THERAPEUTIC VACCINATION AGAINST CERVICAL CANCER – ARE WE NEAR?

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Abstract

Therapeutic vaccines developed to target the papillomavirus antigens that are expressed by cervical cancer induce immune responses, but have yet to show clinical efficacy. Transplantable tumour models expressing human papillomavirus antigens do not predict vaccine outcome in the clinic. Understanding how immune responses are influenced in a tumour antigen experienced host, and what surrogate marker or markers reflect the potential efficacy of therapeutic vaccines in the clinic, will be necessary to provide new approaches to immunotherapy for cervical cancer.

Human papillomavirus (HPV) causes cervical cancer

HPV infections are very common and it has been estimated that the lifetime risk for genital HPV infection is over 50% for sexually active women.[1] Human papilloma viruses are the main etiological agent in cervical cancer and more than 99% of cervical cancers contain human papillomavirus DNA.[2] Worldwide it is estimated that there are about 500,000 cancers of the cervix uteri diagnosed each year and 270,000 deaths, mainly in developing countries. Cervical cancer is the second most common malignant disease in women, with nearly 80% of the cases arising in developing countries[3]. Despite the fact that millions of women have papillomavirus infection every year, most of them clear the infection within 18 months (Fig 1).[4-6]

More than 100 different HPV types have been described and they can be grouped into high and low-risk types, depending on their oncogenicity.[7] Of these, about 30 types infect genital tract or other mucosal sites. In cervical cancer, HPV16 is the most prevalent (50-60%), followed by types 18, 31, 33 and 45[8-10]. Papillomavirus infection can also lead to anal, vulvar, penile, oral and tonsillar cancers.[11-12]

Papillomavirus oncogenes as targets for immunotherapy

Human papillomaviruses are small DNA viruses that infect the basal cell layer of epidermis and their protein coding sequences (open reading frames) can be divided into early (E1-7) and late (L1, 2) according to their expression in the viral life cycle. L1 and L2 code for viral capsid proteins and are expressed only in differentiating keratinocytes. HPV has two major oncogenes, E6 and E7. These genes are expressed early in the basal layer of the epidermis and are the major targets for therapeutic approaches. E7 has been shown to be more highly expressed in cancer cells and is more immunogenic than E6 and is widely used in therapy models.[13]

The effectiveness of human papillomavirus oncogenes lies in the fact that they prevent the infected epithelial cells from differentiating. E6 protein binds to p53 and E7 to pRb preventing exit from the cell cycle. Cells continue to divide, permitting ongoing replication of the virus genome. Papillomavirus is a non-lytic virus that divides only in epithelial keratinocytes and encodes mainly short-lived proteins in the cells in which the virus replicates. These characteristics make it a hard target for immunotherapy.

