Chapter 7

Therapeutic Human Papillomavirus (HPV) Vaccines

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First Published July 15, 2016

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Introduction

Cervical cancer trends to occur in midlife. Most cases are found in women younger than 50. More than 15% of cases of cervical cancer are found in women over 65 but, these cancers rarely arise in women who have regular tests for cervical cancer screening before they were 65. The most important etiological factor in cervical cancer and precursor lesions is persistent infection by HPV. Thus, an effective HPV vaccine program will be decreasing mortality and morbidity of diseases mediated HPV. FDA [Food and Drug Administration of the United States] approved two profilactic vaccines for prevention of HPV dissemination. These vaccines have no therapeutic efficacy on infections and lesions of HPV. Studies on therapeutic vaccines have been proceeding. Therapeutic vaccines purposes be developed cellular immunity against infected cells by stimulating the patients’ immune responses [1].

HPV types are non-enveloped, icosahedral symmetric viruses about 55 nm in size. Viral genome consists of circular double-stranded DNA, encodes for early proteins [E1, E2, E4, E5, E6, E7] and for late [L1, L2] proteins. Early proteins play role in virus replication and cell transformation. E6 causes to p53 suppression. E7 proteins interact with retinoblastoma [pRb] proteins. Late proteins are the structural units of viral capsid. Late genes encode viral capsid proteins, participate to packing of new synthesized virions. E2, E5, E6 and E7 are regarded as being crucial for malignant progression and HPV immune escape. E2 and E5 are expressed soon after infection, initiating carcino-
genic progression. E6 and E7 are the major transforming proteins. Recently identified genes E3 and E8 are located in early gen region. These genes are found only in a few papillomavirus types [HPV 1, 11, 16, 31, 33] [2-5].

HPV infects to basal cells of cervical epithelium. Integration of HPV DNA into the host cell chromosome is important to viral persistence and carcinogenic effects of virus. Integration may be randomly along cell genome and this case may lead to increase of E6/E7 gen expression leading to oncogenesis. However, increased of E6/E7 expression can occur also without integration. HPV integration in the most of cervical cancer cells causes to breaking in E2 gen region. This situation have been leading to increased of E6/E7 oncogen expressions. E6 and E7 proteins of especially high risk HPV types [e.g. types 16 and 18] interact with tumor supressor proteins such as p53 and retinoblastoma [pRb] proteins, respectively and inhibit to their functions. Thus, it causes uncontrolled proliferation and immortalization of cells. The binding of E6 protein to p53 leads to eclipse in the G1 phase of cell, apoptosis and DNA repairing are stopped. On the other hand, E7 protein interacts with pRb and mitotically interactive cellular proteins such as cyclin-E. Thus, it provides to stimulation of cellular DNA synthesis and proliferation [6-9].

HPV E6/E7 antigens are ideal and target vaccines to be developed therapeutic vaccine because of expression in the infected cells HPV mediated. Nowadays, therapeutic HPV vaccines had been developed to targeted E6 and E7 antigens. E7 has been used more frequently than E6. Because it is more abundantly expressed and is more highly conserved than the E6 protein. These vaccines; peptid-protein based vaccines, live-attenuated vector vaccines, cellular-based vaccines and nucleic acid-based vaccines. All of them have various advantage and disadvantages. These vaccines try to control of HPV infection by cellular immunity [10].

**Protein-Based Therapeutic HPV Vaccines**

Protein-based vaccines contain all of antigenic epitopes. These vaccines have no the suppression problem of MHC. Unfortunately, these vaccines are introduced to immune system by MHC Class 2-mediated because of some special feature. This event composes a dominant antibody response rather than CTL responses. New strategies have been generated in order to presentation MHC 1 mediated. To MHC1 mediated presentation, to avoid this disadvantage and to improve immunogenicity, adjuvant [liposome-polycationic DNA transporter particles and ISCOMATRIX] have been added into vaccines and/or proteins for therapeutic vaccination have been linked to molecules able to better induce APC presentation [The binding of Bordetella pertussis adenin cyclase to HPV 16 E7 antigen interacted with integrin receptors of dendritic cells and the binding of translocation region of Pseudomonas aeruginosa exotoxin to HPV 16 E7 antigen].
Therefore, the dendritic cell maturation will be stronger to stimulate and cytokine expression will be inducted [11-13].

