1 VLPs are capable of protecting women from HPV infection and HPV-associated CIN (26). In this study, the incidence of persistent HPV-16 infection was 3.8 per 100 woman-years-at-risk in the placebo group and 0 per 100 woman-years-at-risk in the vaccine group. In short, the L1 VLP vaccine was 100% effective (CI = 90 to 100%) at preventing persistent HPV-16 infection in the population of

Table 1. Prophylactic HPV Vaccine Clinical Trials

HPV Type	Vaccine Type	Ag	Dose (adjuvant)	Vaccination schedule	Sponsor	Subject population
HPV-11	VLP	L1	3, 9, 30, 100 micro-g (alum)	0, 4, 16 wks	MedImmune	Phase I trial in healthy volunteers (22)
HPV-16	VLP	L1	10 or 50 micro-g (none, alum or MF59)	0, 4, 16 wks	NCI	Phase I trial in healthy volunteers (23)
	VLP	l1	10, 20, 40, 80 micro-g	0, 2, 6 mos		2-yr randomized controlled multicenter clinical trial in healthy nonpregnant women aged 18 to 26 yrs (25)
	VLP	L1	50 micro-g (none)	0, 1, 6 mos	NCI	Phase II trial in healthy volunteers (27, 162)
	VLP	L1	40 micro-g	0, 2, 6 mos	Merck	Randomized, placebo-controlled, multicenter trial in young women aged 16 to 23 years (26)
	peptide	L2 (aa 108-120)	100 or 500 micro-g (none)	0, 4, 12 wks	Ministry of Education, Science, Culture and Sports of Japan	Placebo-controlled trial in healthy volunteers (55)
HPV-18	VLP	L1	80 micro-g (alum)	0, 2, 6 mos	Merck	Double-blind, randomized, placebo-controlled phase I trial in healthy women aged 16-23 years (163)
HPV-16, 18	VLP	L1	20 micro-g type 16, 20 micro-g type 18 (ASO4)	0, 1, 6 mos	GlaxoSmithKline (GSK)	Phase II trial in healthy volunteers aged 15-25 years (40)
HPV-6, 11, 16, 18	VLP	L1	20 micro-g type 6, 40 micro-g type 11, 40 micro-g type 16, and 20 micro-g type 18	0, 2, 6, mos	Merck	Phase II trial in healthy volunteers (41)

aa, amino acids; VLP, virus-like-particle

females tested over this relatively short period. Additionally, new HPV-16-related CIN only occurred among the placebo recipients (26). These studies suggest that serum antibodies can in fact transfer from plasma to the cervical mucosa where they protect throughout the menstrual cycle against HPV infection. However the longevity of protection is currently under investigation.

It has been hypothesized that cell-mediated immunity may play a role in the protective effect of the VLP-based vaccine. Although the study result showed 100% protection against persistent HPV infections and CIN, some patients in the treatment group developed transient HPV infections which later resolved during the vaccine trial. L1-specific CD4+ and CD8+ T-cells were subsequently observed in these HPV-16 VLP vaccination experiments (27). These results indicate the possible contribution of cellular immunity in preventing persistent infection. Alternatively, cell mediated immunity may not be relevant to protection, and these transient PCR detections of HPV DNA may reflect detection of deposited but neutralized virus or aborted infections.

More clinical trials of VLP vaccines are currently under way (see Table 1), including a large, randomized, double-blind, placebo-controlled trial of HPV-16 and HPV-18 L1 VLPs in 21,000 Costa Rican women (28). An important aim of these trials is assessing the long-term protective efficacy of the VLP vaccination. While human and nonhuman primate studies suggest that these antibodies are quite durable (7, 29), tracking the level of the antibodies induced by the vaccines or natural infection in human subjects long-term is a critical direction in vaccine research. In particular, it will be important to define the threshold protective titer of neutralizing antibodies. Further studies in men are also critical, but await better assays of infection.

3.1. Alternative routes of vaccine administration

Although parenteral vaccination has been shown to be able to induce neutralizing antibodies in the cervical mucosa alternative routes of administration are under consideration in an effort to induce maximal mucosal immune response across the menstrual cycle while simultaneously decreasing invasiveness and cost of vaccination. Possible alternate routes include nasal and oral delivery. Mucosal immunoglobulin (Ig)G and IgA antibodies specific for HPV have been shown to be generated by nasal inoculation with VLPs in mice (16, 30). A clinical trial showed that inoculation by HPV-16 VLP nasal spray or aerosol can generate specific IgG and IgA antibodies in the cervical secretions of female subjects (31). A new clinical trial further confirms the efficacy of respiratory mucosal immunization, showing that the induced serum antibody titer is comparable to intramuscular vaccination (6). Oral vaccination was tested in yet another preclinical study and was demonstrated to induce levels of systemic HPV-specific IgG and IgA antibody response comparable to parenteral vaccination (32). Another oral vaccine in development uses yeast as a vector (17). However, these edible vaccines still require more research to optimize their potency.

3.2. Live vaccine vectors

A preventative vaccine is most urgently needed in developing nations that lack the resources for cervical cytology screening programs. In settings that lack the necessary infrastructure and financial resources, the ability to vaccinate in the absence of a cold chain to maintain vaccine stability becomes important. Furthermore, vaccines that do not require the use of needles and provide immunity after a single dose are highly desirable. Live vaccine vectors offer these advantages, and also the possibility of simple, inexpensive manufacture. While live

vaccines have been used very successfully (e.g. vaccinia for smallpox), they may carry additional risk to the patients as compared to a subunit vaccine. This is particularly pertinent in immunocompromised individuals and the safety and ethics of this approach must be carefully considered. However, despite these issues, the potential advantages of live vectors warrant their consideration. One of the most attractive approaches is to incorporate papillomavirus antigen expression within already existing live vectors (e.g. measles) without reducing their original efficacy. An innovative vaccination strategy involves the use of adenovirus as a live vector carrying the papillomavirus L1 gene with production of VLPs *in vivo*. Adenovirus has been used as a vaccine by the US military for its recruits. A study using canine oral papillomavirus L1 gene showed that VLPs can be generated in large quantities while the adenovirus gene is expressed in culture cells (33). Furthermore, adenovirus can be administered orally, thus representing another potential noninvasive approach to vaccine administration. Experimentation in canine animal models has already shown protection against wart formation using this adenovirus vaccine model (34).

Adeno-associated virus has been used extensively in gene-therapy and is another candidate for *in vivo* VLP production. A report demonstrated that a single intramuscular co-injection with recombinant adeno-associated virus encoding HPV-16 L1 and recombinant adenovirus encoding GM-CSF can achieve the same vaccine effect as the VLP vaccine which requires three booster injections in mice (35). Vesicular stomatitis virus vector has similarly been shown to be effective in rabbits (36). *Salmonella* bacterium has also been proposed as a potential live vector and mouse experiments show that nasal or oral vaccination with HPV 16 L1-expressing *Salmonella* bacteria induces high titer of neutralizing antibodies (37).

3.3. Polyvalent VLP prophylactic vaccines

One drawback of HPV L1 VLP vaccines is that although they can induce effective neutralizing antibodies, the antibodies are type-specific and induce very limited cross protection (20, 32). In the trial described by Koutsky *et al*, researchers observed type-specific protection, documenting equal numbers of *non*-HPV-16-related CIN in both the placebo and vaccine arms with HPV-16-positive CIN only in the placebo arm (26). Because there are at least 15 types of high-risk HPV, modification must be taken to broaden the protection range of the vaccine, polyvalent vaccines being a potential solution.

Two pharmaceutical companies, GlaxoSmithKline (GSK) and Merck, have begun clinical trials of polyvalent HPV L1 VLP vaccines to protect against multiple HPV types (see Table 1). The GSK vaccine contains both HPV-16 and HPV-18 L1 VLPs (38) while the Merck product currently undergoing clinical trial is tetravalent, containing HPV-6, -11, -16 and -18 L1 VLPs. Initial studies for the vaccines indicate that the vaccine is well tolerated and can generate neutralizing antibody titers to each of the HPV types in quantities similar to those produced by monovalent L1 VLP vaccines (39, 40). Recently, two clinical studies concluded that a polyvalent vaccine could substantially reduce the acquisition of infection and clinical disease caused by common HPV types (40, 41).

The use of plasmid-based vaccines is an intriguing possibility for highly polyvalent vaccines. Vaccination with CRPV L1 expressing plasmid DNA partially protects against experimental infections (42). However this approach requires extensive codon modification to provide high level L1 expression. Several advances in DNA delivery, such as gene gun, significantly boost the immunogenicity of these vaccines.

Another relatively new technology under investigation is hypervariable epitope constructs (HECs). HECs are composed of sequence variants of immunodominant B-cell epitopes of the major capsid protein, L1, of HPV. Mouse experiments show that antibodies generated by this kind of vaccine can cross-react with HPV-16 and 18 (43). With the continued clinical development of VLP vaccines for HPV types other than HPV-16 (HPV-6b (44) and HPV-11 (22, 45, 46)) and of polyvalent VLP vaccines and novel technologies such as HECs, protection may be possible against a wider range of HPV types. An alternative approach to polyvalent vaccines is the identification of a single, conserved antigen that confers broad immunity. In this regard L2 protein shows great promise, as detailed below.

3.4. L2-based vaccines

Although L1 is the immunodominant antigen in the generation of neutralizing antibodies *in vivo* (47), L2 has also arisen an important target for vaccine development (see Table 2). Several previous reports find that vaccination with L2 induces cross-neutralizing antibodies (47-49). Though the ability of L2 vaccination to provide cross type protection has not yet been demonstrated, vaccination with L2 peptides protects animals from experimental challenge with the homologous type papillomavirus (50-53) via neutralizing antibodies (54).

The possibility of an L2 VLP vaccine has recently been tested in preclinical and clinical situations. One clinical study demonstrated that nasal inoculation with an epitope of HPV-16 L2 produces L2-specific cross-neutralizing antibodies that bind both HPV-16 and HPV-52 L1/L2 capsids (55). However, despite superior cross-neutralization, immunization with L2 generally stimulates production of low titers of antibodies while L1 VLPs generate much higher titers (54, 56). If immunogenicity of L2 within VLPs and other L2-based vaccines can be improved, L2 vaccines may be an attractive alternative strategy to polyvalent vaccines to prevent multiple type HPV infection.

4. THERAPEUTIC HPV VACCINES

Although the HPV VLP and L2 vaccines have been shown to be highly protective, they are ineffective in the elimination of disease in the present population of infected women. Moreover, it is still possible that some

Table 2. Comparison of Preventative Vaccine Targeting HPV L1 and L2 Antigens

Antigen	Vaccine Approach	Advantages	Disadvantages
L1	Protein VLP	1. Safe, immunogenic and effective 2. Large scale production is possible in a number of systems 3. Not oncogenic 4. Elicits high titers of HPV neutralizing antibodies 5. HPV neutralizing antibodies reach the cervical mucosa 6. Nasal, aerosol, or oral inoculations have been used for non-invasive vaccination	1. Limited cross-neutralizing power 2. Multivalent vaccines needed to protect against multiple types of HPV
L2	Protein monomer, Peptide	1. Safe 2. Elicits cross-neutralizing antibodies 3. Not oncogenic 4. Nasal inoculation has been used for non-invasive vaccination	1. Immunologically sub-dominant to L1 2. Elicits low titers of neutralizing antibodies

VLP, virus-like-particle

viruses may successfully breakthrough neutralizing antibodies induced by the vaccine and establish new infection. Given these potential shortcomings, additional measures to deal with established HPV infection and HPV-associated diseases are needed. Cell-mediated immunity is likely to be a solution. Current data provide evidence that cell-mediated immunity is important in anti-HPV immune response. Several studies report that HPV-related infection and malignancies have increased incidence in patients with impaired cell-mediated immunity, such as transplant (57) or AIDS patients (58-60). It has also been observed that when immunosuppressive therapy is discontinued in previously immunosuppressed patients, HPV-associated warts often regress (61). In spontaneously regressing HPV warts, infiltrating CD4+ and CD8+ T-cells are often observed, while in non-regressing warts the T cells are rarely seen, thus indicating the role of T cells in anti-HPV immune response (62).

Though there is evidence to suggest a role for cell-mediated immunity with prophylactic type vaccines, generating cellular immunity from HPV VLPs is not an ideal strategy as the infected basal epithelial cells usually do not express L1 or L2 proteins. Additionally, the L1 and L2 genes are frequently lost in HPV-associated malignancies. In order to prevent the development of lesions, eliminate existing lesions or even eliminate malignancies, a therapeutic vaccine should target HPV antigens that are continuously expressed in the infected cells and cancer cells. The early viral proteins (E1 to E7) are potential target antigens as they are expressed early in viral infection and help regulate progression of the disease. During integration of HPV into the host genome, E2, a negative regulator of E6 and E7, is often deleted leading to upregulation of E6 and E7 in cervical cancer cells. E6 and E7 in particular are critical for HPV replication and cervical epithelial transformation, and are expressed throughout the viral life-cycle. This notion has popularized E6 and E7 as a favorite target for HPV therapeutic vaccines (63).

