Vaccination against cervical cancer

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The link between human papillomavirus (HPV) and cervical cancer raises the question, can the burden of cervical cancer be reduced using HPV vaccination strategies? The clinical trials to date will be reviewed, along with the challenges and potential for future development.

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Worldwide, cervical cancer is the second most common cancer to affect women, with almost 500 000 new cases annually (Ferlay et al, 2001). The majority of deaths from cervical carcinoma occur in the developing world, where incidence figures often equate with mortality.

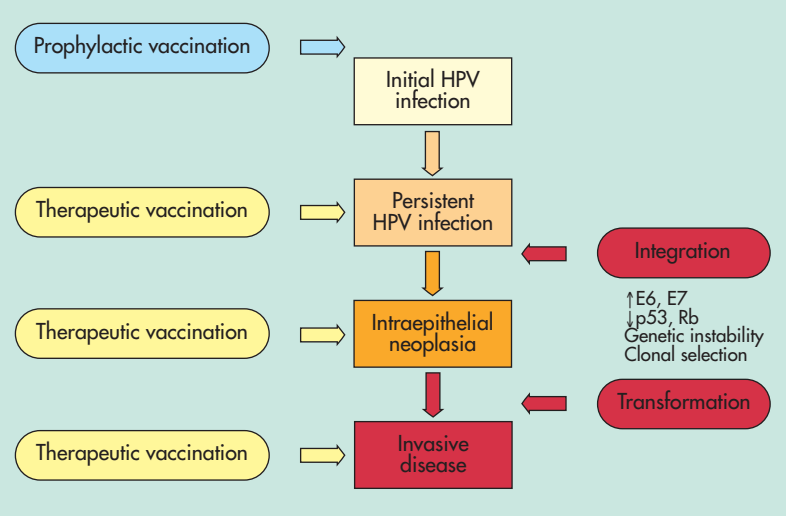
In the UK the national cervical screening programme has been successful in reducing the incidence of cervical cancer. However, the screening programme is expensive, costing approximately £130 million per year. Despite coverage rates of up to 80%, the annual incidence of cervical cancer is still approximately 3000. Approximately 50% of women diagnosed with cervical cancer have an adequate screening history.

TABLE 1. HPV types affecting mucosal epithelium

	Commonest types	Additional types
Low risk	6,11	42, 43, 46
High risk	16, 18	31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68

HPV = human papillomavirus

Figure 1. A model of human papillomavirus (HPV) natural history and vaccination strategies. Rb = retinoblastoma gene product.



HUMAN PAPILLOMAVIRUS

Human papillomavirus (HPV) is an intracellular DNA virus that infects terminally differentiated keratinocytes. There are over 100 types, those affecting mucosal surfaces are divided into two groups: low risk (LR) types that cause anogenital warts and high risk (HR) types that can cause anogenital neoplasia (*Table 1*). Neoplasia is detected most often in the cervix, although the vulva, vagina and anal region may also be affected. Worldwide 99.7% of cervical cancers contain HR HPV (Walboomers et al, 1999). HPV 16 and 18 have both been designated as carcinogenic agents by the International Agency for Research on Cancer (1995). A recent pooled analysis of 11 case-control studies calculated odds ratios for squamous cell cervical carcinoma associated with HPV 16 and 18 DNA positivity as 434.5 and 248.1 respectively (Munoz et al, 2003).

NATURAL HISTORY OF HPV INFECTION AND CERVICAL CANCER

There appear to be four stages in the natural history of HPV-related neoplasia (*Figure 1*):

1. Transient infections occur very commonly
2. Persistent infection increases the risk of developing neoplasia significantly
3. Cervical intraepithelial neoplasia (CIN) is the precursor lesion for cervical cancer. Regression may occur in up to 30% of CIN 3, but if left untreated the progression rate to invasive disease is approximately 2% per year
4. Invasive cervical carcinoma is seen in less than 2% of women infected with HR HPV.

