New Developments in Therapeutic HPV Vaccines

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Abstract Human papillomavirus (HPV) infection is the central causal factor in cervical cancer. For years efforts were undertaken to control HPV-associated disease by the development of HPV vaccines. While prophylactic HPV vaccination represents a major public health breakthrough, it is limited to new infection by certain HPV types and does not have therapeutic effects or prevent progression of pre-existing HPV infections to malignancy. Thus, there is an urgent need for therapeutic HPV vaccines. Therapeutic vaccines aim to induce cytotoxic T cells, facilitating clearance of HPV high-grade lesions and cancers. HPV E6 and E7 oncogenes represent ideal antigens for therapeutic HPV vaccination because of their constitutive expression in HPV-containing carcinomas and their critical role in the induction and maintenance of HPV-associated disease. This review discusses the most recent advances in HPV therapeutic vaccines and summarizes effects of new basic immunology findings and vaccine delivery technologies.

Keywords Cervical cancer · Cervical intraepithelial neoplasia · CIN · Immunization · Clinical trials · Immunotherapy · Lesions

Introduction

Human Papillomavirus (HPV) is the most common sexually transmitted agent worldwide and comprises more than 150 genotypes. Persistent infection with high-risk (oncogenic) HPV genotypes, most commonly type 16 and 18, is responsible for all of the cervical carcinogenesis, and 5.2 % of the total global cancer burden, including cancers of the cervix, penis, vagina, vulva, anus, and oropharynx [1]. Recently, an association between HPV and breast cancer has also been proposed [2]. However, not all persistent infections progress to precancerous (high-grade) lesions, and not all infections with high-risk HPV develop into cancer. Several studies of prevalent HPV infection have concluded that HPV is mainly a transient infection [3–5]. For women who do develop cervical cytology abnormalities, approximately 75 % of low-grade lesions in adults and 90 % of low-grade lesions in adolescents resolve spontaneously [6]. The longer an HPV infection persists, the less probable is a spontaneous clearance of the infection [7].

The most successful strategy for cervical cancer prevention has been the implementation of screening strategies for precancerous cervical lesions (i.e., high-grade dysplasia or cervical intraepithelial neoplasia [CIN] 2/3) utilizing conventional Pap smear tests or, more recently, liquid-based cytology (LBC) tests. Cervical cancer incidence rates in the United States decreased by 75 % and mortality by 74 % in the 50 years following the introduction of cervical cytology in 1949 [8]. However, all screening programs require health infrastructure and financial resources that are not available in most developing countries [9]. In addition, they reduce mortality from cervical neoplasia, but in the first place do not prevent development of precancerous high-grade (CIN 2/3) and persistent low-grade lesions (CIN 1), requiring treatment that mainly consists of extirpative or

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ablative procedures aimed at removing visible lesions. These procedures do not target the causative HPV infection and patients with CIN who have undergone ablative surgery or even a hysterectomy may still have HPV infection, which may result in recurrent or persistent CIN [10, 11]. Taken together, HPV-targeted, less invasive treatment methods for precancerous lesions are urgently needed.

HPV infection of the genital tract gives rise to a slow and modest but measurable serum or local antibody response. However, not all individuals infected with HPV develop measurable HPV antibodies, but more than half of all infected subjects will seroconvert [12]. A detectable seroconversion requires sufficient antigenic exposure associated with either a high viral load or persistent infection with HPV [13]. Humoral immunity is directed against conformational epitope(s) derived from the major capsid protein L1 displayed on the outer surface of the intact virus particle and is merely genotype specific [14]. Seropositive individuals are only partially protected against further HPV infection.

Recently available prophylactic vaccines have proven to prevent nearly 100 % of infections and dysplasia induced by the respective vaccine type HPVs. However, they do not target all high-risk HPV types, have a limited cross-protection to other HPV types that cause cancer, have no therapeutic effect, or protect women who are already infected with HPV at the time of vaccination. In addition, their efficacy depends on high vaccination coverage and is not likely to be realized for many years [15].