Live-vector vaccines, peptide or protein vaccines, nucleic acid vaccines, cell-based vaccines, and combined approach vaccines have all been tested in the development of HPV therapeutic vaccines. Table 3 is a summary of the advantages and disadvantages of these strategies. In the following sections, the current progress of these strategies will be discussed separately.

4.1. Live vector vaccines

Recombinant viruses and bacteria are the common candidates of live vector vaccines. The live vector vaccines, especially those that are capable of replication in the host, are generally highly immunogenic and can induce strong immune responses. There are a variety of live vectors available, and researchers have the ability to choose the ideal vector according to the target immune responses desired. For example, the *Salmonella* bacterium is capable of delivering either antigen proteins or mammalian DNA expression vectors encoding the antigen of interest to antigen presenting cells (APCs) in the host organism. While properties like these make live vectors an attractive candidate for therapeutic vaccines, there are several concerns with this methodology, including the prevalence of pre-existing antiviral or antibacterial neutralizing antibodies. Pre-existing immunity may decrease the effectiveness of the vaccine and inhibit repeated vaccination. Additionally, vector antigens may become immunodominant over the HPV antigen carried on the vector, thus interfering with the formation of an efficacious anti-HPV immune response. Using live vectors that can replicate in the host carries the additional risk of toxicity. Recently, it was proposed that live vector vaccines may generate regulatory T cells which could potentially interfere with immune responses decreasing efficacy of the vaccine (64). However, another report shows that an adenoviral vector vaccine can be used to break the tolerance of tumor antigen (65). Taken together, it is clear that an effective and safe live vector vaccine for HPV treatment needs to be developed thoughtfully with careful control of these potentially adverse effects.

The current experimental live vector vaccines for treatment of HPV mainly target viral E6 and E7 proteins and have mostly been tested in preclinical models. The examples of recombinant viral vectors in use include vaccinia virus, adenovirus, adeno-associated virus, alpha-virus RNA replicon particles and HPV pseudovirions (for a review, see reference (66)). A new kind of virus vector, Semliki Forest virus (SFV), has been recently reported to be capable of inducing strong cell-mediated immunity and of breaking tolerance to HPV antigens (67, 68). Such feature of a vaccine may be especially valuable when treating advanced cancer. Another papillomavirus protein, E2, has also been tested in an adenoviral vaccine model. Therapeutic effect (reduced papilloma forming sites) of this vaccine has been observed in rabbits (69). Bacterial examples of live vector HPV vaccines include *Listeria*

Table 3. Characteristics of HPV vaccine approaches

Vaccine Approach	Advantages	Disadvantages
Vector-based: Viral (i.e. vaccinia, Adv, AAV, alphavirus)	1. Highly immunogenic 2. Different immunological properties of viruses 3. A wide variety of vectors are available 4. Can potentially be engineered to express cytokines or other stimulatory molecules	1. Risk of toxicity when using live viruses 2. Potential pre-existing immunity 3. Inhibited repeat immunization 4. Immunodominance of viral vector antigens over HPV tumor antigens
Vector-based: Bacterial (i.e. Listeria, Salmonella, BCG, Lactococcus)	1. Highly immunogenic 2. Can deliver either engineered plasmids or HPV tumor proteins to APCs 3. A wide variety of vectors are available	1. Risk of toxicity when using live bacteria 2. Potential pre-existing immunity 3. Inhibited repeat immunization
HPV Pseudovirions	1. Highly immunogenic 2. Non-replicating 3. Protects and delivers encapsulated DNA encoding HPV tumor antigen 4. Acts as an adjuvant 5. Can induce DC maturation	1. Cumbersome to prepare 2. Inhibited repeat immunization
Peptide-based	1. Stable and easy to produce 2. Safe 3. Can combine multiple epitopes 4. Can enhance peptides for MHC binding	1. Weakly immunogenic 2. Must determine epitopes 3. Must match patient's HLA
Protein-based (incl. VLPs)	1. Easy to produce 2. Multiple known adjuvants 3. No HLA restriction 4. Can produce fusion proteins for enhanced immunogenicity	1. Usually better induction of antibody response than CTL response
DNA	1. Easy to produce, store, and transport 2. Versatility in ability to add targeting and/or co-stimulatory genes 3. Capable of multiple immunizations 4. A variety of modes of administration exist: direct injection, gene gun, intranasal, and biojector 5. Sustained expression of antigen on MHC-peptide complexes (compared to peptide/protein vaccines)	1. Intrinsically weak immunogen 2. Concern of integration or cellular transformation
RNA	1. Non-infectious and transient so no risks of chromosomal integration or cellular transformation 2. Capable of multiple immunizations 3. RNA replicons replicate within the cell to enhance antigen expression 4. Multiple vectors are available	1. Unstable to store and handle 2. Cumbersome to prepare 3. Difficult to prepare large amounts
Dendritic cell-based	1. Highly immunogenic; uses the most potent APC 2. Methods available to generate large numbers of DCs 3. Multiple methods of Ag loading are available 4. Potency can be enhanced by gene transduction or cytokine treatment	1. Labor-intensive, costly, ex vivo, individualized cell processing 2. Variable quality control and a lack of standard criteria for quality of vaccines 3. Do not necessarily home to draining lymph nodes 4. Possibility of tolerization by immature DCs
Tumor cell-based	1. Useful if tumor antigen is unknown 2. Potency can be enhanced by gene transduction or cytokine treatment 3. Likely to express relevant tumor antigens	1. Safety concerns about injecting tumor cells into patients 2. Labor-intensive procedure 3. Weak antigen presentation by tumor cells 4. Requires availability of tumor cell lines or autologous tumor cells

Ag, antigen; APC, antigen presenting cell; CTL, cytotoxic T lymphocyte; DC, dendritic cells; HLA, human leukocyte antigen; MHC, major histocompatibility complex; VLP, virus-like particle

monocytogenes, *Salmonella* and bacillus Calmette-Guérin (BCG) (for a review, see reference (66, 70)). More recently, *Lactococcus lactis* has also been used as a vector for HPV vaccine development (71), and various methods of E7 expression have been developed (72). These bacterial vaccines are also mainly under development in preclinical models.

In the preclinical models, the live vector vaccines usually can generate strong E7-specific T-cell-mediated immune responses and antitumor effects. Their potency can be further enhanced by various adjuvants and fusion-protein strategies. For example, *Listeria monocytogenes* has recently been engineered to secrete HPV-16 E7 fused to the *Listeria* protein ActA (Lm-ActA), a protein necessary for the assembly of the actin filaments that propel *Listeria* to the periphery of an infected cell. Intraperitoneal administration of Lm-ActA-E7 elicited strong E7-specific cytotoxic T-lymphocyte (CTL) responses and caused the complete regression of HPV-positive tumors in mice (73). Fusion to another *Listeria* protein, listerioloyisn (LLO), has also been shown to be able to enhance anti-tumor immunity through facilitation of proteasome digestion (74). This enhancement is shown to be correlated with the stimulating effect of LLO on dendritic cells (75). Another example is recombinant vaccinia virus. It has been shown that the potency of a vaccinia live vector vaccine can be further enhanced by expressing the immunogenic E7 protein linked with calreticulin to promote MHC class I antigen presentation (76). An adenoviral vaccine carrying E7 and recombinant IL-12 was also reported to induce stronger anti-tumor immunity (77).

Some of the live vector vaccines have passed preclinical evaluations and proceeded into clinical trials (see Table 4). For example, a recombinant vaccinia virus

Table 4. Therapeutic HPV Vaccine Clinical Trials

HPV Type	Vaccine Type	Ag	Dose (Adjuvant)	Vaccination Schedule	Sponsor	Patient Population	Reference
HPV-16 and 18	Recombinant Vaccinia Virus -- TA-HPV	E6 and E7 mutated to inactivate Rb binding	Variable	variable	Xenova/ Cantab	(79): Phase I trial in patients with early-stage cervical cancer; (78): Phase I/II trial in patients with advanced cervical cancer; (81): Phase II trial in patients with high-grade VIN or VgIN; (82): Clinical trial in patients with high grade VIN	78, 79, 81, 82
HPV-16 and 18	Recombinant Vaccinia Virus -- MVA E2	E2	10^7 viruses per dose	0, 1, 2, 3, 4, 5 weeks	Instituto Mexicano del Seguro Social (IMSS)	Phase I/II trial in patients with CIN1-3	84
HPV-16	Peptide	E7 (HLA- A*0201 CTL epitopes a.a. 11-22 and 86-93) and pan-DR binding T helper peptide	100 micro-g, 300 micro-g, or 1000 micro-g (Montanide ISA 51)	0, 3, 6, 9 weeks	Netherlands National Institute of Public Health and Environmental Protection	(87): Phase I/II trial in patients with cervical cancer; (88): Phase I/II trial in patients with advanced cervical cancer	87, 88
HPV-16	Peptide	E7 (a.a. 12-20 alone or a.a. 12-20 mixed with a.a. 86-93 linked to a T-cell epitope peptide)	Escalating doses (Montanide ISA 51)	0, 3, 6, 9 weeks	NCI	Phase I trial in patients with CIN2/3 or VIN2/3	89
HPV-16	Peptide (lipopeptide)	Lipidated E7 (HLA-A*0201-restricted epitope, a.a. 86-93 lipopeptide)	n/a	0, 3, 6, 9 weeks	NCI	Phase I trial in patients with cervical or vaginal cancer	90
HPV-16	Fusion Protein -- PD-E7 or D16E7	E7 linked to the first 108 a.a. of Haemophilus influenzae protein D	n/a (AS02B)	0, 2, 4 weeks	GlaxoSmithKline	Phase I/II trial in patients with CIN1 or 3	98
HPV-16	Fusion Protein -- SGN-00101 or HspE7	E7 linked to HSP65 from Mycobacterium bovis	500 micro-g (none)	0, 4, 8 weeks	StressGen	Phase III trial in patients with AIN and Phase II trial in patients with AGW and RRP	100
HPV-16	DNA -- ZYC101	E7 epitope (a.a. 83-95)	50, 100, 200, or 400 micro-g (DNA encapsulated in biodegradable polymer microparticles)	0, 3, 6, 9 weeks	Zycos, Inc.	(127): Phase I trial in patients with anal HSIL; (128): phase I trial in patients with CIN2/3	127, 128
HPV-16 and 18	DNA -- ZYC101a	E6 and E7 epitopes	100 or 200 micro-g (DNA encapsulated in biodegradable polymer microparticles)	0, 3, 6 weeks	Zycos, Inc.	Phase II trial in patients with CIN2/3	129
HPV-16	DNA	E7 contained in the pNGVL4a-Sig/E7(detox)/HSP70 plasmid	Dose escalating	n/a	NCI	Clinical trials have begun for patients with CIN2/3	C Trimble, personal communication
HPV-16	DNA	E7 contained in the pNGVL4a-Sig/E7(detox)/HSP70 plasmid	Dose escalating	n/a	NCI	Clinical trials to begin soon for patients with advanced HNSCC	M Gillison, personal communication
HPV-16	DCs pulsed with HPV +ve tumour lysate	HPV positive tumor lysate (E6 and E7)	107 DCs per injection	6x weekly	n/a	Phase Ib trial in patients with advanced cervical cancer	148
HPV-18	DCs pulsed with E7 protein	E7	3 to 5 million DCs per injection	Vaccinations 1-5 at intervals of 10-14 days; vaccinations 6-14 at intervals of 30-60 days	n/a	A case report of a single patient with cervical cancer	147

a.a., amino acids; AGW, anogenital warts; AIN, anal intraepithelial neoplasia; CIN, cervical intraepithelial neoplasia; CRT, calreticulin; CTL, cytotoxic T lymphocyte; DCs, dendritic cells; HLA, human leukocyte antigen; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; HSIL, high grade squamous intraepithelial lesion; HSP, heat shock protein; n/a, not available or not applicable; NCI, national cancer institute; RRP, recurrent respiratory papillomatosis (warts of the upper airways); VgIN, vaginal intraepithelial neoplasia; VIN, vulvar intraepithelial neoplasia

encoding HPV-16 and HPV-18 E6/E7 (called TA-HPV) was tested in phase I/II clinical trials and was shown to be well tolerated. T cell immune responses were observed after vaccination in some patients with CIN-3, early invasive cervical cancer, and even advanced cervical cancer (78-80). The vaccine was also given to patients with HPV-associated vulvar or vaginal intraepithelial neoplasia with specific immune responses observed (81, 82). In a study conducted by Baldwin and colleagues, five out of 12 patients had at least a 50% reduction in lesion diameter over 24 weeks, and one patient showed complete regression of lesion after vaccination (81). Another study tested a prime-boost strategy on patients with high grade HPV 16-associated vulvar intraepithelial neoplasia (VIN). The researchers primed the patients with TA-HPV and boosted their immune responses with a HPV 16 L2E6E7 fusion protein. Nine out of the ten patients developed HPV 16-specific proliferative T-cell and/or serological responses and three showed lesion shrinkage or symptom relief (83). Another recombinant vaccinia virus encoding E2 (called MVA E2) has also been tested in patients with CIN-1, CIN-2, or CIN-3, though presently it is too early to conclude whether the vaccine can generate an E2-specific immune responses (84). Table 4 lists these and other therapeutic vaccines in clinical trials.

