

Progress in the development of a cervical cancer vaccine

Ursula Winters¹
Richard Roden²
Henry Kitchener³
Peter Stern¹

¹CRUK Immunology Group, Paterson Institute for Cancer Research, Manchester, UK; ²Department of Pathology, The Johns Hopkins School of Medicine, Baltimore, USA; ³Department of Gynaecological Oncology, University of Manchester, St Mary's Hospital, Manchester, UK

Abstract: Persistent infection by 'high risk' genotypes of human papilloma virus (HPV) is necessary but not sufficient for the development of over 98% of cervical cancers. Thus the development of vaccines that prevent HPV transmission represent an important opportunity to prevent cervical cancer. There are several prophylactic HPV vaccine formulations based upon L1 virus-like particles (VLPs) currently in phase III trials and recently released data are extremely promising. However, many practical issues surrounding implementation of these vaccines need to be addressed including, who and when to vaccinate, duration of protection, and integration with current screening programs. The vaccines currently being evaluated target the two most prevalent high risk HPV types which are responsible for approximately 70% of cervical cancers. To increase the breadth of protection, it is likely that L1 VLPs of other viral subtypes must be included, although vaccines targeting the conserved regions of the L2 minor capsid protein warrant further exploration in this regard. In addition the vaccines nearing licensing will not combat established HPV-related disease and a therapeutic vaccine, of which there are several candidates in early stages of development, would be desirable. This review discusses the background to and progress in vaccine development and the issues surrounding the introduction of HPV vaccines.

Keywords: HPV, cervical cancer, vaccine

Pathology

Since Harold zur Hausen (1981) first linked papillomavirus to cervical cancer, two decades of studies have confirmed that persistent infection with certain human papilloma virus (HPV) genotypes termed 'high risk' is a necessary first carcinogenic step, or 'hit' in the pathogenesis of cervical cancer (Walboomers et al 1999).

The complete papillomaviral life cycle is restricted to the human keratinocyte and strictly dependant upon their differentiation. The virus is transmitted through skin to skin contact and is thought to reach the basal keratinocyte via tiny breaches in the epithelium to initiate infection; viral DNA is then replicated episomally using the viral E1 and E2 proteins and the cellular DNA replication machinery. Expression of the viral oncoproteins E5, E6, and E7 delays cell cycle arrest and the differentiation which occurs as the epithelial cell parts from the basal layer and matures. In the superficial layers of the epithelium, the structural proteins L1 and L2 are expressed and assemble around the viral episomes to form mature virions in the cell nucleus. Virions are released from the epithelium within the superficial epithelial cells.

Oncogenic progression is infrequent, but is associated with integration of high risk type HPV viral DNA into the host genome. HPV DNA integration leads to prolongation of the cell lifespan and results in dysplasia. Integration promotes progression, but dysplasias (at least low grade) are also induced by the normal life cycle of the virus, when the genome is episomal. Integration generally disrupts the E2 gene which derepresses the expression of E6 and E7. High risk HPV E6 and E7

Correspondence: Peter Stern
CRUK Immunology Group, Paterson
Institute for Cancer Research, Christie
Hospital, Wilmslow Road, Withington,
Manchester, M20 4BX, UK
Tel +44 161 446 3127
Fax +44 161 446 3109
Email pstern@picr.man.ac.uk

products modulate a number of other pathways critical to the transformed state, most notably the human suppressor gene products p53 and pRb respectively. This leads to failure of cell cycle check point control and genomic instability allowing accumulation of damaged genes and facilitating the evolution of invasive cancer (Frazer 2004; Doorbar 2005). Since the E6 and E7 proteins are driving the neoplastic process, and their loss triggers apoptosis, they are obvious therapeutic vaccine targets.

Epidemiology

It has been estimated that 5.5% of the worldwide incidence of cancer in 2002 was attributable to HPV infection. The majority of HPV-related cancers derive from the uterine cervix, although HPV is probably also a causal factor in some head and neck cancers and a number of other anogenital cancers (Parkin 2005).

Cervical cancer is the second most common malignant neoplasm affecting women worldwide and accounts for nearly 10% of all cancers in women. It is estimated that 493 000 new cases were diagnosed in 2002, 83% of these in developing countries (Parkin et al 2005). In terms of mortality, an estimated 273 000 deaths from cervical cancer occur worldwide each year again with over three quarters of them in developing countries (Ferlay et al 2001).

Although less prevalent in developed countries with effective screening programs, cervical cancer still accounts for 3.6% of all new cancers (Parkin et al 2005). Indeed in 2003 there were 2312 new registrations of invasive cervical cancer in England (ONS 2005a)

Although most of the more than 100 HPV types produce benign lesions, a small subset of genotypes are strongly associated with the development of cervical cancer. This subset has been classified as 'high risk' and includes HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58. More recently genotypes 59, 68, 73, and 82 have been newly identified as high risk, while types 26, 53, and 66 have been designated as probable high risk types (IARC 2005). It has been estimated that a vaccine completely effective against only the two most common high risk HPV types, namely types 16 and 18 could prevent 71% of cervical cancers worldwide. A vaccine containing the seven most common high risk HPV types might prevent around 87% of cervical cancers worldwide with little regional variation (Munoz et al 2004).

Current prevention strategies and treatments

The dysplastic process develops over 1–2 decades, providing a window for structured screening programs to detect and ablate pre-invasive disease.

Since the introduction of call and recall screening in the UK in 1988, cervical cancer rates have halved from 16.5 per 100 000 in 1988 to 8.6 per 100 000 in 2001 (ONS 2005b). It has been estimated that cervical screening prevents around 5000 deaths per year in the UK. Indeed given the rising rates of HPV infection attributable to changes in patterns of sexual behavior this may be an underestimate (Peto et al 2004).