Various protein vaccines have moved to clinical trials. Only few protein-based vaccines have reached the clinical phase. In 31 patients, immunization with HPV-16 E6/E7 fusion protein mixed with the ISOMATRIX adjuvant was reported to be safe and immunogenic [13]. Another vaccine is TA-CIN, was tested to 40 healthy volunteers by intramuscular. This vaccine has been obtained from fusion with E6 and E7 antigens of HPV16 L2 proteins. It has been reported that 8 of 11 volunteers have antigen specific T cell responses. However, when it was tested again in combination with the TLR7-agonist imiquimod in a clinical phase 2 study in 19 women with VIN2/3, complete histological regression of VIN2/3 was observed in 32% of patients at week 10 post-vaccination, increasing to 58% at week 20, and 63% at week 52. HPV-16 E7 fused to Haemophilus influenzae protein D, mixed adjuvant AS02B has been evaluated in and was reported to induce significant E7 specific CTL responses on CIN1-CIN3. Einstein reports that 13 of 58 patients are used hsp 65-E7 antigen from Mycobacterium Bovis for 3 months have complete pathological response. That is, CIN is no found in the LEEP specimens of these patients. 32 patients have partial clinical response [regression of colposcopic lesion by more than 50%] and 11 patients have stable disease. Not one of cases revealed progression. Another study using the same vaccine in 20 CIN 3 patients shown 35% of complete regression. 320 patients who have HPV6-mediated genital warts were vaccinated with an HPV6 L2-E7 fusion protein mixed with the AS02A adjuvant. It reports that antigen specific antibody response, but no difference in wart recurrence was observed between the vaccine and placebo group. HPV 16 L2/E6/E7 fusion has effect of tumor protection and cytotoxic T cell responses while HPV6 L2-E2 fusion has effect on genital warts [14-17].

The future of protein-based vaccines depend on increase of immunogenic characteristics and support of CD8 cell response mediated adjuvants and fusion proteins. The possibility of combined prophylactic and therapeutic vaccines may offer the best chance for a significant reduction in the incidence of death from cervical cancer worldwide.

Peptid-Based Therapeutic HPV Vaccines

Peptid-based vaccines offer several advantages over whole proteins, particularly with regards to safety and ease of production. However, these vaccines are low immunogenic and require to patients’ human leukocyte antigen [HLA] typing. Adjuvant use, lipopeptide conjugation and direct delivery to dendritic cells [DCs] are some of the approaches employed to overcome these problems. Synthetic peptides used in vaccination can be classified in
two categories: Synthetic long peptides [SLPs] and specific epitope [short] peptides. Long peptides reserve CD8+ CTL and CD4+ T helper cell [Th] epitopes, whereas short peptides usually involve only a single defined CTL epitope [18-20].

Several long peptide-based therapeutic HPV vaccines have been tested in both experimental and clinical studies. Kenter reports that the observation of a durable and complete regression in 47% of VIN3 patients treated with a HPV16 E6 and E7 SLP vaccine is a major breakthrough for the whole cancer vaccination. Solares shows that CIGB-228 vaccination was well tolerated and capable to induce IFNγ-associated T cell response in women with high-grade CIN. He observed lesion regression and HPV clearance in several patients. A phase 1 study with synthetic long peptide vaccine in advanced cervical cancer demonstrated low toxicity and high immunogenicity. Takeuchi presents a phase 2 study of multiple peptides cocktail vaccine for treatment-resistant cervical and ovarian cancer, in May 2015. In 21 cervical cancer patients they observed two complete responses, good tolerability and survival of 15.4 months [21-24].

These new vaccines need to be evaluated in larger clinical studies. Researchers highlight the importance of using innovative adjuvants, could significantly increase not only immunological but also clinical responses to the vaccines.

Live-Attenuated Vector-Based Vaccines

Viral Vector-Based Vaccines

Viral vector-based vaccines have both high infection efficacy and high antigens expression, encoded by the virus in the infected cells. Many viral vectors, such as adenoviruses, alphaviruses, vaccinia viruses have been tested. These are the most priming virus-based vaccines [25,26]. The first clinical study was reported in eight patients with advanced cervical cancer. A live recombinant vaccinia virus expressing HPV-16 and 18 E6/E7 proteins was carried out as a single dose to patients. 3 out of 8 patients had an HPV-specific antibody response. No significant side-effects were observed [26].

In 2002, two vaccinations at least 4 weeks apart, 2 weeks before surgery with a live recombinant vaccinia virus expressing modified forms of the HPV-16 and 18 E6/E7 proteins [TA-HPV] was performed to 29 patients with early-FIGO stage cervical cancer to assess the safety and immunological effects of vaccination. Vaccination was well tolerated in all patients, and no serious side effects were observed. After a single vaccination, HPV-specific CTLs were found in four patients. Eight patients had HPV-specific serological responses [25]. In 2006, fifty-four women with high grade lesions [CIN2 and CIN3] were either with an MVA-E2 therapeutic vaccine [modified vac-
cinia Ankara virüs] or conization. Histological analysis showed complete regression in 20 out of 34 women after treatment with MVA-E2, with the development of HPV-specific antibodies. 11 women had a 50% reduction in lesion size. In two other women, the lesion was reduced to CIN2 and in one patient the lesion was reduced to CIN 1. In 80% of patients treated with conization were no observed the lesion but, three patients had recurrence of lesions one year later. This phase 2 study reports that vaccination with MVA-E2 establishes to be very effective in stimulating the immune system against papillomavirus and in regressing of CIN2 and CIN3 lesions[27].