4.2. Peptide vaccines

HPV antigenic proteins are processed by antigen presenting cells (APCs) in the body into peptides. These peptides become associated with major histocompatibility complex (MHC) molecules on the surface of the APCs, which together, stimulate lymphocytes to mount an immune response against the pathogen. Viral peptides have thus been considered as potential candidates for vaccine development. Peptides are easy to manufacture, safer and more stable than live vector vaccines. Though several groups have attempted to utilize these beneficial properties to develop HPV therapeutic vaccines, several limitations have been encountered. The first is that peptide vaccines are MHC specific. A specific vaccinated peptide can only be presented by a specific type of MHC molecule. Since the human MHC molecules are highly polymorphic, a single peptide may not be able to induce immune response in all the recipients of the vaccine, and not every HPV-derived peptide will be immunogenic for a single person. During the vaccine developmental process, different peptides specific for different common human MHC types must be defined and considered as possible components of the vaccine. It is even possible to select peptides that direct either T-helper and/or CTL immune responses. An additional limitation to peptide vaccines is their weak immunogenicity. Addition of adjuvants, such as cytokines, chemokines or costimulatory molecules may be essential for therapeutic efficacy. Epitope enhancement, which increases the affinity of the peptide for the MHC molecules, increasing T-cell receptor binding, creating a booster vaccination schedule, preventing peptide degradation or linking peptides to lipids are additional strategies which have been considered to enhance peptide HPV vaccine potency (for a review, see reference (66)).

Various MHC restricted CD4+ and CD8+ T-cell epitopes of HPV early proteins, such as E2, E6 and E7, have been identified in both mice and humans. Several potency enhancement strategies, including the addition of adjuvants and the formation of lipopeptides, have been tested in preclinical models, (for a review, see reference (66)). One group has recently reported the effect of vaccination of mice with an HPV-16 E5 peptide emulsified with CpG oligodeoxynucleotide (CpG ODN). CpG ODN is a nucleotide sequence found in bacterial DNA which has been shown to induce a series of immune responses, including the induction of antigen-specific CD8+ T-cells. The peptide vaccine emulsified with CpG ODN has been shown to be effective in eradication of E5-containing tumor growth. Additionally, the induced CTL response was found to be stronger than E5 peptide vaccine emulsified with the traditional Freund's adjuvant (85). A new study using a recombinant protein, HPV-16 E7 peptide (amino acids (aa) 38 to 61) linked with an immunoglobulin G fragment, found that a recombinant vaccine could induce E7-specific immune responses and protect mice against challenge of HPV16-positive tumor. The vaccine was also found to be effective for eradicating developed tumor (86), validating the notion that a peptide vaccine can be created to achieve both protective and therapeutic effects.

As predicted by the nature of peptide vaccine strategy, experimental HPV peptide vaccines have been shown to be both safe and well tolerated in humans in recent Phase I/II clinical trials (87, 88). In one study, women were vaccinated with HPV-16 E7 peptides, either amino acids (aa) 12 to 20 alone, or aa 12 to 20 mixed with aa 86 to 93 covalently linked to a T-helper stimulating peptide. Three of the 18 patients with high-grade cervical or vulvar intraepithelial neoplasia had their dysplasia cleared, and six of the same group showed measured regression of lesions (89). A clinical trial using lipidated HPV-16 E7 peptide also demonstrated that CTL responses could be generated in some patients with HPV-associated cancer. However, the vaccine did not elicit regression of the tumor burden (90).

4.3. Protein vaccines

Protein-based vaccines possess some similar advantages, such as safety and stability, to peptide vaccines. Moreover, protein vaccines can potentially overcome the limited specificity of MHC responses with peptide-based vaccines by nature of a protein's more abundant MHC epitopes. In an individual patient's cells, peptides suitable for their own MHC molecules can be generated such that neither the antigen's immunogenic epitope nor the patient's MHC type need to be determined prior to vaccination. Many of the strategies used to enhance peptide vaccine potency can also be applied to protein vaccines, such as adding liposome-polycation-DNA adjuvant (91), ISCOMATRIX (92) or *B. pertussis* adenylate cyclase (93). Strategies aimed at promoting protein uptake by APCs are also important as exogenously administered protein vaccines are not usually efficiently engulfed and presented by APCs. Fusing antigens of interest with special proteins, such as heat shock proteins (Hsp), may help target antigen to APCs. Because protein-based vaccines may elicit better antibody responses than CTL responses, researchers interested in the protein-based vaccine for therapeutic purposes against HPV infection and HPV-associated neoplasia must consider mechanisms to strengthen CTL responses.

Protein-based HPV vaccines have been tested in HPV oncogenic protein-expressing preclinical tumor models (such as the TC-1 and C3 tumor models), and E7 protein vaccine induced CTL responses have already been reported. Many researchers are currently focusing on strategies to improve therapeutic potential of E7 vaccination. Addition of adjuvants, fusion proteins and other approaches have been applied to these preclinical models (for a review, see reference (94)).

The most promising strategies shown in preclinical protein vaccine studies have been translated into clinical trials. One example is the fusion protein containing HPV capsid proteins and HPV early proteins. These fusion vaccines can potentially induce both prophylactic and therapeutic immune responses. The current trials testing the efficacy of fusion protein vaccines are listed in Table 5. One of the experimental vaccines, TA-GW, a fusion of HPV-6 L2 and E7 adsorbed onto Alhydrogel, has been tested in two clinical trials and was found to be well tolerated and effective in clearing HPV genital warts in a subset of patients (95, 96). Another protein vaccine, TA-

Table 5. Prophylactic/Therapeutic HPV Vaccine Clinical Trials

HPV Type	Vaccine Type	Ag	Dose (Adjuvant)	Vaccination Schedule	Sponsor	Patient Population	Reference
HPV-6	Fusion Protein -- TA-GW	L2/E7	0, 3, 30, or 300 μg (Alhydrogel)	0, 1, 4 weeks or 0, 4, 8 weeks	Xenova/Cantab	Phase I trial in healthy volunteers	95
HPV-6	Fusion Protein -- TA-GW	L2/E7	300 μg (Alhydrogel)	0, 1, 4 weeks	Xenova/Cantab	Phase IIa trial in patients with genital warts	96
HPV-16	Fusion Protein -- TA-CIN	L2/E6/E7	25, 125, or 500 μg (none)	0, 4, 8 weeks	Xenova/Cantab	Phase I trial in healthy volunteers	97
HPV-16 and 18	Prime boost -- Fusion protein (TA-CIN) + recombinant vaccinia virus (TA-HPV)	L2/E6/E7 (TA-CIN) + E6 and E7 mutated to inactivate Rb binding (TA-HPV)	n/a	n/a	Xenova	Phase IIa trial in patients with AGIN	82

AGIN, ano-genital intraepithelial neoplasia

CIN, a fusion of HPV-16 L2, E6 and E7, was also shown to be safe in a clinical trial. Antibodies were induced in all the women tested, and T cell immunity was produced in a subgroup of them (97). In order to further enhance vaccine potency, a 'prime-boost' strategy, which combined TA-CIN and TA-HPV (vaccinia virus encoding HPV 16/18 E6E7) vaccinations, was tested in ten women with HPV 16-positive high-grade vulvar intraepithelial neoplasia (VIN). They first received a TA-HPV vaccine and then were boosted three times with TA-CIN. HPV 16-specific proliferative T-cell and/or serological responses were observed in nine of the women, and three women showed lesion shrinkage or symptom relief. However, no direct correlation between clinical and immunological responses was observed (83).

Other fusion protein vaccines that have been tested in clinical trials include fusion of *Haemophilus influenzae* lipoprotein D and HPV-16 E7, fusion of HPV-16 E6 and HPV-16 E7, fusion of BCG Hsp65 and HPV-16 E7, and HPV-16 L1/L2-E2-E7 chimeric VLPs (see Table 4). *Haemophilus influenzae* lipoprotein D and HPV-16 E7 fusion protein (called PD-E7 or D16E7) with adjuvant was administered in a small phase I/II clinical trial. The vaccination was shown to induce potent E7-specific CD8+ T-cell responses. Regression of lesions could be observed in some patients with CIN-1 or CIN-3 (98). An E6E7 fusion protein complexed with ISOMATRIX adjuvant was shown to be safe and immunogenic in a phase I study (99). HspE7 (Stressgen Biotechnologies Corp), the fusion of BCG Hsp65 and HPV-16 E7, is now also being tested in patients with HPV-associated anal dysplasia (100). Chimeric VLPs containing HPV-16 L1/L2-E2-E7 are in production and being prepared for a phase I clinical trial [D Lowy and J Schiller, personal communication]. The chimeric VLP vaccine will be incorporated into the present Costa Rican HPV-16 VLP clinical trial (for a review, see reference (101)).

4.4. DNA vaccines

DNA vaccines, most common in the form of naked DNA expression plasmids, have several beneficial features for HPV therapeutic vaccine development. The DNA vectors, which are easily producible, can be engineered to express tumor antigenic peptides or proteins. The stability and purity of DNA vaccines are even higher than peptide or protein vaccines. Several methods can be used to specifically target the delivery of DNA vaccines to APCs, thus enhancing the antigen presentation to T cells and increasing the vaccine efficacy (for a review, see reference (102)). DNA vaccines can produce antigenic proteins and peptides in the APCs continuously such that the amount of antigen delivered to the immune system is potentially higher than peptide and protein vaccines. Using DNA vaccines to express proteins, thus allowing the cell to generate its own peptide-MHC complex, bypasses the MHC restriction while maintaining greater CTL responses than current protein vaccines. DNA vaccines can also be repeatedly applied to the same patient safely and effectively, unlike vaccines which utilize a live vector. The major limitation of the DNA vaccines is their low immunogenicity. Unlike live vector vaccines, naked DNA vaccines cannot replicate or spread from one cell to another. Additionally, they evoke fewer innate immune responses. A further concern is the possibility that the DNA vaccines may integrate into chromosomal DNA of the transfected cell, carrying the undesirable risk of cellular transformation.

The potency of HPV DNA vaccines have been significantly improved in animal models using several strategies. An important example of such a strategy is targeted delivery of DNA vaccines to the most potent professional APCs, dendritic cells (DCs). DCs can present antigens via both the MHC-I and MHC-II pathways, thus stimulating both CD8+ and CD4+ T-cell immune responses. Using a gene gun (a ballistic device for delivering DNA-coated gold particles into the epidermis) to shoot DNA vaccines directly into the animal skin is an ideal route of delivery to target the intradermal dendritic cells. Additionally, promoting intercellular spreading of the vaccines has also been considered as a means to enhance DNA vaccine potency. Because naked DNA vectors cannot spread from a transfected cell to other cells spontaneously, constructing DNA vaccines which encode the target antigen fused with a protein that can promote the spreading process may increase the number of HPV antigen-presenting DCs. Herpes simplex virus type 1 VP22 protein and its homologues are candidate proteins in this endeavor. Mice experiments show that a fusion DNA vaccine containing HPV-16 E7 and VP22 generated better E7-specific CD8+ T-cell responses and antitumor effects than E7 DNA alone (103, 104). Constructing DNA vaccines with signal peptides for endoplasmic reticulum can also

promote antigen targeting to DCs. E7 antigen and Hsp70 (Sig/E7/Hsp70), a vaccine utilizing such a strategy, has been found to lead to secretion of the E7 antigen-Hsp70 fusion protein by transfected cells. Enhanced CTL response and antitumor activity have been observed in mice vaccinated with this construct(105-107).