MECHANISMS OF HPV CARCINOGENESIS

Replication of cells with damaged DNA is usually prevented by the tumour suppressor proteins Rb and p53 (*Figure 2*). HR HPVs produce two onco-

proteins (E6 and E7) that are able to inhibit these tumour suppressor proteins (*Figure 3*). During initial infection these proteins are expressed at low levels. Neoplastic transformation is associated with integration of part of the HPV genome into host cell DNA, leading to overexpression of the E6 and E7 oncoproteins. In this way HPV not only damages the host cell DNA, but is able to override the usual measures for prevention of replication and eventual clearance of the abnormal cell. This leads to the accumulation of genetic damage, genetic instability and eventual emergence of a malignant phenotype.

IMMUNE RESPONSES TO HPV INFECTION

Neutralizing antibodies to the L1 or L2 capsid proteins are HPV-type specific. Although they are able to prevent an infection, they are not sufficient for eradication.

Once infection has occurred, viral proteins synthesized within the host cell are processed into peptides and presented by major histocompatability complex (MHC) molecules on the cell surface. Which peptide is displayed depends on the MHC molecules. These are polymorphic and therefore different peptides from the same protein will be displayed by different individuals. The viral peptides displayed on the cell surface are clearly distinguishable as foreign antigens and are the targets for cell-mediated immune responses involving virus-specific CD8+ cytotoxic T lymphocytes (CTL) and CD4+ helper T lymphocytes (Th).

Antigen displayed on neoplastic epithelial cells is recognized by activated effector cytotoxic T cells. Effector T cell activation requires presentation by professional antigen-presenting cells, such as dendritic cells.

HPV VACCINES

Vaccination against cervical cancer has been targeted at different stages of the natural history. Prophylactic vaccinations aim to prevent primary infection and would need to be administered before the onset of sexual activity. Therapeutic vaccination aims to treat patients with HPV-related lower genital tract neoplasia, either intraepithelial or invasive disease.

Prophylactic HPV vaccines

Background: A major breakthrough in prophylactic vaccine design came with the discovery that late proteins (L1 and L2) in the viral capsid can self-assemble into virus-like particles (VLPs), capable of inducing virus-neutralizing antibodies.

Trials: The first randomized placebo-controlled double blind trial (RCT) of an HPV 16 L1 VLP vaccine (Merck, New Jersey, USA) showed 100% efficacy for persistent HPV 16 infection ($P<0.001$). A total of 2392 young women were studied, with a median follow up of 17.4 months (Koutsky et al, 2002). Nine cases of HPV 16-related CIN all occurred in the placebo group.

A second RCT of 1100 women demonstrated a 100% efficacy for a HPV 16 and 18 VLP vaccine (GSK Biologicals, Rixensart, Belgium) in preventing persistent HPV infection at 1 year (Ferris, 2003). A 99.8% seroconversion rate was seen, with induced titres of antibody 80–100 times higher than those seen in natural infections.

An international multicentre RCT of a quadrivalent VLP vaccine incorporating two HR HPVs (16 and 18) as well as two LR HPV (6 and 11) is currently underway (FUTURE II study).

Discussion: These early clinical trials are very encouraging, but leave many questions unanswered: how long protection will be conferred for; whether booster doses will be required, or at what age to commence vaccination need defining. In order to cover 80% of cervical cancer cases a quadrivalent vaccine would be needed; which two extra HR HPV types would be required would depend on geographical location. Whether vaccinating against some HPV types will have a permissive effect resulting in an increased incidence of other types is not yet known.

Therapeutic vaccines

The aim of therapeutic responses is to produce HPV-specific cytotoxic T cells to the early protein antigens displayed by neoplastic cells, commonly

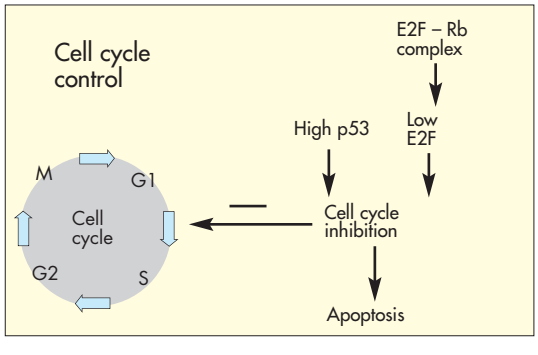


Figure 2. Mechanism to prevent replication of cell with DNA damage. Rb = retinoblastoma gene product.