Therefore, therapeutic HPV vaccines will continue to be an area of intense investigation as a treatment modality for persistent infection and inducing regression of precancerous lesions or remission of advanced cancer.

Challenges and Advancements

The past two decades have seen a multitude of clinical trials with experimental candidate therapeutic vaccines. Many vaccination strategies were employed and generally proved to be safe and, in most trials, also immunogenic. Clinical responses, however, were sparse or completely lacking. This was not expected initially because cervical cancer has unique HPV-derived antigens that are not tolerized due to thymic negative selection like self-antigens and immune responses had been demonstrated in patients before. It turned out that specific immune evasion and immune resistance mechanisms acquired by the tumor cells during persistent infection and the year-long progression to cancer are responsible for the disappointing clinical results. These mechanisms and how they can be overcome were investigated in detail recently (see further in article), and to some extent, the use of potent adjuvants and the performance of

clinical trials in patients with premalignant (not yet immune-escaped) lesions led to more promising clinical results.

In the past 2 years, a more detailed knowledge of the role of the immunosuppressive regulatory T cells (Tregs) and toll-like receptors (TLRs) in the progression and treatment of HPV-associated diseases has created new strategies for the development of immunotherapy.

Tregs are CD25^{hi} Foxp3⁺ CD4⁺ T cells that function to suppress the pathogenic immune responses mediated by effector T cells and play key roles in controlling immunoreactions [16]. Recently, their interaction with HPV therapeutic vaccines has been further explored. Tregs are found with higher frequency in persistently HPV-infected patients. They play a pivotal role in the suppression of antiviral T cell responses and severely hamper the efficacy of the therapeutic vaccines [17, 18]. CD4⁺ HPV-specific Treg clones have been isolated from HPV-induced intraepithelial lesions, HPV-induced cancers, and their draining lymph nodes [19]. In addition, analyses showed that HPV therapeutic vaccines themselves can induce and/or boost Tregs and that these vaccine-induced Tregs are associated with early treatment failure [20•]. Moreover, it has been shown that repeated vaccinations may increase Treg frequencies [21]. In contrast, selective depletion of Tregs *in vivo* has already proved effective for tumor rejection in mice and humans. In this respect, our own research showed that combining an adenovirus-based therapeutic vaccination and Treg depletion can lead to the complete and permanent eradication of HPV-expressing tumors in a mouse tumor model [22•]. In patients with condylomata acuminata, Treg depletion using low-dose cyclophosphamide ameliorates the local immune milieu of the lesion site and successfully prevents recurrence of such lesions [23•]. In addition, Liu et al. [24] reported that the combination of adenovirus-mediated expression of immunostimulatory proteins with systemic Treg depletion resulted in a significant delay of tumor growth and even suppressed distant tumor outgrowth in a cervical cancer mouse model. These results showed that therapeutic vaccines in combination with agents capable to target and eliminate Tregs-mediated immunosuppression might be a promising approach to improve HPV-directed immunotherapy. However, other studies in this direction indicated that depletion of Treg in a mouse model of cervical carcinoma did not enhance the immune response induced by the Semliki Forest viral vaccine SFVeE6/7 immunization, suggesting that the SFVeE6/7 vaccine may not require additional immune clearance interventions to modulate Treg activity [25].

A complementary approach is to further enhance vaccine-induced immune responses by potent adjuvants. TLRs are the best-characterized family of pattern-recognition receptors that detect microbe-derived substances. When activated, they stimulate the production of

proinflammatory cytokines and chemokines to increase the host's ability to eliminate pathogens [26]. TLRs are expressed on a wide variety of cells, including B cells, specific types of T cells, monocytes, dendritic cells (DC), macrophages, certain epithelial cells, and many other innate immune cells [27]. In recent years, the role of TLRs in HPV persistence, clearance, and HPV-associated cancer progression has become an active field of research with many exciting results. Dampened TLR expression in the cervical mucosa is a type-specific mechanism by which HPV16 interferes with innate immune responses. This contributes to viral persistence. On the other hand, TLR upregulation and resultant cytokine induction is important in HPV clearance [28•]. In a similar study, the downregulation of TLR4 was found to be associated with the progression of cervical neoplasia [29]. Moreover, upregulation of TLR in the stroma and not in the epithelium might contribute to the persistence of HPV and ultimately promote the progression of the HPV associated lesions [30••]. Therefore, TLRs and their signaling pathways may act as attractive targets for therapeutics.