Following reports from pilot studies, in 2003 the National Institute for Health and Clinical Excellence (NICE) recommended that liquid based cytology become the primary screening method in the UK. Newer technologies including HPV DNA testing by hybrid capture are currently under investigation as a primary screening test in a number of trials including the ARTISTIC (A Randomised Trial of HPV Testing in Primary Cervical Screening) trial in Manchester (Kitchener et al 2005).

HPV testing appears to be more sensitive but less specific for detecting high grade cervical intraepithelial neoplasia (CIN) than cytology (Cuzick et al 2003). Its lack of specificity lies in the fact that it will detect many transient infections. HPV testing as a primary screening test with cytological triage of those found to be HPV positive has been proposed as a possible screening model. A repeat testing interval of 12 months for those women found to be HPV positive who have normal or borderline cytology has been found to be as effective as immediate colposcopy (Cuzick et al 2003). This has the potential to improve the detection of high grade CIN without increasing the number of women referred for colposcopy. However the social acceptability of a nationwide screen for a sexually transmitted infection and the psychological impact of a positive test are factors which may impact on uptake rates. Some of these issues are being addressed by the ARTISTIC study. Currently the main obstacle to HPV testing is its high cost which would need to be offset by savings resulting from less pap smears being performed and/or longer screening intervals.

Screening is currently aimed at detecting CIN and treating high grade disease. This is because over 70% of mild dysplasia including CIN I resolves spontaneously, however 25% of CIN II progresses to severe dysplasia within

5 years and about 1/3 of CIN III progresses to cancer. Thus, CIN I does not necessarily require treatment when detected. However cytological and colposcopic follow up should be performed until spontaneous regression has occurred or treatment is required (NHSCSP 2004). In the future it may be possible to limit follow up to only those women known to have high risk HPV types.

In terms of current treatments, a Cochrane review of 28 trials suggested that there was no superior surgical technique for treating CIN when considering knife cone, laser cone, large loop excision of the transformation zone (LLETZ), laser ablation, and cryotherapy. However LLETZ appeared to provide the most reliable specimens for histology with the least morbidity (Martin Hirsch et al 1999). Excisional techniques are highly effective and one of the most widely used techniques, the LLETZ has a success rate of 91%–94% (Prendiville et al 1989). However this treatment does not necessarily eliminate HPV from the upper genital tract and there is a risk of recurrence. One can monitor residual disease in follow up using cytology, histology of the margins of excision, or even by high risk HPV detection. Any treatment that can address the underlying HPV infection is clearly preferable since physical removal has already failed. Prevention of infection, by prophylactic vaccination, would potentially eliminate the development of cervical cancer whilst a successful therapeutic immunization would be valuable in treating premalignant and malignant disease.

Report of studies of vaccine developments

Vaccination is historically the most effective and inexpensive approach to combat infectious disease. Thus there is a large body of literature relating to vaccination against HPV and cervical cancer (Frazer 2004; Kadish and Einstein 2005; Mahdavi and Monk 2005; Stern 2005), but herein we will highlight some of the most clinically relevant studies on the topic.

Prophylactic vaccines

The discovery that the major viral capsid protein L1 self assembles to form virus-like particles (VLPs) antigenically similar to the native virus has driven preventative HPV vaccine development (Zhou et al 1991; Ghim et al 1992; Kirnbauer et al 1992; Rose et al 1994). These VLPs lack the oncogenic viral DNA but elicit high titers of neutralizing antibody that protect against experimental viral infection. This was confirmed in several different animal models

(Breitburd et al 1995; Suzich et al 1995; Kirnbauer et al 1996).

Two vaccines currently under development, Gardasil® (Merck, Whitehouse Station, NJ, USA) and Cervarix™ (GlaxoSmithKline [GSK], Rixensart, Belgium), use this technology. They both use HPV 16 and 18 L1 VLPs as the basis of their cervical cancer preventive vaccines, but in addition Merck has added HPV 6 and 11 to prevent benign condylomata acuminata (90% of which are HPV 6 or 11 positive). The vaccines are highly immunogenic and appear safe, with recipients displaying no significant adverse effects. The efficacy of both vaccines in large phase II studies has been impressive. Merck's original formulation which contained HPV 16 VLPs only was shown to have 100% protection against persistent HPV infection. In their landmark study of 1500 women published in 2002 all of the 41 women who acquired persistent infections, defined as two detections within 4 months, had received placebo. The 9 cases of HPV 16-related CIN also occurred in placebo recipients (Koutsky et al 2002). Notably, 22 cases of CIN related to types other than HPV16 was reported in both the placebo and vaccine arms, suggesting a considerable degree of type specificity in protection. The 4 year follow up data for this study has now been published. Among 755 vaccine recipients there were no cases of HPV 16-related CIN 2 or 3 in the per protocol analysis in comparison with 12 cases in the placebo recipients (Mao et al 2006). GSK reported remarkably similar efficacy for their HPV16/18 vaccine in 2004, reporting 100% protection against persistent infection in 700 vaccinated women. CIN was identified in 6 placebo recipients and in one vaccinated woman who was infected with a non-16/18 HPV type (Harper et al 2004). Evidence of partial cross-protection against very related HPV types (in prevention of abnormal cytology) was reported at the International Papillomavirus Meeting (Dubin et al 2005) and is consistent with findings of low titer cross-neutralizing antibodies in vitro (Roden et al 1996).

Subsequent to these reports Merck have published results of 500 women vaccinated with the quadrivalent vaccine containing HPV16, 18, 6, and 11 VLPs, reporting an 89% efficacy against persistent infection (Villa et al 2005). Only one of the four cases in the vaccine group was a confirmed persistent infection; other three were single time detections at the last visit. Current studies by both companies aim to show protection against development of intermediate and high grade CIN which is more closely linked with development of cervical cancer.

