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RECENT ADVANCES IN STRATEGIES FOR IMMUNOTHERAPY OF HUMAN PAPILLOMAVIRUS-INDUCED LESIONS

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Abstract

Human papillomavirus (HPV)-induced lesions are distinct in that they have targetable foreign antigens, the expression of which is necessary to maintain the cancerous phenotype. Hence, they pose as a very attractive target for “proof of concept” studies in the development of therapeutic vaccines. This review will focus on the most recent clinical trials for the immunotherapy of mucosal and cutaneous HPV-induced lesions as well as emerging therapeutic strategies that have been tested in pre-clinical models for HPV-induced lesions. Progress in peptide-based vaccines, DNA-based vaccines, viral/bacterial vector-based vaccines, immune response modifiers, photodynamic therapy and T cell receptor based therapy for HPV will be discussed.

1. INTRODUCTION

The human papillomaviruses are a family of sexually transmitted, double-stranded DNA viruses with over 100 different genotypes identified till date. HPV genotypes are divided into the low-risk and high-risk categories based on the spectrum of lesions they induce. Fifteen HPV types are classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82); 3 are classified as probable high-risk types (26, 53, and 66); and 12 are classified as low-risk types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108)¹. The low-risk types primarily induce benign genital condylomas and low-grade squamous intraepithelial lesions whereas the high-risk types are most frequently associated with the development of anogenital cancers and can be detected in 99% of cervical cancers², with HPV16 found in about 50% of cases³. Infection by the low-risk types is not confined to the anogenital area and can cause other diseases such as recurrent respiratory papillomatosis. Similarly, infection by the high-risk types is also not confined to the anogenital area, since 18.3% of cancers of the oropharynx contain DNA from these types⁴. In the United States, an estimated 75% of the sexually active general population ages 15 to 49 years acquires at least one genital HPV type during their lifetime⁵. Though most individuals remain asymptomatic and spontaneously clear their infections, a small percentage of patients develop clinically or histologically recognizable lesions that develop into invasive cancer. The widespread use of cervical cytological screening using Papanicolaou (Pap) smear tests has reduced the mortality rate from cervical cancer in developed countries. However, in developing countries

where screening programs are minimal, cervical cancer remains the second leading cause of cancer-related deaths among women⁶. Furthermore, Pap smear tests will not help identify HPV infection in males, where it can cause penile and anal cancers as well as cancers of the head and neck. Thus, it is essential to develop effective prophylactic and therapeutic strategies directed against HPV.

2. PROPHYLACTIC VACCINES

While therapeutic vaccines aim to develop a strong cellular immune response to HPV antigens that are expressed in transformed cells, prophylactic vaccines aim to prevent infection with HPV by inducing a neutralizing antibody response. Although this review will focus on the recent advances made in the development of therapies targeted towards HPV, it would be remiss to not to discuss the most recent information on prophylactic vaccines for HPV.

Prophylactic vaccines for HPV are based on HPV virus-like particles (VLP), which are obtained by over-expressing the major capsid protein L1 alone, mimic infectious virions in structure and induce high titers of HPV-neutralizing antibodies. Gardasil, the quadrivalent vaccine by Merck & Co., is the first FDA approved prophylactic vaccine developed to prevent cervical cancers, precancerous genital lesions and genital warts due to human papillomavirus (HPV) types 6, 11, 16 and 18. Very recently, the results of a phase 3 randomized, double-blind clinical trial that assessed prophylactic efficacy in 5305 Gardasil-vaccinated women with the primary composite end point of cervical intraepithelial neoplasia (CIN) grade 2 or 3, adenocarcinoma in situ, or cervical cancer related to HPV16 or 18 were published⁷. In an average 3-year follow-up, vaccine efficacy for prevention of primary composite end-point was 98% in the susceptible population and 44% in the intention-to-treat population of all women who had undergone randomization. The efficacy against all high-grade cervical lesions, regardless of HPV type, in the intention-to-treat population was 17%⁷. It should be noted that the 17% efficacy in this population included a mixture of lesions that could be caused by both prevalent and incident infection, and may therefore underestimate protection conferred against high-grade lesions caused by incident infection. Cervarix is a vaccine that was developed by GlaxoSmithKline (GSK) in partnership with MedImmune. It has been approved for use in Australia and is currently awaiting approval from the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Cervarix is a bivalent HPV16 L1/HPV18 L1 VLP vaccine administered in AS04 adjuvant (3-*O*-desacyl-4'-monophosphoryl lipid A and aluminium hydroxide). A randomized control trial that assessed prophylactic efficacy in 393 Cervarix-vaccinated women with the combined end point of HPV16 infection, HPV18 infection, or HPV16 and HPV18 co-infection, and associated CIN (grades 1, 2, and 3), showed complete prevention of 12-month persistent infections, high efficacy through 4.5 years of follow-up and sustained levels of antibodies against HPV16 and HPV18⁸. Initial data from a phase 3 trial showed that at 18 months after the first of a three-dose regimen, 100% of women up to age 55 vaccinated with Cervarix had antibodies present against HPV 16 and 18⁹. Most recently, GSK reported a prophylactic efficacy of 90.4% in an interim analysis of an ongoing phase 3 randomized, double-blind clinical trial that assessed the efficacy of Cervarix against CIN2, CIN3, adenocarcinoma in situ, and invasive carcinoma associated with HPV16 or HPV18

and against persistent infections with HPV16 or HPV18 in 9319 Cervarix-vaccinated women¹⁰.

Despite the very promising results in females that have not encountered HPV, thus far, all available data show poor efficacy in females who have been infected with the specific HPV type(s) prior to vaccination, and women with prevalent HPV infections are not protected from the development of HPV-induced lesions. A phase 3, masked, community-based randomized trial was conducted to evaluate whether vaccination against HPV 16 and 18 increases the rate of viral clearance in women infected with HPV and found no evidence of accelerated viral clearance at 6 or 12 months in the vaccinated group as compared to the control group¹¹. These data illustrate the lack of therapeutic function in VLP-based vaccines and indicate inefficacy in the vast majority of the sexually active population. Furthermore, with the long incubation time of HPV, multiple mechanisms of immune evasion (reviewed in 12) and only a few years of observation in these studies, sustained efficacy of prophylactic vaccines on cancer prevention has yet to be determined. In addition to the above-mentioned limitations of current HPV-vaccines, there are controversies that have affected worldwide implementation of vaccination against HPV such as high cost of the vaccines and social resistance to mandatory vaccination of pre-teenage girls and boys. Though there is insufficient evidence to support that social resistance has led to a substantial reduction in vaccine implementation, there is frequent social debate on acceptability of HPV vaccines for pre-teenage girls and boys; and individuals that remain unvaccinated have a greater risk of developing persistent infections that have the potential to lead to cancers. The demand for HPV vaccines in several of the developing countries appears to be widespread despite the high cost. However, high cost poses a serious problem for implementation of HPV vaccines in developing countries where screening programs are minimal and need is significant. Meanwhile, the need for developing therapeutic strategies to treat HPV induced lesions remains.

3. THERAPEUTIC VACCINES

As mentioned previously, therapeutic vaccines target antigens present in the cancer cells. In the case of HPV-induced cancers, the most frequently targeted antigens are the E6 and E7 proteins, because they are oncogenic and sustained expression is required for the maintenance of the cancerous phenotype. Several therapeutic vaccines that target the E6 and/or E7 proteins have been developed over the last fifteen years (extensively reviewed in 13). These vaccines aim to control HPV-associated malignancies by activating the patient's own cellular immune response to recognize and kill cancer cells that express the foreign proteins. Table 1 summarizes the results of several more recent and ongoing immunotherapy trials for mucosal and cutaneous HPV-induced lesions, which include cervical and vaginal intraepithelial lesions, anal intraepithelial lesions, condylomas and anogenital warts, head and neck squamous cell carcinomas, and recurrent respiratory papillomatosis.

a) Clinical Trial Design for HPV-Associated Disease

In the past, treatment of patients with end-stage disease, limited patient numbers, absence of randomized placebo-controlled studies and spontaneous regression rates of early-stage

disease presented difficulties in interpreting the results of clinical trials. Many trials were designed such that patients underwent definitive treatment after a three month monitoring period due to the ethical and safety issues of leaving lesions untreated, when the patient had viable options for therapy such as surgery or cryotherapy. However, in recent years, this has changed and some of the noteworthy emerging themes of the clinical trials summarized in this review are studies in which patients are not suffering from end-stage disease and thus have the best chance for immunotherapeutic intervention, studies which are placebo-controlled and have large patient cohorts, and studies utilizing a combination of conventional treatment either before or along with immunotherapeutic treatment to determine whether antigen-specific immunity achieved after conventional therapy can lead to a decrease in recurrent or metastatic disease.

b) HPV-Specific Tumor-Directed Therapies

The continued expression of the E6 and E7 proteins in human HPV-induced tumors makes them excellent targets for HPV-specific immunotherapy, which has been confirmed many times over in preclinical animal models for HPV-induced tumors. Vaccine platforms used to target HPV proteins in human clinical trials include peptide/protein-based therapies, viral vector-based therapies, DNA-based therapies, and dendritic cell-based therapies (Table 1). HspE7, a fusion of heat shock protein 65 (Hsp65) from *Mycobacterium bovis* and HPV16 E7 has resulted in some clinical responses when administered to patients with cervical and anal precancerous lesions^{14–16}. However, when used in combination with de-bulking surgery in patients with recurrent respiratory papillomatosis, HspE7 treatment significantly extended the time in which patients needed to return again for subsequent de-bulking surgeries¹⁷. In the past, TA-HPV, a recombinant vaccinia virus vector expressing HPV16 E6/E7 and HPV18 E6/E7, has not had much success eliciting complete clinical responses when used on its own¹³. However an ongoing trial is designed to test whether recurrence of lesions after surgery can be minimized when immunotherapy is used as an adjunctive treatment¹⁸ (Table 1). DNA-based therapies and dendritic cell-based therapies are still being pursued, but without significant advances in either delivery methods or combination therapies which address immune evasion strategies by HPV, local immune suppression in the patients, or baseline tumor de-bulking surgeries, these platforms will likely fall short as their predecessors have.

c) Non HPV-Specific Tumor-Directed Therapies

Two of the therapies that are currently being tested in human clinical trials do not involve vaccination against HPV tumor antigens, but are nonetheless tumor-directed immunotherapies. These are topical immune stimulatory agents and humanized monoclonal antibody therapy. Immune stimulatory agents, such as Imiquimod, act nonspecifically to elicit either innate or adaptive immunity against antigens in the tumor or lesion. Imiquimod, an agent that has been tested in many clinical trials^{19–23}, activates toll-like receptor (TLR)-7 on antigen-presenting cells (APC) (reviewed in 19) and results in the induction of pro-inflammatory cytokines, chemokines, and other immune mediators, which in turn leads to the generation of a Th1-biased cell-mediated immune response and a concomitant generation of cytotoxic effector cells¹⁹. Imiquimod-treated lesions display significant inflammatory cell infiltrates composed of CD4⁺ and CD8⁺ T lymphocytes, granzyme B⁺

myeloid DC, and plasmacytoid DC expressing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)^{24, 25}. Additionally, Imiquimod has been shown to exert pro-apoptotic activity towards tumor cells that is dependent on Bcl-2 and caspase activation²⁶. Patients with VIN or AIN, lesions that can be easily accessed for cream or suppository-based treatments, who were treated with Imiquimod showed significant clinical responses as well as some HPV16-specific immune responses^{20–23}, suggesting that induction of local inflammation and immunity is an important aspect of successful immunotherapy.