For example, agonists that target the TLRs recently have been used as adjuvants to enhance the efficacy of vaccines. Imiquimod was the first approved topically active TLR7 agonist against HPV. It was demonstrated to change the microenvironment of HPV-induced vulvar intraepithelial neoplasia (VIN) and this resulted in viral clearance, normalization of immune cell counts, and a complete regression of the lesion [31]. In a phase 2 study, therapeutic HPV vaccination (TA-CIN, fusion protein HPV16 E6E7L2) in conjunction with imiquimod in patients with VIN grades 2 and 3 led to clinical response in some of the patients with significant systemic HPV-specific immune response and local infiltration of CD4+ and CD8+ T cells but not Tregs in the lesions of these patients [32]. In addition, it was described that some TLR3/4 agonists could promote the maturation of E7-loaded myeloid-derived DCs and their ability to induce T helper type 1 polarization, augmenting an E7-specific T cell response [33]. These TLR3/4 agonists could significantly enhance antitumor activity and might be a promising adjuvant for HPV therapeutic vaccines against HPV infections and lesions.

Recently Applied Therapeutic Vaccine Strategies

Initial vaccine strategies used HLA-restricted minimal peptide epitopes, recombinantly expressed proteins, recombinant viral vectors, autologous DCs, and, recently, DNA expression plasmids. Recently investigated strategies are summarized in Table 1. The choice of the target antigen is extremely important for designing therapeutic vaccines. Until now, most therapeutic HPV vaccines have targeted one

genotype of HPV, or at most two. The viral oncogenes (HPV E6 and E7) represent ideal antigens for therapeutic HPV vaccination because their sustained expression is required for the maintenance of the cancerous phenotype [34]. However, some studies using highly sensitive and multiplexed HPV tests are beginning to reveal that in many cases of advanced cervical dysplasia [35], and even in cervical cancer [36], there are multiple genotypes of high-risk HPV present in the infected tissue. In such a scenario, targeting only a single HPV genotype would likely be ineffective as a therapeutic approach in some cases. Thus, researchers are seeking to develop novel broad-spectrum vaccines. A novel, broad-spectrum, recombinant fusion protein-based vaccine is directed at the E7 proteins of five of the most prevalent high-risk HPV genotypes worldwide (HPV16, 18, 31, 45, and 52). Together they account for more than 80 % of all HPV-associated cancers and elicit strong, multigenotype specific CD8 T cell immunity when administered to mice in combination with adjuvants comprised of agonists of the TLR3 or TLR9 family of innate immune receptors [37•].

Protein- and long peptide-based vaccines have become attractive approaches in generating antigen-specific immunotherapy because of their safety, simplicity, and coverage of many human leukocyte antigen (HLA) restrictions for presentation [38]. However, the use of protein or long peptides as vaccines has been slowed by the need for strong adjuvants because of their low immunogenicity. Recently, several new protein and peptide-based vaccines against HPV E6 and/or E7 have overcome this obstacle and showed satisfying results in preclinical models. A HPV16E6/E7 long peptide vaccine adjuvanted with Montanide ISA-51 (SEPPIC, Puteaux, France), a detoxified Freund's adjuvant, demonstrated an extraordinary efficacy of 79 % of clinical responses in a clinical trial in patients with persistent VIN lesions. The most important observation is that a measurable antigen-specific T cell response that clearly correlated with the clinical responses was induced [39••]. Progress also has been demonstrated in preclinical models. A new protein vaccine using autologous HSP70 as HPV16 E7 antigen vector could elicit a potent E7-specific anti-tumor effect without any adjuvantation in the mouse cervical cancer model TC-1 [40]. In another study, a novel fusion protein-based vaccine comprising LALF32–51, a cell-penetrating and immunostimulatory peptide, linked to HPV16 E7 antigen could significantly improve the presentation of E7-derived peptides to T cells in vitro, and consequently induced a potent E7-specific cell-mediated immune response and inhibiting TC-1 tumor growth [41].

