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FAST TRACK

EUROGIN 2008 roadmap on cervical cancer prevention

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The EUROGIN 2008 Roadmap represents a continuing effort to provide updated information on primary and secondary prevention of cervical cancer. The report addresses several areas including the progress made toward global implementation of currently licensed human papillomavirus (HPV) vaccines, the possibilities and value of future-generation HPV vaccines, endpoints under consideration for evaluation of candidate HPV vaccines, and monitoring impact of HPV vaccination programmes that can be implemented within developed and less-developed countries. For the sake of completeness, a short update on the evolution of HPV testing in primary screening programmes at present and after HPV vaccine introduction has also been included. The report is available on the EUROGIN website (www.eurogin.com).

Key words: human papillomavirus; vaccine; screening; cervical cancer

Update of new findings in 2008 and human papillomavirus vaccine implementation

The EUROGIN 2007 Roadmap on cervical cancer prevention was produced soon after the publication of decisive trials of quadrivalent and bivalent virus-like particle (VLP) human papillomavirus (HPV) vaccines (against HPV16, 18, 6 and 11 and HPV16 and 18, respectively). These included efficacy trials involving virological and precancerous cervical disease endpoints among women aged 15–26 years, and immunologic bridging trials with endpoints of safety and VLP serum antibody levels in female and male adolescents (reviewed in Schiller *et al.*[1]). For both vaccines, efficacy against all considered endpoints associated with HPV vaccine types, including cervical intraepithelial neoplasia grade 2 or worse (CIN2+), among young women who were not yet infected at the time of vaccination was greater than 95%.

As expected, more limited protection was noted for both vaccines among women infected with HPV vaccine types before vaccination, and against endpoints associated with any HPV type. Neither vaccine was found to clear existing HPV infection[2] nor slow the rates of progression from infection to CIN.[3] These trials therefore demonstrated that both HPV vaccines are most effective when given to females naïve to HPV vaccine types, *i.e.*, prior to the onset of sexual activity.

Important progress was made in 2008 in understanding experience with HPV vaccine introduction in many countries[4] and in the evaluation of the cost-effectiveness of different vaccination strategies.[5] More than 100 world countries approved one or both vaccines, and those implementing mass vaccination programmes are rapidly increasing. Developed countries such as the United States, Canada, Australia and New Zealand were the first to make this move.[6] Australia has already reported an encouraging coverage of school-based vaccination in different regions (70% or more).[7] The decision to introduce HPV vaccines was also unusually rapid among the European Union (EU) Member States.[8] Indeed, a majority of the 27 EU Member States made a recommendation to integrate HPV vaccination into their respective national immunization programmes, and many of these have started to provide vaccine. HPV vaccines have already been offered, free of charge, to young adolescent girls in Italy, Luxembourg, Norway and the United Kingdom, and reimbursement for the vaccines is available for this age group in France, Germany and Sweden.

The recommended age for routine vaccination was 11–13 years except in France (14–23 years, according to sexual history) and Germany (12–17 years). Temporary catch-up vaccination, up to age 17 or 18, has also been recommended or initiated in a few countries.[8,9] It is of concern that data on HPV vaccination acceptance in many EU countries are not available, and that market sales data suggest that in some countries (*e.g.*, France and Germany) women in the catch-up age groups have received more vaccine than younger girls who are the primary target.

In developing countries, where 80% of cervical cancer cases occur, the potential benefits of HPV vaccines are enormous. HPV vaccines are licensed in many low- and middle-income countries but few such countries have recommended HPV vaccine introduction for national immunization programmes due to other programmatic and financial priorities. In 2009, routine use of HPV vaccines was recommended by World Health Organization (WHO) in countries where prevention of cervical cancer and/or other HPV-related diseases constitutes a public health priority, vaccine introduction is programmatically feasible, sustainable financing can be secured and the cost-effectiveness of vaccination strategies in the

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country or region is considered. In 2008, the Global Alliance for Vaccines and Immunization (GAVI, www.gavialliance.org) that subsidizes vaccines for the world's poorest countries, pledged to make subsidies for HPV vaccines available if they were recommended by WHO and funds were secured. Funding is a profound challenge given currently high cost of HPV vaccines and the global financial crisis (www.who.int/immunization/sage_conclusions/en/index.html). Ultimately, GAVI hopes to make HPV vaccine subsidies available to GAVI-eligible countries interested in vaccine introduction between 2010 and 2020.