In addition to the extracellular DC-targeting strategies, intracellular targeting strategies to enhance antigen presentation via the MHC-I and MHC-II pathways are also important methods to strengthen the induced T cell response. Generally, cytosolic or nuclear proteins are presented via the MHC-I pathway and can stimulate CD8+ T cells, while extracellular proteins are processed in the lysosomes of professional APCs and presented via the MHC-II pathway, stimulating CD4+ T cells. Linking the HPV antigen to various proteins that target antigen to proteasomes for degradation, or to the endoplasmic reticulum, generally enhance the MHC-I presentation of the vaccine antigens (108-112). Fusing the HPV antigen with the sorting signal of the lysosomal-associated membrane protein type 1 or other MHC-II targeting molecules can redirect the antigen to the MHC-II presentation pathway (for a review, see reference (102)). Another way to enhance antigen presentation is to use a single-chain trimer (SCT) molecule as a vaccine. A SCT molecule is composed of the antigenic peptide, beta2-microglobulin (beta2m) and MHC class I heavy chain. This kind of vaccine can bypass antigen processing and lead to stable presentation of peptides by the antigen presenting cells. Animal study has shown that this kind of vaccine can achieve improved tumor control potency (113). The protein expression level of the antigen from the DNA vaccine is also important, and codon optimization has been shown to be effective in increasing the CTL response induced by DNA vaccine (114, 115). Furthermore, polyepitopes can be coded in the DNA vaccine to broaden the effect of induced immune response (116). Each of these is a possible method under consideration to enhance the antigen presentation of the DNA HPV therapeutic vaccines.

Improving the interaction between DCs and T-cells is yet another way to enhance DNA vaccine potency. Co-stimulatory molecules play an important role in the activation of naive T-cells by MHC-peptide complexes on DCs. Transfecting DCs with co-stimulatory molecule genes or adding cytokines to stimulate co-stimulatory signals can enhance the co-stimulatory environment (117, 118). In addition, other strategies such as linking the E7 gene with an *E. coli* beta-glucuronidase could improve vaccine potency (119). Prolonging the survival of DCs may increase the time and frequency of DC-naïve T cell interaction, thus augmenting the immune response generated. This strategy has been used by vaccinating mice with DNA encoding HPV antigens and anti-apoptotic molecules such as Bcl-x_L. The anti-apoptotic proteins helped DCs to resist effector T-cell induced apoptosis activated by the DCs themselves, thus prolonging the DC survival and enhancing E7-specific CD8+ T-cells activation (104, 120-125). (For a review, see references (66, 126)).

Several experimental DNA vaccines have proceeded into clinical trials. A phase I clinical trial in patients with high-grade anal intra-epithelial lesions (127) and another phase I trial in CIN-2/3 patients (128) tested a plasmid DNA vaccine, ZYC-101 (ZYCOS Inc), which encodes multiple HLA-A2-restricted epitopes derived from the HPV-16 E7 protein encapsulated in biodegradable polymer microparticles. No subjects in any dosage group tested showed problems with tolerating the vaccine. The immune response to the peptide epitopes encoded within the DNA vaccine was increased in ten of the 12 individuals with anal dysplasia (127). Five out of 15 women with CIN-2/3 had complete histological responses and 11 out of 15 women with CIN-2/3 had human papillomavirus-specific T-cell responses (128). The next generation ZYC-101 vaccine, ZYC-101a (ZYCOS Inc), contains a plasmid DNA encoding both HPV-16 and HPV-18 E6 and E7 epitopes. A recent phase II trial demonstrates that a prospectively defined subgroup of younger women have a significantly higher rate of disease resolution when treated with ZYC-101a than when administered placebo (129). However, when comparing the effect of placebo and ZYC-101a in the whole population studied, no significant difference was observed. Another clinical trial at Johns Hopkins Hospital tested a DNA vaccine encoding E7 linked to *Mycobacterium tuberculosis* Hsp70 (pNGVL4a-Sig/E7(detox)/Hsp70). Present results show that the vaccine is well tolerated [C Trimble, personal communications].

4.5. RNA replicon vaccines

Naked RNA molecules that replicate within transfected cells are called RNA replicons. RNA replicons express the encoded antigen at high levels, can be immunized in patients repeatedly, and persist in the transfected cells for long periods of time (130-132), making these molecules more immunogenic than conventional DNA vaccines. Furthermore, unlike DNA vaccines, RNA replicons don't raise concerns of cell transformation, which is especially important to consider given the use of potentially oncogenic proteins such as HPV E6 and E7. RNA replicons are, however, less stable than DNA and transfected cells ultimately go through apoptosis due to the activity of the replicon. In order to combine both the benefits of DNA vaccines and RNA replicon, researchers have created naked 'suicidal' DNA encoding the RNA replicon. This kind of vaccine is not only stable and efficiently transfected but also has the strong potency and safety of RNA replicon (130).

Several preclinical studies of HPV vaccines have used an alpha-virus RNA replicon administered as either RNA or DNA encoding HPV-16 E7. Researchers showed that they could induce strong immune responses. If combined with fusion protein strategies to enhance intracellular localization of antigen or intercellular spread of antigen, tumor eradication has been observed (133-137). Combination of E6 and E7 genes is also a plausible strategy to enhance the potency of alpha-virus RNA replicon-based vaccines (138). Additionally, a new replicon system utilizing a stable, non cytopathic RNA replicon, flavivirus *Kunjin* (KUN), has been recently created. Vaccination of mice with HPV-16 E7 CTL epitope expressing KUN replicons could induce specific T-cell responses and

protection from tumor challenge (139). One important advantage of KUN replicon based vaccines over alpha-virus replicon based vaccines is that KUN replicon does not initiate cell death. This may increase the time during which antigen presentation can occur while still maintaining a low risk of cell transformation (140).

4.6. Dendritic cell-based vaccines

As mentioned above, DCs are the most potent antigen presenting cells. Extensive studies have revealed the mechanisms of the development of DCs, the process of antigen presentation and DCs' migration and maturation control signals. This understanding has become the basis for the development of DC-based vaccines (for a review, see reference (141)). To create a DC-based HPV vaccine, it is necessary to load DCs with viral antigens and deliver them into the patients. Antigen-loading strategies include pulsing DCs with synthetic peptides or tumor lysate or transferring nucleic acids encoding tumors antigens directly into DCs. Infecting DCs with tumor antigen-expressing vaccinia virus (142) or fusing DCs with tumor cells (36) has also been shown to be plausible. Reagents such as CpG oligonucleotides or sorbitol may be added to stimulate DCs to enhance their immunogenicity (143, 144). Introducing co-stimulatory molecule or cytokine genes into DCs can enhance the DC-T cell interaction, which may also stimulate a stronger immune response. However, preparing a DC-based vaccine is labor intensive. Cells must be cultured *ex vivo* for each individual patient. The culture process as well as the antigen loading and administration methods may bring great variation in quality of DC vaccines. An even more serious concern is that immature DCs that are mixed in the DC vaccine may potentially result in antigen tolerance (for a review, see reference (145)).

In the vaccine production process, most preclinical DC vaccine researchers either pulse the *ex vivo* cultured DCs with HPV antigen or transfect HPV antigen-coding DNA, RNA or viral vector into the cultured cells. Both strategies have been shown to be able to induce specific CTL immune responses in mice, and regression of pre-established tumors was also observed in some cases. Various routes of DC vaccine administration have also been studied in preclinical models. Among intramuscular, subcutaneous or intravenous delivery of E7-expressing DC vaccine, Wang *et al* discovered that intramuscular administration is the most efficient way to generate large number of E7-specific CD8+ T-cell precursors and antitumor immunity (146). (For a review, see reference (66)).

The DC-based vaccines are just beginning to be translated into clinically relevant studies. A recent case report pointed out that an HPV-18 E7 protein-pulsed DC vaccine inhibited tumor progression in a female patient with metastatic cervical cancer. Complete remission didn't occur but the patient's health status was improved and no significant side effects were observed (147). A recent clinical trial used DCs from nine patients with advanced cervical cancer pulsed with tumor lysate. The DCs were prepared as autologous vaccines for the patients, and the patients received six weekly subcutaneous injections. CTL responses were generated in some patients, and the DCs were well tolerated by their original donor (148). Other clinical trials have employed a similar strategies (149).

4.7. Tumor cell-based vaccines

Tumor cells, with some modification (i.e. transfection with cytokine genes), may become immunogenic and serve as a form of cancer vaccine. The cytokine genes that have been used in HPV-transformed tumor vaccine development include IL-2 (150), IL-12 (151, 152) or granulocyte-macrophage colony-stimulating factor (GM-CSF) (152, 153). Many of these constructs have been shown to generate antitumor effects in mouse models. One benefit of tumor cell-based vaccines is the convenience that tumor antigens need not be clearly identified, though in the case of HPV-associated cervical cancer, tumor antigens are well known. Using tumor cell-based vaccines, however, carries the risk of introducing new cancers, which is not acceptable for relatively healthy HPV carrier or patients with mild cervical neoplasia. The application of tumor cell-based vaccines may thus be limited to patients with advanced cancers. Additionally, for the tumor vaccines to be most effective, developing the vaccine from the patients' own tumor cells is thought to be ideal, limiting the ability to achieve large scale production. The process of isolating tumor cells from patients is very challenging in itself. The number and uniformity (including antigen presentation and transformation) of the cells is important as is preventing contamination of fibroblasts infiltrating tumor. Additional work, such as transfecting the cells with cytokines, is required to enhance the immunogenicity of the tumor cells to the immune system. Thus, autologous tumor vaccines are impractical for clinical use. The development of allogenic tumor cell lines that express common tumor antigens is a possibility for tumor cell-based vaccines to be widely useful, though using adjuvants or engineering tumor cells to express cytokines or co-stimulatory molecules is still required to make the vaccine immunogenic.

4.8. Combined approaches

Priming and boosting the immune system with different kinds of HPV vaccines may generate better immune responses than using a single vaccine modality. For example, priming the immune system with an HPV DNA vaccine and subsequently boosting the immune system with a live viral vector vaccine has been shown to achieve stronger anti-tumor effects than either vaccine alone (154, 155). Priming with DNA vaccine and boosting with tumor vaccine has also been reported to moderately increase immune response (156). RNA replicon boosted with vaccinia vector is another combination that has been tested. Mice were primed with replication-defective alpha-virus replicon particles encoding the HPV-16 E7 antigen, and received HPV E7 vaccinia virus boosters. This combination elicited E7-specific CD8+ T-cells and antitumor effects in mice (157). In addition, strategies that combine DNA vaccination or tumor cell vaccination with antiviral or anticancer agents, such as those tested for treatment of rabbit papillomas and mouse tumor models, may also be useful for treating HPV infections (158-160). However, a prime-boost treatment experiment using a TA-

CIN (HPV-16 L2/E6/E7 protein) vaccine combined with vaccinia HPV-16/18 E6/E7 boosting showed that although the regimen is immunogenic, no simple relationship can be observed between induction of HPV-16-specific immunity and clinical outcome (161). Future study is required to optimize the combinatory vaccination strategy.

5. CONCLUSION

Eradication of cervical cancer and other HPV-related anogenital cancers is an exciting possibility because a necessary causal agent, high-risk human papillomavirus, has been identified and characterized. Therefore, prophylactic and therapeutic vaccines against HPV hold great promises in decreasing worldwide morbidity and mortality associated with the virus. The L1 VLP vaccines have achieved a high efficacy regarding prevention of HPV infection in clinical trials. The potentially cross-protective L2 vaccines are also showing promising development. Efforts in dealing with established HPV infection and potential breakthrough infection in preventive vaccinated women have been the focus of therapeutic vaccine studies. Several of different types of these therapeutic vaccines, including live vector vaccines, peptide or protein vaccines, nucleic acid vaccines, cell-based vaccines and combined approach vaccines, have been shown to induce HPV-specific cellular immunity and antitumor responses in preclinical animal models. The most promising strategies have even been pushed into clinical testing with mixed results, possibly reflecting immune tolerance in patients with persistent HPV infection and disease. Thus, optimizing the immunotherapy against HPV and HPV-associated malignancies requires further study.

Research of the next generation of prophylactic HPV vaccines will probably focus on protection against multiple HPV types and development of inexpensive, heat-stable, single dose formulations for use in low resource settings. Although many of the therapeutic vaccine studies are still trying to elucidate the anti-HPV immune response generated by the vaccinations in preclinical models, completion of the many head-to-head clinical trials of various therapeutic vaccine types will help determine which strategies are best suited for use in humans. It is quite plausible, that in the future, we may be able to control HPV infection and HPV-associated disease in humans through the dual protection of both prophylactic and therapeutic HPV vaccines.

Finally, a useful HPV vaccine should meet the requirements of the people who need it most. That is the population in developing countries, wherein 78% of all cervical cancers occur. Vaccine delivery, cost, requirement of a cold chain system for storage and transportation may all become potential obstacles for the vaccine to be available for these people in need. Future vaccine development must take these criteria into consideration, and safety should not be compromised in these vulnerable populations.

6. ACKNOWLEDGMENTS

This review is not intended to be an encyclopedic one and the authors apologize to those not cited. Special thanks go to Drs Keerti V Shah and Robert J Kurman for their helpful discussions and Mr. David Boyd for his critical review of the manuscript.