Recently, a new HPV fusion peptide composed of Tat49–57 (amino acids 49–57) and the cytotoxic T lymphocyte (CTL) epitope HPV16E7 (amino acids 49–57) combined with GM-CSF expression plasmid application has been

Table 1 Recent therapeutic HPV vaccine developments

Vaccine type	Study	Vaccine	Antigen	HPV type(s)	Status of progress	Immunological response	Antitumor response
Protein	Wick and Webb [37•]	Pentaxix + TLR3/TLR9	E7	HPV16, 18, 31, 45, 52	Preclinical	CD8 T cell immunity	TC-1 tumors underwent rapid and complete regression
	Li et al. [40]	E7-mHSP70	E7	HPV16	Preclinical	ND	Protected mice against growth of the TC-1 tumor cells prophylactically and inhibited TC-1 tumor spread to lungs
	Granadillo et al. [41]	LALF32–51	E7	HPV16	Preclinical	ND	Induced a potent suppression of tumor growth in mice
Peptide	Muderspach et al. [44]	Tat-E7/pGM-CSF	E7	HPV16	Preclinical	CD8+ memory T cells	Protected mice against growth of tumor cells prophylactically and decrease tumor growth
Long peptide	Kenter et al. [39••]	HPV16E6E7 long peptide	E6, E7	HPV16	Clinical	CD4+ IFN- γ	79 % clinical response; 47 % complete response, good correlation of T cell and clinical responses
Virus	Brun et al. [47]	TG4001	E6, E7	HPV16	Clinical phase II	ND	7/10 patients became HPV negative and underwent CIN 2/3 regression
DNA	Dimiz and Ferreira [50]	pgD-E7E6E5	E5, E6, E7	HPV16	Preclinical	Specific CD8+ T cell responses	Three vaccine doses inhibited tumor growth in 70 % of mice with established tumors
	Lee et al. [51]	AcHERV-HP16L1	ND	HPV16	Preclinical	Immunized mice have a high secretion level of IFN- γ and neutralizing antibody	ND
	Cho et al. [52•]	HPV16L1MBP/mPEI/pHPV16L1	ND	HPV16	Preclinical	CD8+ T cell and humoral immune responses	ND
	Ohlschlager et al. [53••]	HPV16E6E7SH	E6, E7	HPV16	Preclinical	CD8+ T cell responses	ND
	Kim et al. [68•]	DC vaccine with siRNA targeting PTEN	E7	ND	Preclinical	Tumor antigen-specific CD8+ T cells	Decreased tumor growth
	Kim et al. [69]	DCs with siRNA targeting IL-10R	E7	ND	Preclinical	HPV E7-specific CD8+ T cells	Generated stronger antitumor effects against E7-expressing TC-1 tumor cells

HPV human papillomavirus; ND no data; IFN- γ interferon gamma; CIN cervical intraepithelial neoplasia; DC dendritic cell; siRNA short interfering RNA; PTEN phosphatase and tensin homolog; IL-10R interleukin-10 receptor

shown to be effective in eliciting potent long-lasting CD8⁺ memory T cells immune responses *ex vivo* and *in vivo* [42]. In patients suffering from HPV-associated lesions, peptide-based vaccines have been tested for their therapeutic efficacy but showed only moderate immune and clinical responses [43, 44]. It will be interesting to see if the new approaches will be more effective.

