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Summary

Persistent infection with oncogenic human papillomavirus (HPV) is a necessary cause of cervical cancer. Moreover, HPV type 16 (and to a lesser degree HPV type 18) is linked with more rare cancers, namely cancer of the vulva, vagina, penis, anus, oropharynx and larynx. Effective prophylactic vaccines have been developed. In this review, we briefly address immunological aspects of HPV infection and the results of HPV vaccination trials. Internationally standardized monitoring and evaluation of prophylactic HPV vaccination programmes will be essential for arriving at the most (cost-)effective strategies for cancer control.

Keywords: cancer prevention, surveillance, virus-like particles

Immunity against human papillomaviruses

Humoral immunity

Human papillomavirus (HPV) infection is restricted to epithelial cells; therefore presentation of viral antigens to the host immune system is limited. Natural HPV infection of the genital tract gives rise to a slow and modest but measurable serum antibody response in most but not all infected individuals [1,2]. The intensity of the antibody response depends on viral load and persistence [3]. The presence of HPV antibodies is long-lasting, but does not contribute to the clearance of established infections [4]. HPV serology is an important tool in epidemiological studies to assess past exposure [5–8].

The capsid of papillomaviruses is composed of two viral proteins: the major capsid protein, or L1, and the minor capsid protein, or L2 [9]. Virus-neutralizing anti-L1 antibodies are essentially type-specific [2,10,11]. The L2 protein is situated more internally of the capsid, but a small segment is exposed at the surface and can also be recognized by virus-neutralizing antibodies [12–14]. These anti-L2-antibodies are less potent than anti-L1 antibodies [12,14,15], but they appear to show some cross-reactivity to heterologous HPV types [16,17].

The discovery that the L1 capsid protein could be expressed in eukaryotic cells and could self-assemble into so-called virus-like particles (VLPs) was a critical step in the development of HPV vaccines [18]. Correct conformation of the capsid proteins is necessary to elicit protective antibodies [19]. Denaturation or improper folding of the L1 protein alters the presentation of epitopes, resulting in that mainly unprotective antibodies are induced. HPV L1 VLPs contain the same conformationally dependent neutralizing epitopes that are present on infectious viruses. Essentially, all neutralizing antibodies against HPV16 can be blocked using a monoclonal antibody designated HPV16.V5 [20]. This antibody recognizes a surface-exposed loop called the FG loop, with an adjacent loop (the HI loop) contributing to maintaining correct conformation of the FG loop [21]. Although the region recognized by HPV16.V5 appears to be essential for neutralization, deletion of the HPV16.V5 contact residues affects the binding of immune sera only marginally, indicating the presence of several different neutralizing epitopes in the region [22].

Cellular immunity

Clearance of a naturally acquired HPV infection is triggered by a specific cell-mediated immune (CMI) response

(reviewed in [23]. Dendritic cells or Langerhans cells, present in the cervical epithelium, play an important role in recognizing HPV-infected cells and stimulating T helper 1 (Th1) cells, which elicits the production of cytotoxic T lymphocytes (CTL) [24]. These cytotoxic effector cells attack infected cells, resulting in resolution of the infection [25]. However, little is known about how to modulate these immune responses.

HPV vaccination

Prophylactic vaccination

Vaccination with VLPs gives rise to virus-neutralizing antibodies in serum. Vaccination by intramuscular injection of L1 VLPs has been shown to be highly immunogenic and well-tolerated in Phase I trials. Three randomized placebo-controlled Phase II trials with, respectively, a monovalent HPV16 vaccine, a bivalent HV16/18 vaccine and a quadrivalent HPV6/11/16/18 vaccine candidate, have demonstrated consistently almost complete protection against persistent infection with the targeted HPV types [26–30]. Moreover, these trials confirmed the safety of the vaccines and showed strong immunoresponses that were several orders of magnitude higher than those observed after natural infections. The characteristics and the main reported results of these studies have been summarized previously [31].

Two pharmaceutical companies [Merck Sharp & Dohme (MSD) and GlaxoSmithKline (GSK)] are currently conducting large multi-centre Phase III vaccine trials in all continents except Africa [32]. In addition, the National Cancer Institute (USA) is conducting a population-based trial in Costa Rica. All these Phase III trials aim to demonstrate that vaccines protect against histologically confirmed high-grade cervical intraepithelial neoplasia (CIN) associated with the targeted HPV types.

Therapeutic HPV vaccines

Development and maintenance of cervical precursors and their progression to invasive cancer requires the continued intracellular expression of the viral oncoproteins of E6 and E7 [33,34]; therefore, therapeutic vaccines have aimed at stimulating T cell responses against these viral early oncogenes. Currently, different methods and formats of therapeutic vaccines such as administration of peptide antigens or recombinant proteins, plasmid DNA vaccines, viral vector vaccines and administration of E7-pulsed dendritic cells are being evaluated [35]. These vaccines have been variably immunogenic, and there has often been no correlation with clinical outcomes [25].