Although HPV vaccination has been included in the immunization programmes of only a few low- or middle-income countries, demonstration projects providing vaccine through schools and/or community campaigns have shown coverage of >85% among young adolescent girls in Peru and Uganda (www.path.org). Provided high coverage of adolescent women is achieved, and vaccine costs are substantially lower than in high income countries, models suggest that vaccination against HPV16 and 18 could be very cost-effective even in the poorest countries.[10]

With respect to vaccine efficacy, new trial results were reported at several scientific conferences in 2008, but none have been published in peer-reviewed journals, precluding changes in conclusions about vaccine efficacy since the EUROGIN 2007 Roadmap.[11] Assessment of HPV infection to distinguish women who can benefit from vaccination continues, for instance, to be discouraged,[12] as limitations of markers of prior/current infection have not been overcome. Indeed, some new data have emerged showing that infection with the same HPV type can persist, but become occasionally undetectable.[13]

Similarly, no new evidence supports modifying the EUROGIN 2007 conclusions about optimal age for the primary target group for HPV vaccination,[14] *i.e.*, routine vaccination for girls aged 9–14 years is recommended and catch-up vaccination of females aged 15–18 years is worth considering if resources permit.[11] Recent cost-effectiveness models have clearly shown that the cost per cervical cancer case averted increases steeply with age at vaccination as a greater proportion of vaccinees are infected with HPV vaccine types before vaccination. In the United States, for instance, one model estimated that the cost per case estimates rises from 43,600 US dollars for vaccination of 12-year-old girls to 97,300 and 152,700 US dollars, respectively, if females of 13–18 years or 13–26 years were also vaccinated.[5]

EUROGIN's 2007 call for further evaluation of the efficacy of vaccination of women above age 26 years is also still valid.[11] As expected, both vaccines showed satisfactory safety and immunogenicity in trials of women aged 25–45 years for the quadrivalent vaccine, and 26–55 years for the bivalent vaccine.[1] Data from the quadrivalent vaccine trial also suggested excellent protection from developing incident HPV infection and low-grade cervical and external genitalia lesions associated with HPV vaccine types in women not previously infected with these HPV types but neither prevention of CIN2+ nor significant reductions in persistent HPV 16 or 18 infections were reported in the intent to treat analyses.[15] In fact, current vaccine trials of women above age 26 years may well be too small to assess these endpoints, owing to the rare occurrence of CIN2+ in ''older'' women who have never been infected with HPV16 or 18. Whereas immunobridging studies were used to compare the antibody levels of girls younger than 15 years to those of older females included in clinical efficacy trials of precancerous and cancerous cervical lesions, the same immunobridging assumptions will not be applicable in women over 26 for a number of reasons (see ''Endpoints for evaluation of next-generation vaccines'' section later). In addition, prevention of HPV infection and CIN1 cannot be considered sufficient evidence for cervical cancer prevention in middle-aged women.[1] On an average, the progression from new infection to invasive cervical cancer takes decades and middle-aged women who are negative for HPV vaccine types may have demonstrated an ability to resolve HPV infection without the need for vaccination. Everywhere, women above a certain age would have much more to gain from improvements in cervical cancer screening than vaccination programmes.

In 2008, the bivalent vaccine was shown to provide limited cross-protection against infection from HPV31 and 45.[16] In addition, recently the quadrivalent vaccine was shown to provide partial protection against CIN2+ caused by a combination of high-risk types other than HPV16 and 18.[17,18] However, it remains unknown if partial cross-protection will be long-lasting or clinically relevant, especially in vast populations where the majority of cervical cancer pre-cancers and cancers are due to HPV 16 and 18. With respect to duration of vaccine efficacy, long-term community-based vaccine trials in Costa Rica[2] and the Nordic countries that will continue at least 9 years after vaccination will determine if booster doses are needed after the 3-dose primary series. As of 2008, clinical protection for both vaccines has been demonstrated through 5–6 years after vaccination. A quadrivalent vaccine booster 5 years after vaccination induced a strong B-cell memory response, a property of other vaccines with durable efficacy (*e.g.*, hepatitis B virus vaccine).[1]

