Therapeutic human papillomavirus vaccination

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Dysplastic lesions and cancer of the cervix are distinct from most other malignancies in that they harbor foreign antigens derived from human papillomavirus (HPV). The expression of those viral oncogenes is necessary to maintain the cancerous phenotype. Therefore, these antigens are unique and very attractive targets for 'proof-of-concept' studies in the development of therapeutic vaccines. In this review, we focus on the most recent developments in therapeutic vaccines and clinical immunotherapy trials for mucosal HPV-induced lesions, and some emerging therapeutic strategies that have been tested in preclinical models. Progress in peptide-based vaccines, DNA-based vaccines and viral or bacterial vector-based vaccines for HPV will be discussed, as well as the possible reasons for their success and failure.

Cervical cancer is the second leading cause of cancer deaths in women worldwide. The disease and its premalignant stages are associated with human papillomavirus (HPV) infection, creating a unique opportunity to prevent and treat cervical cancer through antiviral vaccination. The HPVs are a family of double-stranded DNA viruses with over 100 different genotypes. HPV genotypes are divided into low-risk and high-risk for their potential to transform cells. A total of 15 HPV types are classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82), three are classified as probable high-risk types (26, 53 and 66) and 18 are classified as low-risk types (6, 11, 34, 40, 42, 43, 44, 54, 55, 57, 61, 67, 70, 71, 72, 81, 83 and 84) [1]. While low-risk types induce benign genital condylomata and low-grade squamous intraepithelial lesions, the high-risk types are associated with anogenital cancers and can be detected in 99% of cervical cancers, with type HPV 16 found in approximately 50% of cases [2,3]. However, the low-risk types can cause other diseases such as recurrent respiratory papillomatosis. 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 types [4]. In general, approximately 75% of the sexually active population acquires at least one genital HPV type during their lifetime [5]. Most individuals remain asymptomatic and clear HPV infections spontaneously. This is probably owing to an active immune response. A small percentage of patients develop clinically or histologically recognizable precancers that can persist and may develop over time into invasive cancer. By cervical cytological screening using Papanicolaou (Pap) smears,

cervical precancers can be detected; this has successfully reduced the mortality rate from cervical cancer in developed countries. True precancers, the so-called high-grade cervical intraepithelial neoplasias (CIN), have to be removed surgically. Removal of part of the uterine cervix can have an adverse effect on pregnancy outcomes, resulting in an increased risk of prematurity and low birth weight in future pregnancies [6]. This effect is independent of the surgical technique used. By contrast, cytologic screening and prophylactic and therapeutic vaccination have the potential for primary and secondary prevention. In addition, therapeutic vaccination does not lead to loss of cervical tissue. Most importantly, vaccination concepts are especially attractive for developing countries, where screening programs are minimal and cervical cancer remains the second leading cause of cancer-related deaths among women [7].

Prophylactic vaccines have been proven to be highly efficacious. They have been introduced since 2006 and have the potential to reduce the burden of cervical cancer by approximately 70% [8–10]. However, they have no therapeutic potential owing to their mechanism of action via antibodies targeting the L1 capsid antigen, which is not expressed in the persistently HPV-infected basal epithelial cells [11]. Therefore, therapeutic vaccines may still offer benefits for the treatment of high-grade lesions (or worse), which will still develop despite the availability of prophylactic vaccines because prophylactic vaccines do not protect against all carcinogenic HPV types. In addition, women who are already infected might still progress to cancer if left untreated: when protective immunity fades, breakthrough infections may arise, and not everyone will be vaccinated.

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Therapeutic vaccine strategies