Monoclonal antibody therapies aim to mobilize the patient's innate immune system. Passively administered antibodies can be used to track down tumor cells that express or over-express specific surface markers and destroy them by recruiting components of the immune system such as complement proteins and leukocytes involved in antibody-dependent cytotoxicity. Both these types of tumor-directed therapies have the potential to be used alone or in combination with active immunization against HPV tumor-specific antigens. The results of ongoing clinical trials with monoclonal antibodies (Table 1) should reveal how efficacious this strategy can be against HPV-associated lesions.

d) Immunological Versus Clinical Endpoints

The success rates of single-agent immunotherapeutic vaccines for advanced HPV-associated lesions have been historically disappointing. Thus far therapeutic clinical trials have not been able to provide strong evidence that clinical responses are a direct result of immunological responses to vaccination. Some patients exhibit clinical responses and yet have no detectable immunological responses to vaccination whereas others exhibit strong immunological responses but negligible or limited clinical responses. Many trials measure systemic anti-HPV immunity as an immunological endpoint. While this measurement is certainly important and may even be beneficial, a more significant measurement would be the local immune response at the disease site. Absence of clinical efficacy may be related to failure of systemic tumor-specific cytotoxic T-lymphocytes (CTL) to correctly traffic to the tumor site as well as immune suppressive mechanisms in place within the tumor microenvironment (reviewed in 12).

e) Role of Regulatory T cells

Recently it has been shown that in patients with persistent HPV16 infection, there is an increase in the frequency of circulating CD4⁺CD25^{hi}CTLA4⁺FoxP3⁺ regulatory T cells²⁷. Moreover, cervical lesions in patients with HPV16+ or HPV18+ tumors are highly infiltrated with CD4⁺FoxP3⁺ T cells whereas they are mostly absent in the normal cervix²⁸. This suggests that amongst the population of tumor-infiltrating lymphocytes, there is a significant population that can suppress anti-tumor immune responses within the tumor itself if the endogenous or vaccine-induced CTL response to HPV antigens is not great enough. Therefore, modulation of CD4⁺ T regulatory cells that down-regulate activated effector T lymphocytes by cell-specific depleting antibodies or general lympho-depletion is a potential strategy for achieving increased therapeutic efficacy. Significant regression in VIN, AIN, and anogenital warts has been reported after topical treatment with Imiquimod (Table 1). Thus, the use of topical adjuvants at the disease site to create an appropriate cytokine environment to direct HPV-specific T cells and other inflammatory cells to the disease site

after conventional therapy or vaccination against HPV antigens is another possible strategy to improve clinical outcome.

f) Choice of Antigen

The choice of HPV antigen for therapeutic vaccination can also impact the clinical outcome depending on whether the lesion is early or advanced. Studies on healthy women with no evidence of CIN disease reveal that HPV E2 and E6-specific memory T helper lymphocytes are present and may represent natural immunity against previous viral encounter^{29–31}. In contrast, impaired CD4+ T cell proliferative responses against the E2 and E6 antigens are detected in women with precancerous lesions or cervical cancer patients³². CTL and T helper lymphocytes recognizing E7 epitopes are rarely found in HPV-negative patients or patients that clear the virus, however they can be found in patients with advanced disease or those with persistent virus infection^{33, 34}. Although this may seem to suggest that immunity against E2 and/or E6 results in clearance of virus and early lesions while immunity against E7 may represent failure of the immune response to control cancer progression, other studies have shown that CTL against both E6 and E7 are present in both healthy HPV+ women and women with advanced cervical cancer^{35–38}. Interpretation of these results is complicated by the fact that most of the studies use limited patient numbers and do not analyze both CD4+ and CD8+ responses at the same time and most clinical trials target either E7 alone or a combination of E6/E7 for both early and advanced lesions. However, based on the above referenced natural history studies, it makes sense to include E2 and E6 as targets in early stage disease. The clinical trials targeting the E2 protein in patients with CIN2/3 or genital warts have reported significant promise in terms of clinical responses^{39, 40}. However, due to the lack of controls, it is not clear whether some of the clinical responses are due to 1) natural spontaneous regression of the lesions, 2) the local delivery route of the vaccine (intra-uterine/intraurethral), 3) the choice of the antigen (bovine papillomavirus (BPV) E2), or 4) the immune-stimulatory adjuvant effects of repeated viral delivery with MVA. In addition, the rationale for vaccinating against the E2 protein from BPV instead of an HPV genotype is not clear, though it is theoretically possible that the patient's immune system is tolerized against HPV E2, but can still respond to epitopes derived from BPV E2. Therapeutic cross-reactivity between HPV types or within groups of related HPVs is theoretically possible, but is much more likely to be limited to species- and type-specificity rather than group- or pan-HPV-specificity. However, this question cannot be answered adequately or accurately due to the lack of availability of T cell clones. Nevertheless, future research efforts and clinical trials that include patients with early stage lesions should also aim to include genotype-specific E2 and E6 as therapeutic targets, as well as E7.

The majority of clinical trials using therapeutic agents have shown limited efficacy in eradicating established tumors in humans. This lack of clinical outcome seen in these studies may involve the general state of the patients' immune system or be related to the advanced stage of the patient's cancer. Although the design of recent and future clinical trials may have improved to examine efficacy of therapy in patients with early stage cancers and pre-invasive lesions when their immune system is more competent, the necessity to focus efforts on the development of new and improved therapeutic strategies remains. The following sections will review the most recent investigations into therapeutic strategies that have been

tested in pre-clinical animal models for HPV-induced lesions as well as discuss potential therapies that have been investigated *in vitro*. However, before discussing emerging therapies, it is worthwhile to discuss the appropriateness of the most commonly used animal models for experimental HPV-induced lesions and describe the latest advances in the development of such models.

4. ANIMAL MODELS FOR HPV-INDUCED LESIONS

Papillomaviruses are strictly species-specific and do not infect hosts other than their natural one, with the only known exception being the infection of horses and other equids by BPV types 1 and 2⁴¹. Due to this species-specificity and the fact that human papillomaviruses cannot be produced in sufficient quantities *in vitro*, no *in vitro* system of HPV infection is readily available and no animal model of HPV infection exists. However, preclinical models are necessary for initial vaccine and immunotherapeutic testing that target HPV and HPV-induced lesions. Researchers have had to rely either on animal papillomavirus systems or on tumor-transplantation models, which use cells transformed by oncogenic papillomavirus proteins such as E6 and E7, as a substitute for developing therapeutic strategies to target HPV-induced lesions. The current preclinical models of natural infection include rabbit, dog and bovine models, and have been discussed in detail elsewhere (reviewed in 41). Till date, there are no natural papillomavirus infection models for small laboratory rodents. Dog and bovine models are difficult to study, particularly for immunological assessment, as they must be studied in outbred populations. This review will focus on some of the most commonly used preclinical animal models and the newest preclinical animal models that have been generated for use in the development of therapeutic strategies for HPV-induced lesions, particularly in mice, rabbits and monkeys.

a) Murine Models

The most well studied animal models used as substitutes for HPV-induced tumors are based on challenging C57Bl/6 mice with tumor cells. The C3 line is one of the most commonly used tumor cell lines and was transformed using a pRSVneo-derived plasmid containing the HPV16 genome and the activated ras oncogene⁴². The C3 cell line expresses an H-2Db-bound peptide - HPV16 E7 (49–57) (RAHYNIVTF) and CTL that recognize E7 (49–57) were capable of eradicating HPV16-transformed cells *in vitro* and *in vivo*^{42, 43}. The C3 cells also express the ampitope (SSPVNSLRNVV)–peptide, which is a highly immunogenic H-2Db restricted epitope that was encoded by a cryptic ORF in the antisense strand of the ampicillin resistance gene of the plasmid backbone⁴⁴. Vaccination of immuno-competent mice with the ampitope peptide protects them against challenge with C3-derived cell lines⁴⁴ and therefore this epitope functions as a tumor-specific antigen. The other most common tumor cell line used in such mouse models is TC-1, which was established by co-transforming primary lung epithelial cells with the HPV16 E6, HPV16 E7, and activated ras oncogenes⁴⁵. These systems are appropriate in evaluating the safety of a therapeutic intervention and examining whether it can induce HPV-specific immune responses that can eradicate a transplanted tumor. However, an important difference between the C3 and TC-1 cell lines is that in C3, the HPV genes are under the control of their natural promoters, whereas in TC-1, the E6 and E7 genes are under the control of a viral promoter. This

difference affects level of expression of the HPV proteins, which in turn, may affect antigen processing and presentation.

HLA-A*0201 transgenic mice represent a versatile animal model for preclinical studies of HLA-A*0201 restricted CTL responses. Since human MHC class I epitopes can be tested in this system, HLA-A*0201 mice represent a model that is closer than C57Bl/6. Independent reports have demonstrated that HLA-A*0201 (AAD) transgenic mice recognize CTL epitopes detected by human CD8+ T cells that express the same MHC^{46, 47}. However, straight HLA-A*0201 mice are more likely to mount high affinity CTL responses against peptides since the mouse CD8 molecules have a low affinity for human HLA α 3 domains. There are two HLA-A*0201 expressing HPV16 E6 and E7 expressing tumorigenic cell lines that can be used to study therapeutic interventions namely TC1/A2 and HLF16. The TC1/A2 line was generated by transforming TC1 cells with HLA-A*0201 from HLA-A*0201 (AAD) transgenic mice⁴⁸. The limitation of this line is that the CD8+ T-cell immune response is predominantly directed to against the H-2Db-restricted HPV16 E7 (49–57) epitope in HLA-A*0201 (AAD) transgenic mice. The HLF16 line was generated by transfecting heart lung fibroblasts from HLA-A*0201 transgenic mice with the HPV16 E6 and E7 oncogenes and H-Ras V12⁴⁹. Furthermore, the dominant H-2Db-restricted HPV16 E7 (49–57) epitope was removed from HPV16 E7 to ensure that all anti-tumor responses were mediated through the HLA-A*0201-restricted epitopes. Animal tumor models such as these are more appropriate in evaluating whether responses can be generated to human HLA-A*0201-restricted epitopes *in vivo* following therapeutic intervention.