7. REFERENCES

1. Walboomers, J. M., M. V. Jacobs, M. M. Manos, F. X. Bosch, J. A. Kummer, K. V. Shah, P. J. Snijders, J. Peto, C. J. Meijer & N. Munoz: Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*, 189, 12-19 (1999)
2. Steenbergen, R. D., J. de Wilde, S. M. Wilting, A. A. Brink, P. J. Snijders & C. J. Meijer: HPV-mediated transformation of the anogenital tract. *J Clin Virol*, 32 Suppl 1, S25-33 (2005)
3. Devaraj, K., M. L. Gillison & T. C. Wu: Development of HPV vaccines for HPV-associated head and neck squamous cell carcinoma. *Crit Rev Oral Biol Med*, 14, 345-62 (2003)
4. Parkin, D. M., F. Bray, J. Ferlay & P. Pisani: Global cancer statistics, 2002. *CA Cancer J Clin*, 55, 74-108 (2005)
5. Bosch, F. X., M. M. Manos, N. Munoz, M. Sherman, A. M. Jansen, J. Peto, M. H. Schiffman, V. Moreno, R. Kurman & K. V. Shah: Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer Inst*, 87, 796-802 (1995)
6. Munoz, N., F. X. Bosch, S. de Sanjose, R. Herrero, X. Castellsague, K. V. Shah, P. J. Snijders & C. J. Meijer: Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*, 348, 518-27 (2003)
7. Ho, G. Y., Y. Y. Studentsov, R. Bierman & R. D. Burk: Natural history of human papillomavirus type 16 virus-like particle antibodies in young women. *Cancer Epidemiol Biomarkers Prev*, 13, 110-6 (2004)
8. Ferenczy, A. & E. Franco: Persistent human papillomavirus infection and cervical neoplasia. *Lancet Oncol*, 3, 11-6 (2002)
9. zur Hausen, H.: Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer*, 2, 342-50 (2002)
10. Mahdavi, A. & B. J. Monk: Vaccines against human papillomavirus and cervical cancer: promises and challenges. *Oncologist*, 10, 528-38 (2005)
11. Mandic, A. & T. Vujkov: Human papillomavirus vaccine as a new way of preventing cervical cancer: a dream or the future? *Ann Oncol*, 15, 197-200 (2004)
12. Tomson, T. T., R. B. Roden & T. C. Wu: Human papillomavirus vaccines for the prevention and treatment of cervical cancer. *Curr Opin Investig Drugs*, 5, 1247-61 (2004)
13. Trimble, E. L. & M. Schoenfeldt: Current clinical trials of HPV vaccines. *Oncology (Williston Park)*, 18, 859-60, 862 (2004)
14. Hagensee, M. & D. Galloway: Growing Human Papillomaviruses and Virus-Like Particles in the Laboratory. *Papillomavirus Rep*, 4, 121--124. (1993)
15. Kirnbauer, R., F. Booy, N. Cheng, D. R. Lowy & J. T. Schiller: Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly

immunogenic. *Proc Natl Acad Sci U S A*, 89, 12180-4 (1992)
16. Nardelli-Haefliger, D., R. B. Roden, J. Benyacoub, R. Sahli, J. P. Kraehenbuhl, J. T. Schiller, P. Lachat, A. Potts & P. De Grandi: Human papillomavirus type 16 virus-like particles expressed in attenuated Salmonella typhimurium elicit mucosal and systemic neutralizing antibodies in mice. *Infect Immun*, 65, 3328-36 (1997)
17. Sasagawa, T., P. Pushko, G. Steers, S. E. Gschmeissner, M. A. Hajibagheri, J. Finch, L. Crawford & M. Tommasino: Synthesis and assembly of virus-like particles of human papillomaviruses type 6 and type 16 in fission yeast Schizosaccharomyces pombe. *Virology*, 206, 126-35 (1995)
18. Zhou, J., X. Y. Sun, H. Davies, L. Crawford, D. Park & I. H. Frazer: Definition of linear antigenic regions of the HPV16 L1 capsid protein using synthetic virion-like particles. *Virology*, 189, 592-9 (1992)
19. Kirnbauer, R., L. M. Chandrachud, B. W. O'Neil, E. R. Wagner, G. J. Grindlay, A. Armstrong, G. M. McGarvie, J. T. Schiller, D. R. Lowy & M. S. Campo: Virus-like particles of bovine papillomavirus type 4 in prophylactic and therapeutic immunization. *Virology*, 219, 37-44 (1996)
20. Breitburd, F., R. Kirnbauer, N. L. Hubbert, B. Nonnenmacher, C. Trin-Dinh-Desmarquet, G. Orth, J. T. Schiller & D. R. Lowy: Immunization with viruslike particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. *J Virol*, 69, 3959-63 (1995)
21. Suzich, J. A., S. J. Ghim, F. J. Palmer-Hill, W. I. White, J. K. Tamura, J. A. Bell, J. A. Newsome, A. B. Jenson & R. Schlegel: Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. *Proc Natl Acad Sci U S A*, 92, 11553-7 (1995)
22. Evans, T. G., W. Bonnez, R. C. Rose, S. Koenig, L. Demeter, J. A. Suzich, D. O'Brien, M. Campbell, W. I. White, J. Balsley & R. C. Reichman: A Phase 1 study of a recombinant viruslike particle vaccine against human papillomavirus type 11 in healthy adult volunteers. *J Infect Dis*, 183, 1485-93 (2001)
23. Harro, C. D., Y. Y. Pang, R. B. Roden, A. Hildesheim, Z. Wang, M. J. Reynolds, T. C. Mast, R. Robinson, B. R. Murphy, R. A. Karron, J. Dillner, J. T. Schiller & D. R. Lowy: Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. *J Natl Cancer Inst*, 93, 284-92 (2001)
24. Nardelli-Haefliger, D., D. Wirthner, J. T. Schiller, D. R. Lowy, A. Hildesheim, F. Ponci & P. De Grandi: Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. *J Natl Cancer Inst*, 95, 1128-37 (2003)
25. Poland, G. A., R. M. Jacobson, L. A. Koutsky, G. M. Tamms, R. Railkar, J. F. Smith, J. T. Bryan, P. F. Cavanaugh, Jr., K. U. Jansen & E. Barr: Immunogenicity and reactogenicity of a novel vaccine for human papillomavirus 16: a 2-year randomized controlled clinical trial. *Mayo Clin Proc*, 80, 601-10 (2005)
26. Koutsky, L. A., K. A. Ault, C. M. Wheeler, D. R. Brown, E. Barr, F. B. Alvarez, L. M. Chiacchierini & K. U. Jansen: A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med*, 347, 1645-51 (2002)
27. Pinto, L. A., J. Edwards, P. E. Castle, C. D. Harro, D. R. Lowy, J. T. Schiller, D. Wallace, W. Kopp, J. W. Adelsberger, M. W. Baseler, J. A. Berzofsky & A. Hildesheim: Cellular immune responses to human papillomavirus (HPV)-16 L1 in healthy volunteers immunized with recombinant HPV-16 L1 virus-like particles. *J Infect Dis*, 188, 327-38 (2003)
28. Herrero, R., A. Hildesheim, C. Bratti, M. Schiffman, A. C. Rodriguez, S. Wacholder, E. Freer, J. Schiller & D. Lowy: Rationale and design of the NCI-Costa Rica HPV 16 vaccine trial. *19th International Papillomavirus Conference*, P-94 (2001)
29. Ruiz, W., W. L. McClements, K. U. Jansen & M. T. Esser: Kinetics and isotype profile of antibody responses in rhesus macaques induced following vaccination with HPV 6, 11, 16 and 18 L1-virus-like particles formulated with or without Merck aluminum adjuvant. *J Immune Based Ther Vaccines*, 3, 2 (2005)
30. Balmelli, C., R. Roden, A. Potts, J. Schiller, P. De Grandi & D. Nardelli-Haefliger: Nasal immunization of mice with human papillomavirus type 16 virus-like particles elicits neutralizing antibodies in mucosal secretions. *J Virol*, 72, 8220-9 (1998)
31. Nardelli-Haefliger, D., F. Lurati, D. Wirthner, F. Spertini, J. Schiller, D. Lowy, F. Hartmann & P. De Grandi: Mucosal vaccination of female volunteers with purified HPV 16 VLPs. *19th International Papillomavirus Conference*, P-103 (2001)
32. Rose, R. C., C. Lane, S. Wilson, J. A. Suzich, E. Rybicki & A. L. Williamson: Oral vaccination of mice with human papillomavirus virus-like particles induces systemic virus-neutralizing antibodies. *Vaccine*, 17, 2129-35 (1999)
33. Berg, M., J. Difatta, E. Hoiczyk, R. Schlegel & G. Ketner: Viable adenovirus vaccine prototypes: high-level production of a papillomavirus capsid antigen from the major late transcriptional unit. *Proc Natl Acad Sci U S A*, 102, 4590-5 (2005)
34. Johnston, K. B., J. M. Monteiro, L. D. Schultz, L. Chen, F. Wang, V. A. Ausensi, E. C. Dell, E. B. Santos, R. A. Moore, T. J. Palker, M. A. Stanley & K. U. Jansen: Protection of beagle dogs from mucosal challenge with canine oral papillomavirus by immunization with recombinant adenoviruses expressing codon-optimized early genes. *Virology*, 336, 208-18 (2005)
35. Liu, D. W., J. L. Chang, Y. P. Tsao, C. W. Huang, S. W. Kuo & S. L. Chen: Co-vaccination with adeno-associated virus vectors encoding human papillomavirus 16 L1 proteins and adenovirus encoding murine GM-CSF can elicit strong and prolonged neutralizing antibody. Int J Cancer, 113, 93-100 (2005)
36. Roberts, A., J. D. Reuter, J. H. Wilson, S. Baldwin & J. K. Rose: Complete protection from papillomavirus challenge after a single vaccination with a vesicular stomatitis virus vector expressing high levels of L1 protein. J Virol, 78, 3196-9 (2004)
37. Baud, D., F. Ponci, M. Bobst, P. De Grandi & D. Nardelli-Haefliger: Improved efficiency of a Salmonella-based vaccine against human papillomavirus type 16 virus-like particles achieved by using a codon-optimized version of L1. J Virol, 78, 12901-9 (2004)
38. PATRICIA: Trial launched of new vaccine aimed at preventing cervical cancer [GSK News]. GlaxoSmithKline (2004)