Data from Merck's 'Future II' study was presented at the Infectious Diseases Society of America in October 2005 (Skjeldestad 2005). This phase III study of Gardasil involving more than 12 000 women aged 16 to 26 showed no cases of CIN in the per protocol vaccine arm and 21 cases of CIN in the placebo arm, suggesting 100% efficacy against HPV types 16 and 18 associated CIN.

All the available data points to the vaccines being extremely effective in preventing infection and CIN over a relatively short period of time, but these studies rely on prevention of CIN as a surrogate for cancer. Only after large populations have been vaccinated, with many years of follow up, could estimates of direct impact on cancer prevention be made. Licensure of prophylactic vaccines is likely in late 2006 and key questions regarding the practical implementation of these vaccines remain unresolved (Table 1).

An important issue that needs to be addressed before vaccination programs can be successfully implemented is public education. There is a lack of awareness of the link between HPV and cervical cancer; for example, in a study of 1032 women attending a well women clinic in the UK, only 30% had heard of HPV and even among those who had their knowledge was generally poor (Waller et al 2003).

Another difficult issue is the question of the age at which to vaccinate. In order to be most effective the vaccine should be given before first sexual intercourse. This would entail gaining parental approval for vaccination of their children which requires careful discussion and well coordinated public education campaigns.

The duration of vaccine-induced immunity is as yet unknown and vaccinating at 10–12 years old may be too early to offer protection from the virus during the time when

most women are exposed. Only relatively short term follow up data is available from the current studies. Data at 36 months post vaccination with Gardasil showed 94% seropositivity for HPV 6, 11, and 16, and 76% of women had antibody responses against HPV 18 (Villa et al 2005). Longer follow up data and bridging studies are required to clarify whether booster vaccinations will be required. However, it is possible that T cell responses, including those against early proteins, and not just neutralizing antibody, are required for long term protection against HPV infection.

In addition to the oncogenic HPV types 16 and 18, Merck have added HPV types 6 and 11 VLPs to their formulation. The addition of these non-oncogenic types might provide an incentive for men to receive the vaccine. However, evidence that this vaccine is effective in men per se, or effective in preventing benign genital warts and other HPV-related disease in men is not yet available. Merck currently have an ongoing trial to address the specific issue of the efficacy of their quadrivalent vaccine in men. Even if efficacy in men is proven, acceptability of male vaccination may still prove a contentious area given the low rate of HPV-associated malignancy in men. Will the essentially altruistic motivation of preventing disease in their future partners be sufficient to encourage parents to allow vaccination of their boys?

A potential limitation of the current vaccines under development is their HPV type specificity. Approximately 70% of cancers i.e. those caused by HPV types 16 and 18 may be prevented by the current vaccines. Adding protection against HPV types 45 and 31 could prevent a further 10% (Bosch et al 1995) assuming no cross protection.

Since prophylactic vaccines do not protect against all HPV subtypes nor do they address the current burden of pre-invasive and invasive disease, a role for continued cervical screening programs integrated with vaccination in a two pronged approach remains. One mathematical model looking at clinical benefits and cost-effectiveness in the US assumed conservatively that vaccination would not impact on current screening practice; using this assumption the model predicted that a HPV 16/18 vaccine ranging in efficacy from 70%–100% would reduce the lifetime risk of cancer by 46%–66%. The absolute lifetime risk would be reduced from 0.86% to 0.3% (if 100% effective) and to 0.47% (if 70% effective) compared with current screening. This model also examined the incremental cost-effectiveness ratio (ICER) of introducing a HPV 16/18 vaccine using various scenarios; this is the additional cost of a strategy (in this case vaccination) divided by its additional clinical

Table 1 Contentious issues surrounding prophylactic vaccines

Prophylactic vaccines

Implementation issues

Age at wish to vaccinate
Vaccination of men
Duration of infection/need for booster doses
Coordination with screening programmes
Public education

Technology issues

Expense of manufacturing
Expense of distribution – cold chain
Type specificity
Combined therapeutic effect

benefit compared with the next most expensive strategy. Again using the assumption that vaccination would not alter current screening practice they predicted that the ICER of a HPV 16/18 vaccine would vary from \$20 600 (100% vaccine efficacy) –\$33 700 (70% vaccine efficacy) per quality adjusted life year depending on vaccine efficacy. Various strategies combining primary prevention with vaccination and secondary prevention with cytological screening were also examined. The most effective strategy with an incremental cost-effectiveness ratio of less than \$60 000 per quality adjusted life year is combining vaccination at age 12 years with triennial conventional cytological screening beginning at age 25 years. This would reduce the lifetime risk of cancer by 94% compared with no intervention (Goldie et al 2004).

The main financial benefit of HPV vaccines in developed countries would be the cost savings made from a reduction in the number of screen detected abnormal cervical smears requiring further investigation each year. In developing countries the main impact would be in a reduction of the actual number of cervical cancer cases and hence the overall financial burden of treating this disease. However these benefits must be offset against the expense of manufacturing and of distributing the current HPV VLP vaccines.

A theoretical possibility is that once vaccines have eliminated the commonest types HPV 16 and 18, the rarer subtypes could expand in frequency to refill this ecological niche. Although unlikely it will be necessary for health providers to monitor for this possibility with post vaccination surveillance. The natural history study being carried out by the US National Cancer Institute in Costa Rica using the bivalent 16/18 vaccine may go some way to answering this and other questions.

An ideal vaccine to circumvent this problem would protect against not only those HPV types contained in the vaccine, but also induce antibodies that would protect against other HPV types ie, a pan-oncogenic HPV vaccine. One possible target is the L2 minor capsid protein. In its normal context of a virion or VLP, the L2 capsid protein is immunologically subdominant to the highly immunogenic L1 capsid protein. However, the amino terminus of L2 produced in *Escherichia coli* has been shown to induce broadly cross neutralizing antibodies (Pastrana et al 2005) and to protect against infection (Chandrachud et al 1995). Therefore using an L2 vaccine might provide a generic HPV-neutralizing antibody to protect against many high risk HPV types and warrants further investigation.