The addition of early antigens (E6 or E7 in particular) to the L1 virus-like particle (VLP) vaccines is also being investigated to determine if a cell-mediated immune response could be elicited along with the antibody response to the L1 VLP component [17]. If so, this would open the way to development of chimeric vaccines with a therapeutic component included for combined use in treatment and prophylaxis [36,37].

Licensure of VLP vaccines

On 8 June 2006, the US Food and Drug Administration (FDA) approved Gardasil®, the quadrivalent vaccine, developed by MSD, containing VLP L1 of HPV types 6, 11, 16 and 18, for use in females 9–26 years of age (press release P06-77, 8 June 2006, *FDA News*; available at: http://www.fda.org). The FDA recognized the indications of protection against cervical cancer, genital warts (condyloma acuminata), cervical adenocarcinoma *in situ*, CIN (grades 2, 3 and 1), vulvar intraepithelial neoplasia (grades 2 and 3) and vaginal intraepithelial neoplasia (grades 2 and 3) caused by the vaccine types. The FDA press release stated that the vaccine is effective if administered prior to HPV infection.

The Advisory Committee for Immunization Practices (ACIP) recommended routine vaccination of girls aged 11–12 years, but also allowed the administration of the vaccine to girls aged 9 or 10 years and girls and young women aged 13–26 years (available at: http://www.cdc.gov/nip/vaccine/hpv/).

On 27 July 2006, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicine Agency (EMEA) adopted a positive opinion, recommending to grant a marketing authorization of Gardasil for the prevention of high-grade cervical dysplasia (CIN 2/3), cervical carcinoma, high-grade vulvar dysplastic lesions (VIN 2/3) and external genital warts (press release doc.ref. EMEA/CHMP/274938/2006, available at: http://www.emea.eu.int/pdfs/human/opinion/Gardasil27493806.pdf). On 20 September 2006, EMEA has provided the official authorization for marketing of the vaccine in the European Union, specifying that its use should be in accordance with official recommendations.

An application has also been filed with the EMEA for licensure of Cervarix (the bivalent VLP L1 HPV16/18 vaccine manufactured by GSK).

Current HPV vaccination issues

Vaccination against non-oncogenic HPV

HPV types 6 and 11 jointly cause approximately 90% of genital warts [38]. These types also cause some of the low-grade dysplastic cervical lesions. Moreover, in rare circumstances HPV types 6 and 11 can cause serious disease. HPV6, and in particular HPV11, are the major causes of recurrent respiratory papillomatosis, a severe disease that may be fatal. So-called giant condylomas or Buschke–Löwenstein tumours of the vulva, penis and anus are also associated with these HPV types [39]. These tumours rarely metastasize, but may sometimes be fatal. The vaccine manufactured by Merck contains L1 VLPs of both HPV 6 and HPV 11. High clinical

and statistically significant protection was confirmed in Phase III trials (press release P06-77, 8 June 2006, *FDA News*; available at: http://www.fda.org).

Intermediate end-points

Prevention of cervical cancer is the most important expected clinical benefit if HPV vaccination. Trials have used surrogate end-points because cancer develops slowly and cancer as an end-point requires unrealistically large and lengthy studies, and state-of-the-art clinical management requires that premalignant lesions are treated immediately, making cancer both unfeasible and unethical as end-point in a clinical trial setting [40]. Protection against infection seems to be an obvious end-point for an infectious disease. However, HPV infection is extremely common, with a majority of the entire female population having experienced HPV infection at some point in their lives, but with most infections resolving spontaneously. Because HPV-induced cancer occurs in only a small proportion of exposed individuals, estimates of vaccine efficacy against infection cannot be extrapolated to be valid against cancer.

A World Health Organization (WHO) expert group consensus report proposed histologically confirmed high-grade CIN or worse (i.e. including cervical cancer) associated with one of the target vaccine types as an acceptable surrogate end-point for Phase 3 vaccination trials [40]. Type-specific persistence of infection, defined as the presence of the same HPV type at two or more consecutive visits separated by 6–12 months, is another interesting outcome measure that is a later and thus more informative end-point than protection against any infection [41].

Duration and consistency of the antibody response to VLPs

Type-specific L1 VLP-antibodies reach maximum titres at month 7, i.e. 1 month after administration of the third dose. Titres decline until month 24 and remain stable thereafter [28,42]. At 3 years, antibody titres remain two- to 20-fold higher than in placebo controls [42]. Complete protection against HPV16-associated CIN lesions was observed over the whole follow-up duration of two Phase IIb trials: 48 months for the monovalent HPV16 vaccine and 53 months for the bivalent HPV16/18 vaccine [26,27].

Optimal target age range for vaccination

The incidence of HPV infection is very high among sexually active women [43–45]. Therefore, vaccination before initiation of sexual contacts is the safest strategy for complete protection. However, vaccination programmes targeting 12-year-olds will, compared to programmes targeting 15-year-olds, delay the cancer prevention gains by 3 years [46]. The highest HPV incidences are between 16 and 20 years of age, with a peak incidence at 18 years [46]. 'Catch-up' vaccination programmes that target the age groups that are spreading the infection most actively will contribute to effective infection control. Large cancer-preventive gains are expected from catch-up vaccination up to 18 years of age and diminishing, but noteworthy gains are still seen up to 24 years of age [44].