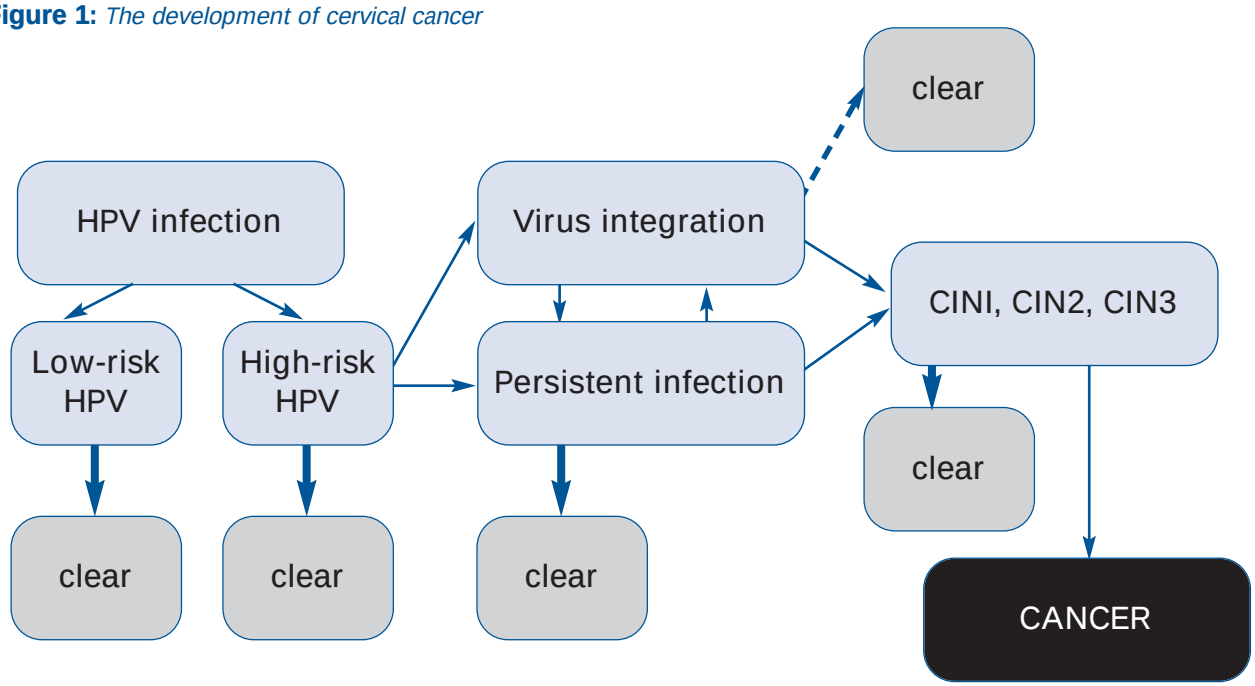
Prophylactic vaccines (Gardasil® HPV6/11/16/18; Cervavix® HPV16/18), in which L1 capsid protein of the virus is used as an antigen, have been so far efficacious in clinical trials, providing 100% protection against infection with the HPV types incorporated in the vaccines.[14] About 30% of cervical cancers are due to HPV types other than HPV16/18. However, these vaccines do not assist with the virus in already infected women. The duration of protection is not currently known and there are millions of HPV-infected women in the world who cannot benefit from the prophylactic vaccine. HPV is prevalent already in newborn babies and the effect of this early infection on prophylactic vaccine efficacy is unknown.[16-18]

An HPV therapeutic vaccine would therefore assist to help reduce the global burden of papillomavirus-related cancers. There are several challenges for development of therapeutic vaccines. These include viral and tumour determined immune escape mechanisms, poor immunogenicity of some viral antigens and the immuno-compromised state of cancer patients.

Advantages of immunotherapy

The main advantage of antigen specific immunotherapy is that it is specific towards tumour cells and does not harm healthy cells. The body's immune system can, in principle, recognise a tumour as non-self and attack cells expressing tumour specific antigen. Systemic immune responses can target microscopic metastases anywhere in the body. William Coley is often regarded

Figure 1: *The development of cervical cancer*



After papillomavirus infection, most infections clear between 9-18 months. A small group of women either get persistent infection or the virus gets integrated into the genome. It is unclear how these two events impact each other or which comes first more often. It is not known if and how cells, where virus has been integrated, are cleared. Part of persistent infections progress to cervical precancerous lesions. Of these, most CIN1 lesions regress, also some of CIN2 lesions, but few CIN3 lesions regress and eventually this leads to cervical cancers years or decades later.

as the father of immunotherapy and developed immuno-therapeutics which activate the innate immune system.[19] He treated sarcomas with massive bacterial infection and immune effector mechanisms associated with bacterial infection were able to control tumour growth. After his time, several researchers tried to treat cancers with similar non-antigen specific immunotherapy with limited success. Animal models of specific immune compromise have however, established that the innate and adaptive immune systems play a role in controlling the development of some tumours.[20-21]

What would be the clinical opportunity for using a HPV therapeutic vaccine? Cervical cancer develops through precancerous lesions, called cervical intraepithelial neoplasia (CIN). Most CIN1 lesions regress spontaneously and some CIN2 lesions regress. New vaccines are relatively expensive and the cost/benefit-ratio might be very high if early CIN lesions, that could regress naturally, are treated. However, trials of HPV therapeutic vaccines in cervical cancer have shown poor results and this may be due to initiation of treatment when patients are already severely immuno-compromised. Recent vaccine trials have aimed to treat CIN2/3 lesions and have shown more encouraging results. It could be beneficial to direct immunotherapy to CIN2/3 lesions since after conventional surgical excision, recurrence occurs in about 5-20% of patients.[30-31]

Route and adjuvants

Muscle is not well provided with antigen presenting cells and novel methods are being considered to create better immune response for therapeutic vaccines.[32] Since skin and mucosa contain greater numbers of dendritic cells, the transdermal route may be a better method of vaccine delivery. Many of the recent clinical trials (Table 1) have used subcutaneous immunisation.