Therapeutic vaccines target antigens present inside the infected cell. In the case of HPV-induced cancers, the predominantly targeted antigens are the viral oncogenes E6 and E7, because their sustained expression is required for the maintenance of the cancerous phenotype [12]. Several therapeutic vaccines target the E2, E5, E6 and/or E7 proteins by many different strategies and were tested in clinical trials over the past 15 years [13]. These vaccines activate the patient's cellular immune response to recognize and kill cells that express HPV proteins. Initial trials in patients with advanced cervical cancer, who tend to have decreased immune function, showed vaccine safety. In more recent and ongoing immunotherapy trials, patients with earlier invasive or premalignant disease were included. Patients with lesions induced by mucosal or cutaneous HPVs, such as cervical and vaginal intraepithelial lesions, anal intraepithelial lesions, condylomas and anogenital warts, head and neck squamous cell carcinomas and recurrent respiratory papillomatosis were treated. These trials were placebo-controlled and blinded, with higher patient numbers and fewer immunosuppressed individuals. This facilitates interpretation of antigen-specific immunity and its correlation with clinical efficacy. Performing therapeutic vaccination trials in patients with precancerous lesions will need very close monitoring when lesion development is to be observed for longer periods of time. To participating patients, an effective definitive treatment is withheld that is, on the other hand, not without side effects and is probably an overtreatment in many of the patients, who could experience spontaneous lesion regression. Therefore, performing such vaccination trials is not unethical.

In addition, the choice of antigens is changing: initially, and for pragmatic reasons, the HPV E7 protein was regarded as an ideal target. Recently, it was shown that the larger E6 and E2 proteins may be more immunogenic and more effective targets [14–16].

In addition, new basic immunology findings, such as the presence and characterization of immunosuppressive regulatory T cells, have influenced the strategies of immunotherapy [17].

Since the local tumor environment is immunosuppressive or not immunostimulatory, a combination of vaccination with topical immune stimulatory agents such as imiquimod, or other proinflammatory mediators such as interferons, may improve the performance of systemic immunization by vaccines [18].

Examples of vaccines in clinical trials

Vaccination strategies used to target HPV proteins in clinical trials were mostly based on peptides/proteins, viral vectors, DNA or dendritic cells, as exemplified in Table 1 [19]. For example, Muderspach and colleagues vaccinated women with high-grade vulval intraepithelial neoplasia (VIN) with two minimal T-cell epitopes of the HPV 16 E7 antigen in four escalating doses emulsified in Freund's adjuvans [20]. Only three of the 18 patients cleared VIN until 3 weeks after the last immunization. Partial regression was observed by colposcopy in six additional patients. Immunological responses were detected in ten of 16 patients. Virus DNA clearance in cytological samples was detected in 12 of 18 patients; however, in biopsies, viral RNA was still detectable. The short follow-up period may have prevented better regression results.

The recombinant protein HspE7, a fusion of heat shock protein 65 (Hsp65) from *Mycobacterium bovis* and HPV 16 E7, has shown clinical responses when administered to patients with cervical and anal precancerous lesions [21–23]. Patients with recurrent respiratory papillomatosis, who were treated with HspE7 after debulking surgery, had a significantly extended time until repeat surgery [24].

Recombinant protein technology takes advantage of the ability of truncated L1 proteins to form virus-like particles (VLP) identical to the manufacturing techniques for prophylactic vaccines. Heterologous antigens can be fused to the truncated L1 and are incorporated into the particles now termed chimeric virus-like particles (CVLP) [25]. CVLPs show high prophylactic and therapeutic efficacy in animal models and immunogenicity in humans, where they induce cytotoxic CD8+ T cells to E7 [26,27]. We have used a CVLP consisting of HPV16 L1 fused to amino acids 1–55 of HPV16 E7 in a placebo-controlled, blinded Phase I/II clinical trial [28]. A total of 36 patients with CIN grade 2/3 lesions received four applications, and were equally randomized to receive placebo or two different dosages of the vaccine. The vaccine was safe and well tolerated at both dosages, with minimal side effects comparable with the prophylactic vaccines. Importantly, the CVLP vaccine induced high antibody titers to the L1 and also to E7. The response followed an anamnestic pattern in these actively HPV 16-infected patients. T-cell responses to L1 were measured in 90% of patients, while induced or enhanced T-cell responses to E7 were found in 50% of the vaccinees. Although not statistically

Table 1. Exemplified published clinical immunotherapy trials for human papillomavirus-induced lesions .

