FEATURE ARTICLE

When Hope Meets Reality

Vaccination Against Cervical Cancer[1]

Executive summary

Cervical cancer is the second most common gynecological malignancy and the third leading cause of cancer-related death in women worldwide. The causal relationship between human papillomavirus (HPV) infection and cervical cancer has stimulated development of both preventive and therapeutic vaccines against high-risk HPV genotypes. Prophylactic vaccination strategies focus on the induction of high-titer neutralizing antibodies that protect against HPV infection.

Two major phase II clinical trials of vaccines in development with pharmaceutical companies have demonstrated 100% vaccine efficacy in preventing persistent HPV infection and its associated cellular abnormalities; however, whether the vaccines induce long-lasting protective immunity is yet to be determined. Both vaccines – Gardasil™ from Merck & Co. and Cervarix™ from GlaxoSmithKline – are now in pivotal phase III clinical trials and at least one is expected to be on the market within the next 2–3 years. However, vaccine-elicited reduction in the incidence of HPV-related lesions and cervical cancer is not expected to become apparent for decades due to existing infections and the long latency period between infection and development of clinical disease.

Therapeutic vaccination is intended to control HPV-associated malignancies by stimulating cellular immune responses that destroy HPV-infected cells. Progress in the field of HPV immunotherapy has remained elusive, largely because the precise immune responses critical to elimination of HPV-infected cells have not been clearly defined. However, the theoretical advantages of therapeutic vaccines over standard surgical treatments for cervical lesions – attacking the root cause of lesion development and leaving the patient with long-term protective immunity – will continue to stimulate research and development.

Cervical cancer remains one of the leading causes of cancer-related death among women worldwide, with nearly 500 000 new cases each year and 250 000 deaths. According to the American Cancer Society, approximately 10 400 new cases of invasive cervical cancer will be diagnosed in the US in 2005 and about 3700 women will die of the disease. The widespread use of cervical cytological screening based on the Papanicolaou (Pap) smear test has resulted in a 70% decline in mortality from cervical cancer in developed countries with effective Pap-screening programs over the past 50 years. However, in developing countries where screening programs are minimal, cervical cancer remains the second leading cause of cancer-related deaths among women, after breast cancer.

Viral villain

Cervical cancer originates from epithelial cells lining the cervix. Although most cervical infections with human papillomavirus (HPV) spontaneously self-resolve with no symptoms, it is well established that persistent infection with high-risk HPV genotypes is the cause of high-grade cervical lesions (cervical dysplasia), which can develop into invasive cervical cancer. Following infection of cervical epithelial cells, the HPV genome directs the host cell to produce new infectious virus particles. In a small percentage of cases, the genome of the virus integrates into the host chromosome, ultimately leading to the growth of malignant tumors. The prevalence of low-grade squamous intraepithelial lesion (SIL), an indication of HPV infection, and high-grade SIL, the precursor of invasive cervical cancer, is 1.0–2.5% within screened populations.

Fifteen HPV genotypes are now classified as high-risk, of which the most prevalent are HPV16 and HPV18. DNA from high-risk HPV genotypes is detected in virtually all cases of cervical cancer, with HPV16 or HPV18 being found in almost 70% of HPV-positive biopsies, regardless of geographical region.

In the long term, effective global implementation of both prophylactic and therapeutic vaccination regimens could result in a significant reduction in both healthcare costs and worldwide cervical cancer incidence.

Although Pap-based cervical screening programs have dramatically reduced the incidence of cervical cancer in developed countries, economic forces restrict their use in most developing nations. For the latter countries, a prophylactic vaccine targeting HPV offers the greatest hope in reducing cervical cancer. Because of their high

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Table I. Prophylactic HPV vaccines in late-stage development[a]

Vaccine	Composition	Company	Indication	Clinical status
Gardasil™	Virus-like particles (VLP) containing L1 capsid protein from HPV6, 11, 16, 18	Merck & Co.	Genital warts, Cervical cancer	Phase III (US, Latin America, Europe, Asia, Australia)
Cervarix™ (MEDI-517)	VLP containing L1 capsid protein from HPV16 and HPV18	GlaxoSmithKline, MedImmune	Cervical cancer	Phase III (US, UK)

a Source: R&D Insight (Adis International)

prevalence in cervical lesions, HPV16 and HPV18 are the focus of HPV vaccine development.

Prophylactic HPV vaccines – VLP technology holds the key

Development of prophylactic HPV vaccines has mostly focused on the use of virus-like particles (VLPs) to generate neutralizing antibodies. VLPs are essentially empty viral shells lacking genetic material; therefore, although they resemble the virus in structure, they are incapable of replicating and causing infection and cancer. The development of traditional heat-inactivated or attenuated vaccines is restricted by the inability to efficiently isolate HPV from natural lesions or propagate the virus in cell culture or animal hosts.