All these potentially controversial issues will be considered by the Advisory Committee on Immunization Practices (ACIP) prior to implementation of the vaccine in the US. ACIP is the federal advisory committee that formulates recommendations for use after a vaccine is approved by the US Food and Drug Administration (FDA). Data considered before recommendations are made address epidemiology of the infection, immunogenicity, efficacy, and safety of the vaccine. Acceptability data, programmatic considerations, and cost-effectiveness data are also reviewed. Elements of recommendations include appropriate age, whether booster doses are needed, indications, and contraindications (Markowitz et al 2005).

In Europe the centralized licensing of new vaccines is under the remit of the European Medicines Evaluation Agency (EMA). Application to a reference member state (RMS) can also be made as part of a decentralized or mutual recognition procedure (Wood and Levandowski 2003). Issues surrounding vaccination policy in the UK are then under the remit of the Department of Health's joint committee on vaccination and immunization (JCVI) which advises the Secretaries of State for Health (DOH 2005).

Therapeutic vaccines

While an effective prophylactic vaccine appears within reach, the impact of such a vaccine on cervical cancer rates will probably not be detectable for several decades after implementation. This is because of the long latency period from HPV infection through dysplasia to cervical cancer, and evidence that a prophylactic vaccine will be of little or no benefit to women already infected and on this pathway of disease. Hope for women with established HPV infection would come in the form of a therapeutic vaccination and this is likely to be dependant on the induction of cell-mediated immunity.

The development of therapeutic vaccines is much more challenging than that of prophylactic vaccines for a variety of reasons. High grade CIN lesions do express the E6 and E7 oncoproteins, but at a low level. The lesions are heterogeneous and genetically unstable and immune evasion strategies by the virus and evolution of the tumor, such as human leukocyte antigen (HLA) class I down regulation, can compromise the effectiveness of any cytotoxic T cells induced by a therapeutic immunization (Garrido et al 1997; Stern et al 2001).

The aim of therapeutic vaccines would be to eradicate high grade CIN lesions and even cervical cancers; however

it would clearly be unethical to offer women this type of disease treatment with a vaccine of unproven efficacy when effective surgical treatments exist. Therapeutic vaccines are therefore often initially trialed as adjuvants to conventional surgical treatment or in cases of advanced disease. Vulval intraepithelial neoplasia (VIN) is a high risk HPV-associated lesion which frequently recurs regardless of all currently available treatments. Although there are some differences in cervical and vulval HPV-associated lesions, VIN offers an alternative disease system for testing the efficacy of HPV vaccination.

There are many different types of therapeutic vaccine candidates including those based on viral gene-derived peptides and proteins, DNA, RNA, and various viral and bacterial vectors. They all aim to induce specific cell-mediated immunity and, in most cases, the targets are the E6 and E7 proteins. Therapeutic vaccines have generally proven safe and immunogenic although proof of significant clinical efficacy is less evident.

Recent clinical trials which have reported on therapeutic HPV vaccines are listed in Table 2. This is not intended as an exhaustive list, but highlights some relevant studies using a variety of vaccine delivery systems.

One way to present an oncogenic protein to the immune system is to introduce the gene encoding the protein into the genome of a recombinant virus. This delivery system exploits the capability of viruses to infect eukaryotic cells. Vaccines using viral vectors are highly immunogenic. The most extensively studied viral vector vaccines use recombinant vaccinia virus. These have been shown to be safe and to induce both antibody and T cell responses (Kaufmann et al 2002; Davidson et al 2003; Corona Gutierrez et al 2004; Hallez et al 2004; Smith et al 2005).

T cells recognize antigens as peptides presented in conjunction with major histocompatibility (MHC) molecules. Peptide fragments of the E6 and E7 proteins can be directly inoculated as vaccines, such peptide vaccines are safe and relatively easy to produce. However, their major drawback lies in the fact that only certain HLA types can present specific peptides necessitating HLA matching. Since 40% of Caucasians carry the HLA-A2 alleles, the peptides presented by this allele have been the most widely studied (Ressing et al 2000). The immunogenicity of peptide vaccines has been improved by the use of adjuvant (Muderspach et al 2000).

Researchers at the University of Leiden (Leiden, The Netherlands) have developed a vaccine consisting of long overlapping peptides encompassing the complete amino acid

sequences of HPV 16 E6 and E7. Prototypes of this vaccine were able to induce full regression of papillomavirus-induced premalignant lesions in rabbits (Vambutas et al 2005) as well as the eradication of established tumors in mice (Zwaveling et al 2002). A recently completed phase I study in women with cervical carcinoma showed that 4 vaccinations with this vaccine were safe and lead to a strong systemic type I T cell immune response in almost all patients. Currently this vaccine is being tested in 3 different phase II studies in patients with different stages of disease (van der Burg, personal communication).

Protein vaccines have the advantage over peptide vaccines in that they contain all potential epitopes of the oncogenic protein; in this case usually E6 or E7, thus circumventing the need for HLA matching. They have been shown to produce antibody and T cell responses in patients (de Jong et al 2002). Use of protein-based vaccines with adjuvants or following modification of the target antigens by fusion to other molecules such as heat shock proteins have also been investigated in relation to clinical efficacy (Frazer et al 2004).

Another vaccine approach uses plasmid DNA-encoding antigen to provoke immune responses and the vector itself can exhibit immunostimulatory properties deriving from the CpG DNA content. This allows for more sustained presentation of the antigen to the immune system. A currently active trial employs a DNA vaccine fused to HSP70 (NCI 2005) (see Table 3).

