Prophylactic HPV vaccines

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Two HPV L1 VLP vaccines have been developed, providing protection for at least 5 years and reducing the risk of cervical cancer

The ability to generate human papillomavirus (HPV) virus-like particles (VLPs) by the synthesis and self-assembly in vitro of the major virus capsid protein L1 has transformed our prospects for preventing both benign and malignant HPV-associated genital disease and, in particular, for significantly reducing the incidence of cervical carcinoma in women. Two HPV L1 VLP vaccines have been developed. Both of these vaccines have been shown to be safe and highly immunogenic, generating high titres of neutralising antibody that persist at measurable levels higher than those measured in natural infections for at least 60 months post-vaccination. This suggests that strong immune memory is generated. At present, the assumption is that the protection achieved by these vaccines against HPV-induced ano-genital pathology is mediated via serum-neutralising IgG. However, since there have been no vaccine failures thus far, immune correlates of protection have not been established. The available evidence is that the immunodominant neutralising antibodies generated in natural infections are type-specific and are not cross-neutralising, although highly homologous HPV pairs share cross-neutralisation epitopes. Cross-reactive and cross-neutralising antibodies are generated in HPV L1 vaccines. At lower concentrations, cross-protection against incident infection has been shown, but the duration of any cross-protection that might be elicited is uncertain. L1 VLP vaccines are prophylactic, not therapeutic, vaccines and for maximal population effectiveness should be delivered before sexual activity begins—that is, to pre-pubertal females (or males). Robust antibody responses have been demonstrated in immunogenicity bridging studies in 9–15-year-old boys and girls. However, social and cultural issues may be important in determining vaccine take-up in the optimal cohort.

Papillomaviruses are small, double-stranded DNA viruses that infect the squamous epithelia (skin and internal mucosae) of both animals and humans. Human papillomaviruses (HPVs) are a large branch of this family: at least 200 known HPVs have been isolated from tissue biopsies and, of these, approximately 100 have been fully sequenced. Despite the daunting size, HPVs fall basically into two groups: those that infect skin, or cutaneous surfaces, and those that infect the internal wet squamous mucosal surfaces, particularly the genital tract. Within these groups, there are low-risk types (lrHPV), which generate benign lesions (in other words, warts), and high-risk or oncogenic types (hrHPV), which are associated with cancers and their precursor lesions. Primary intervention by vaccination is the most effective strategy for the control of disease caused by viruses. In 2006, a prophylactic vaccine that protects against the most common low-risk and high-risk HPV types in genital infections was licensed and a second vaccine targeting hrHPV only is expected to receive a license early in 2007.

BURDEN OF DISEASE ATTRIBUTED TO GENITAL HPVS

Approximately 40 HPV types regularly or sporadically infect the mucosal epithelial surfaces of the lower genital tract, causing both warts and cancers. The lrHPV types HPV6 and 11 cause more than 90% of external genital warts, with minor types (HPV42, 44) and some hrHPVs contributing to the remaining 10%.[1] HPV-associated malignant disease in the genital tract is dominated by HPV16 and HPV18 which, with their close relatives (31, 33, 35, 52, 58, 39, 45, 59, 56, 66 and 51), are the cause of virtually all cervical cancer[2] and the majority of the high-grade cervical cancer precursor lesions CIN2/3 (cervical intra-epithelial neoplasia). Thus, 99% or more of biopsies of invasive cervical cancer worldwide,[3] and approximately 80% of CIN2/3, contain hrHPV DNA sequences.[4] HPV16 dominates, and is present in at least 50% of cancers irrespective of geographical location, followed by HPV18 (7–20%). However, invasive cervical cancer is not the only malignant disease associated with hrHPV infection; HPV DNA sequences are found in a proportion of anal, vulvar, vaginal, penile, and head and neck cancers (table 1); again, HPV16 is the dominant oncogenic type, followed by HPV18. Overall, the global malignant burden attributable to HPV infection is calculated to be 3.71% of all cancers.[5]

Benign disease caused by genital lrHPVs is not trivial. Genital warts are the most common viral sexually transmitted disease in the UK, with 79,618 new cases reported from STD clinics in 2004. They are highly infectious, result in significant morbidity and cost the UK healthcare system £25–30 million per annum. A maternal history of genital warts[6] is associated with a 231-fold risk for recurrent respiratory papillomatosis (RRP), which is an uncommon but potentially devastating disease that is characterized by the growth of wart-like benign neoplasms throughout the aero-digestive tract that often requires repeated surgery.[7]