VLPs can be obtained by overexpression of the major HPV capsid protein L1 alone, or by co-expression with the minor capsid protein L2, and subsequent spontaneous self-assembly of the protein(s) into VLPs. Several early clinical trials showed that VLP vaccines based on L1 from HPV16 or HPV18 are well tolerated and elicit strong antibody responses. In terms of preventing persistent HPV16 or HPV18 infection, vaccine efficacy in some studies was as high as 90–100% compared with placebo. This early work paved the way for the two VLP vaccines currently in pivotal late-stage clinical trials – a quadrivalent vaccine (Gardasil™) in development with **Merck & Co**. and a bivalent vaccine (Cervarix™) in co-development with **GlaxoSmithKline** and **MedImmune** (see table I).

Gardasil

Merck's HPV vaccine contains L1 VLPs from HPV16 and HPV18, the two high-risk HPV types associated with the development of cervical lesions, and HPV6 and HPV11, two genotypes associated with genital warts. Thus, the quadrivalent vaccine is intended to prevent genital warts as well as cervical cancer. Merck licensed the vaccine from Australian-based **CSL**, which had previously licensed it from researchers at the University of Queensland. Merck holds exclusive global rights to the vaccine except in Australia and New Zealand where CSL has retained ownership. A phase III trial of the quadrivalent vaccine formulated with alum adjuvant (Gardasil) is ongoing in approximately 25 000 patients in several countries. In May 2005, the first data released indicated a three-dose regimen administered over 6 months elicited nearly 100% seroconversion to vaccine-contained HPV types in adolescents aged 10–15 years and females aged 16–23 years. Antibody levels were significantly higher in the younger age groups than in the older females. In April 2005, results were released from a phase II clinical trial in females aged 16–23 years. After 3 years' follow-up, the vaccine had 100% efficacy compared with placebo at preventing clinical disease associated with HPV6, 11, 16, or 18, and 90% efficacy in preventing persistent infection or disease due to HPV6, 11, 16, or 18. It is anticipated that Merck may file for US FDA approval of Gardasil in late 2005.

Cervarix

GlaxoSmithKline and MedImmune are jointly developing a bivalent vaccine containing L1 VLPs from HPV16 and HPV18 that was originally developed by researchers at the University of Rochester, NY. A phase III trial of the bivalent vaccine formulated in AS04 adjuvant (aluminum hydroxide and 3-deacylated monophosphoryl lipid A) – Cervarix – began in mid-2004 and is scheduled to enroll several thousand females aged 10–55 years. Results from phase II clinical trials indicated the vaccine was 100% effective at preventing persistent infection with HPV16 and HPV18. Unlike Gardasil, Cervarix is designed to prevent cervical cancer only. GlaxoSmithKline anticipates filing for approval of Cervarix in Europe and other non-US markets in 2006. A date for FDA filing has not yet been announced.

Antibody titers

Studies with animal papillomaviruses have shown that the production of virus-neutralizing antibodies on the mucosal surfaces of the genitals is necessary for protection against infection. As HPV does not spread systemically, mucosal immune responses will probably also be required for protection in humans. In general, clinical trials have

indicated that VLP vaccines elicit serum antibody titers that are 40–60 times higher than those induced by natural infection and can persist for years with only a slight decline. Preliminary data indicate that HPV VLP vaccines formulated in AS04 adjuvant induce substantially higher and more persistent antibody levels compared with alum-adjuvanted formulations. Importantly, VLP-based vaccination also induces local mucosal immunity in the cervix of immunized women in the form of both IgG and secretory IgA antibodies.

Potential issues with prophylactic vaccines

Type-specific protection

One concern regarding the HPV vaccines in development is their lack of protection against HPV genotypes not contained in the vaccine formulation. Neutralizing antibodies against VLPs of a certain genotype are remarkably type-specific and cross-neutralize only a very limited number of other genotypes. This indicates that vaccination will probably elicit type-specific protection, meaning protection against most or all of the 15 high-risk HPV types would require vaccines containing many more antigens than those currently in development. Theoretically, a vaccine containing VLPs from the seven most oncogenic HPV types could prevent 87% of cervical cancers with little geographical variation. However, it may be logistically difficult to produce a vaccine containing so many VLP types; furthermore, including multiple VLP types in one formulation might result in low antibody responses against certain types – so-called 'antigenic competition'. Indeed, in a phase II trial of Merck's quadrivalent vaccine, antibody titers to HPV16 were dominant over those targeting the other HPV types.