An effective T cell mediated immune response requires antigen presentation by specialized antigen-presenting cells (APCs). Dendritic cells are one of the most efficient antigen-presenting cells and vaccines based on dendritic cells which have been loaded with tumor antigen have been tested in clinical trials and shown to produce specific T cell responses in a proportion of recipients (Ferrara et al 2003).

To increase the clinical efficacy of vaccines currently under development, a combined approach involving two or more vaccination technologies has been employed. One such strategy involves priming of the immune system with a viral vector vaccine and subsequently boosting with a fusion protein vaccine (Davidson et al 2004; Smyth et al 2004)

Assessing the immunological response to therapeutic vaccines can be problematic. This is because the immune system of an individual with established HPV infection has already had exposure to viral antigens and in many cases the tumor induces mechanisms of tolerance that render antigen-specific immunity ineffective. Pre-existing immunity means that the definition of a novel immunological

Table 2 Published clinical trials of therapeutic HPV vaccines

Delivery system	Antigen	Participants	Key findings	Phase
Viral vector				
	TA-HPV, Xenova E6-E7 fusion protein	11 patients CIN3, VIN3, VAIN	5 patients made novel HPV specific T cell responses after vaccination. No clinical responses	Phase I/II
	MVA E2 recombinant vaccinia	36 women CIN1, 2 and 3	34/36 complete responses	Phase I/II
	Fusion protein PD-E7. E7 linked to Hemophilus influenza	7 patients with CIN1 and 3.	5/7 CTL responses	Phase I/II
	TA-HPV, Xenova, E6-E7 fusion protein	18 women with high grade VIN	7/7 antibody responses 8 women had partial clinical responses, 13 women had HPV specific immune responses after vaccination	Phase II
	TA-HPV, Xenova, E6-E7 fusion protein	29 women stage 1b or 2a cervical cancer prior to radical hysterectomy	Correlation between response and pre-vaccination local immune status HPV specific CTL in 4 women Serological response in 8 women	Phase I/II
Peptide	HPV 16 E7 peptides	15 women with advanced cervical cancer	T helper responses in 4 patients, No specific CTL responses	Phase I/II
	E7 Peptide + IFA	18 women with high grade CIN or VIN	10/16 CTL responses	Phase I
Protein	HSP-E7 fusion protein, Stressgen SGN-00101	32 women with CIN3 prior to LEEP	9/17 clinical responses	Phase II
	Protein/Iscomatrix adjuvant E6E7-IMX, CSL	31 women with CIN randomized to vaccine or placebo	Ongoing study 10/21 complete responses, 4/21 partial responses Most developed antibody responses	Phase I
	TA-CIN L2, E6, E7 Fusion protein, Xenova	40 healthy volunteers randomized to vaccine or placebo	Approx half developed new CTL responses	Phase I
Prime boost	TA-HPV+TA-CIN, Xenova	29 women with high grade VIN	TA-CIN specific IgG antibody and T cell proliferative responses in majority	Phase I/II
DNA	Plasmid DNA encoding fragments derived from E6 and E7 proteins in microparticles	161 women with CIN2/3 randomized to vaccine/ placebo prior to cone	Antibody and CTL responses, no simple correlation with the clinical responses	Phase I/II
DC	Autologous DC pulsed with recombinant HPV16E7 and HPV18E7	15 women stage IV cervical cancer	43% of vaccinated women cleared disease versus 27% of placebo recipients (not statistically significant). Significant disease resolution in under 25yo Specific cellular responses in 4/11	Phase I/II

Abbreviations: CIN, cervical intraepithelial neoplasia; CTL: cytotoxic T lymphocyte; DC, dendritic cell; DNA, deoxyribonucleic acid; HPV, human papilloma virus; IFA, incomplete Freund's adjuvant; LEEP, loop electro-surgical excision procedure; MVA, modified vaccinia Ankara; PD, hemophilus influenza protein D; VAIN, vaginal intraepithelial neoplasia; VIN, vulval intraepithelial neoplasia.

Table 3 Active clinical trials of HPV vaccines

Trial name	Phase	Type	Age	Sponsor	Vaccine type
Phase III randomized study of vaccine therapy comprising human papillomavirus 16/18 L1 virus-like particle/AS04 vaccine versus hepatitis A vaccine (Havrix) for the prevention of grade 2 or 3 cervical intraepithelial neoplasia, adenocarcinoma in situ of the cervix, or invasive cervical cancer in younger healthy participants.	Phase III	Prevention	18 to 25	NCI	VLP
A study to evaluate the efficacy of quadrivalent HPV (types 6, 11, 16 and 18) L1 VLP in reducing the incidence of HPV 6, 11, 16, and 18-related external genital warts, PIN, penile, perianal or perineal cancer, and the incidence of HPV 6, 11, 16 and 18 related genital infections in young men	Phase III	Prevention	16 to 23	Merck	VLP
Phase I/II study of pNGVL4a-Sig/E7(detox)/HSP70 DNA vaccine in preventing cervical cancer inpatients with human papillomavirus-16-positive grade 2 or 3 cervical intraepithelial neoplasia	Phase I/II	Prevention	Over 18	NCI	DNA
Phase II study of SGN-00101 immunotherapy in patients with grade III cervical intraepithelial neoplasia	Phase II	Prevention	18 and over	NCI	HSP
Phase II randomized study of SGN-00101 vaccine in human papillomavirus-16-positive patients with atypical squamous cells of undetermined significance or low grade squamous intraepithelial lesions of the cervix.	Phase II	Prevention	18 to 50	NCI	HSP
Surviving peptide vaccination for patients with advanced melanoma, pancreatic, colon and cervical cancer.	Phase I/II	Treatment	19 to 90		Peptide
Three clinical trials in progress in patients at different stages of HPV-related disease	Phase II	Treatment		University of Leiden	Long Peptide
Phase II adjuvant study of the recombinant vaccinia virus vaccine expressing the human papilloma virus 16, 18, E6, and E7 in early cervical cancer	Phase II	Treatment	19 and above	EORTC	Viral vector
Phase II clinical trial of MVA-HPV1L2 in women with CIN 2/3 (incomplete title)	Phase II	Treatment		Transgene	Viral vector
A pilot study for the immunotherapy of recurrent cervical cancers using DCs pulsed with human papillomavirus type 16 E7 antigen	Phase I	Treatment	18 to 75	National Taiwan University Hospital	DC

Note: Information sourced from the NCI and US NIH websites.