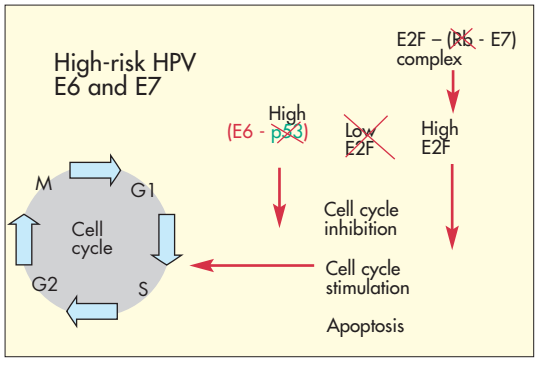


Figure 3. Mechanism of action of high-risk human papillomavirus (HPV) oncoproteins. Rb = retinoblastoma gene product.

E6 and E7. A number of different approaches towards achieving this have been investigated.

Vaccines and trials: ***Recombinant viruses:*** The establishment of recombinant vaccinia virus technology has allowed the delivery of E6 and E7 in a virus vector that, unlike HPV, is extremely immunogenic.

The first clinical trial of an HPV vaccine used TA-HPV (Xenova, Cambridge, UK) in eight patients with recurrent or advanced cervical cancer (Borysiewicz et al, 1996). One patient, who developed an HPV-specific CTL response, showed disease remission and is disease free 8 years later. As the majority of women with late stage disease appear immunocompromised (Fiander et al, 1995), the next studies with TA-HPV were in women with earlier stage cancer or pre-invasive disease. Evidence of immunological responses to vaccination in some recipients was confirmed and three subsequent trials attempted to assess the clinical response in women with high grade anogenital intraepithelial neoplasia (HG AGIN) (Tristram et al, 2002; Baldwin et al, 2003; Davidson et al, 2003). Immune responses were detected in the majority of patients, although clinical responses were seen only in the minority.

Recombinant bacteria: Bacteria can also stimulate T cell responses and have properties that facilitate mucosal delivery, allowing direct application to mucosal lesions and oral or intranasal administration. No clinical trials have yet been carried out.

Proteins and peptides: The administration of viral peptides alone is likely to be poorly immunogenic and might induce tolerance, requiring adjuvants to be given. The need for an individual to have the particular MHC molecule required to present a particular peptide can be circumvented by using longer peptides or full length proteins to deliver all the potential peptide epitopes to the immune system.

An MHC-restricted E7 peptide vaccine with adjuvant has been trialled in 18 women with an appropriate HLA type. Three weeks after the last dose, a clinical response was seen in nine recipients (three complete, six partial) (Muderspach et al, 2000).

The study demonstrating the best clinical responses presented to date used a combination of a BCG heat shock protein (BCGhsp) and an HPV 16 E7 fusion protein (Stressgen, Victoria, BC Canada) in 80 patients with HG anal intraepithelial neoplasia. At 6 months there was a 75% response rate (all partial), at 15 months this had increased to 95% (51% partial and 44% complete) (Palefsky et al, 2002).

DNA vaccines: DNA vaccines can be tailored to include genes encoding for cytokines or molecules involved in antigen processing or presentation.

An encapsulated plasmid DNA vaccine (ZYC101, Zycos Inc, Lexington, MA, USA) was used in the largest reported RCT of a therapeutic vaccine, involving 161 women with CIN 2/3 (Petry et al, 2003). The increased resolution rates in the treatment arm did not reach statistical significance, unless the analysis was restricted to women under the age of 25 years (43 *vs* 27% and 70 *vs* 23% respectively).