Class of vaccine	Composition	Target antigen	Trial Phase	Patients included	Immunological responses	Clinical responses	Ref.
Peptides/proteins	Peptides of HPV 16 E7 aa 12–20, and aa 86–93, emulsified with incomplete Freund's adjuvant	HPV 16 E7	I	18 patients with CIN 3, HPV 16-positive and HLA-A2-positive	10/16 increased reactivity in cytokine release or cytotoxicity assay	3/18 CR, 6/18 PR, 9/18 NR, 12/18 HPV-DNA negative	[20]
	Fusion protein of *Mycobacterium bovis* Hsp65 and HPV 16 E7 protein (HspE7, SGN-00101)	HPV 16 E7	II	21 patients with LSIL, HSIL, ASCUS or AGUS	9/17 IFN-γ ELISPOT	7/20 CR, 1/20 PR, 11/20 SD, 1/20 NR	[21]
	HspE7 (SGN-00101)	HPV16 E7	II	58 patients with CIN 3	ND	13/58 CR, 32/58 PR, 11/58 SD, 2/58 NR	[22]
	HspE7 (SGN-00101)	HPV 16 E7	I/II	15 HIV I-positive patients with AIN 2/AIN 3	ND	1/15 CR, 4/15 PR, 10/15 NR, 3/5 responders HPV-DNA-negative after 48 weeks	[23]
	HspE7 (SGN-00101)	HPV 16 E7	I/II	27 patients with recurrent respiratory papillomatosis with baseline lesion debulking	ND	Significant increase in median intersurgical interval (55–106 days)	[24]
	HPV 16 L1E7 chimeric VLP or placebo	HPV 16 L1 and E7 (aa 1–55)	I/II	39 patients with CIN 2 or CIN 3	Vaccine: 24/24 positive/enhanced antibody; Placebo: 0/12 positive/enhanced antibody	Vaccine: 10/23 PR (histology); 6/23 HPV 16 DNA-negative; Placebo: 4/12 PR (histology); 1/12 HPV 16 DNA-negative	[28]
Heterologous prime/boost protein/viral vector	Prime: HPV 16 L2/E6/E7 fusion protein (TA-CIN) Boost: recombinant vaccinia virus (TA-HPV) expressing HPV 16 and HPV 18 deactivated E6/E7	HPV 16 E6/E7	II	27 patients with VIN 3 and two patients with VAIN 3	14/24 antibody enhanced; 10/27 proliferative response; 11/25 IFN-γ ELISPOT	1/29 CR, 5/29 PR, 18/29 SD, 5/29 NR	[30]

aa: Amino acid; 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; ELISPOT: Enzyme-linked immunospot; HLA: Human leukocyte antigen; HPV: Human papillomavirus; HSIL: High-grade squamous intraepithelial lesion; HSP: Heat-shock protein; IFN-γ: Interferon-γ; LSIL: Low-grade squamous intraepithelial lesion; ND: Not determined; NR: Nonresponder; PR: Partial responder; SD: Stable disease; VAIN: Vaginal intraepithelial neoplasia; VIN: Vulval intraepithelial neoplasia; VLP: Virus-like particle.

Table 1. Exemplified published clinical immunotherapy trials for human papillomavirus-induced lesions (cont.).

Class of vaccine	Composition	Target antigen	Trial Phase	Patients included	Immunological responses	Clinical responses	Ref.
Viral vector	Modified vaccinia virus Ankara, with BPV E2	BPV E2 (possibly cross-reacting to HPV)	II	54 patients with CIN 2 or CIN 3, intralesional injection	Vaccine: 34/34 positive antibody and CTL responses; Conization alone: ND	Vaccine: 19/34 CR, 15/34 PR; Conization: 16/20 CR, 4/20 NR (recurrence)	[16]
	Recombinant vaccinia virus (TA-HPV) expressing HPV 16 and HPV 18 deactivated E6/E7	HPV 16 and HPV 18 E6 and E7	I/II	29 patients with untreated stage Ib or IIa once before and once after radical hysterectomy	8/29 with elevated antibody response to HPV E6 and/or E7 4/29 with specific CTL	ND	[29]
Dendritic cell-based	Mature autologous DC pulsed with recombinant HPV 16 E7 or HPV 18 E7 protein	HPV 16 E7 or HPV 18 E7	I	15 stage IV cervical cancer patients	3/11 antibody response; 4/11 proliferative response; 3/11 IFN-γ ELISPOT	None	[32]
	Mature autologous DC pulsed with HPV 16 E7 or HPV 18 E7 and KLH	HPV 16 E7 and HPV 18 E7	I	Ten tumor-free patients after radical hysterectomy	10/10 antibody responses; 10/10 CD4+ T cells by IFN-γ ELISPOT; 8/10 CD8+ T cells by IFN-γ ELISPOT; 10/10 DTH to E7 and KLH	ND	[33]