Abbreviations: CIN, cervical intraepithelial neoplasia; DC, dendritic cells; DNA, deoxyribonucleic acid; EORTC, European organisation for research and treatment of cancer; MVA, modified vaccinia Ankara; NCI, National Cancer Institute; NIH, National Institutes of Health; PIN, Penile intraepithelial neoplasia; VLP, virus-like particles.

response may have to be defined as an arbitrary increase in an immune marker. In addition a proportion of clinical lesions show spontaneous regression and therefore any putative vaccine efficacy must be above and beyond that predicted to occur spontaneously (Stern et al 2001; Stern 2005).

Prophylactic and therapeutic

An ideal vaccine would have both prophylactic and therapeutic effects. Such a vaccine would have immediate benefit in contrast to the inevitable delay before the clinical impact of prophylactic vaccines. Several candidates are in development.

Chimeric VLPs build on the technology used to create VLP vaccines that induce neutralizing antibody. Introducing E7 fused to the L1 capsid protein into these VLPs induces CD8 mediated responses in addition to neutralizing antibody and thus generates a vaccine with potential therapeutic and prophylactic capability. A phase I clinical trial was performed in Germany by Medigene (Martinsried, Germany) and demonstrated both safety and immunogenicity.

Recent animal studies indicate that as for L1 VLP vaccines, protection against experimental papillomavirus challenge by L2 vaccination can be mediated by neutralizing antibodies (Embers et al 2002). However unlike L1 VLP vaccination, vaccination with HPV L2 induces antibodies that cross neutralize diverse mucosal HPV genotypes in vitro (Pastrana et al 2005). This important distinction from L1 VLP vaccines suggests the possibility of a simple pan-HPV prophylactic vaccine derived from L2 sequences. Neither L1 nor L2 vaccines are expected to induce effective clearance of established HPV lesions probably because of the lack of capsid protein expression by cervical carcinomas and infected basal keratinocytes. This is in contrast with the consistent expression of E6 and E7 proteins through the spectrum of HPV lesions. TA-CIN is a fusion protein of HPV 16L2E6E7 it combines the pan HPV neutralizing potential of L2 with E6 and E7 oncogene directed therapeutic T cell activity. It has been shown to be safe and induce both antibody and T cell responses against E6 and E7 (de Jong et al 2002). The serum of healthy volunteers vaccinated with TA-CIN has been shown to neutralize not only HPV16 but also HPV 18 (Gambhira et al 2005). TA-CIN with an appropriate adjuvant might therefore be a candidate vaccine with combined prophylactic and therapeutic potential worthy of further study.

Prospects

A vaccine for preventing persistent HPV infection, the first necessary step for cervical carcinogenesis, is tangible. Two major pharmaceutical companies are conducting global phase III studies and are within sight of regulatory approval for their prophylactic vaccines. Whilst development of first generation prophylactic vaccines nears completion there is scope for an improved second generation of vaccines which addresses some of the shortcomings of the first generation. These include expense in manufacturing and distribution which may reduce impact in the countries where such a vaccine is most needed, and type specificity such that screening programs must remain in place.

For the vaccines in late stages of development there are many issues surrounding implementation yet to be resolved including who and when to vaccinate, duration of protection, and synchronization with current screening and other pathogen vaccination programs.

Many candidate vaccines with therapeutic potential that use a variety of delivery systems have been trialed and are the subject of ongoing trials; there are low expectations that any of the current therapeutic vaccines will have a substantial public health impact in the near future. An ideal vaccine would be pan-oncogenic and have both therapeutic and prophylactic potential. Whilst there are such candidates in development, the reality is some way off.

Disclosure

Richard Roden is a paid consultant of Knobbe, Martens, Olsen and Bear, LLC, California, USA.

References

- Bosch FX, Manos MM, Munoz N, et al. 1995. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer Inst*, 87:796-802.
- Breitburd F, Kirnbauer R, Hubbert NL, et al. 1995. Immunization with viruslike particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. *J Virol*, 69:3959-63.
- Chandrachud LM, Grindlay GJ, McGarvie GM, et al. 1995. Vaccination of cattle with the N-terminus of L2 is necessary and sufficient for preventing infection by bovine papillomavirus-4. *Virology*, 211:204-8.
- Corona Gutierrez CM, Tinoco A, Navarro T, et al. 2004. Therapeutic vaccination with MVA E2 can eliminate precancerous lesions (CIN 1, CIN 2, and CIN 3) associated with infection by oncogenic human papillomavirus. *Hum Gene Ther*, 15:421-31.
- Cuzick J, Szarewski A, Cubie H, et al. 2003. Management of women who test positive for high-risk types of human papillomavirus: the HART study. *Lancet*, 362:1871-6.