The EUROGIN 2008 Roadmap will address new topics, namely the development of next-generation HPV vaccines, study designs needed to evaluate these vaccines effectively and swiftly, and possible approaches to monitoring the impact of HPV vaccination programmes. The new Roadmap will also briefly revisit cervical cancer screening with a focus on new evidence supporting the excellent negative predictive value of HPV testing. A comprehensive review of screening and its integration with primary vaccine prevention was already included in the 2007 EUROGIN Roadmap.[19]

Cervical cancer screening after HPV vaccine introduction

New tests for primary cervical cancer screening

Although the present report deals with vaccination, some improvements in screening are worth mentioning. The availability of HPV vaccines will reinforce the need to screen for high-risk HPV infections. The EUROGIN 2007 Roadmap concluded that HPV DNA testing has substantially greater sensitivity than cytology as a stand-alone primary screening test. Cytology typically has a sensitivity of about 50–60% for detecting CIN2+ and never more than 75%, whereas HPV testing has consistently shown sensitivity more than 95%.[20–22] Recently, 4 studies have clearly proven that a negative HPV test affords a substantial increase in duration of protection (*i.e.*, low risk of CIN2+ compared to a negative cytology test). The longest follow-up is for the Hammersmith Study,[23] where risk of developing CIN2+ 5 years after an HPV-negative test result was 0.42%, compared to 0.83% for a negative cytology result. A larger Dutch study[24] also noted that compared to cytology, using HPV DNA test as a primary screening test, yielded a 70% increase in detection of CIN3 or worse at initial screening and a 55% decrease at a second screening 5 years later, indicating that more disease was detected initially and that this was persistent CIN3. Similar results were found by the Swedescreen study.[25] An analysis of several European cohorts[26] showed a much lower risk of CIN3 or worse 6 years after an HPV-negative test (0.27%), which was about half the risk associated with screening by cytology after only 3 years (0.51%, Figure 1). This is powerful evidence in support of using infrequent HPV testing in place of frequent cytology testing.

Together these data provide another major confirmation of the value of HPV testing as a primary screening test (followed by cytologic triage of HPV-positive women) and further support for a screening algorithm similar to that shown in Figure 2. When a highly sensitive test is used, there is no justification for performing any screening among women under the age of 25 years, among whom over-treatment and possible consequences (*e.g.*, premature delivery)[27,28] suggest that more harm than good is done. A challenge for this approach is to avoid unnecessary investigation and treatment of HPV-positive/cytology-negative women, for whom repeat testing is currently recommended after 6–12 months. This is

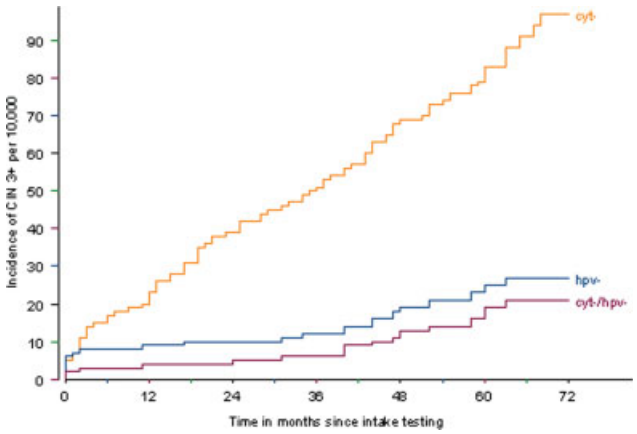

Figure 1 – Cumulative incidence rate for cervical intraepithelial neoplasia 3 or worse (CIN3+) according to baseline test results excluding Denmark and Tubingen, Germany.[26] HPV, human papillomavirus.