HPV VACCINES: RATIONALE

Traditionally, prophylactic vaccines that generate virus-specific neutralising antibody have represented a cost-effective means to control viral diseases. HPV should, in theory, be no exception, but the exquisite host and tissue tropism and complex biology of the papillomaviruses differentiates them from most other viruses–against which vaccination has proven successful. The HPV life cycle is exclusively intra-epithelial and only a fully differentiated squamous epithelium supports the complete infectious cycle and the production of infectious particles. There is no detectable viraemia; virus particles are shed from mucosal surfaces far from lymphatics and vascular channels and, not surprisingly, systemic cellular and humoral immune responses to HPV antigens are poor. Serum-neutralizing antibody to the major capsid protein L1 is generated in genital HPV infections, but neutralizing antibody titres are low and only about 40–50% of infected individuals sero-convert.[8] Furthermore, the degree of protection and the duration afforded by antibody in natural infections is not known[9], and re-infections with the same genotype are thought to occur.

Would, therefore, vaccines that generate neutralising antibody protect? The evidence from animal papillomavirus infections, including some of the earliest published works from Shope (the founding father of papillomavirus research), clearly showed that neutralizing antibody was protective.[10] In Shope's experiments, if rabbits were infected systemically with the cotton-tail rabbit papillomavirus (CRPV) by direct injection of virus into

Table 1 The burden of malignant disease attributable to HPV infection[5]

Site	Attributable to HPV (%)	Developed countries		Developing countries	
		Total cancers	Attributable to HPV	Total cancers	Attributable to HPV
Cervix	100	83,400	83,400	409,400	409,400
Penis	40	5,200	2,100	21,100	8,400
Vulva, vagina	40	18,300	7,300	21,700	8,700
Anus	90	14,500	13,100	15,900	14,300
Mouth	⩾3	91,200	2,700	183,100	5,500
Oro-pharynx	⩾12	24,400	2,900	27,700	3,300
Other	0				
All sites		5,016,100	111,500	5,827,500	449,600

HPV, human papillomavirus.

the muscle or bloodstream, papillomas did not arise on the skin of the challenged animals, but neutralizing antibody was generated and the animals were completely resistant to viral challenge by abrasion of the epithelium. This and other data strongly suggested that generating neutralizing antibody to virus capsid protein would be an effective prophylactic vaccine strategy. Neutralising antibodies are directed against the L1 capsid protein, and the generation of this antibody requires the tertiary or native structure of the protein. As these viruses cannot be grown in bulk in tissue culture, and viral particles (particularly of the oncogenic types) are sparse in lesions, the generation of native structure, or properly folded L1 protein, was challenging. The challenge was met by the demonstration that if the L1 gene was expressed via a recombinant baculovirus,[11 12] the L1 protein was produced in large amounts and self-assembled into a virus-like particle (VLP) or empty capsid that is geometrically and antigenically almost identical to the native virion. These VLPs were shown to generate neutralizing antibody in the animal models and immunized animals were protected against high-level virus challenge.[13–15]

CURRENT HPV VACCINES

Two HPV prophylactic vaccines have been developed. The first is Cervarix™, a bivalent HPV16/18 VLP vaccine from GlaxoSmithKline; the second is Gardasil™, also known as Silgard, a quadrivalent HPV16/18/6/11 VLP vaccine from Merck Vaccines. The details of these vaccines and their trials are shown in table 2. The vaccines are sub-unit vaccines consisting of VLPs, produced by recombinant technology; they do not contain any live biological product or DNA and thus are non-infectious. In 2006, Gardasil™ was licensed in many countries, including the USA and Europe.

Vaccine endpoints

A critical issue for HPV vaccines against oncogenic HPVs is how to ascertain vaccine efficacy, and the advantages and disadvantages of the possible endpoints have been reviewed extensively.[16] The conventional measurable endpoint of vaccine efficacy—disease incidence—is not feasible in the case of cervical cancer for both practical and ethical reasons. High-grade CIN (CIN2/3) is accepted as the immediate precursor of invasive cervical cancer and for vaccine licensure; the endpoint of CIN 2/3 or worse has been accepted widely as an ethically acceptable proxy for cervical cancer.