In the vaccination trials, women who were vaccine-type HPV DNA- or seropositive at enrolment or who became HPV DNA-positive during the vaccination period were not part of the per-protocol population. Preliminary analysis of the large Phase III trial with the quadrivalent vaccine observed that protection against HPV16/18-associated $CIN2^+$ was absent among women who were baseline HPV DNA-positive and sero-positive for HPV16 or 18 and was strongly reduced (efficacy of 31·2; 95% CI: < 0–54·9%) for women who were HPV DNA-positive but seronegative at the time of vaccination [see GARDASIL (Human Papillomavirus [types 6, 11, 16, 18] Recombinant Vaccine, Vaccines and Related Biological Products Advisory Committee (VRBPAC); briefing document', available at http://www.fda.gov]. These data suggest a potential utility of testing for the HPV DNA and antibody status before vaccinating older women who have already initiated sexual contacts.

Immunization of males

Immunization of boys with VLPs elicits a serum immune response similar to that in girls. Because genital HPV infection is sexually transmitted, immunization of men may help to prevent infection of women. Modelling studies on herd immunity, i.e. indirect protection of those who remain susceptible, owing to a reduced prevalence of infections in the risk group for disease, have been published [47–49]. The utility of immunization of males depends on the assumed population coverage of vaccination, with successively smaller additional benefits seen in scenarios with high population coverage [50].

Vaccination programme strategies as a randomized health care policy

Design of HPV vaccination programmes will need to be based on estimations of the impact of HPV vaccination on the burden of cervical cancer incidence and mortality using mathematical modelling of projected effects from the observed surrogate endpoint effects [46,50,51]. For programme design issues that are ambiguous, notably which age groups should be targeted age and whether vaccination of males is required, randomization of vaccination programmes is an interesting option. That the incidence of cervical and other HPV-associated cancers does eventually decrease in vaccinated populations should then be verified by monitoring prevalence of HPV infection and

Table 1. Cumulative proportion of cervical cancers in Europe that are attributed to a ranked combination of human papilloma virus (HPV) types and the number of cervical cancers occurring each year expected to be caused by these types. According to recent estimates, 51 000 cases of cervical cancer occur yearly [59]. Sixty-five per cent, or 33 400 of cancer cases, are attributed to HPV16; 71·5% (or 6·1% more) can be attributed to HPV16 or HPV18. Almost 88% of cervical cancers are attributed to one of eight HPV types. Adapted from Munoz *et al.* [57] and Arbyn *et al.* [59].

HPV types prevented	Proportion of cervical cancers prevented	Number of annual cases prevented in Europe (rounded to hundreds)
16	65·4%	33 400
16 + 18	71·5%	36 500
16 + 18 + 33	77·1%	39 300
16 + 18 + 33 + 31	81·2%	41 400
16 + 18 + 33 + 31 + 45	84·1%	42 900
16 + 18 + 33 + 31 + 45 + 56	85·6%	43 700
16 + 18 + 33 + 31 + 45 + 56 + 35	86·8%	44 300
16 + 18 + 33 + 31 + 45 + 56 + 35 + 52	87·8%	44 800
All HPV	100%	51 000

incidences of HPV-associated diseases by registry-based follow-up [52–55].

HPV types

Antibody responses elicited by VLP immunization are specific for the individual HPV type, with limited cross-neutralization even for closely related HPV types. There are 13–18 different HPV types that have been proposed as oncogenic [39,56]. Although it would be technically feasible to add additional VLPs to the second-generation HPV vaccines, there is probably a limit for how large amounts of antigen can be included in combined vaccines without risking deteriorating responses against the major oncogenic HPV type, HPV16.

Table 1 shows the cumulative proportion of the main HPV types present in cervical cancer, estimated for Europe from studies conducted by the International Agency for Research on Cancer (IARC) [55]. Approximately 51 000 new cases of cervical cancer occur yearly in Europe [58,59]. Thus, by complete vaccination with a 100% effective HPV16 vaccine, 33 400 incident cases of cervical cancer could be avoided. An HPV16/18 vaccine could potentially avoid 36 500 cases per year (71·5%) and an octavalent vaccine could potentially reduce the incidence with 88%. This simple calculation assumes absence of 'type replacement' or cross-protection which, respectively, should decrease or increase vaccine efficacy.

Type replacement – what is meant and is it likely?

There is a theoretical concern that eradication of some HPV types will cause post-vaccination emergence of disease caused by types not included in the vaccine: 'type replacement'.

Type replacement is a viral population dynamics phenomenon and is defined as elimination of some types, causing an increase in the incidence of other types. This effect can occur only if two conditions apply: (1) there exists partial competition of different types during natural infection and (2) the vaccine does not afford cross-protection against types naturally competed against [60].