In addition to models of tumor transplantation, there are some transgenic models for HPV-induced tumors. Transgenic mice expressing HPV oncogenes are thought to provide a valuable model for exploring the immune response to HPV during the process of transformation. One such strain of transgenic mice express the HPV16 E6 and E7 oncogenes under the control of the keratin-14 promoter in keratinized epithelia and are called K14E6E7 mice⁵⁰. While no cancer is observed, these mice develop progressive cutaneous lesions, including papillomatosis and dysplasia. Very recently, another transgenic mouse tumor model was described in which expression of the HPV16 E6 and E7 proteins is restricted to thyroid tissue⁵¹. While this system is an interesting one to study T-cell avidity in an immune response against self-antigens, the relevance of this model for developing therapeutic strategies for HPV-induced tumor lesions is unclear, since there is no evidence to show deletion of high-avidity HPV-specific T-cells in infected individuals.

b) Rabbit Models

Though many studies using natural infection of rabbits have been carried out in outbred populations, several inbred strains of rabbits are available and have been used to study immune responses to natural papillomavirus infection. The commonly used cottontail rabbit papillomavirus (CRPV)/rabbit model has many advantages, the most significant of which is that papillomas can be generated by direct infections of the skin with viral DNA in the absence of encapsidation by the viral coat proteins. Since both active and latent infections can be established with CRPV, this model is appropriate to study potential therapies for persistent HPV infection. However, studies on viral immunity using rabbit major

histocompatibility antigens (MHC) are uninformative in the context of human immune responses. In order to assess immunity in the context of a human MHC allele, a transgenic rabbit model expressing HLA-A*0201 was generated⁵², which is particularly advantageous because epitopes from HPV proteins can be engineered into similar genes in the CRPV genome, which retains the ability to generate papillomas. This model is very interesting and appropriate to evaluate anti-HPV immune responses after natural infection.

c) Monkey Models

Animal papillomaviruses are generally associated with benign cutaneous or mucosal papillomas or fibropapillomas. Papillomavirus-associated genital dysplasias in animals have been documented in only a few reports. In non-human primates, cervical dysplasias were described many decades ago, but only recently have they been associated with papillomaviruses⁵³. Cervical neoplasia occurs relatively frequently in female macaques and includes benign vaginal papillomas, mild to severe intraepithelial dysplasias as well as invasive cervical carcinomas. The lesions express papillomavirus antigens and share distinctive histopathologic similarities with those found in women⁵³. Most recently, it was found that transfer of cervical cytobrush samples from donor animals naturally carrying rhesus papillomaviruses resulted in new infections in 4 of 12 previously virus-free animals and abnormal cytology and histology in 1 of 4 infected animals after 18 weeks of infection⁵⁴. This new macaque model of papillomavirus infection should prove useful in the study of viral persistence, carcinogenesis, and development of both new prophylactic and therapeutic treatment regimens. There are limitations to using monkeys as models for developing immunological interventions some of which are 1) the expense involved in maintaining large colonies, and 2) research reagents that are not as readily available as they are for small rodents. The other potential limitation of using monkeys is that they are outbred, and therefore show large variations in their immune responses. However, this may also be advantageous for the testing of therapeutic regimens because it approximates humans more closely. Thus, while monkey models are not useful for the testing of immune interventions that depend on MHC molecules, they are quite interesting for testing strategies that induce humoral immunity or use the host's antigen processing machinery to induce cellular immune responses.

5. EMERGING THERAPEUTIC STRATEGIES

a) Peptide Immunization-Based Therapies

The use of peptides as therapeutics is experiencing renewed enthusiasm due to advances in delivery, stability and design of peptides and there is a growing emphasis on the use of peptides to gain insight into tissue-specific processing of the immunogenic epitopes of proteins. Furthermore, the use of long or overlapping peptides broadens the range of antigenic epitopes. Peptides can also be synthesized with known post-translational modifications and/or deliberately introduced protease-resistant peptide bonds to regulate their processing and have successfully been used to generate tumor antigen-specific T-cells *in vitro*⁵⁵. However, many of these innovations have been made using tumor antigens other than those derived from HPV proteins.

Progress in the therapy of HPV-induced lesions with peptides as antigens has mostly been had with the use of novel reagents and methods to encapsulate them or by combining them with different immune-stimulatory reagents. A study with transplanted tumor cells in C57Bl/6 mice that compared the efficacy of oligodeoxynucleotide (CpG) with Freund's adjuvant in inducing a response to the HPV16 E5 (25–33) peptide showed that CpG stimulated stronger CTL responses. Furthermore, the study found that the effector/memory/recall phase induced by E5 (25–33) peptide was superior to that induced by HPV16 E5 protein delivered using recombinant adenovirus, though the chronological patterns of immune response were similar⁵⁶. Another novel adjuvant that has been used to enhance peptide immunogenicity is very small size proteoliposomes (VSSP). VSSP are obtained by using anionic detergents to incorporate gangliosides into the outer membrane protein complex of *Neisseria meningitides*. The results of the study showed that vaccination with the HPV16 E7 (49–57) minimal CTL peptide and VSSP protected mice against tumor challenge, induced regression of established transplanted tumors and produced E7-specific CD8+ T-cell responses⁵⁷. The most recent therapeutic vaccine studies with peptide immunization use VacciMax, a proprietary combination of encapsulated CTL epitopes fused to the universal T-helper epitope, PADRE and combined with an adjuvant^{58, 59}. The results of the first study with VacciMax showed that a single administration of the vaccine induced a long-lasting CTL response and complete protection against a tumor challenge as well as a rapid decrease in tumor size that led to tumor eradication in a therapeutic setting in young mice⁵⁸. A follow-up study was done to test therapeutic efficacy in aged mice with very large sized transplanted tumors (>700mm³) and showed tumor eradication in less than 3 weeks post-immunization after a single treatment⁵⁹. In contrast to the majority of studies on peptide immunizations that test adjuvant combinations, one study tested the therapeutic efficacy of long overlapping peptides. Cutaneous infection of rabbits with CRPV was used as a pre-clinical model for persistent HPV-infection, including recurrent respiratory papillomatosis, and the study was designed to test therapeutic efficacy of long CRPV E6 and E7 peptides containing both CD4+ T-helper and CD8+ CTL epitopes. Therapeutic peptide vaccination was able to significantly control wart growth and abrogate latent infection with CRPV⁶⁰.

Despite the renewed enthusiasm in the potential of peptides as therapeutic cancer vaccines, in general, the potential difficulties of using peptide antigens for treatment of naturally occurring HPV-induced tumors are the types of HPV causing the tumors and the genetic immunological makeup of the patient (HLA type). However, it is possible to screen patients for the types of HPV present as well as HLA type and it is possible to create a patient-specific vaccine with the appropriate peptide antigens, based on this information.

b) DNA Vector-Based Therapies

DNA vaccines have generated widespread global interest for a variety of applications, including cancer immunotherapy, due to several potential advantages: (a) the presence of full-length cDNA provides multiple epitopes, thereby overcoming the limitation of MHC restriction; (b) the plasmid DNA itself contains unmethylated CpG motifs that may act as potent immunological adjuvants; (c) plasmid DNA is relatively inexpensive and easy to purify in large quantities (d) plasmid DNA is very stable, making it convenient to handle,

store and distribute world-wide; (e) DNA vaccines are very safe and have few side-effects or safety concerns and (f) plasmid DNA-based vaccines are not accompanied by pre-existing immunity or the induction of anti-vector immunity which enables repetitive vaccination. Despite all these attractive advantages and the many efforts by laboratories world-wide to develop DNA-based cancer vaccines, human clinical trials to date have been disappointing in terms of immunogenicity^{61, 62}. Thus far, these initial clinical trials have shown that DNA vaccines are safe and well tolerated, but objective clinical or tumor responses are rare. In recent years, advances in DNA vaccine based approaches for HPV-induced lesions have been minimal and investigations have been limited to combining DNA vaccines with different adjuvants or testing efficacy in different model systems. One of the most recent studies exploring DNA-based approaches combines a DNA vaccine with heat-shock protein (Hsp) 70 or Hsp110 and demonstrates that the use of autologous Hsp70 potentially enhances antigen-specific immune responses⁶³. Another study uses an adjuvant-free approach to test the efficacy of a DNA-based vaccine against a human HPV16+ esophageal squamous cell carcinoma cell line in Hu-PBL-SCID mice produced by engrafting the immuno-competent human peripheral blood lymphocytes into SCID mice. The data from this study suggests that prophylactic vaccination delayed tumor growth through CD8+ T cell-dependent CTL-induced apoptosis⁶⁴. One inherent risk in the use of DNA vectors encoding whole proteins such as E6 and E7, which are most frequently targeted due to their sustained expression in cancer cells, is the potential for transformation due to their oncogenic activity. In order to circumvent this, an interesting DNA vaccine was developed in which the HPV16 E7 gene was “shuffled” such that it contained all the putative HLA epitopes and still exhibited enhanced safety features⁶⁵. The results of the study showed that the rearranged primary sequence was devoid of transforming properties and induced moderate E7-specific CTL in mice⁶⁵. Another novel approach called “insertional replication” was used to design a DNA-based vaccine. In this approach, the native HPV16 E7 protein was split into three arbitrary regions designated as a, b and c; and all three regions, a, b, and c were replicated to enhance expression. This approach resulted in enhanced immunity as evidenced by the ability to prevent tumor formation and therapeutically treat existing tumors in C57BL/6 mice⁶⁶.

c) Viral/Bacterial Vector-Based Therapies

Delivery of HPV antigens in bacteria or recombinant virus-based delivery systems poses an attractive approach for therapeutic vaccination. It offers an advantage over peptide immunization in that CTL epitopes can be processed and presented naturally and can be delivered more efficiently to target cells. It also has an advantage over DNA-based approaches because alongside increasing the efficiency of introducing heterologous genes into target cells, viruses like vaccinia and vesicular stomatitis virus or bacteria such as *Listeria monocytogenes* are extremely immunogenic and provide the “danger signals” required to initiate an immune response. A novel bacterial-based vaccine that was recently developed uses live *Lactococcus lactis* strains expressing cell wall-anchored HPV16 E7 and a secreted form of IL-12. The vaccine was administered mucosally and the study assessed both prophylactic and therapeutic efficacy in a murine tumor model using transplanted tumor cells that were transformed with HPV16 E7. Immunized mice showed full protection against tumor growth, even after a second tumor challenge, suggesting that prophylactic immunization may provide long-lasting immunity. Therapeutic immunization with the

vaccine induced regression of palpable tumors in treated mice, which occurred through CD4+ and CD8+ dependent T cell responses⁶⁷. The most well studied bacterial vector for the therapy of experimental HPV-induced lesions is based on *Listeria monocytogenes* as the delivery system. In this vaccine approach, the bacterium is genetically engineered to secrete the tumor-associated antigen fused to a molecular adjuvant that enhances the overall immune response. Initial pre-clinical studies with *Listeria* have been discussed in previous reviews on this topic⁶⁸. The most recent study with *Listeria*-based vaccines assesses the efficacy this approach in a transgenic model system with tissue-restricted expression of HPV16 E6 and E7 in the thyroid. This model allows examination of the immune response to HPV antigens as “self” antigens. The results showed regression of solid implanted tumors, although at a lower frequency than in wild type mice. E7-specific CD8+ T cells induced in transgenic mice are of both lower avidity and lower frequency when compared to the wild type mice⁵¹. This is interesting because it shows that *Listeria*-based vaccines against E7 appear to be overcoming central tolerance by expanding low avidity CD8+ T cells specific for E7 that are not deleted during thymopoesis. However, there is no evidence to suggest that high affinity T cells specific for foreign antigens are deleted in patients with HPV and therefore raises questions as to the significance of this finding for therapy of naturally occurring HPV-induced lesions.