aa: Amino acid; 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; ELISPOT: Enzyme-linked immunospot; HLA: Human leukocyte antigen; HPV: Human papillomavirus; HSP: Heat-shock protein; IFN-γ: Interferon-γ; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion; ND: Not determined; NR: Nonresponder; PR: Partial responder; SD: Stable disease; VAIN: Vaginal intraepithelial neoplasia; VIN: Vulvral intraepithelial neoplasia; VLP: Virus-like particle.

significant, owing to low numbers of patients, there was a clear trend for clinical improvement in the vaccine versus placebo group. Histological improvement was observed in only 25% of placebo recipients, but in 39% of vaccines, and 56% of those were free of HPV DNA by the end of the study. Since the vaccine was not adjuvanted with additional immunostimulatory agents, there is room for improvement.

As an example for a recombinant virus, a vaccinia-based vector, termed TA-HPV, expressing mutated HPV 16 E6/E7 and HPV 18 E6/E7 was given to cervical cancer patients with early invasive disease around surgery [29]. Safety, tolerability and immunogenicity were acceptable. Currently, the efficacy to prevent recurrence is investigated when TA-HPV is given in an adjuvant setting after surgery [29,101]. In addition, the vaccine was tested in conjunction with protein-based strategies in order to circumvent any anti-vector immunity by heterologous prime-boost immunization [30].

Dendritic cell-based therapies and, more recently, DNA-based therapies will not suffer from such difficulties. Pre-existing or induced immunity is not expected to pose a problem. Both strategies are still being pursued. Despite very promising preclinical results with dendritic cell-based vaccines, the current data from clinical trials underline the problem of immune evasion and immune suppression in patients with advanced cervical cancer [31–33]. Obstacles to be solved are the compromised antigen presentation by loss of HLA expression or components of the antigen-processing machinery, the strongly immunosuppressive cytokine microenvironment within the tumor, and the recruitment of immunosuppressive cells such as regulatory T cells. Only when solving these specific problems by combination therapies, such as tumor debulking, reversion of immune evasion, and a powerful immune stimulation, can this strategy possibly be successful in late-stage patients. It will, however, inherently have the disadvantage of a highly individualized therapy.

The immunogenicity of DNA vaccines in larger animals and humans has been disappointing in clinical trials so far [34,35]. Initial clinical trials have demonstrated their safety and that they are well tolerated, but objective tumor or clinical responses were rare. Recently, their advantages and new developments became obvious, as is discussed below.

New strategies in preclinical & early clinical testing

Peptide-based vaccines

Advances in delivery, stability and design of peptides have lead to promising results and have fostered renewed enthusiasm. The use of long or overlapping peptides broadens the range of antigenic epitopes presented and the applicability irrespective of the HLA-makeup of the patient. Peptides can be synthesized with modifications conferring protease-resistant peptide bonds to regulate their processing, or with targeting signals to enhance presentation [36].

Progress in the therapy with peptides as antigens has mostly been made with their combination with novel immune stimulatory reagents and methods to encapsulate them for delivery. A study with transplanted tumor cells in mice compared the efficacy of oligodeoxynucleotides (CpGs), which are strong activators of innate and adaptive immune responses, with classical Freund's adjuvant to induce a response to the HPV 16 E5 (25–33) peptide, and demonstrated that CpGs stimulated stronger cytotoxic T lymphocyte (CTL) responses. Furthermore, the effector/memory/recall phase induced by the E5 (25–33) peptide was superior to that induced by the HPV 16 E5 protein delivered by vaccination with a recombinant adenovirus, although similar chronological patterns of immune response were observed [37].