Several epidemiological studies have addressed the question of possible competition between infection with different HPV types. The presence of type-specific antibodies (a marker of past or present infection) for one HPV type is associated with a strongly increased risk for also being seropositive for other HPV types, also when adjusted for determinants of sexual behaviour. For example, the OR for being seropositive for HPV16/18/33 is 2·9 (95% CI: 1·6–5·3) if a woman is seropositive for HPV6/11, even when the risk is adjusted for sexual behaviour and other sexually transmitted infections [61]. This is the opposite tendency to the expected finding, had there been competition.

Furthermore, studies of multiple positivities of HPV DNA in the same samples have, in general, not found clear examples of types of HPV DNA that do not go together, as would have been expected had there been competition [62]. If anything, past infection with HPV appears to increase the likelihood to acquire a new infection. For example, Mendez *et al.* [63] reported on a cohort study where baseline HPV6/11 DNA positivity was associated with a 14·1-fold (95% CI 2·1–95·4) increased risk for incident infection with HPV18 at subsequent visits, where baseline HPV16/18 DNA was associated with a 5·7-fold (95% CI: 2·2–15·1) risk for HPV58 acquisition and no statistically significant decreased HPV incidences.

Viral dynamics could also be affected if the duration of infectivity is affected, i.e. if prior infection with one HPV type would affect the time it takes to clear infection with another HPV type. In a population-based cohort study of > 6000 women, baseline HPV seropositivity did not affect the clearance rate of other HPV types [64].

Thus, it seems that the first prerequisite for type replacement – natural competition – does not apply and that type replacement is therefore unlikely. However, it should be pointed out that most of the studies that have investigated viral type competition effects on incidence and/or clearance have had limited statistical power to detect small effects, particularly for rare HPV types.

Although it is not yet clear if there will be any cross-protection of the VLP vaccines, there are preliminary data from a trial vaccinating with an HPV16/18 VLP vaccine, where cross-protection was seen for related HPV types [94·2% (95% CI: 63·3–99·9%) protection against HPV45 and 54·5% (95% CI: 11·5–77·7%) protection against HPV31, respectively] [26] further decreasing the likelihood for replacement phenomena.

Viral escape mutants

Apart from the risk of changes in population dynamics of already existing types, the possibility exists that viral escape mutants forming new serotypes could occur. However, the fact that HPV replicates using the cellular DNA polymerases and thus has a very slow mutation rate suggests that this risk is low. This is also indicated by the fact that so far all different viral strains and variants of HPV16 from all over the world have been found to constitute a single serotype [65].

Attributable proportion/number of healthy women at risk

Because many women will be saved from cervical cancer caused by HPV16/18 by vaccination, the amount of healthy women who will be at risk for cervical cancer caused by other HPV types will increase. The proportion of cases prevented if an HPV type is eliminated is therefore not exactly the same as the proportion of positive cases but is given by S*1/1-RR, where S is the proportion of positive cases and RR is the relative risk. When HPV-related relative risks for cancer are increased about 100-fold, this effect is so small that it is usually ignored. However, for specific rare 'oncogenic' HPV types, the relative risks are not so high when compared to a reference category of all women without that specific HPV type. Consideration of attributable proportions is therefore of particular relevance when discussing benefits and caveats of including additional HPV types in second-generation HPV vaccines.

Monitoring of HPV vaccination programmes

HPV differs from most other vaccine-preventable diseases in that the major diseases to be prevented occur many decades after infection. Whereas the clinical research setting has provided approved vaccines that prevent infection and early clinical disease such as condylomas and CIN, it will be up to the surveillance setting to provide data that the expected cancer prevention gains will materialize as they should if there is appropriate population coverage and provided that type replacement or escape mutants do not occur. Other important tasks for the HPV surveillance include monitoring of long-term safety and actual effects on health care cost consumption.

As different countries will have different priorities, health care infrastructure and differences in their HPV epidemiology the HPV vaccination strategies chosen are likely to differ considerably. It would be particularly important to evaluate HPV vaccination programmes with regard to whether they result in an effective control of the infection, as inadequate control of infection will result in inadequate cancer control a few decades later.

What should be monitored?

Levels of protective antibodies in vaccinated subjects

As has been mentioned, the initial decline of the levels of HPV antibodies has levelled off and appears to be stable after 5 years. It is not known whether waning of HPV antibody levels in the long term will necessitate a vaccine booster (either as a general recommendation or directed to those who have developed insufficient levels). Because there have so far been almost no cases of vaccination failure, it has not been possible to define the minimum level of antibody levels that is required for protection. If a reliable immunological correlate of protection can be identified, this will help not only in assessing the requirement for booster vaccinations, it will also greatly facilitate evaluation of second-generation vaccines.

Population coverage of HPV vaccination

Many countries are likely to implement identifiable HPV vaccination registries to facilitate evaluation. If this is not conducted, the option exists to perform sero-epidemiological surveys to establish the vaccine-induced level of immunity in the population.