The development of virus-based delivery systems has focused on using viral envelopes, without the genetic material of the virus. One such study uses envelopes derived from the influenza virus to deliver encapsulated HPV16 E7 protein and showed that immunized animals developed a strong cellular immune response, which could prevent the outgrowth of transplanted tumors in mice, in a prophylactic setting⁶⁹. Another recent study describes an adenovirus based delivery system and showed that immunization with replication deficient adenovirus particles carrying calreticulin linked to HPV16 E7 resulted in a dramatic increase in the frequency of functional E7-specific CD8+ T cells that were able to prevent tumor growth in a prophylactic setting as well as eradicate well-established tumors and induce long-term immunological memory⁷⁰. A study that used chimeric HPV16 VLP to induce immunity to E2 and E7 showed that certain combinations of immune-modulators could significantly enhance responses to E7, but cellular responses to E2 remained low even after repeated immunizations⁷¹. A significant problem with using bacteria or virus-based delivery systems is the generation of anti-vector humoral immunity, which inhibits enhancement of responses after repeat immunizations with the same vector. In order to overcome this, heterologous prime-boost strategies for immunization in which different delivery methods for the initial inoculation and subsequent boosts have been used successfully. In the study of therapy with chimeric E2 and E7 HPV16 VLP, priming with the VLP and boosting with E2 peptides was used to overcome low T cell responses to E2⁷¹. Heterologous prime-boost immunizations have also been used in the clinic to treat patients with HPV-induced lesions⁷² and have shown promise. However, it should be noted that an exception for limited homologous boosting in the presence of existing anti-vector immunity is highly attenuated variants of vaccinia virus such as MVA, in which genetic deletions and point mutations result in low levels of circulating anti-vaccinia antibodies following vaccination⁷³.

Another viral-vector based strategy that has been adopted to induce anti-tumor immunity is the use of alphaviruses such as Sindbis virus, Venezuelan Equine Encephalitis virus (VEE)

or Semliki Forest Virus (SFV). Since alphaviruses express the RNA of the E6 and E7 oncogenes in the cytosol, this eliminates the potential integration of these oncogenes into host cell chromosomes. Furthermore, there is no preexisting immunity to these viruses in a vast majority of the population. The most recent studies using alphavirus-based vectors to treat HPV-induced lesions have been conducted with SFV or VEE vectors. An optimized SFV construct encoding a fusion protein of HPV16 E6 and E7 and a translational enhancer (SFV-enhE6,7) was shown to induce that high frequencies of specific-CTL, which resulted in the eradication of established tumors in mice challenged with tumor cells and the frequencies of CTL induced depended on the route of immunization⁷⁴. This strategy was also tested in immune-tolerant HPV16 E6/E7-transgenic mice and showed that depending upon the route of immunization, specific CTL could be induced⁷⁵. In addition to the above-mentioned advantages, VEE replicon particles (VRP) have dendritic cell tropism, which makes them more attractive for delivering HPV antigens and inducing anti-tumor immunity for HPV-induced lesions⁷⁶. Studies utilizing VRP that contained mutated, fused E6 and E7 genes of HPV-16 demonstrated 100% protection from tumor challenge in vaccinated mice. Eradication of established tumors was observed in 90% of C57BL6 and HLA-A*0201 transgenic mice challenged with tumor cells, in a therapeutic setting⁷⁷. Eradication of tumors in the HLA-A*0201 transgenic mice is significant because these mice bear the most common human leukocyte antigen in the human population. The encouraging results from these studies provide incentive for the development of alphavirus-based immunotherapies for use in clinical trials of HPV-induced lesions.

d) Immune Response Modifiers

HPV VLP have been used as a substitute for virus particles in studies of virus-cell interactions due to the unavailability of methods to produce the virus in large quantities. Research on VLP interactions with dendritic cell types have shown that epithelial dendritic cells that are located at the site of natural HPV infection, the Langerhans cells (LC), do not get activated when incubated with VLP and do not initiate epitope-specific immune responses against VLP-derived antigens⁷⁸. This is because LC present HPV-derived peptides in the absence of co-stimulation, thereby becoming immune-suppressive⁷⁹. On the other hand, myeloid dendritic cells are activated by VLP and once activated, they are able to stimulate HPV-specific T cells⁸⁰. Different intra-cellular signaling cascades are initiated in DC and LC upon uptake of HPV VLP. When stimulated with HPV-VLP, the mitogen-activated protein kinase (MAPK) pathway is activated in DC whereas it is inactivated in LC. However, the phosphoinositide 3-kinase (PI3-K) pathway is activated in LC, leading to a signaling cascade that results in the inactivation of Akt. Inhibiting PI3-K with specific molecules such as LY294002 reverses this inactivation of Akt⁸¹. Blocking of PI3-K also reverses the phenotype of HPV-VLP stimulated LC from immune-suppressive to immune-stimulatory, indicating that HPV inhibits LC from inducing an immune response by activating PI3-K while down-regulating MAPK upon infection⁸¹. Taken together, the data further suggest that the suppressive phenotype of LC is not absolute and can be reversed by targeting the PI3-K pathway. Thus, using inhibitors of PI3-K may be a potential strategy to reverse the immune-suppressive phenotype of HPV VLP-treated LC.

PI3-K is a term that is applied to a family of lipid kinases that are expressed in almost every cell type and provide a critical signal for many diverse biological functions such as cell proliferation, cell survival, membrane trafficking, glucose transport, neurite outgrowth, actin reorganization, chemotaxis, superoxide production and membrane ruffling⁸². Given their wide range of activities, developing specific and selective inhibitors for the various family members and isoforms becomes essential; and so far, inhibitors of the PI3-K family have not been developed for clinical trials⁸². The first generation of PI3-K inhibitors, in particular the fungal metabolite wortmannin and the flavone-based compound LY294002 have been used widely as pharmacological tools to provide evidence for the involvement of the PI3-K pathway⁸³. These reagents have several limitations for use in human trials such as poor selectivity, limited potency *in vivo*, severe organ toxicity, poor stability and problematic pharmacokinetics. However, recently published work gives cause for optimism. There are several patents published that describe novel PI3-K inhibitors, some of which are isoform-specific (reviewed in 84) and the development of these reagents for clinical applications is eagerly awaited. A recent initial pre-clinical study evaluated the *in vivo* efficacy and toxicity of ZSTK474, a PI3-K inhibiting compound that showed potent anti-tumor activity. ZSTK474 was administered orally to nude mice subcutaneously challenged with the human cancer cell lines A549, PC-3 and WiDr. Oral administration of ZSTK474 showed strong anti-tumor activity against the human cancer xenografts without toxic effects in critical organs⁸⁵. The results of this study provide strong support for the development of specific and selective inhibitors of the PI3-K pathway, which when developed, may be of use for therapy of HPV-induced lesions.

Another potential strategy to reverse the immune-suppressive phenotype of HPV VLP-treated LC may be via modulation of toll-like receptor activity. TLR are a conserved set of receptors that trigger innate immune activation and distinct immune-surveillance roles for individual TLR are taking shape. Differential expression of TLR in distinct innate immune cell subsets has been shown to correlate with induction of different types and quantities of cytokines by different TLR agonists⁸⁶. Human peripheral blood DC have particularly well-defined roles in TLR-based immune-surveillance. However, in LC, the expression pattern of TLR is controversial^{87, 88} and TLR-signaling pathways have not been well characterized. Complementary sets of TLR direct either plasmacytoid dendritic cell (TLRs-7 and -9) to secrete anti-viral IFN- α or myeloid dendritic cell (TLRs-2, -3, -4, -5, -6 and -8) to secrete IL-12 and promote expansion of antigen-specific T cells⁸⁹. A variety of synthetic TLR-7, TLR-8 and TLR-7/8 agonists were recently characterized for their ability to induce cytokines from isolated plasmacytoid dendritic cell and myeloid dendritic cell⁹⁰. Though the effects of TLR agonists on LC are not well understood, we anticipate that manipulation of TLR signaling through the use TLR-agonists may modulate HPV immunity such that it may be possible to reverse the suppressive phenotype of LC at the site of the lesion through topical application.

In the context of HPV16 infection, the presence of HPV capsids and expression of the E6 and E7 proteins in dendritic cells and keratinocytes has both activating and inhibitory effects on several TLR pathways that can affect immune recognition. HPV16 L1 VLP have been shown to stimulate secretion of cytokines by murine bone-marrow derived DC via MyD88, a signaling adapter protein that is utilized by a number of cell surface-expressed TLR⁹¹.

Recognition of HPV6b L1 VLP by human monocyte-derived DC involves TLR-4, and results in activation of the NF- κ B signaling pathway. However the presence of TGF- β , such as would be found in the epithelium, inhibits TLR-4-mediated activation of human LC⁹². In human primary keratinocytes, ectopic expression of HPV16 E6/E7 leads to up-regulation of the TLR-3 and -5 pathways, but inhibition of the TLR-9 pathway which responds to the presence of viral and bacterial dsDNA CpG motifs⁹³. From these studies, it is clear that HPV can not only be recognized by various TLRs, but can also modulate their activity, depending on which strain of HPV is used and in what context. Therefore, additional modulation of these pathways during persistent infection or immunotherapeutic strategies could also help to activate the local immune response against HPV antigens.

e) Photodynamic Therapy

Photodynamic therapy (PDT) uses drugs called photo-sensitizers that collect in tumor cells and kills them primarily via the generation of reactive oxygen species such as singlet oxygen and free radicals that mediate cellular toxicity, when they are exposed to specific wavelengths of light. In addition to directly killing cancer cells, PDT appears to shrink or destroy tumors in two other ways. The photo-sensitizer can damage blood vessels in the tumor and PDT may activate the immune system to attack the tumor cells⁹⁴. Two studies on PDT as a therapy for HPV-associated malignancy have shown that topical PDT treatment of multi-focal high-grade VIN produced a short-term response in only 37% and 27% of these lesions whereas low-grade or mono-focal and bifocal higher grade lesions were much more responsive. A study that investigated the reason for such poor response rates correlated the incidence of high-risk HPV infection, HLA class I loss, and numbers of infiltrating immune cells with the clinical response of VIN patients treated with topical PDT and showed that high-risk HPV infection and lack of cell-mediated immunity play a significant role⁹⁵. A study of 31 patients with CIN (2 with CIN2, 29 with CIN3) concluded that PDT is effective not only in improving the cytological and histological measures when treating CIN but also for eradicating cervical HPV⁹⁶.

Studies on PDT in various animal models have suggested that photo-oxidative lesions produced by PDT-treated tumors are recognized by the host as altered self and prompt a strong inflammatory immune response mediated by T-cells and tumor-associated macrophages. Due to the induced immune response, this therapy may be particularly suitable for combination with immunotherapy-based treatments, including angiogenic growth factors, cytokines and adoptive transfer of immune cells. The most recent study on PDT as therapy for HPV+ lesions was done in a pre-clinical transplanted tumor model and assessed the efficacy of combining PDT-treated tumor cell lysates with CpG. PDT-cell lysates co-injected with CpG showed significant suppression of tumor growth in mice at both prophylactic and therapeutic levels as compared with each treatment alone and appeared to be mediated by CD8+ T cells⁹⁷. These data suggest that PDT-cell lysates along with CpG may be another possible strategy for HPV immunotherapy.

f) T Cell Receptor Based Therapy

One final approach in immunotherapy for HPV that has the potential to be utilized in future clinical trials is the genetic transfer of antigen-specific T cell receptors (TCR) with high

affinity for tumor-associated antigens into T cells from patients. Though the safety and efficacy of this approach has been tested in other cancers such as melanoma, investigations into TCR transfer and adoptive therapy for HPV-induced lesions is restricted by the limited availability of TCR with high affinity for HPV antigens^{98, 99}. However, efforts to isolate high affinity HPV-specific TCR are ongoing and once the relevant TCR are isolated, this approach will confer the appropriate tumor killing specificity and has the potential to generate a bank of TCR genes with specificity to various viral tumor antigens that may be selected for any patient with tumor cells expressing the appropriate antigen.