Vaccine-associated emergence of rare HPV strains

Ideally, widespread vaccination against HPV16 and HPV18 would result in a significant reduction in the worldwide incidence of cervical cancer. However, there is concern that due to type-specific protection, such a strategy might simply lead to the emergence and spread of the less common high-risk HPV types and thus no overall decrease in disease incidence. At least 30% of cervical cancers are caused by HPV genotypes other than HPV16 and HPV18. But, reassuringly, these HPV types are less likely to cause persistent infection and neoplastic disease.

Efficacy

While the efficacy of L1 VLP-based vaccines in preventing persistent HPV infection, as measured by the detection of HPV DNA, has been as high as 100% in some cases, the efficacy against transient infection appears somewhat lower. However, it remains unclear whether the detection of HPV DNA represented true productive infections or a recent encounter with an infected partner. In addition, in a phase II trial of Cervarix, women who did not receive all three doses of the vaccine were less protected than those who did complete the vaccination regimen; therefore, if this vaccine becomes commercially available, it will be very important to ensure patients receive all doses. It will be difficult to estimate vaccine efficacy against clinical disease based on efficacy against HPV infection, because HPV-induced disease occurs only in a small percentage of infected individuals. Furthermore, as vaccine protection is likely to be type-specific, disease must be shown to be attributable to the specific HPV type(s) present in a vaccine before a conclusion can be drawn about its protective efficacy.

As a consequence of existing HPV infection, even if prophylactic vaccination were to begin immediately, there would still be an estimated 10 million new cases and 5 million deaths from cervical cancer throughout the world over the next 20 years.

Target population

The current target population for prophylactic vaccination is adolescent girls and boys. However, there could be significant resistance among the general public to routinely vaccinating pre-pubescent females against a sexually transmitted disease. Clearly, education programs about HPV and its association with genital warts and cancer will be important if this strategy is to succeed.

Epidemiological impact

Gardasil and/or Cervarix may be on the market within 2–3 years. However, even if vaccination is widespread and eliminates the most prevalent HPV types, a reduction in the incidence of cervical dysplasia and cancer is not expected to become apparent for 10–25 years, since it generally takes this long for existing HPV infection to progress to disease. It is estimated that a vaccine that prevents 98% of persistent infections with HPV16 or HPV18 will be associated with an equivalent reduction in HPV16- or HPV18-associated cervical cancer but a lesser reduction (51%) in total cervical cancer, because of cancers associated with other oncogenic non-HPV16 and non-HPV18 types. Mathematical models estimate that a 50% reduction in the incidence of cervical cancer would

not occur for 25–55 years, assuming that 75% of females are vaccinated and protected against HPV16 alone. Cost-analysis modeling has shown that vaccination against HPV in combination with delayed and less-frequent screening can be more cost effective than screenings alone.

Overall, it is likely that cytological screening practices in developed countries will not change significantly for decades. As a consequence of existing HPV infection, even if prophylactic vaccination were to begin immediately, there would still be an estimated 10 million new cases and 5 million deaths from cervical cancer throughout the world over the next 20 years. Therefore, there is a compelling case for developing therapeutic vaccines against existing and breakthrough HPV infections, as virus-specific immunotherapy could have an immediate impact on the incidence of cervical cancer.

Therapeutic HPV vaccines – activating the cellular immune response

Unlike prophylactic vaccines – which are designed to elicit a neutralizing antibody response to prevent HPV infection in uninfected individuals – therapeutic HPV vaccines are intended to stimulate cellular immune responses that destroy HPV-infected cells in patients with cervical dysplasia or cancer. A large body of evidence suggests that cellular immunity, especially activation of antigen-specific CD4+ and CD8+ T lymphocytes, is most likely the critical defense mechanism against HPV-infected cells. The main target of experimental therapeutic vaccines is the viral oncoproteins E6 and E7, which are expressed at all stages of cervical neoplasia and play important roles in transforming the epithelial cell to a cancerous phenotype. Their expression is also essential for maintenance of the transformed state and is not likely to be lost as a result of immunological pressures. For these reasons, E6 and E7 are considered to be the best targets for HPV-specific immunotherapy.

Variety of vaccines

Various types of vaccine targeting the E6 and/or E7 proteins have been produced, including candidates based on peptides, proteins, DNA, viral and bacterial vectors, chimeric VLPs or dendritic cells. Several vaccines have been tested in early clinical trials, mostly in treatment-refractory patients with late-stage cervical cancer who have already undergone surgery, radiotherapy and/or chemotherapy. Not unexpectedly, given the health status of such patient populations, proof of clinical efficacy remains to be demonstrated for any therapeutic HPV vaccine.