HPV DNA prevalences in sexually active teenage populations

As HPV incidences are very high, the effect of a successful HPV vaccination programme should be possible to measure rapidly by sentinel sampling in sexually active teenage populations. As it is particularly important to reach the most active populations when evaluating control of infection, strategies with sampling in clinics offering sexual counselling to the youth may be preferable to sampling in secondary schools. The outcomes to be measured include both whether there is efficient control of HPV types included in the vaccines and whether the prevalence of non-vaccine HPV types is stable.

Condyloma incidence

England and Wales implemented registration of condylomas in the 1970s, but surveillance of condylomas has been absent in other countries. Consequently, the epidemiology and public health burden of condylomas is not well known. However, symptomatic condylomas appear to be common

and the age-specific incidence curve of first-attack condyloma appears to be somewhat similar to the Chlamydia incidence. As the incubation time from exposure to clinical condyloma is between 3 and 12 months, and because some 90% of condylomas are caused by HPV types included in the quadrivalent HPV vaccine, the disappearance of condylomas from the sexually active youth population is expected to be the first clinical outcome of HPV vaccination programmes. If condylomas do not disappear, this will be a very early clinically noticed warning that the control of HPV infection is not adequate in the condyloma outbreak region and will necessitate investigation of possible reasons for this (such as insufficient population coverage, type replacement, waning immunity or problems in quality of the vaccination).

Cervical screening results

For Europe, the proportion of low-grade cervical dysplasia attributable to HPV vaccine types has been estimated at 24% and the proportion of high-grade cervical dysplasia, 57% [66]. With incubation times from 1 to 4 years, effective control of HPV should result in a significant decline in the burden of screen-detected precursor lesions requiring follow-up and treatment at medium-term follow-up.

For informative monitoring of whether or not remaining screen-detected lesions are attributable to HPV vaccine types, HPV typing of lesions will be required. In the case of low-grade lesions, the fact that triaging of borderline lesions (i.e. HPV testing with atypical squamous cells of uncertain significance (ASCUS) and referral only of those positive for oncogenic HPV) is now a recommended standard of care [67] may result in that informative HPV-type data for surveillance can be obtained from local laboratories performing HPV testing as a part of the screening programme. A prerequisite for this to happen is, of course, that HPV tests that include HPV typing are used.

For high-grade lesions, HPV typing of a random subsample of lesions is likely to be required. The fact that liquid-based cytology is gradually replacing conventional cytology is likely to simplify the issue, as the remaining left-over samples are of better quality for HPV typing. Surveys of type-specific HPV infections using cytological biobanks (stored Pap smears or residual liquid based cytological material) linked to vaccination registries will be an important tool to address type replacement, incidence of breakthrough infections, breakthrough lesions and duration of protection.

HPV-associated malignancies

A recent IARC review concluded that while cervical cancer is caused entirely by HPV, other sexually transmitted cancers are caused by HPV to a varying extent: penis 40%, anus 90%, vulva/vagina 40% and oropharynx 12% [68]. While HPV16/18 are responsible for only about 70% of cervical cancers, the type diversity in the non-cervical HPV-associated cancers is less: HPV16/18 are responsible for about 90% of the HPV-positive anal, vulvar/vaginal and oropharyngeal cancers [68]. However, whereas cervical cancer has been studied extensively the total amount of high-quality HPV typing observations is much lower for the other HPV-associated cancers, making the estimates less reliable. It seems likely that routine HPV typing of all cases of HPV-associated cancer forms will become an essential part of the long-term evaluation/monitoring of HPV vaccination programmes.

Combination of HPV vaccination and screening programmes

Current HPV vaccines include only the major oncogenic types, responsible for only 70% of cervical cancers. Moreover, as the vaccines are aimed at protecting HPV-naive individuals, and their effect on already exposed women is questionable, screening will continue to be necessary.

Nevertheless, the reduced background risk may, after just a few decades, allow an increase of the screening intervals. For example, it has been estimated that conventional cytological screening every 5 years starting at 30 years of age results in a 67% reduction in lifetime cervical cancer risk. Adding HPV16/19 vaccination to this programme resulted in a risk reduction of 89% [69]. Clearly, several aspects of monitoring and evaluation are the same or strongly interrelated for screening and vaccination, arguing that these complementary strategies need to be co-ordinated in a cervical cancer prevention programme [70].

Internationally comparable methods for monitoring of HPV vaccination programmes

The global HPV LabNet has been launched by the WHO as an initiative towards global quality assurance and standardization of HPV testing methods used in the follow-up of HPV vaccination programmes. The results of the international comparison of HPV serological methods found that the methods used are comparatively robust, provided that measurements are related to the same international standard serum that is assayed in parallel [71]. Self-defined 'titres' varied by orders of magnitude and self-interpreted results varied greatly in terms of sensitivity and specificity. However, when assay data were related to the international standard and when the standard was used to set the 'cut-off level' for positivity there was good agreement, both in terms of antibody levels and sensitivity/specificity [71]. International comparison of HPV DNA detection methods demonstrated the urgent need for quality assurance, as 29 global expert laboratories reported results with sensitivity for HPV DNA detection varying by 5 orders of magnitude and with both false positives and incorrect HPV typings being common [72].