A novel adjuvant used to enhance peptide immunogenicity is very-small-size proteoliposomes, which are produced by using anionic detergents to incorporate gangliosides into the outer membrane protein complex of *Neisseria meningitidis*. Detergents and gangliosides facilitate uptake of peptides in proteoliposomes by professional antigen-presenting cells, the key activators of adaptive immune responses. Vaccination with the HPV 16 E7 (49–57) minimal CTL peptide and very-small-size proteoliposomes protected mice against tumor challenge, induced tumor regression and induced E7-specific CD8+ T-cell responses [38].

Another recent therapeutic peptide vaccine used VacciMax® (ImmunoVaccine Technologies Inc., Nova Scotia, Canada), a proprietary combination of encapsulated CTL epitopes fused to the universal T-helper epitope PADRE® and combined with an adjuvant. Activating a T-helper response is beneficial, if not a prerequisite, for a sustained CTL response. A single administration of VacciMax

induced a long-lasting CTL response, complete protection against tumor challenge and rapid tumor eradication in a therapeutic setting. The significance of the study was that tumor eradication was achieved in less than 3 weeks postimmunization after a single treatment in aged mice with very-large-sized transplanted tumors (>700 mm^3) [39].

A very attractive approach of vaccination uses long overlapping peptides as antigen. These synthetic peptides represent the complete antigen in several fragments. They are taken up by antigen-presenting cells, are then processed to the respective minimal T-cell epitopes, are loaded onto the major histocompatibility complex (MHC) and presented to T cells. Such activated T cells will find the corresponding naturally processed epitopes on the tumor cells and are activated to exert their cytolytic function. This approach was tested in a clinically relevant rabbit model. Cutaneous infection of rabbits with cotton tail rabbit papillomavirus (CRPV) was used as a preclinical model for persistent HPV infection, and for recurrent respiratory papillomatosis. The study was designed to test the therapeutic efficacy of long CRPV E6 and E7 peptides containing both CD4$^+$ T-helper and CD8$^+$ CTL epitopes. Following peptide vaccination, wart growth was significantly controlled and latent infection with CRPV was abrogated [40]. In humans, peptide vaccines have the disadvantage that the diverse HPV types causing the disease have to be known. Long peptide vaccines circumvent the necessity to know the patient's HLA type, which is obligatory for minimal epitope peptide vaccines.

DNA-based vaccines

The concept of DNA vaccines is the uptake of eukaryotic expression plasmids into cells and expression of an encoded antigen to which an immune response is then induced. Advantages of DNA vaccines potentially include:

- Full-length cDNA of a given antigen provides all potential epitopes, overcoming the limitations of MHC restriction;

- Plasmid DNA is inexpensive to produce and easy to purify;

- The plasmid DNA produced in bacteria contains unmethylated CpG motifs that act as potent immunological adjuvants;

- Plasmid DNA is stable and can be conveniently handled and distributed, also without a cold chain, which is important for developing countries;

- The safety of DNA vaccines has been demonstrated, and only a few mild side effects were observed;

- No pre-existing immunity or antivector immunity to plasmid DNA-based vaccines is seen, enabling repetitive booster vaccination.

Viral oncogenes such as HPV *E6* and *E7* as vaccine genes in DNA vectors carry the potential for transformation of cells owing to their oncogenic activity. It is a mandatory prerequisite to inactivate this transforming activity. To this end, we have developed a generic strategy by reorganization of the genetic sequence, termed 'shuffling'; for example, dissection of the original sequence of the *E7* gene at known sites responsible for transforming activity and fusion of the fragments in a 'nonsense' order. Thereby, all putative HLA epitopes are still contained. We could show that the rearranged primary sequence was devoid of transforming properties and induced E7-specific CTL in mice and in humans after *in vitro* immunization [41]. Immunogenicity of the sequence was significantly enhanced by codon optimization and by adding a Kozack sequence for more efficient translation in human cells. This vaccine awaits clinical testing. Addition of sequences enhancing expression and targeting to MHC class I and II compartments has also been used successfully by other laboratories to modulate DNA vaccines [42]. Moreover, this family of DNA constructs targets dendritic cells and extends their lifespan, which increases immunity significantly.

In accordance with the use of Hsp65 recombinant fusion protein as vaccine, a recent study explored a combination of antigen DNA with sequences of Hsp70 or Hsp110, and demonstrated that the use of autologous Hsp70 potently enhances antigen-specific immune responses [43].