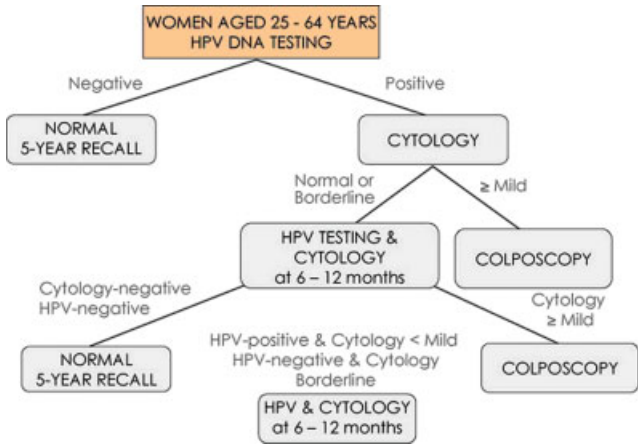

Figure 2 – Proposed cervical cancer screening algorithm—developed countries.[37] HPV, human papillomavirus.

particularly important for women aged less than 30, or even 35 years, among whom transient HPV infections are more common.[29]

New adjunct tests such as HPV type-specific tests may help to determine which of these women need immediate referral and which can be safely monitored at longer intervals. Evidence is mounting that typing for HPV16 is particularly important because of its faster progression time and stronger link with CIN3 or worse,[30] whereas HPV types 18 and 45 are more strongly associated with adenocarcinoma[31] and endocervical lesions. This means that, especially for persistent infections containing these types, endocervical curettage and further exploration of the endocervical canal is warranted when colposcopy does not identify a visible lesion. Routine typing of cytology specimens for HPV16 and 18 is currently being planned or has been implemented for the next generation of HPV tests, but it would seem advisable to include HPV45 with 18 and keep HPV16 as a distinct evaluation so that future assays will routinely identify HPV16 and HPV18 or 45 separately.

As noted earlier, the lower specificity of HPV DNA tests compared to cytology remains an issue, especially for younger women in whom much HPV infection is benign and transient. It is desirable to minimise the number of women allocated to short-term repeat testing (*e.g.*, HPV-positive/cytology-negative women) and several approaches are currently under development to try to improve test specificity while retaining high sensitivity, including

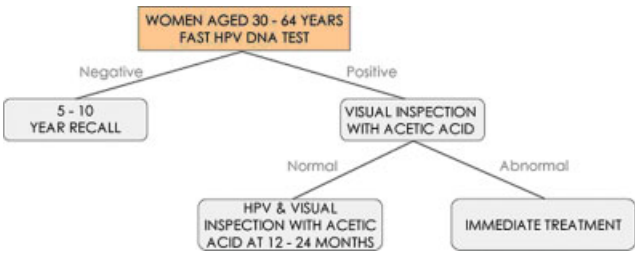

Figure 3 – Proposed cervical cancer screening algorithm—developing countries.[37] HPV, human papillomavirus.

tests that detect persistent HPV DNA, HPV mRNA tests, tests that assess proliferation and cell cycle markers, and p16 detection by staining of cytology slides as well as through detection by ELISA. Initial results from one study of referral patients does suggest that mRNA tests and p16 staining[32] have a higher specificity than consensus HPV DNA testing, but both test need further evaluation in a primary screening context before recommending them for routine use.

Screening in the developing world

A very large screening trial from India published after EUROGIN 2008 has demonstrated the superior test performance of primary screening with a single HPV testing using Hybrid Capture 2 (Qiagen, Gaithersburg, MD, formerly Digene Corporation) over both one cytology test and visual cervical inspection techniques in the prevention of cervical cancer incidence and mortality.[33] In addition, the first clinical results were recently reported on the *care*HPV assay, which requires no electricity or running water, only 2.5 hr to conduct, and is expected to be substantially cheaper than Hybrid Capture 2.[34] Although slightly less sensitive than Hybrid Capture 2 for detection of CIN2+, its performance onsite by laboratory workers from Shanxi, China far exceeded that of visual cervical inspection, and was comparable to liquid-based cytology in Beijing. This test offers a promising approach for screening in remote areas that cannot routinely access laboratories that process screening specimens in high volume. Work is needed to evaluate the performance and cost effectiveness of these tests in large scale screening programs in other countries.