6. FUTURE DIRECTIONS

HPV-induced lesions are distinct in that they have targetable foreign antigens, the expression of which is necessary to maintain the cancerous phenotype. This blatant expression of foreign antigens in a tumor makes HPV a very attractive target for “proof of concept” studies in the development of therapeutic vaccines. Efforts to develop and test novel strategies targeted towards HPV should help to advance the field of immunotherapy significantly.

While considerable progress has been made in the development of prophylactic vaccines against HPV, in recent years, advances in the field of therapeutic vaccines seem to have progressed more slowly. Substantial progress may have been impeded by difficulty in interpreting results due to the design of clinical trials that were restricted by factors such as limited patient numbers, lack of placebo-controlled analysis and evaluation in patients with end-stage disease. Regardless, the results from past and on-going clinical HPV vaccine trials provide an opportunity to at least identify characteristics of the immune response that best correlate with regression of HPV-associated lesions and provide data leading to the rational development of more effective vaccines. In addition to improving the design of clinical trials, future studies should concentrate on addressing local immune suppressive mechanisms and immune evasion strategies utilized by HPV (reviewed in 12). In situ vaccination, local application of immune adjuvants and the continuation of combinatorial therapies remain viable options for enhancing immune responsiveness in patients.

In pre-clinical therapeutic animal models, single agent vaccination may be enough to eradicate tumor cells; however, this strategy is unlikely to succeed in patients where tumor cells have co-evolved with the immune system to evade detection and elimination, which results in long-standing disease status. Heterologous prime-boost vaccination strategies against tumor antigens focus the host immune response on the antigen instead of the delivery system and increase the frequency of circulating antigen-specific cells. However, considering some of the obstacles outlined above, increasing the number of tumor-specific CTL alone may not be enough to cure patients suffering from HPV-induced cancers. Combining new and improved immunotherapeutic strategies with current standard of care treatments may potentially lead to a decline in recurrence rates and may also leave the patient with long-term immunity. This becomes particularly important if recurrence is probable or if and when the patient becomes re-infected with HPV, which is frequent in the case of HPV. In summary, despite the recent advancement in the prevention of HPV

infections, there is a continuing and very significant need to develop better strategies to treat HPV-induced lesions.

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BIBLIOGRAPHY

1. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med.* 2003; 348(6):518–527. [PubMed: 12571259]
2. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999; 189(1):12–19. [PubMed: 10451482]
3. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shah KV. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer Inst.* 1995; 87(11):796–802. [PubMed: 7791229]
4. Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, Balaram P, Rajkumar T, Sridhar H, Rose B, Pintos J, Fernandez L, Idris A, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *J Natl Cancer Inst.* 2003; 95(23):1772–1783. [PubMed: 14652239]
5. Koutsky L. Epidemiology of genital human papillomavirus infection. *Am J Med.* 1997; 102(5A):3–8. [PubMed: 9217656]
6. Parkin DM. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer.* 2006; 10:10.
7. Future II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med.* 2007; 356(19):1915–1927. [PubMed: 17494925]
8. Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, Jenkins D, Schuind A, Costa Clemens SA, Dubin G. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet.* 2006; 367(9518):1247–1255. [PubMed: 16631880]
9. Schwarz TF, Dubin G. HPV Vaccine Study Investigators for Adult Women. Human papillomavirus (HPV) 16/18 L1 AS04 virus-like particle (VLP) cervical cancer vaccine is immunogenic and well-tolerated 18 months after vaccination in women up to age 55 years. *ASCO Meeting Abstracts.* 2007; 25(18 suppl) Abstract 3007.
10. Paavonen J, Jenkins D, Bosch FX, Naud P, Salmeron J, Wheeler CM, Chow SN, Apter DL, Kitchener HC, Castellsague X, de Carvalho NS, Skinner SR, et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *Lancet.* 2007; 369(9580):2161–2170. [PubMed: 17602732]
11. Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, Bratti MC, Schiller JT, Gonzalez P, Dubin G, Porras C, Jimenez SE, Lowy DR, et al. Effect of Human Papillomavirus 16/18 L1 Viruslike Particle Vaccine Among Young Women With Preexisting Infection: A Randomized Trial. *JAMA.* 2007; 298(7):743–753. [PubMed: 17699008]
12. Kanodia S, Fahey LM, Kast WM. Mechanisms used by human papillomaviruses to escape the host immune response. *Curr Cancer Drug Targets.* 2007; 7(1):79–89. [PubMed: 17305480]
13. Brinkman JA, Hughes SH, Stone P, Caffrey AS, Munderspach LI, Roman LD, Weber JS, Kast WM. Therapeutic vaccination for HPV induced cervical cancers. *Disease Markers.* 2007; 23(4):337–352. [PubMed: 17627067]

14. Roman LD, Wilczynski S, Muderspach LI, Burnett AF, Rodriguez AO, Brinkman JA, Kast WM, Facio G, Felix JC, Aldana M, Weber JS. A phase II study of Hsp-E7 (SGN-00101) in women with high grade cervical dysplasia. *Gynecol Oncol.* 2007
15. Einstein MH, Kadish AS, Burk RD, Kim MY, Wadler S, Streicher H, Goldberg GL, Runowicz CD. Heat shock fusion protein-based immunotherapy for treatment of cervical intraepithelial neoplasia III. *Gynecol Oncol.* 2007
16. Palefsky JM, Berry JM, Jay N, Krogstad M, Da Costa M, Darragh TM, Lee JY. A trial of SGN-00101 (HspE7) to treat high-grade anal intraepithelial neoplasia in HIV-positive individuals. *AIDS.* 2006; 20(8):1151–1155. [PubMed: 16691066]
17. Derkay CS, Smith RJ, McClay J, van Burik JA, Wiatrak BJ, Arnold J, Berger B, Neefe JR. HspE7 treatment of pediatric recurrent respiratory papillomatosis: final results of an open-label trial. *Ann Otol Rhinol Laryngol.* 2005; 114(9):730–737. [PubMed: 16240938]
18. A phase II trial in patients with early cervical cancer to study the safety and the immunological effects of vaccination with TA-HPV, a live recombinant vaccinia virus expressing the human papillomavirus 16 and 18 E6 and E7 proteins. 2007. www.clinicaltrials.gov/ct2/show/study?term=NCT00002916
19. Stanley MA. Imiquimod and the imidazoquinolones: mechanism of action and therapeutic potential. *Clin Exp Dermatol.* 2002; 27(7):571–577. [PubMed: 12464152]
20. Van Poelgeest MI, van Seters M, van Beurden M, Kwappenberg KM, Heijmans-Antonissen C, Drijfhout JW, Melief CJ, Kenter GG, Helmerhorst TJ, Offringa R, van der Burg SH. Detection of human papillomavirus (HPV) 16-specific CD4+ T-cell immunity in patients with persistent HPV16-induced vulvar intraepithelial neoplasia in relation to clinical impact of imiquimod treatment. *Clin Cancer Res.* 2005; 11(14):5273–5280. [PubMed: 16033846]
21. Schofer H, Van Ophoven A, Henke U, Lenz T, Eul A. Randomized, comparative trial on the sustained efficacy of topical imiquimod 5% cream versus conventional ablative methods in external anogenital warts. *Eur J Dermatol.* 2006; 16(6):642–648. [PubMed: 17229604]
22. Le T, Menard C, Hicks-Boucher W, Hopkins L, Weberpals J, Fung-Kee-Fung M. Final results of a phase 2 study using continuous 5% Imiquimod cream application in the primary treatment of high-grade vulva intraepithelial neoplasia. *Gynecol Oncol.* 2007
23. Wieland U, Brockmeyer NH, Weissenborn SJ, Hochdorfer B, Stucker M, Swoboda J, Altmeyer P, Pfister H, Kreuter A. Imiquimod treatment of anal intraepithelial neoplasia in HIV-positive men. *Arch Dermatol.* 2006; 142(11):1438–1444. [PubMed: 17116834]
24. Stary G, Bangert C, Tauber M, Strohal R, Kopp T, Stingl G. Tumorcidal activity of TLR7/8-activated inflammatory dendritic cells. *J Exp Med.* 2007; 204(6):1441–1451. [PubMed: 17535975]
25. Wolf IH, Kodama K, Cerroni L, Kerl H. Nature of inflammatory infiltrate in superficial cutaneous malignancies during topical imiquimod treatment. *Am J Dermatopathol.* 2007; 29(3):237–241. [PubMed: 17519620]
26. Schon M, Bong AB, Drewniak C, Herz J, Geilen CC, Reifemberger J, Benninghoff B, Slade HB, Gollnick H, Schon MP. Tumor-selective induction of apoptosis and the small-molecule immune response modifier imiquimod. *J Natl Cancer Inst.* 2003; 95(15):1138–1149. [PubMed: 12902443]
27. Molling JW, de Gruijl TD, Glim J, Moreno M, Rozendaal L, Meijer CJ, van den Eertwegh AJ, Scheper RJ, von Blumberg ME, Bontkes HJ. CD4(+)CD25(hi) regulatory T-cell frequency correlates with persistence of human papillomavirus type 16 and T helper cell responses in patients with cervical intraepithelial neoplasia. *Int J Cancer.* 2007
28. Piersma SJ, Jordanova ES, Van Poelgeest MI, Kwappenberg KM, van der Hulst JM, Drijfhout JW, Melief CJ, Kenter GG, Fleuren GJ, Offringa R, van der Burg SH. High number of intraepithelial CD8+ tumor-infiltrating lymphocytes is associated with the absence of lymph node metastases in patients with large early-stage cervical cancer. *Cancer Res.* 2007; 67(1):354–361. [PubMed: 17210718]
29. Nakagawa M, Stites DP, Farhat S, Judd A, Moscicki AB, Canchola AJ, Hilton JF, Palefsky JM. T-cell proliferative response to human papillomavirus type 16 peptides: relationship to cervical intraepithelial neoplasia. *Clin Diagn Lab Immunol.* 1996; 3(2):205–210. [PubMed: 8991637]
30. de Jong A, van der Burg SH, Kwappenberg KM, van der Hulst JM, Franken KL, Geluk A, van Meijgaarden KE, Drijfhout JW, Kenter G, Vermeij P, Melief CJ, Offringa R. Frequent detection of

human papillomavirus 16 E2-specific T-helper immunity in healthy subjects. *Cancer Res.* 2002; 62(2):472–479. [PubMed: 11809698]