Another study tested the efficacy of a DNA-based vaccine against a human HPV 16$^+$ esophageal squamous cell carcinoma cell line in a xenograft model by engrafting immunocompetent human peripheral blood lymphocytes into severe combined immunodeficiency mice. This study suggests that prophylactic vaccination delayed tumor growth through CD8$^+$ T-cell-dependent CTL-induced apoptosis [44]. Future and current clinical trials will show if this translates into DNA vaccine efficacy.

New viral & bacterial vector-based therapies

An attractive approach for therapeutic vaccination is delivery of HPV antigens in bacteria or

recombinant viruses. Targeting and processing of the antigen follows natural routes, and the vector provides 'danger signals' required to initiate an immune response. These important signals are not efficiently delivered by peptides and DNA.

A novel bacterial-based vaccine uses live *Lactococcus lactis* strains expressing cell wall-anchored HPV 16 E7 and a secreted form of IL-12 as an adjuvant. The vaccine was administered mucosally. The study assessed both prophylactic and therapeutic efficacy in a murine tumor model. Immunized mice demonstrated 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 through both CD4+- and CD8+-dependent T-cell responses [45].

The best studied bacterial vector for the therapy of experimental HPV-induced lesions is *Listeria monocytogenes*. In this approach, the bacterium is genetically engineered to secrete the tumor-associated antigen fused to a molecular adjuvant enhancing the overall immune response. Importantly, *Listeria*-based vaccines were assessed in a transgenic model system with tissue-restricted expression of HPV 16 E6 and E7 in the thyroid, mimicking HPV antigens as 'self' antigens. Regression of solid implanted tumors was observed, although at a lower frequency than in wild-type mice. The E7-specific CD8+ T cells induced in transgenic mice were of lower avidity and lower frequency when compared with the wild-type mice [46].

The significance of this result is that 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 were not deleted during thymopoesis. This is interesting *per se*; however, there is no evidence of deletion of high-affinity T cells against HPV in patients and the significance of this finding is therefore not clear.

As described above, the development of virus-based delivery systems has focused on using viral envelopes, L1 VLPs, without the genetic material of the virus, for prophylaxis and therapy. Heterologous viruses can also be employed.

Envelopes of influenza virus can deliver encapsulated HPV 16 E7 protein. Immunized animals developed a strong cellular immune response, which in a prophylactic setting could prevent the outgrowth of transplanted tumors in mice [47]. Immunization with replication-deficient adenovirus particles carrying HPV16 E7 linked to calreticulin resulted in a dramatic increase in the frequency of functional E7-specific CD8+ T cells. This vaccine was able to prevent tumor growth in a prophylactic setting, was able to eradicate established tumors, and induced long-lasting immunological memory [48].

Another viral-vector-based strategy is the use of alphaviruses such as Sindbis virus, Venezuelan equine encephalitis virus (VEE) or Semliki Forest virus. These recombinant RNA viruses express the RNA of the *E6* and *E7* oncogenes exclusively in the cytosol. This eliminates the potential integration of these oncogenes into the host genome. The most recent studies using alphavirus-based vectors to treat HPV-induced lesions have been conducted with Semliki Forest virus or VEE vectors. VEE replicon particles have dendritic cell tropism, delivering HPV antigens directly to antigen-presenting cells and inducing antitumor immunity for HPV-induced lesions [49]. Studies utilizing VEE containing mutated, fused *E6* and *E7* genes of HPV 16 demonstrated complete protection from tumor challenge. Eradication of established tumors was observed in 90% of *HLA-A*0201* transgenic mice challenged with tumor cells in a therapeutic setting [50]. Eradication of tumors in the *HLA-A*0201* transgenic mice is significant because these mice bear the most common HLA in the human population. The encouraging results from these studies provide the basis for the use of alphaviruses in clinical trials of HPV-induced lesions.