For both HPV antibodies and HPV DNA tests, biological reference standards that will define an international unit of measurement are being launched. For quality assurance and as a basis for certification, global proficiency panels will be made available. Finally, a 'WHO Manual of HPV Diagnosis and Control' that will provide examples of state-of-the-art methods is expected by 2008.

Conclusions and recommendations

L1 VLP HPV vaccines have been found to be safe, well tolerated and to offer HPV-naive women a very high level of protection against HPV persistent infection and cervical intraepithelial lesions associated with the types included in the vaccine.

The reduction in background risk of cervical cancer by elimination of the most important HPV types will affect the cost-effectiveness of screening programmes and may, in the long term, allow increasing screening intervals. Co-ordinated quality assurance/montoring of HPV vaccination and cervical screening is advisable for finding the most (cost-)efficient strategies for cervical cancer control.

The continuous monitoring of which HPV types are spreading in the population will become necessary for early monitoring of 'type replacement' phenomena, inappropriate vaccination strategies or other reasons for vaccination failure. HPV-associated cancers and condylomas are now vaccine-preventable diseases and should from now on be subject to similar surveillance strategies to other vaccine-preventable diseases.

References

1 Carter JJ, Koutsky LA, Wipf GC, Christensen ND, Lee SK, Kuypers J, Kiviat N, Galloway DA. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of University women. J Infect Dis 1996; **174**:927–36.

2 Carter J, Koutsky L, Hughes J, Lee S, Kuypers J, Kiviat N, Galloway D. Comparison of human papillomavirus types 16, 18 and 6 capsid antibody responses following incident infection. J Infect Dis 2000; **181**:1911–9.

3 Ho G, Studentsov Y, Bierman R, Burk R. Natural history of human papillomavirus type 16 virus-like particle antibodies in young women. Cancer Epidemiol Biomarkers Prev 2004; **13**:110–6.

4 Shah KV, Viscidi RP, Alberg AJ, Helzlsouer KJ, Comstock GW. Antibodies to human papillomavirus 16 and subsequent in situ or invasive cancer of the cervix. Cancer Epidemiol Biomarkers Prev 1997; **6**:233–7.

5 Lehtinen M, Luukkaala T, Wallin K-L, Björge T, Luostarinen T, Thoresen S, Dillner J. Human Papillomavirus infection, risk for subsequent development of cervical neoplasia and associated population attributable fraction. J Clin Virol 2001; **22**:117–24.

6 Dillner J. The serological response to papillomaviruses. Semin Cancer Biol 1999; **9**:423–30.

7 Dillner J, Lehtinen M, Björge T *et al*. Prospective seroepidemiologic study of human papillomavirus infection as a risk factor for invasive cervical cancer. J Natl Cancer Inst 1997; **89** (17):1293–9.

8 Dillner J, Kallings I, Brihmer C *et al*. Seropositivity to human papillomavirus types 16 1996, 18 or 33 capsids and to Chlamydia trachomatis are markers of sexual behaviour. J Infect Dis **173**:1394–8.

9 Orth G, Favre M. Human papillomaviruses. Biochemical and biologic properties. Clin Dermatol 1985; **3**:27–42.

10 Roden R, Hubbert N, Kirnbauer R, Christensen N, Lowy D, Schiller J. Assessment of the serological relatedness of genital human papillomaviruses by hemagglutination inhibition. J Virol 1996; **70**:3298–301.

11 Hines J, Ghim S, Christensen N, Kreider J, Barnes W, Schlegel R, Jensen A. Role of conformational epitopes expressed by human papillomavirus major capsid proteins in the serologic detection of infection and prophylactic vaccination. Gynecol Oncol 1994; **55**:13–20.

12 Christensen ND, Kreider JK, Kan NC, DiAngelo SL. The open reading frame L2 of cottontail rabbit papillomavirus contains antibody-inducing neutralizing epitopes. Virology 1991; **181**:572–9.

13 Kawana K, Yoshikawa H, Taketani Y, Yoshiike K, Kanda T. Common neutralization epitope in minor capsid protein L2 of human papillomavirus types 16 and 6. J Virol 1999; **73**:6188–90.

14 Roden R, Yutzy W, Fallon R, Inglis S, Lowy D, Schiller J. Minor capsid protein of human genital papillomaviruses contains subdominant, crossneutralizing epitopes. Virology 2000; **270**:254–7.

15 White W, Wilson S, Palmer-Hill F *et al*. Characterization of a major neutralizing epitope on human papillomavirus type 16, L1. J Virol 1999; **73**:4882–9.

16 Nieland J, Silva DD. 1999. Chimeric papillomavirus virus-like particles induce a murine self-antigen-specifik protective and therapeutic antitumor immune response. J Cell Biochem 1999; **73**:145–52.

17 Greenstone HL, Nieland JD, deVisser KE *et al*. Chimeric papillomavirus virus-like particles elicit antitumor immunity against the E7 oncoprotein in an HPV16 tumor model. Proc Natl Acad Sci USA 1998; **95**:1800–5.

18 Zhou J, Sun XY, Syenzel DJ, Frazer I. Expression of vaccinia recombinant HPV16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. Virology 1991; **185**:251–7.