31. Welters MJ, de Jong A, van den Eeden SJ, van der Hulst JM, Kwappenberg KM, Hassane S, Franken KL, Drijfhout JW, Fleuren GJ, Kenter G, Melief CJ, Offringa R, et al. Frequent display of human papillomavirus type 16 E6-specific memory T-Helper cells in the healthy population as witness of previous viral encounter. *Cancer Res.* 2003; 63(3):636–641. [PubMed: 12566307]
32. de Jong A, Van Poelgeest MI, van der Hulst JM, Drijfhout JW, Fleuren GJ, Melief CJ, Kenter G, Offringa R, van der Burg SH. Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6. *Cancer Res.* 2004; 64(15): 5449–5455. [PubMed: 15289354]
33. Bontkes HJ, de Gruijl TD, van den Muysenberg AJ, Verheijen RH, Stukart MJ, Meijer CJ, Scheper RJ, Stacey SN, Duggan-Keen MF, Stern PL, Man S, Borysiewicz LK, et al. Human papillomavirus type 16 E6/E7-specific cytotoxic T lymphocytes in women with cervical neoplasia. *Int J Cancer.* 2000; 88(1):92–98. [PubMed: 10962445]
34. Welters MJ, van der LP, van den Eeden SJ, Kwappenberg KM, Drijfhout JW, Fleuren GJ, Kenter GG, Melief CJ, van der Burg SH, Offringa R. Detection of human papillomavirus type 18 E6 and E7-specific CD4+ T-helper 1 immunity in relation to health versus disease. *Int J Cancer.* 2006; 118(4):950–956. [PubMed: 16152582]
35. Nakagawa M, Stites DP, Farhat S, Sisler JR, Moss B, Kong F, Moscicki AB, Palefsky JM. Cytotoxic T lymphocyte responses to E6 and E7 proteins of human papillomavirus type 16: relationship to cervical intraepithelial neoplasia. *J Infect Dis.* 1997; 175(4):927–931. [PubMed: 9086151]
36. Nakagawa M, Viscidi R, Deshmukh I, Costa MD, Palefsky JM, Farhat S, Moscicki AB. Time course of humoral and cell-mediated immune responses to human papillomavirus type 16 in infected women. *Clin Diagn Lab Immunol.* 2002; 9(4):877–882. [PubMed: 12093689]
37. Valdespino V, Gorodezky C, Ortiz V, Kaufmann AM, Roman-Basaure E, Vazquez A, Berumen J. HPV16-specific cytotoxic T lymphocyte responses are detected in all HPV16-positive cervical cancer patients. *Gynecol Oncol.* 2005; 96(1):92–102. [PubMed: 15589586]
38. Rensing ME, van Driel WJ, Celis E, Sette A, Brandt MP, Hartman M, Anholts JD, Schreuder GM, ter Harmsel WB, Fleuren GJ, Trimpos BJ, Kast WM, et al. Occasional memory cytotoxic T-cell responses of patients with human papillomavirus type 16-positive cervical lesions against a human leukocyte antigen-A *0201-restricted E7-encoded epitope. *Cancer Res.* 1996; 56(3):582–588. [PubMed: 8564975]
39. Garcia-Hernandez E, Gonzalez-Sanchez JL, Andrade-Manzano A, Contreras ML, Padilla S, Guzman CC, Jimenez R, Reyes L, Morosoli G, Verde ML, Rosales R. Regression of papilloma high-grade lesions (CIN 2 and CIN 3) is stimulated by therapeutic vaccination with MVA E2 recombinant vaccine. *Cancer Gene Ther.* 2006; 13(6):592–597. [PubMed: 16456551]
40. Albarran YC, de la GA, Cruz Quiroz BJ, Vazquez ZE, Diaz EI, Mendez FE, Lopez CM, Andrade-Manzano A, Padilla S, Varela AR, Rosales R. MVA E2 recombinant vaccine in the treatment of human papillomavirus infection in men presenting intraurethral flat condyloma: a phase I/II study. *BioDrugs.* 2007; 21(1):47–59. [PubMed: 17263589]
41. Campo MS. Animal models of papillomavirus pathogenesis. *Virus Res.* 2002; 89(2):249–261. [PubMed: 12445664]
42. Feltkamp MC, Smits HL, Vierboom MP, Minnaar RP, de Jongh BM, Drijfhout JW, ter Schegget J, Melief CJ, Kast WM. Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. *Eur J Immunol.* 1993; 23(9):2242–2249. [PubMed: 7690326]
43. Feltkamp MC, Vreugdenhil GR, Vierboom MP, Ras E, van der Burg SH, ter Schegget J, Melief CJ, Kast WM. Cytotoxic T lymphocytes raised against a subdominant epitope offered as a synthetic peptide eradicate human papillomavirus type 16-induced tumors. *Eur J Immunol.* 1995; 25(9): 2638–2642. [PubMed: 7589138]
44. van Hall T, van de Rhee NE, Schoenberger SP, Vierboom MP, Verreck FA, Melief CJ, Offringa R. Cryptic open reading frames in plasmid vector backbone sequences can provide highly immunogenic cytotoxic T-lymphocyte epitopes. *Cancer Res.* 1998; 58(14):3087–3093. [PubMed: 9679975]

45. Lin KY, Guarnieri FG, Staveley-O'Carroll KF, Levitsky HI, August JT, Pardoll DM, Wu TC. Treatment of established tumors with a novel vaccine that enhances major histocompatibility class II presentation of tumor antigen. *Cancer Res.* 1996; 56(1):21–26. [PubMed: 8548765]
46. Engelhard VH, Lacy E, Ridge JP. Influenza A-specific, HLA-A2.1-restricted cytotoxic T lymphocytes from HLA-A2.1 transgenic mice recognize fragments of the M1 protein. *J Immunol.* 1991; 146(4):1226–1232. [PubMed: 1704033]
47. Shirai M, Arichi T, Nishioka M, Nomura T, Ikeda K, Kawanishi K, Engelhard VH, Feinstone SM, Berzofsky JA. CTL responses of HLA-A2.1-transgenic mice specific for hepatitis C viral peptides predict epitopes for CTL of humans carrying HLA-A2.1. *J Immunol.* 1995; 154(6):2733–2742. [PubMed: 7533182]
48. Peng S, Trimble C, He L, Tsai YC, Lin CT, Boyd DA, Pardoll D, Hung CF, Wu TC. Characterization of HLA-A2-restricted HPV-16 E7-specific CD8(+) T-cell immune responses induced by DNA vaccines in HLA-A2 transgenic mice. *Gene Ther.* 2006; 13(1):67–77. [PubMed: 16107858]
49. Eiben GL, Velders MP, Schreiber H, Cassetti MC, Pullen JK, Smith LR, Kast WM. Establishment of an HLA-A*0201 human papillomavirus type 16 tumor model to determine the efficacy of vaccination strategies in HLA-A*0201 transgenic mice. *Cancer Res.* 2002; 62(20):5792–5799. [PubMed: 12384540]
50. Melero I, Singhal MC, McGowan P, Haugen HS, Blake J, Hellstrom KE, Yang G, Clegg CH, Chen L. Immunological ignorance of an E7-encoded cytolytic T-lymphocyte epitope in transgenic mice expressing the E7 and E6 oncogenes of human papillomavirus type 16. *J Virol.* 1997; 71(5):3998–4004. [PubMed: 9094677]
51. Souders NC, Sewell DA, Pan ZK, Hussain SF, Rodriguez A, Wallecha A, Paterson Y. Listeria-based vaccines can overcome tolerance by expanding low avidity CD8+ T cells capable of eradicating a solid tumor in a transgenic mouse model of cancer. *Cancer Immun.* 2007; 7:2. [PubMed: 17279610]
52. Hu J, Peng X, Schell TD, Budgeon LR, Cladel NM, Christensen ND. An HLA-A2.1-transgenic rabbit model to study immunity to papillomavirus infection. *J Immunol.* 2006; 177(11):8037–8045. [PubMed: 17114477]
53. Wood CE, Borgerink H, Register TC, Scott L, Cline JM. Cervical and vaginal epithelial neoplasms in cynomolgus monkeys. *Vet Pathol.* 2004; 41(2):108–115. [PubMed: 15017023]
54. Wood CE, Chen Z, Cline JM, Miller BE, Burk RD. Characterization and experimental transmission of an oncogenic papillomavirus in female macaques. *J Virol.* 2007; 81(12):6339–6345. [PubMed: 17428865]
55. Blanchet JS, Valmori D, Dufau I, Ayyoub M, Nguyen C, Guillaume P, Monsarrat B, Cerottini JC, Romero P, Gairin JE. A new generation of Melan-A/MART-1 peptides that fulfill both increased immunogenicity and high resistance to biodegradation: implication for molecular anti-melanoma immunotherapy. *J Immunol.* 2001; 167(10):5852–5861. [PubMed: 11698460]
56. Chen YF, Lin CW, Tsao YP, Chen SL. Cytotoxic-T-lymphocyte human papillomavirus type 16 E5 peptide with CpG-oligodeoxynucleotide can eliminate tumor growth in C57BL/6 mice. *J Virol.* 2004; 78(3):1333–1343. [PubMed: 14722288]
57. Torrens I, Mendoza O, Batte A, Reyes O, Fernandez LE, Mesa C, Guillen G. Immunotherapy with CTL peptide and VSSP eradicated established human papillomavirus (HPV) type 16 E7-expressing tumors. *Vaccine.* 2005; 23(50):5768–5774. [PubMed: 16112257]
58. Daftarian P, Mansour M, Benoit AC, Pohajdak B, Hoskin DW, Brown RG, Kast WM. Eradication of established HPV 16-expressing tumors by a single administration of a vaccine composed of a liposome-encapsulated CTL-T helper fusion peptide in a water-in-oil emulsion. *Vaccine.* 2006; 24(24):5235–5244. [PubMed: 16675074]
59. Daftarian PM, Mansour M, Pohajdak B, Fuentes-Ortega A, Korets-Smith E, Macdonald L, Weir G, Brown RG, Kast WM. Rejection of large HPV-16 expressing tumors in aged mice by a single immunization of VacciMax(R) encapsulated CTL/T helper peptides. *J Transl Med.* 2007; 5:26. [PubMed: 17555571]
60. Vambutas A, DeVoti J, Nouri M, Drijfhout JW, Lipford GB, Bonagura VR, van der Burg SH, Melief CJ. Therapeutic vaccination with papillomavirus E6 and E7 long peptides results in the

control of both established virus-induced lesions and latently infected sites in a pre-clinical cottontail rabbit papillomavirus model. *Vaccine*. 2005; 23(45):5271–5280. [PubMed: 16054734]