Vaccine efficacy

Both vaccines have been evaluated in randomised, placebo-controlled, clinical trials. In women who have no evidence of exposure or infection to the HPV genotypes in the vaccine, both vaccines show high efficacy, with more than 90% reduction in persistent infection (HPV DNA of the same type detected on two successive occasions 6–12 months apart in a woman previously HPV DNA-negative) and 100% reduction in high-grade cervical lesions.[17 18] Although the numbers are small, in the according-to-protocol (ATP) groups in the Phase II trial of the bivalent vaccine[17], there was 100% efficacy against the development of HPV16/18-asscociated high-grade CIN2/3 (table 3).

The efficacy of the quadrivalent vaccine has been evaluated against high-grade CIN, vulvar and vaginal precancerous lesions and genital warts (tables 4 and 5). In the ATP group, 100% protection against disease caused by the vaccine HPV types was achieved.

In the quadrivalent trial, 27% of women had evidence of previous exposure or ongoing infection with one or more of the vaccine HPV types. No protective effect of the vaccine against CIN2/3 was seen in women who were HPV16/18 DNA-positive and/or sero-positive—that is, those who had an immune response but not cleared virus. The lack of protection in this group was not surprising as these are prophylactic not therapeutic vaccines. Interestingly, a modest but non-significant reduction in disease was seen in women who were HPV DNA-positive but sero-negative at entry.

Immunogenicity

The measurement of specific serum immunoglobulin G (IgG) anti-L1 VLP antibodies by immunoassays in vaccinated and unvaccinated individuals is the main parameter used in the current vaccine trials to monitor vaccine-induced immune responses. The immunoassay used in the trials of the quadrivalent Gardasil™ vaccine is a competition assay measuring one neutralising antibody species only, whereas that used in the evaluation of the bivalent Cervarix™ vaccine is an ELISA that measures total anti-VLP serum antibody (for review, see[19]). Consequently, neither direct comparisons of antibody responses to the different vaccines nor for the quadrivalent vaccine to the different HPV VLP types can be made.

VLPs are highly immunogenic and, in VLP-immunised individuals, the peak anti-VLP antibody responses are substantially greater (at least 1–3 logs) than those made at sero-conversion in natural infections.[20 21] However, what is the duration of the protection induced by the vaccines? The data from the trials are very encouraging, with serum antibody levels falling from the peak levels achieved after the third immunization to a lower concentration that persists at the same level (at least 10–20 times that of natural infection) for at least 60 months post-vaccination.[17 18 22] The long-term duration of protection depends on immune memory and there is evidence that both vaccines induce good immune memory. Increased numbers of circulating memory cells are generated after immunisation with the bivalent vaccine and this is attributed to the novel adjuvant ASO_4.[23] Anamnestic responses have been shown to the quadrivalent vaccine. Antibody responses in women entering the quadrivalent Phase II study, who were already sero-positive and DNA-negative for vaccine-type HPV, were about twice the response in women who were naïve to the respective HPV types, suggesting an anamnestic response

Table 2 HPV L1 VLP vaccine profiles

	Gardasil™	Cervarix™
L1 VLP antigens	HPV6 20 μg HPV11 40 μg HPV16 40 μg HPV18 20 μg	HPV16 20 μg HPV18 20 μg
Expression system	Yeast [*S cerevisiae*]	Baculovirus
Adjuvant	Proprietary aluminum hydroxyphosphate sulfate (225 ug)	ASO_4 Aluminum hydroxide (500 μg) plus 50 μg 3-deacylated monophosphoryl lipid A
Injection volume	0.5 ml i.m.	0.5 i.m.
Immunisation schedule	0, 2 and 6 months	0, 1 and 6 months
Adolescent safety/immunogenicity bridging trials	Females and males 9–15 years	Females 10–14 years Males 10–18 years (in progress)
	Licensed	License application made

HPV, human papillomavirus; VLP, virus-like particle.

to infection. Early results from a challenge study of 241 women, in which vaccinated women were given a booster 5 years after enrolment, showed rapid and enhanced antibody responses after the fourth immunisation characteristic of an anamnestic response (LL Villa, personal communication, 2006).