- Davidson EJ, Boswell CM, Sehr P, et al. 2003. Immunological and clinical responses in women with vulval intraepithelial neoplasia vaccinated with a vaccinia virus encoding human papillomavirus 16/18 oncoproteins. *Cancer Res*, 63:6032-41.
- Davidson EJ, Faulkner RL, Sehr P, et al. 2004. Effect of TA-CIN (HPV 16 L2E6E7) booster immunisation in vulval intraepithelial neoplasia patients previously vaccinated with TA-HPV (vaccinia virus encoding HPV 16/18 E6E7). *Vaccine*, 22:2722-9.
- de Jong A, O'Neill T, Khan AY, et al. 2002. Enhancement of human papillomavirus (HPV) type 16 E6 and E7-specific T-cell immunity in healthy volunteers through vaccination with TA-CIN, an HPV16 L2E7E6 fusion protein vaccine. *Vaccine*, 20:3456-64.
- [DOH] Department of Health. 2005. Joint committee on vaccination and immunisation [online]. Accessed 3 December 2005. URL: <http://www.advisorybodies.doh.gov.uk/JCVI/index.htm>.
- Doorbar J. 2005. The papillomavirus life cycle. *J Clin Virol*, 32(Suppl 1):S7-15.
- Dubin G, Colau B, Zahaf T, et al. 2005. Cross-protection against persistent HPV infection, abnormal cytology and CIN associated with HPV-16 and 18 related HPV types by a HPV 16/18 L1 virus-like particle vaccine. 22nd International Papillomavirus Conference, April 30 – May 6, Vancouver, Canada. p 32.
- Einstein MH, Kadish AS, Burk RD, et al. 2005. Heat shock protein (HSP)-based immunotherapy (HSPE7) for treatment of CINIII (NCI 5850, NYGOG). *Gynecol Oncol*, 96:912-13.
- Embers ME, Budgeon LR, Pickel M, et al. 2002. Protective immunity to rabbit oral and cutaneous papillomaviruses by immunization with short peptides of L2, the minor capsid protein. *J Virol*, 76:9798-805.
- Ferlay J, Bray F, Pisani P, et al. 2001. GLOBOCAN 2000: Cancer incidence, mortality and prevalence worldwide, Version 1.0. (IARC Cancer Base No. 5) Lyon, France: IARC Pr.
- Ferrara A, Nonn M, Sehr P, et al. 2003. Dendritic cell-based tumor vaccine for cervical cancer II: results of a clinical pilot study in 15 individual patients. *J Cancer Res Clin Oncol*, 129:521-30.
- Frazer IH. 2004. Prevention of cervical cancer through papillomavirus vaccination. *Nat Rev Immunol*, 4:46-54.
- Frazer IH, Quinn M, Nicklin JL, et al. 2004. Phase 1 study of HPV16-specific immunotherapy with E6E7 fusion protein and ISCOMATRIX adjuvant in women with cervical intraepithelial neoplasia. *Vaccine*, 23:172-81.
- Gambhira R, Bossis I, Viscidi R, et al. 2005. Cross-neutralising antibody response of healthy volunteers vaccinated with human papillomavirus (HPV) 16 minor capsid protein L2. 22nd International Papillomavirus Conference, April 30 – May 6, Vancouver, Canada. p 227.
- Garcia F, Petry KU, Munderspach L, et al. 2004. ZYC101a for treatment of high grade intraepithelial neoplasia. A randomised controlled trial. *Obstet Gynecol*, 103:317-26.
- Garrido F, Ruiz-cabello F, Cabrera T, et al. 1997. Implications for immunosurveillance of altered HLA class I phenotypes in human tumours. *Immunol Today*, 18:89-95.
- Ghim SJ, Jenson AB, Schlegel R. 1992. HPV-1 L1 protein expressed in cos cells displays conformational epitopes found on intact virions. *Virology*, 190:548-52.
- Goldie SJ, Kohli M, Grima D, et al. 2004. Projected clinical benefits and cost-effectiveness of a human papillomavirus 16/18 vaccine. *J Natl Cancer Inst*, 96:604-15.
- Hallez S, Simon P, Maudoux F, et al. 2004. Phase I/II trial of immunogenicity of a human papillomavirus (HPV) type 16 E7 protein-based vaccine in women with oncogenic HPV-positive cervical intraepithelial neoplasia. *Cancer Immunol Immunother*, 53:642-50.
- Harper DM, Franco EL, Wheeler C, et al. 2004. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet*, 364:1757-65.
- [IARC] International Agency for Research on Cancer. 2005. Cervix cancer screening. Lyon, France: IARC Pr.
- Kadish AS, Einstein MH. 2005. Vaccine strategies for human papillomavirus-associated cancers. *Curr Opin Oncol*, 17:456-61.
- Kaufmann AM, Stern PL, Rankin EM, et al. 2002. Safety and immunogenicity of TA-HPV, a recombinant vaccinia virus expressing modified human papillomavirus (HPV)-16 and HPV-18 E6 and E7 genes, in women with progressive cervical cancer. *Clin Cancer Res*, 8:3676-85.
- Kirnbauer R, Booy F, Cheng N, et al. 1992. Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. *Proc Natl Acad Sci U S A*, 89:12180-4.
- Kirnbauer R, Chandrachud LM, O'Neil BW, et al. 1996. Virus-like particles of bovine papillomavirus type 4 in prophylactic and therapeutic immunization. *Virology*, 219:37-44.
- Kitchener H, Almonte M, Wheeler P, et al. 2005. The ARTISTIC trial of HPV testing in primary cervical screening: methods and baseline data. 22nd International Papillomavirus Conference, April 30 – May 6, Vancouver, Canada. p 22.
- Koutsky LA, Ault KA, Wheeler CM, et al. 2002. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med*, 347:1645-51.
- Mahdavi A, Monk BJ. 2005. Vaccines against human papillomavirus and cervical cancer: promises and challenges. *Oncologist*, 10:528-38.
- Mao C, Koutsky L, Ault KA, et al. 2006. Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia. *Obstet Gynecol*, 107:18-27.
- Markowitz L, Dunne E, Gilsdorf J. 2005. Development of recommendations for HPV vaccine use in the United States. 22nd International Papillomavirus Conference, April 30 – May 6, Vancouver, Canada. p 35.
- Martin-Hirsch PL, Paraskevaidis E, Kitchener H. 1999. Surgery for cervical intraepithelial neoplasia. *Cochrane Database Syst Rev*, Issue 3.
- Munderspach L, Wilczynski S, Roman L, et al. 2000. A phase I trial of a human papillomavirus (HPV) peptide vaccine for women with high-grade cervical and vulvar intraepithelial neoplasia who are HPV 16 positive. *Clin Cancer Res*, 6:3406-16.
- Munoz N, Bosch FX, Castellsague X, et al. 2004. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. *Int J Cancer*, 111:278-85.
- [NCI] National Cancer Institute. 2005. Clinical trials [online]. Accessed 22 November 2005. URL: <http://www.cancer.gov/clinicaltrials>.
- [NHSCSP] NHS Cervical Screening Programme. 2004. Colposcopy and programme management: guidelines for the NHS Cervical Screening Programme. Sheffield, UK: NHSCSP Publication No. 20.
- [ONS] Office for National Statistics. 2005a. Cancer registrations in England 2003. National Statistics [online]. Accessed 3 December 2005. URL: <http://www.statistics.gov.uk/statbase/Expodata/Spreadsheets/D9096.xls>.
- [ONS] Office for National Statistics. 2005b. Update to cancer trends in England and Wales 1950–1999. National statistics [online]. Accessed 3 December 2005. URL: http://www.statistics.gov.uk/downloads/theme_health/CancerTrendsUpdates.pdf.
- Parkin DM. 2005. World burden of infection-associated cancers. 22nd International Papillomavirus Conference, April 30 – May 6, Vancouver, Canada. p 10.
- Parkin DM, Bray F, Ferlay J, et al. 2005. Global cancer statistics, 2002. *CA Cancer J Clin*, 55:74-108.
- Pastrana DV, Gambhira R, Buck CB, et al. 2005. Cross-neutralization of cutaneous and mucosal papillomavirus types with anti-sera to the amino terminus of L2. *Virology*, 337:365-72.
- Peto J, Gilham C, Fletcher O, et al. 2004. The cervical cancer epidemic that screening has prevented in the UK. *Lancet*, 364:249-56.
- Prendiville W, Cullimore J, Norman S. 1989. Large loop excision of the transformation zone (LLETZ). A new method of management for women with cervical intraepithelial neoplasia. *Br J Obstet Gynaecol*, 96:1054-60.