61. Rosenberg SA, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, Restifo NP, Haworth LR, Seipp CA, Freezer LJ, Morton KE, Mavroukakis SA, et al. Inability to immunize patients with metastatic melanoma using plasmid DNA encoding the gp100 melanoma-melanocyte antigen. *Hum Gene Ther*. 2003; 14(8):709–714. [PubMed: 12804135]
62. Miller AM, Ozenci V, Kiessling R, Pisa P. Immune monitoring in a phase 1 trial of a PSA DNA vaccine in patients with hormone-refractory prostate cancer. *J Immunother* (1997). 2005; 28(4): 389–395.
63. Li Y, Subjeck J, Yang G, Repasky E, Wang XY. Generation of anti-tumor immunity using mammalian heat shock protein 70 DNA vaccines for cancer immunotherapy. *Vaccine*. 2006; 24(25):5360–5370. [PubMed: 16714072]
64. Lu Y, Zhang Z, Liu Q, Liu B, Song X, Wang M, Zhao X, Zhao Q. Immunological protection against HPV16 E7-expressing human esophageal cancer cell challenge by a novel HPV16-E6/E7 fusion protein based-vaccine in a Hu-PBL-SCID mouse model. *Biol Pharm Bull*. 2007; 30(1):150–156. [PubMed: 17202676]
65. Ohlschlager P, Pes M, Osen W, Durst M, Schneider A, Gissmann L, Kaufmann AM. An improved rearranged Human Papillomavirus Type 16 E7 DNA vaccine candidate (HPV-16 E7SH) induces an E7 wildtype-specific T cell response. *Vaccine*. 2006; 24(15):2880–2893. [PubMed: 16472545]
66. Brinkman JA, Xu X, Kast WM. The efficacy of a DNA vaccine containing inserted and replicated regions of the E7 gene for treatment of HPV-16 induced tumors. *Vaccine*. 2007; 25(17):3437–3444. [PubMed: 17241713]
67. Bermudez-Humaran LG, Cortes-Perez NG, Lefevre F, Guimaraes V, Rabot S, Alcocer-Gonzalez JM, Grataadoux JJ, Rodriguez-Padilla C, Tamez-Guerra RS, Corthier G, Gruss A, Langella P. A novel mucosal vaccine based on live Lactococci expressing E7 antigen and IL-12 induces systemic and mucosal immune responses and protects mice against human papillomavirus type 16-induced tumors. *J Immunol*. 2005; 175(11):7297–7302. [PubMed: 16301635]
68. Coukos G, Conejo-Garcia JR, Roden RB, Wu TC. Immunotherapy for gynaecological malignancies. *Expert Opin Biol Ther*. 2005; 5(9):1193–1210. [PubMed: 16120050]
69. Bungener L, de Mare A, de Vries-Idema J, Sehr P, van der Zee A, Wilschut J, Daemen T. A virosomal immunization strategy against cervical cancer and pre-malignant cervical disease. *Antivir Ther*. 2006; 11(6):717–727. [PubMed: 17310816]
70. Gomez-Gutierrez JG, Elpek KG, Montes de Oca-Luna R, Shirwan H, Sam Zhou H, McMasters KM. Vaccination with an adenoviral vector expressing calreticulin-human papillomavirus 16 E7 fusion protein eradicates E7 expressing established tumors in mice. *Cancer Immunol Immunother*. 2007; 56(7):997–1007. [PubMed: 17146630]
71. Qian J, Dong Y, Pang YY, Ibrahim R, Berzofsky JA, Schiller JT, Khleif SN. Combined prophylactic and therapeutic cancer vaccine: enhancing CTL responses to HPV16 E2 using a chimeric VLP in HLA-A2 mice. *Int J Cancer*. 2006; 118(12):3022–3029. [PubMed: 16425257]
72. Fiander AN, Tristram AJ, Davidson EJ, Tomlinson AE, Man S, Baldwin PJ, Sterling JC, Kitchener HC. Prime-boost vaccination strategy in women with high-grade, noncervical anogenital intraepithelial neoplasia: clinical results from a multicenter phase II trial. *Int J Gynecol Cancer*. 2006; 16(3):1075–1081. [PubMed: 16803488]
73. Dallo S, Maa JS, Rodriguez JR, Rodriguez D, Esteban M. Humoral immune response elicited by highly attenuated variants of vaccinia virus and by an attenuated recombinant expressing HIV-1 envelope protein. *Virology*. 1989; 173(1):323–329. [PubMed: 2510402]
74. Daemen T, Riezebos-Brilman A, Regts J, Dontje B, van der Zee A, Wilschut J. 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*. 2004; 9(5): 733–742. [PubMed: 15535411]
75. Riezebos-Brilman A, Regts J, Freyschmidt EJ, Dontje B, Wilschut J, Daemen T. Induction of human papilloma virus E6/E7-specific cytotoxic T-lymphocyte activity in immune-tolerant, E6/E7-transgenic mice. *Gene Ther*. 2005; 12(18):1410–1414. [PubMed: 15843807]

76. Nishimoto KP, Laust AK, Wang K, Kamrud KI, Hubby B, Smith JF, Nelson EL. Restricted and selective tropism of a Venezuelan equine encephalitis virus-derived replicon vector for human dendritic cells. *Viral Immunol.* 2007; 20(1):88–104. [PubMed: 17425424]
77. Cassetti MC, McElhiney SP, Shahabi V, Pullen JK, Le Poole IC, Eiben GL, Smith LR, Kast WM. Antitumor efficacy of Venezuelan equine encephalitis virus replicon particles encoding mutated HPV16 E6 and E7 genes. *Vaccine.* 2004; 22(3–4):520–527. [PubMed: 14670335]
78. Fausch SC, Da Silva DM, Rudolf MP, Kast WM. Human papillomavirus virus-like particles do not activate Langerhans cells: a possible immune escape mechanism used by human papillomaviruses. *J Immunol.* 2002; 169(6):3242–3249. [PubMed: 12218143]
79. Fausch SC, Da Silva DM, Kast WM. Differential uptake and cross-presentation of human papillomavirus virus-like particles by dendritic cells and Langerhans cells. *Cancer Res.* 2003; 63(13):3478–3482. [PubMed: 12839929]
80. Lenz P, Thompson CD, Day PM, Bacot SM, Lowy DR, Schiller JT. Interaction of papillomavirus virus-like particles with human myeloid antigen-presenting cells. *Clin Immunol.* 2003; 106(3):231–237. [PubMed: 12706410]
81. Fausch SC, Fahey LM, Da Silva DM, Kast WM. Human papillomavirus can escape immune recognition through Langerhans cell phosphoinositide 3-kinase activation. *J Immunol.* 2005; 174(11):7172–7178. [PubMed: 15905561]
82. Ward S, Sotsios Y, Dowden J, Bruce I, Finan P. Therapeutic potential of phosphoinositide 3-kinase inhibitors. *Chem Biol.* 2003; 10(3):207–213. [PubMed: 12670534]
83. Workman P. Inhibiting the phosphoinositide 3-kinase pathway for cancer treatment. *Biochem Soc Trans.* 2004; 32(Pt 2):393–396. [PubMed: 15046615]
84. Ward SG, Finan P. Isoform-specific phosphoinositide 3-kinase inhibitors as therapeutic agents. *Curr Opin Pharmacol.* 2003; 3(4):426–434. [PubMed: 12901953]
85. Yaguchi S, Fukui Y, Koshimizu I, Yoshimi H, Matsuno T, Gouda H, Hirono S, Yamazaki K, Yamori T. Antitumor activity of ZSTK474, a new phosphatidylinositol 3-kinase inhibitor. *J Natl Cancer Inst.* 2006; 98(8):545–556. [PubMed: 16622124]
86. Zarembek KA, Godowski PJ. Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. *J Immunol.* 2002; 168(2):554–561. [PubMed: 11777946]
87. Renn CN, Sanchez DJ, Ochoa MT, Legaspi AJ, Oh CK, Liu PT, Krutzik SR, Sieling PA, Cheng G, Modlin RL. TLR activation of Langerhans cell-like dendritic cells triggers an antiviral immune response. *J Immunol.* 2006; 177(1):298–305. [PubMed: 16785525]
88. Flacher V, Bouschbacher M, Verronese E, Massacrier C, Sisirak V, Berthier-Vergnes O, de Saint-Vis B, Caux C, Dezutter-Dambuyant C, Lebecque S, Valladeau J. Human Langerhans cells express a specific TLR profile and differentially respond to viruses and Gram-positive bacteria. *J Immunol.* 2006; 177(11):7959–7967. [PubMed: 17114468]
89. Ito T, Amakawa R, Kaisho T, Hemmi H, Tajima K, Uehira K, Ozaki Y, Tomizawa H, Akira S, Fukuhara S. Interferon-alpha and interleukin-12 are induced differentially by Toll-like receptor 7 ligands in human blood dendritic cell subsets. *J Exp Med.* 2002; 195(11):1507–1512. [PubMed: 12045249]
90. Gorden KB, Gorski KS, Gibson SJ, Kedl RM, Kieper WC, Qiu X, Tomai MA, Alkan SS, Vasilakos JP. Synthetic TLR agonists reveal functional differences between human TLR7 and TLR8. *J Immunol.* 2005; 174(3):1259–1268. [PubMed: 15661881]
91. Yang R, Murillo FM, Delannoy MJ, Blosser RL, Yutzy WH, Uematsu S, Takeda K, Akira S, Viscidi RP, Roden RB. B lymphocyte activation by human papillomavirus-like particles directly induces Ig class switch recombination via TLR4-MyD88. *J Immunol.* 2005; 174(12):7912–7919. [PubMed: 15944297]
92. Yan M, Peng J, Jabbar IA, Liu X, Filgueira L, Frazer IH, Thomas R. Activation of dendritic cells by human papillomavirus-like particles through TLR4 and NF-kappaB-mediated signalling, moderated by TGF-beta. *Immunol Cell Biol.* 2005; 83(1):83–91. [PubMed: 15661045]
93. Hasan UA, Bates E, Takeshita F, Biliato A, Accardi R, Bouvard V, Mansour M, Vincent I, Gissmann L, Iftner T, Sideri M, Stubenrauch F, et al. TLR9 expression and function is abolished