Most vaccines have adjuvants that enhance the immunogenicity of the vaccine. Proteins and peptides are weakly immunogenic and adjuvants are needed to stimulate a strong immune response and possibly to help break the tolerance to tumour antigens. Aluminium hydroxide is widely used in prophylactic vaccines, but biases immune responses toward antibody production, and new adjuvants will be needed for therapeutic approaches, which will require activation of cytotoxic T lymphocyte (CTL) responses. QS-21, a refined saponin from the bark of *Quillaja Saponaria*, can induce strong T Th1 (T helper 1) type responses in animal models.[33] Certain cytokines (eg. IL-2,IFNy) that direct T cells towards a Th1 type response, might also be considered.

Clinical trials against HPV-induced diseases

Previously, clinical trials have been done in late stage cervical cancer patients who are often already immuno-suppressed and results from these trials have been poor; no clinically significant responses have been seen and immune responses were worse than with later clinical trials.[20-21,30-35] Nowadays, the clinical trials have been directed to earlier, pre-cancerous CIN lesions.

One problem with clinical trials for therapy for CIN is that after the vaccination period, patients with high grade CIN cannot be observed long before treatment for CIN lesion

Table 1. *Clinical trials with HPV therapeutic vaccines from latest three years.*

Patients	Controls/placebo	Vaccine	How efficacy was measured	Results in patients	Reference
30 males with flat condylomas	20 males with flat condylomas	MVA E2 (vaccinia virus Ankara [MVA] expressing the E2 gene ***VV**	Colposcopy, histology, HPV test, ab response, CTL response against HPV+ cancer cells	28/30 clearance of condylomas, 30/30 had ab against vaccine, no recurrence in a year. In control group, 13/20 clearance, 3 recurrences in 3 months, no abs were detected	Albarran et al 2007[22]
58 women with CIN3	None	Hsp(65)E7 (SGN00101) ***P**	Histology, colposcopy	13/58 responded (to CIN1 or clearance), 32/58 reduction of lesion size, 1 1/58 no response	Einstein et al 2007[23]
26 women with CIN2/3 (14 high dose and 12 low-dose vaccine). All were HPV16+	13 women with CIN2/3. All were HPV16+	HPV16L1E7 ***P**	Histology, Ab response for L1, CTL response against E7, HPV test	5/23 showed CTL response against E7. All had ab against L1, seroconversion in 10/25, none in placebo group, 10/17 showed reduction in lesion size in vaccine group, 3/5 in placebo group, HPV16 DNA clearance in 6/16 in vaccine group, 1/7 in placebo group	Kaufmann et al 2007[24]
21 women with CIN2/3	None	Hsp(65)E7 (SGN00101) ***P**	Histology, HPV test	7/20 clearance, 1/20 regression to CIN1, 11/20 no response, 1/20 progression, IFNg-ELISPOT positive 9/20	Roman et al 2007[25]
29 women with AGIN3, 27 of them HPV16+	None	Prime TA-CIN, boost TA-HPV ***P,VV**	Histology, visual measurement of size	1/29 clearance, 5/29 reduction of lesion, 18/29 no change, 5/29 progression. 4/29 clear from HPV DNA	Fiander et al 2006[26]
34 women with CIN2/3	None	MVA E2 ***VV**	Colposcopy, histology, Ab response for E2, CTL response against cancer cells, HPV test	Colposcopy:19/34 clearance, 11/34 reduction of lesion, 4/34 minimal reduction, histology: 20/34 clearance, 11/34 reduction in lesion size, 3 downgrade to CIN2/1, ab against E2 in all patients, CTL response against cancer cells in all patients, HPV viral load reduced in all patients	Garcia-Hernandez et al 2006[27]