39. Brown, D. R., K. A. Ault, C. Wheeler, K. U. Jansen, J. F. Smith, E. Barr, A. DiCello, M. R. Nelson & R. Railkar: A dose-ranging study of the safety and immunogenicity profiles of a quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP candidate vaccine in young healthy women. *19th International Papillomavirus Conference*, O-51 (2001)
40. Harper, D. M., E. L. Franco, C. Wheeler, D. G. Ferris, D. Jenkins, A. Schuind, T. Zahaf, B. Innis, P. Naud, N. S. De Carvalho, C. M. Roteli-Martins, J. Teixeira, M. M. Blatter, A. P. Korn, W. Quint & G. Dubin: Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet*, 364, 1757-65 (2004)
41. Villa, L. L., R. L. Costa, C. A. Petta, R. P. Andrade, K. A. Ault, A. R. Giuliano, C. M. Wheeler, L. A. Koutsky, C. Malm, M. Lehtinen, F. E. Skjeldestad, S. E. Olsson, M. Steinwall, D. R. Brown, R. J. Kurman, B. M. Ronnett, M. H. Stoler, A. Ferenczy, D. M. Harper, G. M. Tamms, J. Yu, L. Lupinacci, R. Railkar, F. J. Taddeo, K. U. Jansen, M. T. Esser, H. L. Sings, A. J. Saah & E. Barr: Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol*, 6, 271-8 (2005)
42. Sundaram, P., R. E. Tigelaar, W. Xiao & J. L. Brandsma: Intracutaneous vaccination of rabbits with the E6 gene of cottontail rabbit papillomavirus provides partial protection against virus challenge. Vaccine, 16, 613-23 (1998)
43. Reddy, K. J., B. Banapour, D. E. Anderson, S. H. Lee, J. P. Marquez, M. P. Carlos & J. V. Torres: Induction of immune responses against human papillomaviruses by hypervariable epitope constructs. Immunology, 112, 321-7 (2004)
44. Zhang, L. F., J. Zhou, S. Chen, L. L. Cai, Q. Y. Bao, F. Y. Zheng, J. Q. Lu, J. Padmanabha, K. Hengst, K. Malcolm & I. H. Frazer: HPV6b virus like particles are potent immunogens without adjuvant in man. Vaccine, 18, 1051-8 (2000)
45. Brown, D. R., J. T. Bryan, J. M. Schroeder, T. S. Robinson, K. H. Fife, C. M. Wheeler, E. Barr, P. R. Smith, L. Chiacchierini, A. DiCello & K. U. Jansen: Neutralization of human papillomavirus type 11 (HPV-11) by serum from women vaccinated with yeast-derived HPV-11 L1 virus-like particles: correlation with competitive radioimmunoassay titer. J Infect Dis, 184, 1183-6 (2001)
46. Emeny, R. T., C. M. Wheeler, K. U. Jansen, W. C. Hunt, T. M. Fu, J. F. Smith, S. MacMullen, M. T. Esser & X. Paliard: Priming of human papillomavirus type 11-specific humoral and cellular immune responses in college-aged women with a virus-like particle vaccine. J Virol, 76, 7832-42 (2002)
47. Roden, R. B., W. H. t. Yutzy, R. Fallon, S. Inglis, D. R. Lowy & J. T. Schiller: Minor capsid protein of human genital papillomaviruses contains subdominant, cross-neutralizing epitopes. Virology, 270, 254-7 (2000)
48. Kawana, K., H. Yoshikawa, Y. Taketani, K. Yoshiike & T. Kanda: Common neutralization epitope in minor capsid protein L2 of human papillomavirus types 16 and 6. J Virol, 73, 6188-90 (1999)
49. Kawana, Y., K. Kawana, H. Yoshikawa, Y. Taketani, K. Yoshiike & T. Kanda: Human papillomavirus type 16 minor capsid protein l2 N-terminal region containing a common neutralization epitope binds to the cell surface and enters the cytoplasm. *J Virol*, 75, 2331-6 (2001)
50. Embers, M. E., L. R. Budgeon, M. Pickel & N. D. Christensen: Protective immunity to rabbit oral and cutaneous papillomaviruses by immunization with short peptides of L2, the minor capsid protein. *J Virol*, 76, 9798-805 (2002)
51. Campo, M. S., G. J. Grindlay, B. W. O'Neil, L. M. Chandrachud, G. M. McGarvie & W. F. Jarrett: Prophylactic and therapeutic vaccination against a mucosal papillomavirus. *J Gen Virol*, 74, 945-53 (1993)
52. Christensen, N. D., J. W. Kreider, N. C. Kan & S. L. DiAngelo: The open reading frame L2 of cottontail rabbit papillomavirus contains antibody-inducing neutralizing epitopes. *Virology*, 181, 572-9 (1991)
53. Lin, Y. L., L. A. Borenstein, R. Selvakumar, R. Ahmed & F. O. Wettstein: Effective vaccination against papilloma development by immunization with L1 or L2 structural protein of cottontail rabbit papillomavirus. *Virology*, 187, 612-9 (1992)
54. Embers, M. E., L. R. Budgeon, T. D. Culp, C. A. Reed, M. D. Pickel & N. D. Christensen: Differential antibody responses to a distinct region of human papillomavirus minor capsid proteins. *Vaccine*, 22, 670-80 (2004)
55. Kawana, K., T. Yasugi, T. Kanda, N. Kino, K. Oda, S. Okada, Y. Kawana, T. Nei, T. Takada, S. Toyoshima, A. Tsuchiya, K. Kondo, H. Yoshikawa, O. Tsutsumi & Y. Taketani: Safety and immunogenicity of a peptide containing the cross-neutralization epitope of HPV16 L2 administered nasally in healthy volunteers. *Vaccine*, 21, 4256-60 (2003)
56. Wakabayashi, M. T., D. M. Da Silva, R. K. Potkul & W. M. Kast: Comparison of human papillomavirus type 16 L1 chimeric virus-like particles versus L1/L2 chimeric virus-like particles in tumor prevention. *Intervirology*, 45, 300-7 (2002)
57. Halpert, R., R. G. Fruchter, A. Sedlis, K. Butt, J. G. Boyce & F. H. Sillman: Human papillomavirus and lower genital neoplasia in renal transplant patients. *Obstet Gynecol*, 68, 251-8 (1986)
58. Schafer, A., W. Friedmann, M. Mielke, B. Schwartlander & M. A. Koch: The increased frequency of cervical dysplasia-neoplasia in women infected with the human immunodeficiency virus is related to the degree of immunosuppression. *Am J Obstet Gynecol*, 164, 593-9 (1991)
59. Laga, M., J. P. Icenogle, R. Marsella, A. T. Manoka, N. Nzila, R. W. Ryder, S. H. Vermund, W. L. Heyward, A. Nelson & W. C. Reeves: Genital papillomavirus infection and cervical dysplasia--opportunistic complications of HIV infection. *Int J Cancer*, 50, 45-8 (1992)
60. Sun, X. W., L. Kuhn, T. V. Ellerbrock, M. A. Chiasson, T. J. Bush & T. C. Wright, Jr.: Human papillomavirus infection in women infected with the human immunodeficiency virus. *N Engl J Med*, 337, 1343-9 (1997)
61. Benton, C., H. Shahidullah & J. A. A. Hunter: Human papillomavirus in the immunosuppressed. In: Papillomavirus Report. (1992)
62. Coleman, N., H. D. Birley, A. M. Renton, N. F. Hanna, B. K. Ryait, M. Byrne, D. Taylor-Robinson & M. A.

Stanley: Immunological events in regressing genital warts. *Am J Clin Pathol*, 102, 768-74 (1994)
63. Cubie, H. A., M. Norval, L. Crawford, L. Banks & T. Crook: Lymphoproliferative response to fusion proteins of human papillomaviruses in patients with cervical intraepithelial neoplasia. *Epidemiol Infect*, 103, 625-32 (1989)
64. Hussain, S. F. & Y. Paterson: CD4+CD25+ regulatory T cells that secrete TGFbeta and IL-10 are preferentially induced by a vaccine vector. *J Immunother*, 27, 339-46 (2004)
65. Tang, Y., L. Zhang, J. Yuan, H. Akbulut, J. Maynard, P. J. Linton & A. Deisseroth: Multistep process through which adenoviral vector vaccine overcomes anergy to tumor-associated antigens. *Blood*, 104, 2704-13 (2004)
66. Ling, M. & T. C. Wu: Therapeutic Human Papillomavirus Vaccines. In: Cervical Cancer: From Etiology to Prevention. Eds: T. E. Rohan&K. V. Shah. Kluwer Academic Publishers, Boston (2004)
67. Daemen, T., A. Riezebos-Brilman, J. Regts, B. Dontje, A. van der Zee & J. Wilschut: Superior therapeutic efficacy of alphavirus-mediated immunization against human papilloma virus type 16 antigens in a murine tumour model: effects of the route of immunization. *Antivir Ther*, 9, 733-42 (2004)
68. Riezebos-Brilman, A., J. Regts, E. J. Freyschmidt, B. Dontje, J. Wilschut & T. Daemen: Induction of human papilloma virus E6/E7-specific cytotoxic T-lymphocyte activity in immune-tolerant, E6/E7-transgenic mice. *Gene Ther* (2005)
69. Brandsma, J. L., M. Shlyankevich, L. Zhang, M. D. Slade, E. C. Goodwin, W. Peh & A. B. Deisseroth: Vaccination of rabbits with an adenovirus vector expressing the papillomavirus E2 protein leads to clearance of papillomas and infection. *J Virol*, 78, 116-23 (2004)
70. Hussain, S. F. & Y. Paterson: What is needed for effective antitumor immunotherapy? Lessons learned using Listeria monocytogenes as a live vector for HPV-associated tumors. *Cancer Immunol Immunother*, 54, 577-86 (2005)
71. Bermudez-Humaran, L. G., N. G. Cortes-Perez, Y. Le Loir, J. M. Alcocer-Gonzalez, R. S. Tamez-Guerra, R. M. de Oca-Luna & P. Langella: An inducible surface presentation system improves cellular immunity against human papillomavirus type 16 E7 antigen in mice after nasal administration with recombinant lactococci. *J Med Microbiol*, 53, 427-33 (2004)
72. Cortes-Perez, N. G., V. Azevedo, J. M. Alcocer-Gonzalez, C. Rodriguez-Padilla, R. S. Tamez-Guerra, G. Corthier, A. Gruss, P. Langella & L. G. Bermudez-Humaran: Cell-surface display of E7 antigen from human papillomavirus type-16 in Lactococcus lactis and in Lactobacillus plantarum using a new cell-wall anchor from lactobacilli. *J Drug Target*, 13, 89-98 (2005)
73. Sewell, D. A., D. Douven, Z. K. Pan, A. Rodriguez & Y. Paterson: Regression of HPV-positive tumors treated with a new Listeria monocytogenes vaccine. *Arch Otolaryngol Head Neck Surg*, 130, 92-7 (2004)
74. Sewell, D. A., V. Shahabi, G. R. Gunn, 3rd, Z. K. Pan, M. E. Dominiecki & Y. Paterson: Recombinant Listeria vaccines containing PEST sequences are potent immune adjuvants for the tumor-associated antigen human papillomavirus-16 E7. *Cancer Res*, 64, 8821-5 (2004)
75. Peng, X., S. F. Hussain & Y. Paterson: The ability of two Listeria monocytogenes vaccines targeting human papillomavirus-16 E7 to induce an antitumor response correlates with myeloid dendritic cell function. *J Immunol*, 172, 6030-8 (2004)
76. Hsieh, C. J., T. W. Kim, C. F. Hung, J. Juang, M. Moniz, D. A. Boyd, L. He, P. J. Chen, C. H. Chen & T. C. Wu: Enhancement of vaccinia vaccine potency by linkage of tumor antigen gene to gene encoding calreticulin. *Vaccine*, 22, 3993-4001 (2004)
77. Jin, H. S., E. K. Park, J. M. Lee, S. E. NamKoong, D. G. Kim, Y. J. Lee, H. J. Jun, B. D. Han, S. M. Bae & W. S. Ahn: Immunization with adenoviral vectors carrying recombinant IL-12 and E7 enhanced the antitumor immunity to human papillomavirus 16-associated tumor. *Gynecol Oncol*, 97, 559-67 (2005)
78. Borysiewicz, L. K., A. Fiander, M. Nimako, S. Man, G. W. Wilkinson, D. Westmoreland, A. S. Evans, M. Adams, S. N. Stacey, M. E. Boursnell, E. Rutherford, J. K. Hickling & S. C. Inglis: A recombinant vaccinia virus encoding human papillomavirus types 16 and 18, E6 and E7 proteins as immunotherapy for cervical cancer. *Lancet*, 347, 1523-7 (1996)
79. Kaufmann, A. M., P. L. Stern, E. M. Rankin, H. Sommer, V. Nuessler, A. Schneider, M. Adams, T. S. Onon, T. Bauknecht, U. Wagner, K. Kroon, J. Hickling, C. M. Boswell, S. N. Stacey, H. C. Kitchener, J. Gillard, J. Wanders, J. S. Roberts & H. Zwierzina: Safety and immunogenicity of TA-HPV, a recombinant vaccinia virus expressing modified human papillomavirus (HPV)-16 and HPV-18 E6 and E7 genes, in women with progressive cervical cancer. *Clin Cancer Res*, 8, 3676-85 (2002)
80. Adams, M., L. Borysiewicz, A. Fiander, S. Man, B. Jasani, H. Navabi, C. Lipetz, A. S. Evans & M. Mason: Clinical studies of human papilloma vaccines in pre-invasive and invasive cancer. *Vaccine*, 19, 2549-56 (2001)
81. Baldwin, P. J., S. H. van der Burg, C. M. Boswell, R. Offringa, J. K. Hickling, J. Dobson, J. S. Roberts, J. A. Latimer, R. P. Moseley, N. Coleman, M. A. Stanley & J. C. Sterling: Vaccinia-expressed human papillomavirus 16 and 18 e6 and e7 as a therapeutic vaccination for vulval and vaginal intraepithelial neoplasia. *Clin Cancer Res*, 9, 5205-13 (2003)
82. Davidson, E. J., C. M. Boswell, P. Sehr, M. Pawlita, A. E. Tomlinson, R. J. McVey, J. Dobson, J. S. Roberts, J. Hickling, H. C. Kitchener & P. L. Stern: Immunological and clinical responses in women with vulval intraepithelial neoplasia vaccinated with a vaccinia virus encoding human papillomavirus 16/18 oncoproteins. *Cancer Res*, 63, 6032-41 (2003)
83. Davidson, E. J., R. L. Faulkner, P. Sehr, M. Pawlita, L. J. Smyth, D. J. Burt, A. E. Tomlinson, J. Hickling, H. C. Kitchener & P. L. Stern: Effect of TA-CIN (HPV 16 L2E6E7) booster immunisation in vulval intraepithelial neoplasia patients previously vaccinated with TA-HPV (vaccinia virus encoding HPV 16/18 E6E7) *Vaccine*, 22, 2722-9 (2004)
84. Corona Gutierrez, C. M., A. Tinoco, T. Navarro, M. L. Contreras, R. R. Cortes, P. Calzado, L. Reyes, R. Posternak, G. Morosoli, M. L. Verde & R. Rosales: Therapeutic vaccination with MVA E2 can eliminate precancerous lesions (CIN 1, CIN 2, and CIN 3) associated