In light of the compelling evidence for the test performance of HPV tests for primary screening, there is no rationale to introduce cytology in countries where high-quality cytology-based screening with high coverage of eligible women has not already been implemented. Approaches based on HPV testing as a primary screening test, followed by a ''see and treat'' procedure for HPV-positive women seem most attractive and are being evaluated in new screening trials.[35,36] One possible screening algorithm is shown in Figure 3. As vaccination is primarily for adolescent girls, screening remains the only viable preventive measure for older women. Mother–daughter programmes in which mothers (or other relatives) are screened when their adolescent daughters are vaccinated may be feasible and represent an attractive option in some settings. The need for multiple visits to complete the 3-dose HPV vaccine series also offers the chance to screen mothers at the first visit and then use ''see and treat'' approaches in HPV-positive mothers when their daughters come for subsequent vaccine doses.

Screening of vaccinated women

It is unlikely that screening will be modified in older adolescents and young women receiving HPV vaccines under catch-up programmes. Girls vaccinated before age 15 years will not need screening for another 10 years, so the current priority for women over 25 years of age should be to introduce HPV test-based screening.[19] This will also provide the necessary experience to use this test in vaccinated women when they reach screening age.

Developing efficient screening algorithms for vaccinated women will require periodic evaluation and modifications. For example, cytology-based screening will have a lower positive predictive value for CIN2+ because vaccination with moderate to high coverage is expected to substantially lower the prevalence of high-grade lesions caused by HPV16 and 18. There will be, however, only a minimal reduction of false-positive tests due to lower grade cervical abnormalities resulting from reactive changes related to low-risk HPV types and other agents. Abnormal cytology results will be less common, and this is likely to be a problem for cytologists, since maintaining the concentration to spot a few abnormal cells will be more difficult when they are very rare.

HPV testing followed by triage using cytology, as proposed in the EUROGIN 2007 Roadmap and again in 2008,[19] may be more appropriate because of the opportunity it creates for cytotechnicians to read only smears that are more likely to contain lesions. It has recently been shown that the accuracy in reading smears from HPV-positive women is increased as compared to blind reading.[38] Automated cytology, especially using p16 staining to identify cells that need careful study,[32] may also be a viable approach for triage of HPV-positive women, although this has not yet been established. New testing approaches using persistent HPV DNA detection, mRNA, proliferation markers and cell cycle proteins may also help to refine screening algorithms and merit formal evaluations. Finally, as more options for HPV detection and triage of HPV-positive findings become available, quality-assurance and standardisation of assays is of paramount importance for any test considered for high volume screening programmes.

Future generations of HPV vaccines

Current HPV vaccines may be replaced in the future with second generation vaccines with different antigens, adjuvants, delivery systems, temperature stability, shelf life and presentation that may influence efficacy, reactogenicity, safety, acceptability, cost and feasibility in a wide variety of immunization settings. The quadrivalent and bivalent HPV vaccines should therefore be considered as introductory products that will potentially be replaced by multiple generations of subsequent vaccines. The timely introduction of current vaccines as well as the development of new, lower-cost, broadly protective HPV vaccines that are effective, affordable and feasible in developing countries that bear the highest burden of cervical cancer must be a public health priority. Ideally, future HPV vaccines may be able to offer other attractive attributes: (1) offer prophylactic and possibly therapeutic efficacy against all, or at least the majority of high-risk carcinogenic HPV types, (2) be inexpensive to manufacture, (3) possess stability in the absence of a cold chain and (4) be needle-free, single-dose immunogens.

Improvements in L1 VLP HPV vaccines

Next-generation HPV vaccines likely to become available within the coming 5 years will continue to be VLP based. Reductions in the total number of doses required for a primary series would provide a partial cost reduction and the long-term immunogenicity of 2-dose *versus* 3-dose strategies are currently being evaluated. It will take time, however, to obtain data to assess duration of protection beyond the 5–6 years established for the 3-dose regimens. New methods to make or source antigens, address patent issues or manufacture vaccines may permit high-volume, lower cost vaccine manufacture possible in developing countries.