Because of their high infection efficiency and excellent expression of antigens in the infected cells, recombinant viral vectors are considered an alluring choice for the delivery of HPV protein [45]. The disadvantage of some viral vectors is the development of host-induced neutralizing antibodies to the vector itself, thus limiting its continued use. The nature of viral vaccine platforms renders them exceptionally suitable for multicenter randomized trials [46]. In a recent phase 2 clinical trial, patients with HPV16-related CIN 2/3 were treated three times at weekly intervals with TG4001 vaccine, consisting of an attenuated recombinant vaccinia virus containing the sequence for modified HPV16 E6 and E7, and the human interleukin (IL)-2 gene. At month 6, regression of the CIN 2/3 lesion and the clearance of HPV 16 infection were seen in 7 of 10 patients [47]. Future directions will involve its efficacy in further phase 3 clinical trials of HPV-associated lesions and whether its antitumor response will be enhanced in combination with neoadjuvant chemotherapy or other adjunct forms of therapy.

DNA vaccines have the advantage of being safe and easy to produce, and can be administered repeatedly to the same individual without loss of efficacy [48]. However, naked DNA suffers from insufficient intrinsic specificity for antigen-presenting cells (APCs) and has a limited ability to amplify and spread *in vivo*. Therefore, various strategies to enhance therapeutic HPV DNA vaccines are currently under active investigation, including alternative immunization routes and delivery methods. The gene gun is a ballistic device that enables delivered DNA to directly transfect keratinocytes and epidermal Langerhans cells. These events stimulate DC maturation and migration to the local lymphoid tissue, where they prime T cells for antigen-specific immune responses [48]. The delivery of HPV DNA vaccines by gene gun was shown to be the most dose-efficient method of vaccine administration compared to needle intramuscular (i.m.) and Biojector (Bioject Medical Technologies, Inc., Tualatin, OR) administrations [49]. In a recent study, a newly developed DNA vaccine encoding herpes simplex virus type 1 (HSV-1) glycoprotein D genetically fused to three HPV16 oncoproteins (E5, E6, and E7), by intradermal (i.d.) administration using a gene gun, induced strong antigen-specific CD8⁺ T cell responses in vaccinated mice and conferred enhanced antitumor protection with

much lower DNA loads when compared to the i.m. delivered DNA vaccine. Significantly stronger therapeutic antitumor effects were observed after i.d. immunization with a single dose of pgD-E7E6E5 co-administration of DNA vectors encoding a co-stimulatory signal, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL-12 [50].

A therapeutic vaccine in widespread use should be acceptably cost effective and highly effective in clearing existing HPV infection. Several novel delivery systems appeared to have higher cellular immunogenicity and much less cost compared to the widely studied methods of introducing foreign genes into mammalian cells such as chemical-based procedures, electroporation, gene-gun, and mammalian viral vector-based systems [48]. Recently, a recombinant baculovirus bearing human endogenous retrovirus (HERV) envelope protein, was used as a nano-carrier for HPV-16L1 DNA vaccine (AcHERV-HP16L1). Compared with the virus-like particle-based vaccine Gardasil® (Merck & Co., Whitehouse Station, NJ), mice immunized with AcHERV-HP16L1 showed similar high levels of humoral immunity in IgG/IgA and in neutralization of HPV pseudovirions. Interestingly, a 450- and 490-fold increase in the interferon (IFN)- γ T cell response was observed at 5 and 20 weeks after the first injection, respectively. The recombinant baculovirus used for HPV16L1 gene delivery can induce both humoral and cellular immunity. Therefore, it could potentially be developed as an efficient prophylactic and therapeutic vaccine [51]. In another report, HPV16L1MBP/mPEI/pHPV16L1, simultaneously carrying a protein vaccine along with its corresponding DNA vaccine, could trigger the highest levels of humoral immune responses among various vaccination groups and enhanced the number of interferon-producing CD8⁺ T cells in a mouse model [52•].

Our own approach was to combine the antigen-carrying plasmid (HPV16E7SH) with expression plasmids for cytokines (IL-2, IL-12, GM-CSF, IFN- γ , macrophage inflammatory protein (MIP) 1- α) as adjuvants. Given in different combinations and time points around the vaccination gene-encoded MIP-1 α applied 5 days before E7SH-immunization combined with IFN- γ or IL-12 (3 days) or IL-2 (5 days) postimmunization led to a significantly enhanced tumor response that was clearly associated with granzyme B secretion and target cells lysis [53••].