with infection by oncogenic human papillomavirus. *Hum Gene Ther*, 15, 421-31 (2004)
85. Chen, Y. F., C. W. Lin, Y. P. Tsao & S. L. Chen: Cytotoxic-T-lymphocyte human papillomavirus type 16 E5 peptide with CpG-oligodeoxynucleotide can eliminate tumor growth in C57BL/6 mice. *J Virol*, 78, 1333-43 (2004)
86. Qin, Y., X. H. Wang, H. L. Cui, Y. K. Cheung, M. H. Hu, S. G. Zhu & Y. Xie: Human papillomavirus type 16 E7 peptide (38-61) linked with an immunoglobulin G fragment provides protective immunity in mice. *Gynecol Oncol*, 96, 475-83 (2005)
87. Ressing, M. E., W. J. van Driel, R. M. Brandt, G. G. Kenter, J. H. de Jong, T. Bauknecht, G. J. Fleuren, P. Hoogerhout, R. Offringa, A. Sette, E. Celis, H. Grey, B. J. Trimbos, W. M. Kast & C. J. Melief: Detection of T helper responses, but not of human papillomavirus-specific cytotoxic T lymphocyte responses, after peptide vaccination of patients with cervical carcinoma. *J Immunother*, 23, 255-66 (2000)
88. van Driel, W. J., M. E. Ressing, G. G. Kenter, R. M. Brandt, E. J. Krul, A. B. van Rossum, E. Schuuring, R. Offringa, T. Bauknecht, A. Tamm-Hermelink, P. A. van Dam, G. J. Fleuren, W. M. Kast, C. J. Melief & J. B. Trimbos: Vaccination with HPV16 peptides of patients with advanced cervical carcinoma: clinical evaluation of a phase I-II trial. *Eur J Cancer*, 35, 946-52 (1999)
89. Muderspach, L., S. Wilczynski, L. Roman, L. Bade, J. Felix, L. A. Small, W. M. Kast, G. Fascio, V. Marty & J. Weber: A phase I trial of a human papillomavirus (HPV) peptide vaccine for women with high-grade cervical and vulvar intraepithelial neoplasia who are HPV 16 positive. *Clin Cancer Res*, 6, 3406-16 (2000)
90. Steller, M. A., K. J. Gurski, M. Murakami, R. W. Daniel, K. V. Shah, E. Celis, A. Sette, E. L. Trimble, R. C. Park & F. M. Marincola: Cell-mediated immunological responses in cervical and vaginal cancer patients immunized with a lipidated epitope of human papillomavirus type 16 E7. *Clin Cancer Res*, 4, 2103-9 (1998)
91. Cui, Z. & L. Huang: Liposome-polycation-DNA (LPD) particle as a carrier and adjuvant for protein-based vaccines: Therapeutic effect against cervical cancer. *Cancer Immunol Immunother* (2005)
92. Stewart, T. J., D. Drane, J. Malliaros, H. Elmer, K. M. Malcolm, J. C. Cox, S. J. Edwards, I. H. Frazer & G. J. Fernando: ISCOMATRIX adjuvant: an adjuvant suitable for use in anticancer vaccines. *Vaccine*, 22, 3738-43 (2004)
93. Preville, X., D. Ladant, B. Timmerman & C. Leclerc: Eradication of established tumors by vaccination with recombinant Bordetella pertussis adenylate cyclase carrying the human papillomavirus 16 E7 oncoprotein. *Cancer Res*, 65, 641-9 (2005)
94. Fausch, S. C., D. M. Da Silva, G. L. Eiben, I. C. Le Poole & W. M. Kast: HPV protein/peptide vaccines: from animal models to clinical trials. *Front Biosci*, 8, s81-91 (2003)
95. Thompson, H. S., M. L. Davies, F. P. Holding, R. E. Fallon, A. E. Mann, T. O'Neill & J. S. Roberts: Phase I safety and antigenicity of TA-GW: a recombinant HPV6 L2E7 vaccine for the treatment of genital warts. *Vaccine*, 17, 40-9 (1999)
96. Lacey, C. J., H. S. Thompson, E. F. Monteiro, T. O'Neill, M. L. Davies, F. P. Holding, R. E. Fallon & J. S. Roberts: Phase IIa safety and immunogenicity of a therapeutic vaccine, TA-GW, in persons with genital warts. *J Infect Dis*, 179, 612-8 (1999)
97. de Jong, A., T. O'Neill, A. Y. Khan, K. M. Kwappenberg, S. E. Chisholm, N. R. Whittle, J. A. Dobson, L. C. Jack, J. A. St Clair Roberts, R. Offringa, S. H. van der Burg & J. K. Hickling: Enhancement of human papillomavirus (HPV) type 16 E6 and E7-specific T-cell immunity in healthy volunteers through vaccination with TA-CIN, an HPV16 L2E7E6 fusion protein vaccine. *Vaccine*, 20, 3456-64 (2002)
98. Hallez, S., P. Simon, F. Maudoux, J. Doyen, J. C. Noel, A. Beliard, X. Capelle, F. Buxant, I. Fayt, A. C. Lagrost, P. Hubert, C. Gerday, A. Burny, J. Boniver, J. M. Foidart, P. Delvenne & N. Jacobs: Phase I/II trial of immunogenicity of a human papillomavirus (HPV) type 16 E7 protein-based vaccine in women with oncogenic HPV-positive cervical intraepithelial neoplasia. *Cancer Immunol Immunother*, 53, 642-50 (2004)
99. Frazer, I. H.: Prevention of cervical cancer through papillomavirus vaccination. *Nat Rev Immunol*, 4, 46-54 (2004)
100. Chu, N. R.: Therapeutic vaccination for the treatment of mucosotropic human papillomavirus-associated disease. *Expert Opin Biol Ther*, 3, 477-86 (2003)
101. Berry, J. M. & J. M. Palefsky: A review of human papillomavirus vaccines: from basic science to clinical trials. *Front Biosci*, 8, s333-45 (2003)
102. Moniz, M., M. Ling, C. F. Hung & T. C. Wu: HPV DNA vaccines. *Front Biosci*, 8, d55-68 (2003)
103. Hung, C.-F., W.-F. Cheng, C.-Y. Chai, K.-F. Hsu, L. He, M. Ling & T.-C. Wu: Improving vaccine potency through intercellular spreading and enhanced MHC class I presentation of antigen. *J Immunol*, 166, 5733-5740 (2001)
104. Kim, T. W., C. F. Hung, D. A. Boyd, L. He, C. T. Lin, D. Kaiserman, P. I. Bird & T. C. Wu: Enhancement of DNA vaccine potency by coadministration of a tumor antigen gene and DNA encoding serine protease inhibitor-6. *Cancer Res*, 64, 400-5 (2004)
105. Hauser, H., L. Shen, Q. L. Gu, S. Krueger & S. Y. Chen: Secretory heat-shock protein as a dendritic cell-targeting molecule: a new strategy to enhance the potency of genetic vaccines. *Gene Ther*, 11, 924-32 (2004)
106. Trimble, C., C. T. Lin, C. F. Hung, S. Pai, J. Juang, L. He, M. Gillison, D. Pardoll, L. Wu & T. C. Wu: Comparison of the CD8+ T cell responses and antitumor effects generated by DNA vaccine administered through gene gun, biojector, and syringe. *Vaccine*, 21, 4036-42 (2003)
107. Chen, C. H., T. L. Wang, H. Ji, C. F. Hung, D. M. Pardoll, W. F. Cheng, M. Ling & T. C. Wu: Recombinant DNA vaccines protect against tumors that are resistant to recombinant vaccinia vaccines containing the same gene. *Gene Ther*, 8, 128-38. (2001)
108. Chen, C.-H., T.-L. Wang, C.-F. Hung, Y. Yang, R. A. Young, D. M. Pardoll & T.-C. Wu: Enhancement of DNA vaccine potency by linkage of antigen gene to an HSP70 gene. *Cancer Research*, 60, 1035-1042. (2000)
109. Cheng, W. F., C. F. Hung, C. Y. Chai, K. F. Hsu, L. He, M. Ling & T. C. Wu: Tumor-specific immunity and

antiangiogenesis generated by a DNA vaccine encoding calreticulin linked to a tumor antigen. *J Clin Invest*, 108, 669-78 (2001)
110. Hung, C.-F., W.-F. Cheng, K.-F. Hsu, C.-Y. Chai, L. He, M. Ling & T.-C. Wu: Cancer immunotherapy using a DNA vaccine encoding the translocation domain of a bacterial toxin linked to a tumor antigen. *Cancer Research*, 61, 3698-3703. (2001)
111. Lin, C. T., T. C. Chang, A. Chao, E. Dzeng, Y. K. Soong, C. F. Hung & C. H. Lai: Enhancement of DNA vaccine potency through linkage of antigen gene to ER chaperone molecules, ER-60, tapasin, and calnexin. *J Biomed Sci*, 12, 279-87 (2005)
112. Peng, S., H. Ji, C. Trimble, L. He, Y. C. Tsai, J. Yeatermeyer, D. A. Boyd, C. F. Hung & T. C. Wu: Development of a DNA vaccine targeting human papillomavirus type 16 oncoprotein E6. *J Virol*, 78, 8468-76 (2004)
113. Huang, C. H., S. Peng, L. He, Y. C. Tsai, D. A. Boyd, T. H. Hansen, T. C. Wu & C. F. Hung: Cancer immunotherapy using a DNA vaccine encoding a single-chain trimer of MHC class I linked to an HPV-16 E6 immunodominant CTL epitope. *Gene Ther*, 12, 1180-6 (2005)
114. Steinberg, T., P. Ohlschlager, P. Sehr, W. Osen & L. Gissmann: Modification of HPV 16 E7 genes: correlation between the level of protein expression and CTL response after immunization of C57BL/6 mice. *Vaccine*, 23, 1149-57 (2005)
115. Cheung, Y. K., S. C. Cheng, F. W. Sin & Y. Xie: Plasmid encoding papillomavirus Type 16 (HPV16) DNA constructed with codon optimization improved the immunogenicity against HPV infection. *Vaccine*, 23, 629-38 (2004)
116. Doan, T., K. Herd, I. Ramshaw, S. Thomson & R. W. Tindle: A polytope DNA vaccine elicits multiple effector and memory CTL responses and protects against human papillomavirus 16 E7-expressing tumour. *Cancer Immunol Immunother*, 54, 157-71 (2005)
117. Leachman, S. A., R. E. Tigelaar, M. Shlyankevich, M. D. Slade, M. Irwin, E. Chang, T. C. Wu, W. Xiao, S. Pazhani, D. Zelterman & J. L. Brandsma: Granulocyte-macrophage colony-stimulating factor priming plus papillomavirus E6 DNA vaccination: effects on papilloma formation and regression in the cottontail rabbit papillomavirus--rabbit model. *J Virol*, 74, 8700-8 (2000)
118. Tan, J., N. S. Yang, J. G. Turner, G. L. Niu, H. F. Maassab, J. Sun, M. L. Herlocher, A. E. Chang & H. Yu: Interleukin-12 cDNA skin transfection potentiates human papillomavirus E6 DNA vaccine-induced antitumor immune response. Cancer Gene Ther, 6, 331-9 (1999)
119. Smahel, M., D. Pokorna, J. Mackova & J. Vlasak: Enhancement of immunogenicity of HPV16 E7 oncogene by fusion with E. coli beta-glucuronidase. J Gene Med, 6, 1092-101 (2004)
120. Peng, S., T. W. Kim, J. H. Lee, M. Yang, L. He, C. F. Hung & T. C. Wu: Vaccination with dendritic cells transfected with BAK and BAX siRNA enhances antigen-specific immune responses by prolonging dendritic cell life. Hum Gene Ther, 16, 584-93 (2005)
121. Kim, T. W., C. F. Hung, D. Boyd, J. Juang, L. He, J. W. Kim, J. M. Hardwick & T. C. Wu: Enhancing DNA vaccine potency by combining a strategy to prolong dendritic cell life with intracellular targeting strategies. J Immunol, 171, 2970-6 (2003)
122. Kim, T. W., C. F. Hung, M. Ling, J. Juang, L. He, J. M. Hardwick, S. Kumar & T. C. Wu: Enhancing DNA vaccine potency by coadministration of DNA encoding antiapoptotic proteins. J Clin Invest, 112, 109-17 (2003)
123. Kim, T. W., C. F. Hung, M. Zheng, D. A. Boyd, L. He, S. I. Pai & T. C. Wu: A DNA vaccine co-expressing antigen and an anti-apoptotic molecule further enhances the antigen-specific CD8+ T-cell immune response. J Biomed Sci, 11, 493-9 (2004)
124. Kim, T. W., J. H. Lee, L. He, D. A. Boyd, J. M. Hardwick, C. F. Hung & T. C. Wu: Modification of professional antigen-presenting cells with small interfering RNA in vivo to enhance cancer vaccine potency. Cancer Res, 65, 309-16 (2005)
125. Kim, T. W., J. H. Lee, L. He, D. A. Boyd, C. F. Hung & T. C. Wu: DNA vaccines employing intracellular targeting strategies and a strategy to prolong dendritic cell life generate a higher number of CD8+ memory T cells and better long-term antitumor effects compared with a DNA prime-vaccinia boost regimen. Hum Gene Ther, 16, 26-34 (2005)
126. Hung, C. F. & T. C. Wu: Improving DNA vaccine potency via modification of professional antigen presenting cells. Curr Opin Mol Ther, 5, 20-24 (2003)
127. Klencke, B., M. Matijevic, R. G. Urban, J. L. Lathey, M. L. Hedley, M. Berry, J. Thatcher, V. Weinberg, J. Wilson, T. Darragh, N. Jay, M. Da Costa & J. M. Palefsky: Encapsulated plasmid DNA treatment for human papillomavirus 16-associated anal dysplasia: a Phase I study of ZYC101. Clin Cancer Res, 8, 1028-37 (2002)
128. Sheets, E. E., R. G. Urban, C. P. Crum, M. L. Hedley, J. A. Politch, M. A. Gold, L. I. Muderspach, G. A. Cole & P. A. Crowley-Nowick: Immunotherapy of human cervical high-grade cervical intraepithelial neoplasia with microparticle-delivered human papillomavirus 16 E7 plasmid DNA. Am J Obstet Gynecol, 188, 916-26 (2003)
129. Garcia, F., K. U. Petry, L. Muderspach, M. A. Gold, P. Braly, C. P. Crum, M. Magill, M. Silverman, R. G. Urban, M. L. Hedley & K. J. Beach: ZYC101a for treatment of high-grade cervical intraepithelial neoplasia: a randomized controlled trial. Obstet Gynecol, 103, 317-26 (2004)
130. Berglund, P., C. Smerdou, M. N. Fleeton, I. Tubulekas & P. Liljestrom: Enhancing immune responses using suicidal DNA vaccines. Nat Biotechnol, 16, 562-5 (1998)
131. Ying, H., T. Z. Zaks, R. F. Wang, K. R. Irvine, U. S. Kammula, F. M. Marincola, W. W. Leitner & N. P. Restifo: Cancer therapy using a self-replicating RNA vaccine. Nat Med, 5, 823-7 (1999)
132. Leitner, W. W., H. Ying, D. A. Driver, T. W. Dubensky & N. P. Restifo: Enhancement of tumor-specific immune response with plasmid DNA replicon vectors. Cancer Res, 60, 51-5 (2000)
133. Cheng, W.-F., C.-F. Hung, K.-F. Hsu, C.-Y. Chai, L. He, C. M. Rice, M. Ling & T.-C. Wu: Enhancement of Sindbis virus self-replicating RNA vaccine potency by linkage of Mycobacterium tuberculosis heat shock protein 70 gene to an antigen gene. J Immunol, 166, 6218-26 (2001)
134. Cheng, W. F., C. F. Hung, K. F. Hsu, C. Y. Chai, L. He, M. Ling, L. A. Slater, R. B. Roden & T. C. Wu: Enhancement of sindbis virus self-replicating RNA vaccine