Table 1. *Clinical trials with HPV therapeutic vaccines from latest three years (continued)*

Patients	Controls/placebo	Vaccine	How efficacy was measured	Results in patients	Reference
161 patients with anogenital warts positive for HPV6/11	159 patients with anogenital warts positive for HPV6/11	HPV6L2E7 ***P**	Photography of warts, HPV test, ab response against L2E7	No change of recurrence rate was seen in vaccine v placebo groups. All vaccinated patients had ab response to L2E7	Vandepapeliere et al 2005[28]
10 patients with CC (stage IB)	None	DC pulsed with HPV16 or HPV18 E7 and KLH (as carrier protein) ***DC**	ELISA, ELISPOT, DTH	10/10 showed ab against E7, 5/10 had no previous ab, 5/10 showed increase in E7 ab levels, 10/10 showed ab against KLH	Santin et al 2008[29]

**VV=HPV antigens delivered by viral vectors, P=protein vaccine, DC=dendritic cell vaccine*

has to be undertaken for ethical reasons. Generally, patients are vaccinated and after a short follow-up period, they are treated with conventional methods eg. loop excision of the cervix transformation zone.

Table 1 shows some recent clinical trials of HPV immunotherapy for women with CIN2 or CIN3 or genital warts. Where placebo controls have been used, there has been no evidence of significant efficacy. Comparison between trials is difficult since responses are measured with different indicators. In general, the results have not been astounding and many therapeutic approaches that have succeeded in animals (murine models), have failed in humans.

Vaccine types

There are several vaccine strategies available today. Below, a few of the strategies are discussed briefly.

Peptide-based vaccines

Peptide-based vaccines have been used in clinical trials against human cancers,[36,37] however the problem is their weak immunogenicity, generating low affinity CTL responses and Th1 stimulation. Peptides from either E6 or E7 oncogenes are used in therapeutic vaccines against HPV-related cancers and pre-cancers. Recently, animal models have shown that cytokines might serve as effective adjuvants, increasing the efficacy of the vaccine.[38] This has also been detected in clinical trials with peptide-based melanoma vaccines.[39] Vaccines with long E6 and E7 peptides have been successfully tested with animals,[40-41] and a phase I trial of immunogenicity and safety of these vaccines in humans has just been published.[42] The trial showed that more than 50% of patients had specific CTL response against E6_E7 long peptides, but of 43 patients only one had complete response and five remained stable with a disease.

Protein vaccines

Protein vaccines have mainly used E7 protein fused to heat-shock protein (hspE7) or HPV L2 and E6 (TA-CIN) and they are safe vaccines. Several trials have been done recently,[13,43-44] however the clinical results have not been plausible. Studies by Frazer and Hallez showed reduction in HPV DNA and viral load was seen as well as some CTL responses, however this didn't correlate with clinical outcome (1/23 in CIN2/3 at Frazer, 0/5 in CIN3 at Hallez, cleared the lesion). Goldstone and colleagues showed that three out of 14 patients with warts cleared the lesion.

Dendritic cell vaccines

Dendritic cell (DC) vaccines are cell-based vaccines where patients own naïve antigen presenting cells are pulsed with antigens and cultured to maturity with specific cytokines. These mature dendritic cells are introduced to the patient and theoretically they have the potential to induce both tumour-specific effector and memory T cells. The basics of DC therapy have been reviewed elsewhere.[45] DC vaccines are highly effective at inducing immunity but difficult and expensive to produce. Animal experiments with DC vaccines against cervical cancer have given poor results.[46] Two small DC therapy trials have been conducted against cervical cancer, CTL and/or antibody responses were seen in 3/11[47] or 4/4[48] patients, however neither study showed any clinical responses, possibly due to late stage of the disease. Recently, a phase I clinical trial has been done in cervical cancer patients to test the efficacy and safety of DC therapy vaccine (Table 1)[29] and it remains to be seen if DC therapy proves to be effective in treatment of CIN lesions or early stage cervical cancer.