- by the cervical cancer-associated human papillomavirus type 16. *J Immunol.* 2007; 178(5):3186–3197. [PubMed: 17312167]
94. Dolmans DE, Fukumura D, Jain RK. Photodynamic therapy for cancer. *Nat Rev Cancer.* 2003; 3(5):380–387. [PubMed: 12724736]
95. Abdel-Hady ES, Martin-Hirsch P, Duggan-Keen M, Stern PL, Moore JV, Corbitt G, Kitchener HC, Hampson IN. Immunological and viral factors associated with the response of vulval intraepithelial neoplasia to photodynamic therapy. *Cancer Res.* 2001; 61(1):192–196. [PubMed: 11196160]
96. Ichimura H, Yamaguchi S, Kojima A, Tanaka T, Niiya K, Takemori M, Hasegawa K, Nishimura R. Eradication and reinfection of human papillomavirus after photodynamic therapy for cervical intraepithelial neoplasia. *Int J Clin Oncol.* 2003; 8(5):322–325. [PubMed: 14586759]
97. Bae SM, Kim YW, Kwak SY, Ro DY, Shin JC, Park CH, Han SJ, Oh CH, Kim CK, Ahn WS. Photodynamic therapy-generated tumor cell lysates with CpG-oligodeoxynucleotide enhance immunotherapy efficacy in human papillomavirus 16 (E6/E7) immortalized tumor cells. *Cancer Sci.* 2007; 98(5):747–752. [PubMed: 17425690]
98. Scholten KB, Schreurs MW, Ruizendaal JJ, Kueter EW, Kramer D, Veenbergen S, Meijer CJ, Hooijberg E. Preservation and redirection of HPV16E7-specific T cell receptors for immunotherapy of cervical cancer. *Clin Immunol.* 2005; 114(2):119–129. [PubMed: 15639645]
99. Lyons GE, Roszkowski JJ, Man S, Yee C, Kast WM, Nishimura MI. T-cell receptor tetramer binding or the lack thereof does not necessitate antigen reactivity in T-cell receptor transduced T cells. *Cancer Immunol Immunother.* 2006; 55(9):1142–1150. [PubMed: 16374636]
100. Kaufmann AM, Nieland JD, Jochmus I, Baur S, Friese K, Gabelsberger J, Gieseck F, Gissmann L, Glasschroder B, Grubert T, Hillemanns P, Hopfl R, et al. Vaccination trial with HPV16 L1E7 chimeric virus-like particles in women suffering from high grade cervical intraepithelial neoplasia (CIN 2/3). *Int J Cancer.* 2007
101. Vandepapeliere P, Barrasso R, Meijer CJ, Walboomers JM, Wettendorff M, Stanberry LR, Lacey CJ. Randomized controlled trial of an adjuvanted human papillomavirus (HPV) type 6 L2E7 vaccine: infection of external anogenital warts with multiple HPV types and failure of therapeutic vaccination. *J Infect Dis.* 2005; 192(12):2099–2107. [PubMed: 16288373]
102. MAGE-A3/HPV16 peptide based immunotherapeutic vaccines for squamous cell carcinoma of the head and neck. Identifier NCT00257738. 2007. www.clinicaltrials.gov
103. A Multi-center, double-blind, randomized, placebo-controlled study of Amolimogene (ZYC101a) in the treatment of high-grade cervical intraepithelial lesions (CIN 2/3) of the uterine cervix. Identifier NCT00264732. 2007. www.clinicaltrials.gov
104. Immunotherapy of recurrent cervical cancers using dendritic cells. Identifier NCT00155766. 2007. www.clinicaltrials.gov
105. Achta MS, Ibrahim R, Herrin E, Gause B, Steinberg S, Grollman F, Rahma O, Bernstein S, Maruffi C, Berzofsky JA, Khleif SN. Pre-immature dendritic cells pulsed with Human papillomavirus 16 E7 peptide vaccine in advanced cervical cancer. *J Clin Oncol.* 2005 Jun 1;23(16S Part I of II):2522.
106. Active immunization of patients with carcinoma of oral cavity or oropharynx with autologous dendritic cells transfected with DNA from autologous tumor. Identifier NCT00377247. 2007. www.clinicaltrials.gov
107. A randomized, double-blind, three-arm parallel-group, placebo controlled phase 3 trial to investigate the clinical efficacy and safety of Polyphenon E in the treatment of external genital warts. Identifier NCT00449982. 2007. www.clinicaltrials.gov
108. A limited access phase II trial of cetuximab (C225, NSC #714692) in combination with cisplatin (NSC#119875) in the treatment of advanced, persistent, or recurrent carcinoma of the cervix. Identifier NCT00101192. 2007. www.clinicaltrials.gov
109. A phase II study of bevacizumab in combination with definitive radiotherapy and cisplatin chemotherapy in untreated patients with locally advanced cervical carcinoma.. Identifier NCT00369122. 2007. www.clinicaltrials.gov

110. A phase I trial of tailored radiation therapy with concomitant cetuximab (C225, NSC #714692) and cisplatin (NSC #119875) in the treatment of patients with cervical Cancer. Identifier NCT00104910. 2007. www.clinicaltrials.gov

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Table I
Recent and Ongoing Clinical Trials for Immunotherapy of HPV-Induced Mucosal and Cutaneous Lesions

Vaccine type	Trial Phase	Vaccine composition	Target antigen(s)	Adjuvant/Route	Patient group	Additional treatment	Immunological responses	Clinical responses	Ref.
Peptides /Protein	II	Fusion protein consisting of <i>Mycobacterium bovis</i> Hsp65 and HPV16 E7 protein (HspE7, SGN-00101)	HPV16 E7	SC	21 patients with LSIL, HSIL, ASCUS or AGUS	None	9/17 IFN γ ELISPOT	7/20 CR 1/20 PR 11/20 SD 1/20 NR	14
	II	HspE7 (SGN-00101)	HPV16 E7	SC	58 patients with CIN 3	None	ND	13/58 CR 32/58 PR 11/58 SD 2/58 NR	15
	I/II	HspE7 (SGN-00101)	HPV16 E7	SC	15 HIV+ patients with AIN 2/AIN 3	None	ND	1/15 CR 4/15 PR 10/15 NR 3/5 responders HPV-negative after 48 wks	16
	I/II	HspE7 (SGN-00101)	HPV16 E7	SC	27 patients with recurrent respiratory papillomatosis	Baseline debulking surgery	ND	Significant increase in median intersurgical interval (55 days to 106 days)	17
	I/II	HPV16 L1E7 chimeric VLP or placebo	HPV16 L1 and E7	SC	39 patients with CIN 2 or CIN3	None	24/24 positive antibody in vaccine group; 0/12 positive antibody in placebo group	Vaccine: 10/23 PR (histology) 6/23 HPV16 DNA negative Placebo: 4/12 PR (histology) 1/12 HPV16 DNA negative	100
	II/III	HPV6 L2-E7 fusion protein or placebo	HPV6 E7 protein	AS02A/IM	191 patients with persistent and/or recurrent anogenital warts	Ablative surgery (CO ₂ or laser surgery)	97/97 positive antibody response in vaccine group	No difference in number of recurrences between vaccine and placebo group	101
	II/III	HPV6 L2-E7 fusion protein or placebo	HPV6 E7 protein	AS02A/IM	92 patients with new or recent anogenital warts, 37 with recurrent warts	Topical 0.5% podophylotoxin solution	64/64 positive antibody response in vaccine group	No difference in (partial) clearance between vaccine and placebo group	101

Vaccine type	Trial Phase	Vaccine composition	Target antigen(s)	Adjuvant/Route	Patient group	Additional treatment	Immunological responses	Clinical responses	Ref.
	I	MAGE-A3 and HPV16 peptides	MAGE-A3 and HPV16 (targets not identified)		90 patients HLA-A2+, recurrent, progressive or metastatic HNSCC	None	Ongoing	Ongoing	102
Viral vector	II	Modified vaccinia virus Ankara expressing BPV E2 (MVA-E2)	BPV E2	Intra-uterine	54 patients with CIN 2 or CIN 3	20 patients treated with conization alone	34/34 vaccine positive antibody responses, CTL responses	Vaccine: 19/34 CR 15/34 PR Conization: 16/20 CR 4/20 NR	39
	I/II	MVA-E2	BPV E2	Intra-urethral	50 patients with flat condyloma	20 patients treated with 5% 5-fluorouracil alone	30/30 vaccine positive antibody responses, CTL responses	Vaccine: 28/30 CR 2/30 NR 5-fluorouracil: 10/20 CR 3/20 PR 7/20 NR	40
	II	TA-HPV (Recombinant vaccinia virus expressing HPV16 E6/E7 and HPV18 E6/E7)	HPV16 E6/E7 HPV18 E6/E7	Dermal scarification	44 patients Untreated stage Ib or IIa cervical carcinoma	Surgical removal of tumor	Ongoing	Ongoing	18
DNA-based	II	Plasmid DNA encoding fragments of HPV16 and HPV18 E6 and E7 in biodegradable microparticles (ZYC101a)	HPV16 E6/E7 HPV18 E6/E7	IM	288 patients with CIN 2 or CIN 3	One dose vaccine group, three dose vaccine group, and placebo group	Ongoing	Ongoing	103
Heterologous prime/boost	II	TA-CIN prime (HPV16 L2/E6/E7 fusion protein) and TA-HPV boost	HPV16 L2, E6, E7 HPV18 E6, E7	IM/Dermal Scarification	27 patients with VIN 3 2 patients with VAIN 3	None	14/24 antibody 10/27 proliferation 11/25 IFN γ ELISPOT	1/29 CR 5/29 PR 18/29 SD 5/29 NR	72
Dendritic cell-based	I	Mature autologous DC pulsed with HPV16 E7 peptides	HPV16 E7	Intranodal	12 patients HLA-A*0-201 positive, recurrent cervical cancer, non-responsive to radio- and chemotherapy	Previous exposure to radiotherapy and/or chemotherapy	Ongoing	Ongoing	104
	I/II	Immature DC pulsed with	HPV16 E7	IV	15 patients HLA-A*0-201,	None	6/14 CTL and IFN γ ELISPOT	Extended progression free	105

Vaccine type	Trial Phase	Vaccine composition	Target antigen(s)	Adjuvant/Route	Patient group	Additional treatment	Immunological responses	Clinical responses	Ref.
		HPV16 E7 peptides			advanced cervical cancer			survival in patients with immune responses	
	I/II	DC transfected with autologous tumor-derived DNA	Tumor-derived proteins	Intranodal	17–39 patients with primary advanced HNSCC	Surgery and radiation or chemoradiation	Ongoing	Ongoing	106
Immune stimulator, TLR7 agonist	II	None			29 subjects with high-grade VIN	Topical 5% Imiquimod cream	11/20 proliferative responses to HPV16 E2/E6/E7 8/20 positive IFN γ cytokine 8/17 positive IFN γ ELISPOT	7/17 CR 6/17 PR 1/17 SD 3/17 NR	20
	II	None			39 subjects with VIN2/3	Topical 5% Imiquimod cream	ND	21/33 CR 9/33 PR 3/33 SD	22
	I	None			22 subjects with AIN 1,2, or 3 (18 perianal and 4 intra-anal)	Topical 5% Imiquimod cream or 5% suppositories	ND	17/22 CR 4/22 PR/SD 1/22 NR	23
	III	None			500+ patients with anogenital or perianal warts	Topical 10% or 15% Polyphenon E ointment and placebo group	ND	Not reported	107
	II/III	None			255+ patients with anogenital warts (6 month follow-up)	Topical 5% Imiquimod cream (A), Surgical ablation (B) or Imiquimod following surgery (C)	ND	(A) 89/95 CR (B) 64/87 CR (C) 65/71 CR	21
Monoclonal antibodies	II	Cetuximab (anti-EGFR antibody)	EGFR	IV	28–62 patients with advanced, persistent, or recurrent cervical cancer	Cisplatin therapy	Ongoing	Ongoing	108
	II	Bevacizumab (anti-VEGF antibody)	Vascular endothelial growth factor	IV	57 patients with stage IIB–IIIB cervical cancer or stage IB–IIA with pelvic node metastases	Radiation therapy and Cisplatin therapy	Ongoing	Ongoing	109

Vaccine type	Trial Phase	Vaccine composition	Target antigen(s)	Adjuvant/Route	Patient group	Additional treatment	Immunological responses	Clinical responses	Ref.
	II	Cetuximab	EGFR	IV	30–100 patients with stage IB–IVA cervical cancer	Radiation therapy and Cisplatin therapy	Ongoing	Ongoing	110

AGUS, atypical glandular cells of uncertain significance; AIN, high-grade anal intraepithelial neoplasia; ASCUS, atypical squamous cells of undetermined significance; BPV, bovine papillomavirus; CIN, cervical intraepithelial neoplasia; CR, complete responder; CTL, cytotoxic T lymphocyte; DC, dendritic cell; DTH, delayed type hypersensitivity; EGFR, epidermal growth factor receptor; ELISPOT, enzyme-linked immunospot; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; HSIL, high-grade intraepithelial lesion; HSP, heat-shock protein; IFA, incomplete Freund's adjuvant; IFN γ , interferon γ ; IV, intravenous; LSIL, low-grade squamous intraepithelial lesion; MVA, modified vaccinia virus Ankara strain; ND, not determined; NR, non-responder; PR, partial responder; SC, subcutaneous; SD, stable disease; TLR, toll-like receptor; VAIN, vaginal intraepithelial neoplasia; VIN, vulvar intraepithelial neoplasia; VLP, virus-like particle.