DC-based vaccines are potent options for cancer immunotherapy because DCs are the most important professional APCs capable of priming naïve T cells and can serve as natural adjuvants [54, 55]. A number of strategies have been developed including the use of different vectors, pulsing of DC with proteins and peptides, or transfecting DC with DNA or RNA to enhance the potency of therapeutic

vaccines for HPV [56–61]. However, the immune responses elicited by the first-generation DC vaccines might not be of the quality required to allow the rejection of bulky tumors because of some specific problems such as a short lifespan of DC, few DCs from the vaccination site migrating to the lymph node, the strongly immunosuppressive cytokine microenvironment within the tumor, or the recruitment of immunosuppressive cells like regulatory T cells [62–67]. Several strategies have been used to improve DC-based vaccines. Kim et al. [68•] reported that a DC vaccine combined with a small interfering RNA (siRNA) targeting phosphatase and tensin homologue (PTEN) led to an increase of in vitro T cell activation activity and in vivo migration to a draining lymph node, respectively. Moreover, these PTEN siRNA-transfected DCs (DC/siPTEN) acquired an increased survival and protection from apoptotic death caused by GM-CSF deprivation or antigen-specific CD8⁺ T cell killing. Most importantly, DC/siPTEN generated more tumor antigen-specific CD8⁺ T cells and stronger antitumor effects in vaccinated mice than did control DCs (DC/siGFP). In another report, DCs modified with siRNA targeting against IL-10R generated more HPV E7-specific CD8⁺ T cells and stronger antitumor effects against E7-expressing TC-1 tumour cells in vaccinated mice than DC/siGFP, as well as DC/siIL-10 [69]. These results demonstrated that using siRNA for interference with immunosuppressive cytokines could enhance DC-based vaccine potency. We may infer that the DC vaccines, combined with other therapies that target many different immunosuppressive agents and signaling pathways, may lead to even better antitumor efficacy.

Combinational Approaches to Enhance HPV Therapeutic Vaccine Potency

Each strategy for delivery of therapeutic HPV vaccines has its own advantages and potential disadvantages. Optimally designed cancer vaccines should combine the best tumor antigens with the most effective immunotherapy agents, delivery strategies, and administration routes to achieve positive clinical results. Ideally, this is first investigated in an uncompromised healthy immune system after safety has been proven and then transferred to patients [70, 71].

Adjuvants are incorporated into a vaccine to increase vaccine immunogenicity by potentiating antigen-specific CD8⁺ and/or CD4⁺ T cell responses, or to increase vaccine effectiveness by impairing one or more immunosuppressive mechanisms that operate at the level of the tumor microenvironment [72–76]. Therefore, recent studies were dedicated to seeking better adjuvants to improve the efficacy of therapeutic HPV vaccines. SA-

4-1BBL, a multifunctional immunomodulator, was used in combination with a HPV16 E7 recombinant protein-based therapeutic vaccine, and induced complete eradication of established tumors in approximately 70 % of mice [77]. In two other preclinical studies, the combination of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), a vascular disrupting agent [78], with therapeutic HPV16 E7 peptide-based vaccination [79•] or E7 DNA vaccination [80] were demonstrated to enhance the antitumor effect and generate strong E7-specific CD8⁺ T-cell immune responses. Using the HPV16E7-expressing TC-1 tumor model, mice were vaccinated with HPV16 E7 peptide, together with a pan HLA-DR epitope (PADRE) peptide, which is capable of binding to different major histocompatibility complex (MHC) class II molecules with high-affinity [81] and the TLR3 ligand poly(I:C) adjuvant, a strong inducer of type I IFN [82]. PADRE peptide and poly (I:C) were used to enhance the activation of CD4⁺ T helper cells and DC, respectively, which altogether generated better E7-specific CD8⁺ T cell immune responses as well as antitumor effects against TC-1 tumors [83]. In another study, an adenovirus-based T cell vaccine (Ad-p14) in combination with treatment by antiglycorticoid-induced tumor necrosis factor receptor (GITR) antibody resulted in complete and permanent eradication of 100 % of TC-1 tumors in mice [22•]. Moreover, a recent study has used the natural killer T cell (NKT) ligand α -galactosylceramide (α -GalCer) as an adjuvant with DNA vaccination against HPV16 oncoprotein E7 to generate high numbers of E7-specific CD8⁺ T cells protective against the transplantable TC-1 (HPV-16+) tumor model [84•]. These new adjuvants trigger innate and adaptive immunity by targeting different innate receptors or pathways.