potency by targeting antigen to endosomal/lysosomal compartments. *Hum Gene Ther*, 12, 235-52 (2001)
135. Cheng, W. F., C. H. Hung, C. Y. Chai, K. F. Hsu, L. He, M. Ling & T. C. Wu: Enhancement of sindbis virus self-replicating RNA vaccine potency by linkage of herpes simplex virus type 1 VP22 protein to antigen. *J Virol*, 75, 2368-76 (2001)
136. Hsu, K. F., C. F. Hung, W. F. Cheng, L. He, L. A. Slater, M. Ling & T. C. Wu: Enhancement of suicidal DNA vaccine potency by linking Mycobacterium tuberculosis heat shock protein 70 to an antigen. *Gene Ther*, 8, 376-83. (2001)
137. Kim, T. W., C. F. Hung, J. Juang, L. He, J. M. Hardwick & T. C. Wu: Enhancement of suicidal DNA vaccine potency by delaying suicidal DNA-induced cell death. *Gene Ther*, 11, 336-42 (2004)
138. Cassetti, M. C., S. P. McElhiney, V. Shahabi, J. K. Pullen, I. C. Le Poole, G. L. Eiben, L. R. Smith & W. M. Kast: Antitumor efficacy of Venezuelan equine encephalitis virus replicon particles encoding mutated HPV16 E6 and E7 genes. *Vaccine*, 22, 520-7 (2004)
139. Herd, K. A., T. Harvey, A. A. Khromykh & R. W. Tindle: Recombinant Kunjin virus replicon vaccines induce protective T-cell immunity against human papillomavirus 16 E7-expressing tumour. *Virology*, 319, 237-48 (2004)
140. Varnavski, A. N., P. R. Young & A. A. Khromykh: Stable high-level expression of heterologous genes in vitro and in vivo by noncytopathic DNA-based Kunjin virus replicon vectors. *J Virol*, 74, 4394-403 (2000)
141. Lanzavecchia, A. & F. Sallusto: Regulation of T cell immunity by dendritic cells. *Cell*, 106, 263-6 (2001)
142. Mackova, J., L. Kutinova, P. Hainz, J. Krystofova, V. Sroller, P. Otahal, P. Gabriel & S. Nemeckova: Adjuvant effect of dendritic cells transduced with recombinant vaccinia virus expressing HPV16-E7 is inhibited by co-expression of IL12. *Int J Oncol*, 24, 1581-8 (2004)
143. Kim, T. G., C. H. Kim, E. H. Won, S. M. Bae, W. S. Ahn, J. B. Park & J. I. Sin: CpG-ODN-stimulated dendritic cells act as a potent adjuvant for E7 protein delivery to induce antigen-specific antitumour immunity in a HPV 16 E7-associated animal tumour model. *Immunology*, 112, 117-25 (2004)
144. Freyschmidt, E. J., A. Alonso, G. Hartmann & L. Gissmann: Activation of dendritic cells and induction of T cell responses by HPV 16 L1/E7 chimeric virus-like particles are enhanced by CpG ODN or sorbitol. *Antivir Ther*, 9, 479-89 (2004)
145. Figdor, C. G., I. J. de Vries, W. J. Lesterhuis & C. J. Melief: Dendritic cell immunotherapy: mapping the way. *Nat Med*, 10, 475-80 (2004)
146. Wang, T.-L., M. Ling, I.-M. Shih, T. Pham, S. I. Pai, L. Lu, R. J. Kurman, D. M. Pardoll & T.-C. Wu: Intramuscular Administration of E7-Transfected Dendritic Cells Generates the Most Potent E7-Specific Anti-Tumor Immunity. *Gene Therapy*, 7, 726-733. (2000)
147. Santin, A. D., S. Bellone, M. Gokden, M. J. Cannon & G. P. Parham: Vaccination with HPV-18 E7-pulsed dendritic cells in a patient with metastatic cervical cancer. *N Engl J Med*, 346, 1752-3 (2002)
148. Adams, M., A. de Jong, H. Havabi, C. Lippetz, B. Jasani, S. Man, A. Fiander, R. Bailey-Wood & S. H. van der Burg: *In vivo* induction of HPV 16 specific CTL and T helper responses in patients with advanced cervical cancer using autologous dendritic cells pulsed with tumour lysate. *19th International Papillomavirus Conference*, P-99 (2001)
149. Adams, M., H. Navabi, B. Jasani, S. Man, A. Fiander, A. S. Evans, C. Donninger & M. Mason: Dendritic cell (DC) based therapy for cervical cancer: use of DC pulsed with tumour lysate and matured with a novel synthetic clinically non-toxic double stranded RNA analogue poly [I]:poly [C12U] (Ampligen®) *Vaccine*, 21, 787-90 (2003)
150. Bubenik, J., J. Simova, R. Hajkova, V. Sobota, T. Jandlova, M. Smahel, E. Sobotkova & V. Vonka: Interleukin 2 gene therapy of residual disease in mice carrying tumours induced by HPV 16. *Int J Oncol*, 14, 593-7 (1999)
151. Hallez, S., O. Detremmerie, C. Giannouli, K. Thielemans, T. F. Gajewski, A. Burny & O. Leo: Interleukin-12-secreting human papillomavirus type 16-transformed cells provide a potent cancer vaccine that generates E7-directed immunity. *Int J Cancer*, 81, 428-37 (1999)
152. Mikyskova, R., M. Indrova, J. Simova, T. Jandlova, J. Bieblova, P. Jinoch, J. Bubenik & V. Vonka: Treatment of minimal residual disease after surgery or chemotherapy in mice carrying HPV16-associated tumours: Cytokine and gene therapy with IL-2 and GM-CSF. *Int J Oncol*, 24, 161-7 (2004)
153. Chang, E. Y., C. H. Chen, H. Ji, T. L. Wang, K. Hung, B. P. Lee, A. Y. Huang, R. J. Kurman, D. M. Pardoll & T. Wu: Antigen-specific cancer immunotherapy using a GM-CSF secreting allogeneic tumor cell-based vaccine. *Int J Cancer*, 86, 725-30 (2000)
154. Wlazlo, A. P., H. Deng, W. Giles-Davis & H. C. Ertl: DNA vaccines against the human papillomavirus type 16 E6 or E7 oncoproteins. *Cancer Gene Ther* (2004)
155. Chen, C. H., T. L. Wang, C. F. Hung, D. M. Pardoll & T. C. Wu: Boosting with recombinant vaccinia increases HPV-16 E7-specific T cell precursor frequencies of HPV-16 E7-expressing DNA vaccines. *Vaccine*, 18, 2015-22 (2000)
156. Rittich, S., M. Duskova, J. Mackova, D. Pokorna, P. Jinoch & M. Smahel: Combined immunization with DNA and transduced tumor cells expressing mouse GM-CSF or IL-2. *Oncol Rep*, 13, 311-7 (2005)
157. Lin, C. T., C. F. Hung, J. Juang, L. He, K. Y. Lin, T. W. Kim & T. C. Wu: Boosting with recombinant vaccinia increases HPV-16 E7-Specific T cell precursor frequencies and antitumor effects of HPV-16 E7-expressing Sindbis virus replicon particles. Mol Ther, 8, 559-66 (2003)
158. Christensen, N. D., R. Han, N. M. Cladel & M. D. Pickel: Combination treatment with intralesional cidofovir and viral-DNA vaccination cures large cottontail rabbit papillomavirus-induced papillomas and reduces recurrences. Antimicrob Agents Chemother, 45, 1201-9 (2001)
159. Christensen, N. D., M. D. Pickel, L. R. Budgeon & J. W. Kreider: In vivo anti-papillomavirus activity of nucleoside analogues including cidofovir on CRPV-induced rabbit papillomas. Antiviral Res, 48, 131-42 (2000)
160. Sobotkova, E., M. Duskova, M. Smahel, V. Holan, O. Janouskova & V. Vonka: Chemotherapy and immunotherapy of tumours induced by gene-modified HPV16-transformed cells. *Oncol Rep*, 12, 877-83 (2004)

161. Smyth, L. J., M. I. Van Poelgeest, E. J. Davidson, K. M. Kwappenberg, D. Burt, P. Sehr, M. Pawlita, S. Man, J. K. Hickling, A. N. Fiander, A. Tristram, H. C. Kitchener, R. Offringa, P. L. Stern & S. H. Van Der Burg: Immunological responses in women with human papillomavirus type 16 (HPV-16)-associated anogenital intraepithelial neoplasia induced by heterologous prime-boost HPV-16 oncogene vaccination. *Clin Cancer Res*, 10, 2954-61 (2004)
162. Pinto, L. A., P. E. Castle, R. B. Roden, C. D. Harro, D. R. Lowy, J. T. Schiller, D. Wallace, M. Williams, W. Kopp, I. H. Frazer, J. A. Berzofsky & A. Hildesheim: HPV-16 L1 VLP vaccine elicits a broad-spectrum of cytokine responses in whole blood. *Vaccine*, 23, 3555-64 (2005)
163. Ault, K. A., A. R. Giuliano, R. P. Edwards, G. Tamms, L. L. Kim, J. F. Smith, K. U. Jansen, M. Allende, F. J. Taddeo, D. Skulsky & E. Barr: A phase I study to evaluate a human papillomavirus (HPV) type 18 L1 VLP vaccine. *Vaccine*, 22, 3004-7 (2004)

Key Words: Cervical Cancer, Cytotoxic T-Lymphocyte, Human Papillomavirus, Immunotherapy, L2, Tumor Immunology, Tumor-Specific Antigens, Vaccine, Virus-Like Particles, Review

Send correspondence to: Dr. T-C Wu, Department of Pathology, the Johns Hopkins University School of Medicine, Ross 512H, 720 Rutland Avenue, Baltimore, Maryland 21205, Tel: 410-614-3899. Fax: 443-287-4295, E-mail: wutc@jhmi.edu

http://www.bioscience.org/current/vol12.htm