Plasmid DNA and recombinant viral vector vaccines

Plasmid DNA and recombinant viral vector vaccines contain protein-coding DNA that produces immunologically active antigens in live cells. These vaccines can induce antibody and CD4+ T cell helper responses and they induce strong CD8+ T cell responses because they express antigens intra-cellularly, introducing them directly into the MHC class I

antigen processing and presentation pathway. Most promising results have been achieved with these viral vector vaccines, however only a few studies have been published.

A group of women were treated with MVA E2 (modified virus Ankara + E2, recombinant papillomavirus vaccine) vaccine and 20/34 showed complete response and 11/34 reduction in lesion size. All patients showed CTL and ab responses.[27] In another study, 30 males with condylomas were treated with the same vaccine (MVA E2) and 28/30 showed complete responses; all had antibody responses and no recurrences were detected within a year.[22]

However, all vaccines face the need to break immunological tolerance by vaccination, MHC class I and antigen loss on tumour cells, systemic defects in dendritic cells and secretion of immunosuppressive cytokines etc.[49] More basic immunology studies are needed to clarify the immunological reactions behind the tumour development.

Animal models for cervical cancer vaccine

There are currently three animal models commonly used for studying cervical cancer immunotherapy. Most studies have been done with a model in which E6 and E7 expressing transformed mouse cells (TC-1) are injected into mice.[50] These cells form tumours in normal mice and vaccines can be tested for their ability to prevent, or more preferably to cure, tumours. A problem with the TC-1 model is that it is "too successful"; therapeutic protocols have worked with this mouse model but human studies with the same therapy have shown very poor results.

A further animal model is a mouse that expresses papillomavirus oncogenes E6 or E7 or the whole HPV16 genome in epithelial cells from the keratin 14 promoter. Mice develop spontaneous tumours in old age,[51] and tumours can be also induced by estrogen treatment. A problem with this mouse model is that the viral genes are expressed in all basal epithelial cells of all organs, including the thymus. The animals are therefore partially tolerant of these proteins[52-53] and the model is a tough test for a vaccine.

A third model is a skin graft model where skin from E7 transgenic mice (previous model) is grafted on to a normal mouse. E7 skin grafts are not rejected. Since E7 is presented to the immune system in HPV infections, it is used in this setting as a model antigen. Therapeutic approaches are tested for ability to reject E7 skin graft. A problem in this model is the site of lesion (skin instead of cervix). However, we believe it is a good model to study some of the requirements for, and effectiveness of immunotherapy, because the antigen is expressed in the correct cell types, without the problems of tumour induced immunosuppression, and the efficacy of immunotherapy can be evaluated over the life of the animal.[54-55]

Future directions for therapeutic vaccines against cervical cancer

Efficacy of therapeutic vaccines for cervical cancer remains to be demonstrated by clinical trials. However, there are several approaches that might increase the success of therapeutic vaccines which are discussed below.

1. Increasing effector T cell functions

Cytotoxic T cells specific to tumour antigens are the key players in tumour regressions. Enhancing the effector T cell function by either increasing the efficacy or the number of tumour specific T cells might lead to better eradication of the lesion. It is also possible to use cytokines, like IL-15, that prolong the life span of T cells.[56]

2. Overcoming the suppressive effect of regulatory T cells and macrophages

It is widely accepted that regulatory T cells (Treg) exist and prevent effective immune responses. Treg cells have been described to control autoimmune diseases, infection and transplantations and to regulate immune responses of tumours.[57] They suppress immune responses either through direct cell to cell contact or through secreting suppressive cytokines such as IL10.[58] A further regulatory population of myeloid suppressor cells seen in epithelial cancer are also able to prevent T cell effector function.