Dendritic cells: Dendritic cells are antigen-presenting cells capable of direct activation of CD8+ T cells. Using dendritic cells as a delivery system or cellular adjuvant requires labour intensive isolation and culture of DCs from each patient, limiting vaccination to small cohorts. Trials in advanced cervical cancer have shown some immunological but no clinical responses (Adams et al, 2001).

Combination approaches: Data from animal studies have suggested that heterologous prime-boost vaccination strategies might enhance immune responses. In the first trial of this approach, 29 patients with HG AGIN received three doses of a recombinant fusion protein HPV16 L2E6E7 (TA-CIN, Xenova, Cambridge, UK), followed by a single dose of TA-HPV (Kitchener et al, 2003). At 3 months 15 women had improvement in symptomatology and six clinical responses were seen (one complete, five partial). Immune responses were seen more often than clinical responses and correlation is ongoing.

Discussion: These trials encompass a large variety of vaccines, often in small cohorts, making it difficult to draw comparisons or conclusions; however, they raise one important question: why do the immune responses seen not translate into better clinical responses? Possible explanations include:

- Generation of a 'wrong' response, i.e. to an antigen not expressed by the neoplastic tissue
- Size of response – generation of an immune response sufficient to be detected with modern immunological methods, but insufficient for clinical impact
- Immune response not targeted to the site of disease
- Too short a time period – the trial with the best results also had the longest follow-up period.

Combined prophylactic and therapeutic vaccination

Chimeric VLPs (CVLPs) that contain an early protein gene in addition to a HPV structural protein have the potential to combine prophylactic and therapeutic approaches.

A RCT of an HPV 16 L1E7 CVLP (Medigene, Martinsried, Germany) in 36 women showed histological response in 9/24 vaccinated women *vs* 3/12 placebo, with viral clearance in 6/24 *vs* 1/12 (Nieland et al, 2003). T cell responses were seen more frequently in vaccinated patients.

Although the trial demonstrated antibody (immunoglobulin (Ig)G and IgA) production following vaccination, prophylactic effect was not assessed and larger trials will be required to establish efficacy.

THE FUTURE OF HPV VACCINES

Safe and effective prophylactic HPV vaccines are predicted to be available within 5 years. Those in charge of cervical cancer prevention programmes and health-care budgets will have to assess cost effectiveness within their particular setting. Prophylactic vaccines might be expected to have the biggest impact in developing countries where screening is not currently available; unfortunately the need for cold storage makes the current VLP vaccines prohibitively expensive. Second generation vaccines are required with lower production and distribution costs and multivalent activity.

The issue of whether to vaccinate men needs addressing. In addition to reducing the pool of infection, it would also protect against anal disease in men who have sex with men. Inclusion of the LR HPV types to protect against genital warts might make vaccination a more attractive proposition for men as well as women.

Public knowledge of the relationship between HPV and cervical cancer is very low. Education will be essential in order to achieve acceptance of a 'sexually transmitted disease vaccine' to ensure the coverage levels needed to protect the population.

Although clinical responses have been observed as a result of therapeutic vaccination, they have been at suboptimal frequency. The challenge now is to optimize vaccine design (dose, vector, adjuvant), vaccination strategy and route of administration in order to produce reliable clinical responses. This will require continued close collaboration between scientists and clinicians.

A vaccine that was both prophylactic and therapeutic would be particularly appealing in countries with limited resources, as it would treat the generation of today, while protecting the generation of tomorrow. **HM**

Conflict of interest: none.

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KEY POINTS

- Cervical cancer is caused by persistent infection with high risk types of human papillomavirus (HPV).
- High-risk HPV infection is very common and usually cleared with no long-term consequences.
- Prophylactic vaccines are designed to stimulate neutralizing antibodies to the viral capsid and require to be given before the onset of sexual activity.
- Current prophylactic vaccines are probably too expensive for use where most urgently needed in the developing world.
- Education of the public and health professionals will be essential to obtain the coverage required for population protection.
- Therapeutic vaccines have generated immune responses, while clinical results have been seen to a lesser extent.
- Further research is required to optimize vaccine design, protocols and delivery.