MECHANISM OF PROTECTION

The mechanisms by which protection is being effected by VLP vaccines is not fully understood and, at present, there are no immune correlates of protection as all vaccinated subjects have sero-converted and there have been no obvious vaccine breakthroughs. The assumption is that the high titres of neutralising serum antibody to L1 generated by VLP vaccines provides protection, but what is the evidence to support this? The most convincing evidence is from pre-clinical experiments in rabbits, in which passive transfer of purified IgG from hyper-immune donors immunized with CRPV L1 VLPs, completely protected the naïve rabbit recipient from papilloma development after virus challenge.[24 25] Only animals immunized with intact VLPs generated neutralizing antibody and only purified IgG from these animals protected the naïve recipients. Microtrauma and abrasion of the epithelial surface is widely held to be the mechanism by which the virus infects genital basal keratinocytes and there is experimental evidence to support this (J Roberts, personal communication, 2006). Epithelial denudation with the retention of a basement membrane would probably result in serous exudation and rapid access of serum IgGs to the virus particles. Furthermore, the portio surface of the cervix and the upper vaginal epithelium are bathed in cervical mucous, and the dominant immunoglobulin in cervical mucous is IgG that has been transudated across the endo-cervical and squamo-columnar surfaces.[26] There is evidently rapid and easy access of serum antibody to the virus particles, thereby explaining the extraordinary efficacy of the VLP vaccines.

Cross-protection

The humoral immunity induced by the VLP vaccines appears to be predominantly type-specific, but there is considerable amino-acid sequence homology in L1 between closely related HPV types[27]. This implies that there could be cross-neutralising epitopes. There is evidence from the Phase II trial of Cervarix™ that HPV16/18 vaccinees are partially protected against incident infection with HPV31 and HPV45.[17] The possibility of cross-protection from the VLP vaccines is strengthened by evidence that cross-reactive and cross-neutralising antibodies against HPV31 and 45 are generated after vaccination with Gardasil™, although at antibody concentrations that are 1–2 logs lower than the dominant type-specific neutralizing antibodies (JV Smith, personal communication, 2006). However, it must be emphasized that, at the present time, there is no evidence for cross-protection against HPV45- or 31-induced disease—that is, CIN2/3—and, if such cross-protection does occur, it is likely to be partial and not complete.

It seems likely that second-generation vaccines will need to consist of or include other oncogenic HPV types and a frequently-asked question is, will we need different ''cocktails'' of HPV types for different populations? This seems unlikely. HPV16 and HPV18 are the dominant types worldwide that are consistently detected in 70% of all cervical cancers. A further six types—HPV45/31/56/52/35 and 33—consistently make up the remaining 20% to 30%, irrespective of the geographic region. A polyvalent vaccine that contained these eight types would effectively protect against more than 90% of all cervical cancers.[28]

WHO AND WHEN TO VACCINATE

HPV L1 VLP vaccines are prophylactic, not therapeutic; therefore, they prevent and do not treat infection. The available evidence is clear that immunization with these vaccines will not be effective in individuals who have established HPV infections of the types included in the current vaccines (Hildesheim, personal

Table 3 Vaccine efficacy: Cervarix™[17]

Endpoint	Number of women in vaccine group	Number HPV16 related	Number HPV18 related	Number of women in placebo group	Number HPV16 related	Number HPV18 related
ASCUS	505	1	1	497	32	17
LSIL	505	1	1	497	22	6
CIN1+	481	0	0	481	8	0
CIN2+	481	0	0	470	5	0

ASCUS, abnormal squamous cells of unknown significance; CIN, cervical intra-epithelial neoplasia; HPV, human papillomavirus; LSIL, low grade squamous intra-epithelial lesion.

Table 4 Vaccine efficacy: Gardasil™

Endpoint	HPV vaccine cases (n = 9342)	Placebo cases (n = 9400)	% Efficacy	95% CI
HPV16/18-related CIN3 or AIS	0	52	100	93,100
HPV16/18-related CIN3	0	47	100	92,100
HPV16/18-related AIS	0	9	100	49,100

(http: & www.cdc.gov/nip/acip/slides/jun06/hpv-2-barr.pdf)
HPV-negative modified intention to treat (HN-MITT): endpoint HPV16/18-related CIN3 and/or AIS
AIS, ; CIN, cervical intra-epithelial neoplasia; HPV, human papillomavirus.