Future perspective

Efforts to develop and test novel strategies targeted towards HPV should help to advance the field of cancer immunotherapy significantly in the next 5–10 years. Using this target disease for 'proof-of-concept' studies in the development of therapeutic vaccines has obvious advantages: defined tumor antigens, strong natural and induced immune responses, and the inherent problem of immune evasion can be studied. Considerable progress has been made in the development of prophylactic vaccines against HPV. This may lead to the misconception that development of therapeutic approaches becomes obsolete. By contrast, owing to the heterogeneity of dysplasia-inducing HPV types, the high number of currently infected persons, possible breakthrough infection when prophylactic immunity fades with time, and the need in developing countries that may not have access to widespread prophylactic vaccination, there is a

continued need for therapeutic vaccines. Ideally, HPV vaccines comprise both prophylactic and therapeutic activity to yield best protection. As preclinical studies continue to advance, the prospect of therapeutic vaccination to treat existing lesions seem good in the near future. The positive consequences may include less invasive and less disfiguring treatment and fewer recurrent or progressive lesions.

Future tasks will be to design adequately controlled clinical trials for the new emerging vaccination strategies, with sufficient patient numbers and avoiding end-stage patients in order to be able to identify immune correlates of protection. Until now, certainty has been obtained that HPV vaccination is safe, immunity can be induced, and efficacy – at least in early and premalignant disease – can be expected. Therefore, the promise is that in the next 5–10 years, such trials will be conducted. If the progress made in other fields of immunology is incorporated, progress in therapeutic HPV vaccines will be stimulated. Meaningful animal models that mimic natural disease in humans belong to this progress. In addition, advances in basic immunology will help vaccines to overcome a suppressed immune system, a tolerogenic disease and a target cell that has already evaded the immune system for years and escaped elimination. Then there may also be hope for patients with advanced disease. Increasing the number of antigen-specific T cells alone will probably not be enough. The homing to tumor sites and effector functions or their recovery will also be improved. Potentially, this could be achieved by the combination of vaccination with local application of immune adjuvants supporting the local immune responsiveness in the patients. If immunotherapy is also combined,

Executive summary

Prevention of cervical cancer

- Based on the invariable association of human papillomavirus (HPV) infection and cervical cancer prevention, options are:
  – Screening for precancers by cytology or HPV test, which has been proven to reduce cancer by 80%.
  – Prophylactic vaccination to prevent initial infection by the most prevalent viruses, which has the potential to further reduce cervical cancer incidence by 70%.
  – Therapeutic vaccination to prevent progression and to eliminate virus-infected cells.

Therapeutic vaccine strategies

- Strategies under development have to consider:
  – Choice of antigen: early viral gene products with high immunogenicity, not late genes, are target antigens on transformed cells.
  – Delivery systems have to induce strong immunity of the adequate type, for example, cytotoxic T cells.
  – Adjuvants should deliver 'danger signals' to enhance and direct the immune response.
  – Circumstances reflecting the clinical situation (tumor size, aged patients, suppressed immune system, immune evasion) have to be considered during preclinical development of therapeutic vaccines.
  – Clinical trials should be well designed and performed in a sufficient numbers of patients with early disease stages to be able to interpret the results.

Future promising therapeutic vaccination approaches

- The most promising vaccines are:
  – Long overlapping peptide vaccines owing to their safety, ease of synthetic production and representation of the complete antigen.
  – DNA vaccines owing to their stability, low cost and ease of modification.
  – Chimeric virus-like particles owing to the combination of prophylactic and therapeutic activity.
  – Viral vector vaccines based on alphaviruses owing to their safety by avoiding DNA intermediates and targeting dendritic cells.

Specific problems to be solved

- Development of meaningful model systems reflecting the real clinical situation of the disease.
- Heterogeneity of HPV types: possibly cross-reacting vaccines should be considered.
- The tolerizing feature of HPV infection and cancer leading to immune suppression and immune evasion may be reversed by local treatment with adjuvants or immune-response modifiers.

Conclusion

- The pace and enthusiasm of investigating therapeutic vaccines has increased after a period of disappointment owing to lack of clinical responses and concentration on prophylactic vaccination. Many diverse and sophisticated approaches are under investigation and clinical testing is performed in patients with early invasive and premalignant disease.
- Specific immune responses are regularly induced without significant toxicity. Therefore, the basis is there for successful and well-designed clinical trials and development of a therapeutic vaccine that may further reduce cervical cancer burden.

current treatment results may be further improved and may lead to a decline in cervical cancer incidence and recurrence.

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