19 Kirnbauer R, Hubbert NL, Wheeler CM, Becker TM, Lowy DR, Schiller JT. A virus-like particle ELISA detects serum antibodies in a majority of women infected with human papillomavirus type 16. J Natl Cancer Inst 1994; **86**:494–9.

20 Wang Z, Christensen ND, Schiller JT, Dillner J. A monoclonal antibody against intact human papillomavirus type 16 capsids blocks the serological reactivity of most human sera. J General Virol 1997; **78**:2209–15.

21 Christensen N, Cladel N, Reed C *et al*. Hybrid papillomavairus L1 molecules assemble into virus-like particles that reconstitute conformational epitopes and induce neutralizing antibodies to distinct HPV types. Virology 2001; **291**:324–34.

22 Ryding J, Dahlberg L, Wallen-Öhman M, Dillner J. Deletion of a major neutralizing epitope of Human Papillomavirus type 16 virus-like particles. J General Virol 2007; **88**:792–802.

23 Man S. Human cellular immune responses against human papillomaviruses in cervical neoplasia. Expert Rev Mol Medical 1998, 1998; 1–19.

24 Niedergang F, Didierlaurent A, Kraehenbuhl J, Sirard J. Dendritic cells: the host Achille's heel for mucosal pathogens? Trends Microbiol 2004; **12**:79–88.

25 Stern P. Recent developments in human papillomavirus vaccines. Expert Opin Invest Drugs 2004; **13**:959–71.
26 Harper D, Franco E, Wheeler C *et al*. Sustained efficacy up to 4–5 years of bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised trial. Lancet 2006; **367**:1247–55.
27 Mao C, Koutsky L, Ault K *et al*. Efficacy of Human Papillomavirus 16 vaccine to prevent cervical intraepithelial neoplasia: a randomized controlled trial. Obstet Gynecol 2006; **107**:18–27.
28 Villa L, Costa R, Petta C *et al*. Prohylactic 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 2005; **6**:271–8.
29 Harper D, Franco E, Wheeler C *et al*. 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 2004; **364**:1757–65.
30 Koutsky L, Ault K, Wheeler C, Brown D, Barr E, Alvarez F, Chiacchierini L, Jansen K. A controlled trial of human papillomavirus type 16 vaccine. N Engl J Med 2002; **347**:1645–51.
31 Arbyn M, Dillner J. Review of current knowledge on HPV vaccination: An appendix to the European Guidelines for Quality Assurance in cervical cancer screening. J Clin Virol 2007; **38**:189–97.
32 Cohen J. Public health. High hopes and dilemmas for a cervical cancer vaccine. Science 2005; **308**:618–21.
33 Steenbergen R, deWilde J, Wilting S, Brink A, Snijders P, Meijer C. HPV-mediated transformation of the anogenital tract. J Clin Virol 2005; **32** (Suppl. 1):S25–S33.
34 zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002; **2**:342–50.
35 Stern P. Immune control of human papillomavirus (HPV) associated anogenital disease and potential for vaccination. J Clin Virol 2005; **32** (Suppl. 1):S72–S81.
36 Schiller J, Nardelli-Haefliger D. Chapter 17: Second generation HPV vaccines to prevent cervical cancer. Vaccine 2006; **24**:147–53.
37 Stanley M. Progress in prophylactic and therapeutic vaccines for human papillomavirus infection. Expert Rev Vaccines 2003; **2**:381–9.
38 Lacey C, Lowndes C, Shah K. Chapter 4: Burden and management of non-cancerous HPV-related conditions. HPV-6/11 Dis Vaccine 2006; **24**:35–41.
39 Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, Ghissassi FE. Carcinogenicity of human papillomaviruses. Lancet Oncol 2005; **6**:204.
40 Pagliusi S, Aguado M. Efficacy and other milestones for human papillomavirus vaccine introduction. Vaccine 2004; **23**:569–78.
41 Lowy D, Frazer I. Chapter 16: Prophylactic human papillomavirus vaccines. J Natl Cancer Inst Monogr 2003; **31**:111–6.
42 Villa L, Ault K, Giuliano A *et al*. Immunologic responses following administration of a vaccine targeting human papillomavirus types 6, 11, 16 and 18. Vaccine 2006; **24**:5571–83.
43 Winer R, Lee S, Hughes J, Adam D, Kiviat N, Koutsky L. Genital human papillomavirus infection: incidence and risk factors in a cohort of female University students. J Epidemiol 2003; **157**:218–26.
44 Woodman C, Collins S, Winter H, Bailey A, Ellis J, Prior P, Yates M, Rollason T. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet 2001; **357**:1831–6.
45 Koutsky L, Holmes KK, Critchlow CW *et al*. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. N Engl J Med 1992; **327**:1272–8.
46 French K, Barnabas R, Lehtinen M, Kontula O, Dillner J, Garnett G. Strategies for the introduction of HPV vaccination: modelling the optimum age and sex specific pattern of vaccination in Finland. Br J Cancer 2007; **96**:514–8.
47 Garnett G. Role of herd immunity in determining the effect of vaccines against sexually transmitted disease. J Infect Dis 2005; **19** (Suppl. 1):S97–106.
48 Taira A, Neukermans C, Sanders G. Evaluating human papillomavirus vaccination programs. Emerg Infect Dis 2004; **10**:1915–23.
49 Hughes J, Garnett G, Koutsky L. The theoretical population-level impact of a prophylactic human papilloma virus vaccine. Epidemiol 2002; **13**:631–9.
50 Barnabas R, Laukkanen P, Koskela P, Kontula O, Lehtinen M, Garnett G. Epidemiology of HPV 16 and cervical cancer in Finland and the potential impact of vaccination: Mathematical modelling analyses. Plos Med 2006; **3**:1–9.
51 Garnett G, Kim J, French K, Goldie S. Chapter 21. Modelling the impact of HPV vaccines on cervical cancer and screening programmes. Vaccine 2006; **24**:178–86.
52 Lehtinen M, Idanpaan-Heikkila I, Lunnas T *et al*. Population-based enrolment of adolescents in a long-term follow-up trial of human papillomavirus vaccine efficacy. Int J STD AIDS 2006; **17**:237–46.
53 Lehtinen M, Apter D, Dubin G *et al*. Enrolment of 22 000 adolescent women to cancer registry follow-up for long-term human papillomavirus vaccine efficacy: guarding against guessing. Int J STD AIDS 2006; **17**:517–21.
54 Lehtinen M. Preparations for implementing human papillomavirus vaccination should begin. Euro Surveill 2005; **10**:1–2.
55 Lehtinen M. Vaccination against human papillomaviruses shows great promise. Lancet 2004; **364**:1731–2.
56 Munoz N, Bosch FX, Sanjose Sd Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; **348**:518–27.
57 Munoz N, Bosch F, Castellsague X, Diaz M, Sanjose Sd Hammouda D, Shah K, Meijer C. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J Cancer 2004; **111**:278–85.
58 Ferlay J, Bray F, Pisani P, Parkin D. Globocan 2002. Cancer incidence, mortalitiy and prevalence worldwide. International Agency for Research on Cancer (IARC) Cancerbase No. 5. Lyon: IARC Press 2004.
59 Arbyn M, Raifu A, Autier P, Ferlay J. Burden of cervical cancer in Europe: estimates for 2004. Ann Oncol 2007; doi: 10.1093/annonc/mdm079.
60 Lipsitch M. Vaccination against colonizing bacteria with multiple serotypes. Proc Natl Acad Sci USA 1997; **94**:6571–6.
61 Silins I, Kallings I, Dillner J. Correlates of the spread of human papillomavirus infection. Cancer Epidemiol Biomarkers Prev 2000; **9** (9):953–9.
62 Rousseau M, Villa L, Costa M, Abrahamowicz M, Rohan T, Franco E. Occurrence of cervical infection with multiple human papillomavirus types is associated with age and cytologic abnormalities. Sex Transm Infect 2003; **30**:581–7.
63 Mendez F, Munoz N, Posso H *et al*. Cervical coinfection with human papillomavirus (HPV) types and possible implications for the prevention of cervical cancer by HPV vaccines. J Infect Dis 2005; **192**:1158–65.