- Ressing ME, van Driel WJ, Brandt RM, et al. 2000. Detection of T helper responses, but not of human papillomavirus-specific cytotoxic T lymphocyte responses, after peptide vaccination of patients with cervical carcinoma. *J Immunother*, 23:255-66.
- Roden RB, Greenstone HL, Kimbaurer R, et al. 1996. In vitro generation and type-specific neutralization of a human papillomavirus type 16 virion pseudotype. *J Virol*, 70:5875-83.
- Rose RC, Reichman RC, Bonnez W. 1994. Human papillomavirus (HPV) type 11 recombinant virus-like particles induce the formation of neutralizing antibodies and detect HPV-specific antibodies in human sera. *J Gen Virol*, 75(Pt 8):2075-9.
- Skjeldestad FE. 2005 Prophylactic quadrivalent human papillomavirus (HPV) (Types 6,11,16,18) L1 virus-like particle (VLP) vaccine (Gardasil™) reduces cervical intraepithelial neoplasia (CIN) 2/3 risk. 43rd Annual Meeting of IDSA, October 6–9, San Francisco, USA. p 53.
- Smith KL, Tristram A, Gallagher KM, et al. 2005. Epitope specificity and longevity of a vaccine-induced human T cell response against HPV18. *Int Immunol*, 17:167-76.
- Smyth LJ, Van Poelgeest MI, Davidson EJ, et al. 2004. Immunological responses in women with human papillomavirus type 16 (HPV-16)-associated anogenital intraepithelial neoplasia induced by heterologous prime-boost HPV-16 oncogene vaccination. *Clin Cancer Res*, 10:2954-61.
- Stern PL, Faulkner R, Veranes E, et al. 2001. The role of human papillomavirus vaccines in cervical neoplasia. In: Kitchener HC (ed). Human papillomavirus: best practice in clinical obstetrics and gynaecology. London: Balliere Tindall. p 783-99.
- Stern PL. 2005. Immune control of human papillomavirus (HPV) associated anogenital disease and potential for vaccination. *J Clin Virol*, 32(Suppl 1):S72-S81.
- Suzich JA, Ghim SJ, Palmer-Hill FJ, et al. 1995. Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. *Proc Natl Acad Sci U S A*, 92:11553-7.
- Vambutas A, DeVoti J, Nouri M, et al. 2005. Therapeutic vaccination with papillomavirus E6 and E7 long peptides results in the control of both established virus-induced lesions and latently infected sites in a pre-clinical cottontail rabbit papillomavirus model. *Vaccine*, 23:5271-80.
- Villa LL, Costa RL, Petta CA, et al. 2005. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol*, 6:271-8.
- Walboomers JM, Jacobs MV, Manos MM, et al. 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*, 189:12-19.
- Waller J, McCaffery K, Forrest S, et al. 2003. Awareness of human papillomavirus among women attending a well woman clinic. *Sex Transm Infect*, 79:320-2.
- Wood JM, Levandowski RA. 2003. The influenza vaccine licensing process. *Vaccine*, 21:1786-8.
- Zhou J, Sun XY, Stenzel DJ, et al. 1991. Expression of vaccinia recombinant HPV 16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. *Virology*, 185:251-7.
- zur Hausen H, de Villiers EM, Gissmann L. 1981. Papillomavirus infections and human genital cancer. *Gynecol Oncol*, 12:S124-8.
- Zwaveling S, Ferreira Mota SC, Nouta J, et al. 2002. Established human papillomavirus type 16-expressing tumors are effectively eradicated following vaccination with long peptides. *J Immunol*, 169:350-8.