communication 2006). Genital HPV infection is usually, but not always, sexually transmitted; the most important risk period for acquisition of HPV16 and HPV18 appears to be the mid- to late-teens and early adulthood.[29] To maximize vaccine benefit, vaccine protection must cover this period. Immunologically, the optimal time for immunisation with VLP vaccines is before puberty. Immunogenicity bridging studies from the quadrivalent vaccine—which looked at antibody concentrations achieved after immunisation in 9–15-year-old girls and boys—show that antibody levels after HPV VLP vaccination are higher in 9–15-year-old girls than in 16–23-year-old women. Antibody concentrations in girls remain at constant levels over the 9–11-year-old range, but fall quite sharply at 12–13 years old (the average age of puberty), with a shallow decline thereafter.[30] These considerations imply that the target group for vaccination should be, in the first instance, pre-adolescent girls in the 9–12-year-old age group.

Vaccination in sexually active women

But what about sexually active women who may have been exposed to HPV? The Phase III trials have shown that vaccination of HPV16/18 DNA-negative women—16–26 year olds—does protect against the development of HPV16/18-related CIN2 or 3, implying that women in this age group can be vaccinated with confidence. However, as the current data indicate that the vaccines would only prevent 70% of cervical cancers (even in the ideal vaccination scenario), all women will have to stay in a cervical cancer screening programme if this is available.

Male vaccination

The potential gains from vaccinating males are that herd immunity would be achieved and virus transmission interrupted effectively. The evidence from the immunogenicity bridging studies[30] show that immunising 9–15-year-old boys induces antibody responses equally as effectively (if not more effectively) than immunising 9–15-year-old girls. However, all the efficacy trials have included women only and there is no efficacy data in men that is available, although trials are ongoing. The arguments against vaccinating boys against the oncogenic HPVs are based on health economic considerations and cost-effectiveness. In a heterosexual population, the spread of HPV infection can be stopped entirely by complete protection of one sex alone[31] and dynamic simulation models of HPV transmission show that if high coverage of females can be achieved, there is little additional reduction in cervical cancer to be gained by vaccinating males.[32] However, when external genital warts are factored into the equation, these arguments lose some of their force. Furthermore, vaccine strategies targeting girls only have not been successful previously—for example, for rubella—and the potential acceptability of targeting one sex only will be relevant in deciding public-health strategies.

CONCLUSIONS

The ability to generate HPV VLPs by the synthesis and self-assembly in vitro of the major virus capsid protein L1 has transformed our prospects for preventing benign and malignant disease associated with genital HPV infection. Two HPV L1 VLP vaccines have been developed: a quadrivalent HPV6/11/16/18 and a bivalent HPV16/18 product. Both vaccines are highly immunogenic and well tolerated. The vaccines have been shown in the various trials to be effective at preventing infection and diseases related to the vaccine HPV genotypes in women who were HPV DNA-PCR-negative at baseline. The protection generated by the vaccines persists for at least 5 years and, since antibody levels remain high after 5 years and there is evidence of good immune memory, it is likely that protection will be long lasting. HPV vaccines will reduce, but not eliminate, the risk of cervical cancer, and screening programmes will remain important secondary interventions for cervical cancer in vaccinated populations, although the screening modalities will change. The primary target group for immunisation with the HPV vaccines is likely to be pre-adolescent girls, but there could be benefit in vaccinating other groups (men, sexually active women of all ages) and the cost-effectiveness of these interventions needs to be evaluated.

Table 5 Gardasil™: Vaccine efficacy against external genital lesions (vulval and vaginal intra-epithelial neoplasia and genital warts)

Population	Endpoint	GARDASIL™ cases	Placebo cases	% Efficacy	% CI	p-Value
PPE	HPV6/11/16/18-EGL	0	40	100	88,100	<0.001
	HPV6-related EGL	0	23	100	83,100	
	HPV11-related EGL	0	10	100	55,100	
	HPV16-related EGL	0	10	100	56,100	
	HPV18-related EGL	0	3	100	<0,100	
HN-MITT	HPV6/11/16/18-EGL	3	59	95	84,99	

(http: & www.cdc.gov/nip/acip/slides/jun06/hpv-2-barr.pdf)
Per protocol (PPE) and HN-MITT (HPV-negative modified intention to treat): endpoint HPV6/11/16/18-related external genital lesions (EGL)

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