64 Silins I, Ryd W, Strand A *et al*. Chlamydia trachomatis infection and persistence of Human Papillomavirus. Int J Cancer 2005; **116**:110–5.
65 Pastrana D, Vass W, Lowy D, Schiller J. HPV16 VLP vaccine induces human antibodies that neutralize divergent variants of HPV16. Virology 2001; **279**:361–9.
66 Clifford G, Franceschi S, Diaz M, Munoz N, Villa L. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. Vaccine 2006; **24**:26–34.
67 Arbyn M, Sasieni P, Meijer C, Clavel C, Kolipoulos G, Dillner J. Chapter 9. Clinical applications of HPV testing: a summary of meta-analyses. Vaccine 2006; **24** (S3):78–89.
68 Parkin D, Bray F. Chapter 2. The burden of HPV-related cancers. Vaccine 2006; **24** (Suppl. 3):S11–S25.
69 Goldie SJ, Kim JJ, Wright TC. Cost-effectiveness of human papillomavirus DNA testing for cervical cancer screening in women aged 30 years or more. Obstet Gynecol 2004; **103**:619–31.
70 Schiller J, Davies P. Delivering on the promise: HPV vaccines and cervical cancer. Nat Rev Microbiol 2004; **2**:343–7.
71 Ferguson M, Heath A, Johnes S, Pagliusi S, Dillner J. Results of the first WHO international collaborative study on the standardization of the detection of antibodies to human papillomaviruses. Int J Cancer 2006; **118**:1508–14.
72 Quint W, Pagliusi S, Lelie N, deVilliers E-M, Wheeler C, WHO HPVDNA, International Collaborative Study Group. Results of the first World Health Organization international collaborative study of detection of human papillomavirus DNA. J Clin Microbiol 2006; **44**:571–9.