Tumour specific regulatory T cells

Tumour specific regulatory T cells have been identified and cell lines established from tumour patients. CIN and cervical cancer patients have increased Treg cell frequencies in peripheral blood and CD4(+) T cell fraction.[59] Also, HPV E6 specific Treg cells have been identified in cervical cancer patients.[60] In a study using TCR transgenic T cells specific for influenza virus hemagglutinin (HA) antigen, it was shown that immunotherapy will amplify the tumour specific regulatory T cells and thus reduce the effectiveness of immunotherapy.[61] Similar results were obtained by using dendritic cell immunisation and targeting antigen through specific pathways.[62]

Vaccine induced T regulatory cells

Chimeric papillomavirus like particles have been candidate vaccines for the treatment of cervical cancer,[63] however it has been demonstrated in a clinical trial that no efficacy has been observed.[24] We have shown that vaccination with chimeric PV VLPs can induce Treg cells and this might explain the unresponsiveness for the therapy.[64] Others have also shown that immunisation through stimulate TLR4 also induce regulatory T cells.[65]

Collectively, these results suggest that immunisation may induce and expand existing tumor specific regulatory T cells, inhibiting cytotoxic T cell responses. In future, it needs to be considered how to overcome the inhibition of vaccine induced and expanded regulatory T cells. Current methods to eliminate regulatory T cells are to deplete these cells using antibodies, such as anti-CD25, anti-GITR. However, conventional T cells can change into regulatory T cells, and as currently no specific markers for regulatory T cells have been identified, antibody depletion might also deplete activated cytotoxic T cells. We found that when IL10 is neutralised at the time of vaccination, cytotoxic cells are not inhibited by regulatory cells.[64-66] Recently, this concept has been tested in a mouse chronic viral

infection model (HCMV), where immunisation plus neutralising IL10 can clear HCMV infection. This may provide a method for the development of therapeutic vaccine against chronic HPV infection and cervical cancer.

3. Increasing tumour cells' sensitivity to effector T cells

Clinical trial results often show successful generation of effector T cells that kill tumour cells in vitro but fail to demonstrate efficacy in vivo, even when effector T cells travel to the tumour site. Although increasing effector T cell trafficking to a tumour site is a focus of therapeutic vaccination, it has been well demonstrated that tumour micro-environment is suppressive to the effector T cells.[67-68] Tumour micro-environment contains suppressive cells including regulatory T cells, suppressive macrophages and high levels of IL10 and TGF beta. Recent results have demonstrated that local administration of pro-inflammatory agents such as TLR agonist (like imiquimod) will boost tumour rejection, although its effect could be at boosting effector T cell function at effector stage.[69] At the same time, sensitivity of tumour cells to effector T cells increases, which may be another mechanism.

Suppressive molecules on tumour cells, PD-1/PD-L1, belong to newly identified B7-CD28 family members which regulate the balance between the stimulatory and inhibitory signals for immuno-regulation.[70-72] PD-L1 expression on peripheral tissues prevents autoimmunity.[73] Tumour cells can use PD-1/PD-L1 pathway to facilitate immune evasion. PD-L1 expression on tumour cells is correlated with poor clinical prognosis of many types of cancers and has been found in many tumour tissues including squamous cell carcinoma;[74] all 18 squamous cell carcinoma tumour samples tested express PD-L1. Tumour derived PD-L1 can promote tumour specific T cells apoptosis, through an unidentified receptor on effector T cells, thus resistant to the killing by effector T cells. More inhibitory molecules expressed on tumour cells have been identified and studies on how to overcome the suppressive functions by these molecules will provide better outcomes for therapeutic vaccines.

Interestingly, it was demonstrated that IFNg promotes the expression of PD-L1 on tumour cells.[75] IFNg is also a critical component for tumour killing. Therefore, IFNg may play a dual role for the rejection of established tumour tissues. IFNg can enhance MHC class I restricted antigen presentation by tumour cells and increase T cell effector function. However, at the same time, it has been demonstrated that IFNg promotes the generation of Foxp3+ regulatory T cells, prevents inflammatory cells trafficking and promotes Th1 cell apoptosis in a tumour model.[76-78] More recently, we have shown that IFNg signalling promotes the secretion of IL10 by VLPs induced regulatory T cells and prevents the rejection of tumour antigen expressing skin graft in our tumour model (unpublished data). More work is needed to find out how to reduce the suppressive signals at the same time as trying to amplify tumour killing components like IFNg.

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