Electroporation represents one way of improving DNA penetration into cells and enhances the magnitude of gene expression. Two recent studies have demonstrated that combining adjuvant CpG motifs and electroporation can trigger an enhanced cellular immune responses and stronger regression of tumors [85, 86•, 87•]. Therefore, selecting an appropriate adjuvant and delivery route may maximize the antitumor potency of therapeutic vaccines.

Until now, the delivery of HPV therapeutic vaccines using i.m., subcutaneous, and i.d. administration has been shown to be the most efficient routes of vaccine administration.

However, bone marrow (BM) was recently shown to be a unique microenvironment that supported robust proliferation and recalled responses of memory T cells, particularly within the CD8⁺ T cell subset. The population of CD8⁺ effector memory T cells within the human BM has a hybrid phenotype and enhanced cytotoxic

function (reviewed in [86•]) [88]. Therefore, BM-derived T cells may be the ideal effectors for HPV therapeutic vaccine strategies. Fresnay et al. [89•] reported that direct intra-BM (IBM) vaccination with a class I HPV16 E7 epitope induced large numbers of activated, IFN- γ -producing E7-specific lymphocytes in mice. In the therapeutic setting, IBM vaccination induced tumor regression in 3 of 10 vaccinated mice and delayed tumor growth in the remaining animals significantly.

Mucosal immunization may be important for protection against pathogens whose transmission and pathogenesis target mucosal tissue [90]. Sexually-transmitted HPV types are prototypic mucosal pathogens that infiltrate epithelial lesions and infect cells of mucosal or squamous epithelia [91]. Therapeutic responses against HPV infection and cancer might be induced systemically and/or at mucosal surfaces by activating the mucosal immune system, which has been established as an ideal target site for some vaccines [92]. Recently, C57BL/6 mice were orally exposed to recombinant *Lactobacillus casei* expressing HPV16 E7 (LacE7). The induction of specific mucosal E7 type 1 T cell with responses was greater after oral immunization with LacE7 when compared to subcutaneous or i.m. antigen delivery. Oral immunization with *Lactobacillus*-based vaccines was also able to induce mucosal cytotoxic cellular immune responses [93]. In a similar study, in the TC-1 tumor model, animals receiving the orally administered *L. casei*-PgsA-E6 vaccine generated systemic and local cellular immune responses and showed reduced tumor size and increased survival rates [94]. Thus, new administration routes may potentially enhance therapeutic effects of individual HPV therapeutic vaccines.

Conclusions

In the past few years, a significant progress in therapeutic vaccines has been made in experimental preclinical research and initial clinical trials. However, now it is important to pave the way for further clinical trials to see these vaccines' true efficacy against HPV-associated lesions, especially against advanced cancer. Advances in understanding the mechanisms of the cellular and molecular nature of innate and adaptive immunity have provided insights into how adjuvants might be improved to enhance immunity and achieve an adequate response. The current wealth of preclinical and clinical information predicts that an ideal HPV therapeutic vaccine should activate both innate and adaptive immunity. Therefore, this would prevent persistent HPV infection from progressing toward advanced cancer or even promote clearance of HPV infection.

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Papers of published papers of interest have been highlighted as:

- Of importance
- Of major importance

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