In another study, 21 patients with HPV 16 related CIN2/3 were treated with FG4001, a modified vaccinia Ankara viral vector encoding HPV-16 antigens [E6/E7] and an adjuvant IL-2. The patients received three weekly subcutaneous injections of TG4001. 48% of patients had clinical response at month 6, with seven complete responses. TG4001 is designed to have a two antiviral approach. One of them is to alert the immune system specifically to HPV 16-infected cells and the other one is to further stimulate the infection-clearing activity of the immune system through IL-2. These promising data warrant be assertive about CIN 2/3 treatment in the future [28]. In 2013, recombinant adenovirus p53 combined with chemotherapy was tested to 40 patients. This study reports that this vaccine combined with chemotherapy is an effective treatment for the patients with advanced cervical cancer [29].

### Bacterial Vector-Based Vaccines

Another strategy for tumor-targeted immunotherapy is the use of bacterial vectors. Attenuated strains of Listeria monocytogenes are currently being evaluated for this aim.

The results of a prospective randomized study employing ADXS11-001 vaccine in 110 women with recurrent cervical cancer were presented American Society of Clinical Oncology meeting in 2014. This study suggests that ADXS11-001 is an active agent in recurrent cervical cancer [30]. An international phase 3 study [AIM2CERV] of ADXS11-001 as adjuvant treatment of advanced cervical cancer is ongoing in collaboration with Gynecologic Oncology Group [31].

### Cellular-Based Vaccines

#### Dendritic Cell-Based Vaccines

Dendritic cells [DC]s have ability to initiate and control T-cell response. A pilot study was carried out in 15 women with advanced cervical cancer treated with autologous monocyte-derived DCs pulsed with recombinant HPV-16 E7 or HPV-18 E7 oncoprotein. This study reports that can be induce T cell response and well tolerated, but no objective responses were observed [32]. In early cervical cancer, a phase 2 randomized study was carried out in surgery-treated patients with high risk cervical cancer.
They used as adjuvant treatment cisplatin alone versus cisplatin combined with DC-CIK cells. The immun function was significantly improved in the experimental arm and the cumulative recurrence rate was significantly lower than that in the control arm [33]. Despite some promising results DC-based treatments have several limitations, such as expensive procedures and labor-intensive. Furthermore these vaccines have short half-life and absence of proliferation limit long lasting immun response.

**Tumor Cell-Based Vaccines**

Tumor cells may be genetically modified in-vitro with genes encoding cytokines to increase their immunogenecity. Several HPV-positive tumor cells have been transduced with cytokine genes such as IL-12, IL2, and GM-CSF. Giving modified malignant cells into patients increases confidence doubt. So tumor cell-based vaccines has been not considered to treatment in early stages cervical cancer or CIN [34,35].

**Adoptive T-Cell Therapy [ACT]**

Recently, adoptive T cell therapy [ACT] has been emerging as a promising cancer therapy. In a phase 2 trial, this therapy was utilized in chemotherapy-resistant metastatic cervical cancer. Although this technic is technically difficult, it can show a dramatic efficacy in treatment of advanced cervical cancer [36,37].

**Nucleic Acid-Based Vaccines**

**DNA-Based Vaccines**

DNA-based vaccines are safe and can be easily produced. The main advantage of them is the production of non-attenuate, non-replicate antigens. Furthermore they are capable of inducing both CTL and Th, B cell immunity. DNA vaccination does not induce anti-vector autoimmune in the patient, therefore they may be multiple administered for long-term protection [38, 39]. This case can be useful to therapeutic cancer vaccination.

In 2004, Garcia reported that ZYC101a [HPV-16/18 E6 and E7-derived CTL epitopes] was to be well tolerated and to promote the regression of CIN in 127 patients with CIN2/3 who are younger than 25 years [40]. Another DNA vaccine study showed that 8 patients had significant immun response and complete regression was observed in three patients [41]. Thereafter, a phase 1 study was performed in 12 patients with CIN3 and 5 patients showed complete regression [42]. In 2015, VGX-3100 vaccine is being tested on CIN2/3 patients in phase 1-2 randomized, double-blind placebo controlled study [NCT01304524]. Among 107 evaluable patients, 53 of the treated patients [49.5%] and 11 of the placebo [30.6%] have complete regression. VGX-3100 is the first therapeutic vaccine to support efficacy against CIN2/3 associated with HPV-16 and HPV-18. This study shows that VGX-3100 vaccine may be a non surgical therapeutic approach for this disease [43].
Another ongoing trial with the same vaccine is a prospective, multicenter, follow-up clinical study to establish recurrence of CIN and long term safety [NCT02411019] [31].

A major advancement in DNA vaccination has been also the introduction of electroporation [EP]. EP provides the application of brief electric pulses to the vaccination site post-vaccination. EP increases plasmid uptake and constitutes a local inflammatory cell infiltrate. Thus, it causes a stronger immune response to the vaccine [44-46].

**Conclusion**

Immunotherapy is one of the promising methods for cancer therapy. About 20% of human cancers are linked to infectious diseases. HPVs linked to cancer express E6 and E7 oncogenes. These oncogenes are an excellent target for cancer immunotherapy. Nowadays effective cancer therapies have been lacking in advanced cervical cancer. In the future HPV therapeutic vaccines must be regarded as a therapeutic option together with other currently available therapies.

**Acknowledgments**

The author thanks to Ajlan Okman for assistance.

**References**