In general, the most immunogenic vaccines appear to be those using viruses, bacteria or dendritic cells as vectors to deliver the E6 and or E7 antigens. However, many regulatory safety issues surround the vector-based vaccines, as well as the potential inability of patients to mount a sufficiently strong immune response to these vaccines because of pre-existing immunity to the virus or bacterial vector, or an inability to boost the response after one vaccination. Dendritic cell vaccination involves patient-specific procedures that are time-consuming and costly. Therefore, it is particularly unlikely that dendritic cell-based therapies would be implemented either for women in developing countries who have no access to adequate screening programs or for women in developed countries who have access to more cost-effective standard surgical procedures.

DNA-based vaccines are easy to produce, very versatile in their ability to carry multiple targeting and co-stimulatory genes and can be used in multiple immunizations. However, safety concerns surround the potential for DNA integration and cell transformation, not to mention the introduction of potential oncogenes such as E6 and E7. A strategy employed to minimize oncogenic risk is the introduction of mutations into the DNA sequence that render the protein produced nonfunctional.

A lack of understanding of the immune correlates of protection is the main reason why therapeutic HPV vaccines, unlike their prophylactic counterparts, have not yet progressed to late-stage clinical trials.

In contrast, peptide- and protein-based vaccines are relatively easy to produce and considered very safe as they contain no genetic material. The drawbacks are that peptide vaccines must match to the patient's HLA type and generally have low immunogenicity, and that protein-based vaccines generally elicit stronger antibody responses than cellular immune responses.

Lack of mechanistic knowledge hinders therapeutic vaccine development

Whereas the mechanism by which vaccination can prevent HPV infection clearly involves the generation of high-titer neutralizing antibodies, the precise mechanisms that lead to the successful elimination of HPV-infected cells are not fully understood and appear far more complex. This lack of understanding of the immune correlates of protection is the main reason why therapeutic HPV vaccines, unlike their prophylactic counterparts, have not yet progressed to late-stage clinical trials. The same

Table II. Therapeutic HPV vaccines in clinical development for cervival dysplasia[a]

Vaccine	Composition	Company	Development status
HspE7	Heat-shock protein based, containing E from HPV16	StressGen, Roche	Phase II (US)
TA-CIN	Fusion protein based, containing E6, E7, L2 from HPV 16 and 18	Cancer Research Technology	Phase II (UK)
ZYC-101a	Plasmid-based DNA vaccine encoding E6, E7 from HPV16 and 18	MGI PHARMA Biologics	Phase II (US)
MVA-HPV-IL2	Viral vector encoding E6, E7 from HPV16	Transgene	Phase II (France)

a Source: R&D Insight (Adis International)

problem also hinders the development of vaccines for therapy of other tumor types. Future trials in HPV will likely include combining heterologous vaccination vehicles in the form of prime/boost strategies, which have shown great promise in preclinical murine models and also in the limited clinical trials that have been performed.

Efforts directed towards early-stage disease

The development of therapeutic vaccines for HPV-associated disease is now focused more on patients with cervical dysplasia than on those with late-stage cervical cancer. Although current surgical treatments for precancerous HPV lesions are generally effective and well tolerated, they do not address the cause of the problem – namely HPV infection and viral persistence. Half of all patients who undergo the loop electrosurgical excision procedure (LEEP) – a standard treatment for high-grade cervical dysplasia – return to the clinic with either recurrent or new lesions. The theoretical advantage of therapeutic vaccines over surgery is the direct targeting of HPV proteins through the patient's own immune system, conferring long-term immunity that protects against re-infection. Several candidate therapeutic vaccines are currently in clinical trials with pharmaceutical companies (see table II).

Vax to the future

Advances in HPV vaccine development for the prevention and treatment of cervical cancer have produced encouraging results in clinical trials over the past five years. The next 5–10 years will be particularly exciting because we should gain a clearer understanding of the long-term protective effects of VLP-based vaccines.

There is widespread optimism that at least one prophylactic HPV vaccine will be approved by the FDA and made available to the public within the next 2–3 years. However, several issues will need to be addressed before vaccination can make a substantial public health contribution, including: defining the population to be vaccinated; deciding how or if cervical cancer screening programs should be adjusted; and determining how to implement effective vaccination and screening programs in developing countries.

Clinical development of therapeutic HPV vaccines is at an earlier stage than that of their prophylactic counterparts and is limited by a lack of understanding of the immune correlates of efficacy. Ongoing studies should shed light on the mechanisms that hinder successful treatment, such as immune evasion or suppressive T cells, enabling the design of vaccines that counteract these effects. In the long term, effective global implementation of both prophylactic and therapeutic vaccination regimens could result in a significant reduction in both healthcare costs and worldwide cervical cancer incidence.

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