Studies demonstrate that 7 HPV genotypes (HPV16, 18, 45, 31, 33, 52 and 58) account for nearly 90% of all cervical cancer cases worldwide, with little regional variation.[39] Because studies of both current HPV vaccines suggest only modest cross protection against some of these additional HPV types,[16–18] an increase in the type-specific protection of HPV vaccines from 70 to 90% would be highly desirable. The most simplistic approach to developing next-generation HPV vaccines is based on increasing the valency of the current vaccine formulations. For instance, a candidate nonavalent VLP vaccine including HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58 has been announced by the manufacturer of the quadrivalent vaccine.

Available data have demonstrated that increasing valency does not competitively impair induction of type-specific antibodies,[40] but whether this will hold true as VLP number increases to 9 or more is unknown. Other concerns with respect to increased valency of next-generation VLP vaccines relate to unknown safety and reactogenicity. The most frequent adverse events associated with licensed HPV vaccines are injection-site reactions of pain, erythema or swelling. Increased valency may influence reactogenicity or overall vaccine safety. A modest increase (from 3 to 6%) in adverse events at the injection site was recorded with the highest dose used in phase II dose-escalating studies of the quadrivalent vaccine.[41] Phase III clinical trials of multivalent VLP HPV vaccines in 16- to 26-year-old women are on-going (Clinical trials.gov identifier: NCT00543543).

Improvements in VLP HPV vaccine delivery, including mucosal approaches, are also currently under evaluation. The main goal of mucosal vaccination would be to reduce the number of vaccine doses required, and to generate sustained, high titer neutralising antibodies in optimal local mucosal sites (*e.g.*, cervix, vagina, vulva, mouth, throat, penis and anus). Mucosal delivery of VLP HPV vaccines in humans using a nebuliser are encouraging and have demonstrated antibody responses similar to those produced by intramuscular injections.[42] A close association between nasal immunisation and immunological response in other mucosal sites (*i.e.*, the vagina) has also been demonstrated.[43] Finally, needle-free vaccines may also improve vaccine delivery by avoiding risk of infection from contaminated needles and costs associated with the use of health workers and sterile needle delivery systems. Lyophilised preparations may represent a further improvement in vaccine delivery by circumventing the need for a cold chain.

Alternative L1 VLP HPV vaccines or subunit vaccine production in plants and bacteria

Methods for reducing L1 VLP HPV vaccine costs must consider modified production strategies as a priority. To date, new approaches have primarily targeted production of HPV VLPs or subunit synthesis in plants or bacteria. When compared to production in laboratory-based eukaryotic cell systems, including those being used for current L1 VLP HPV vaccines, production of recombinant proteins in plants and bacteria is generally more economic, with minimal manufacturing and processing requirements.

The ability of plant-based vaccines to deliver sufficient antigen to induce protective immune responses is well established for a wide range of antigens.[41,44] HPV VLPs have been successfully produced in transgenic plants, but the production yield of HPV L1 VLPs has been disappointingly low in most natural plant-based systems.[45–47] A novel and transgenic plant-based system that enhanced the production of L1 VLPs using a human codon-optimising gene linked to a chloroplast-targeted signal has been reported,[48] and plant-based vaccine strategies to produce high-titre HPV-specific neutralising antibodies remain a promising area of research. Vaccines that can be administered orally could improve vaccine coverage in remote areas, reduce delivery costs and enhance compliance, particularly in children. The challenges facing plant-based vaccine development cannot, however, be underestimated and include technical and regulatory aspects as well as public perceptions related to transgenic crops. Furthermore, very large quantities of soluble antigen must be used in ''edible vaccines'' to survive the digestive tract.

Strategies to produce HPV L1 VLP subunit structures in bacteria that could circumvent the requirement for maintaining intact icosohedral VLP structures have also been considered. Specifically, subunits composed of pentameric capsomers produced in *E. coli* have been shown to induce neutralising antibodies.[49]

HPV L2 minor capsid protein vaccines

HPV vaccines targeting the highly conserved linear epitopes within the HPV minor capsid protein, L2, is probably the most promising approach to generating broadly cross-reactive antibodies. Although natural infection and immunisation with L1/L2 VLPs fails to elicit anti-L2 antibody responses, vaccination with bacterially expressed L2 protein or peptides derived from L2, results in the production of neutralising antibodies that are protective in animal models.[50–52] Studies demonstrate that L2 is poorly exposed in native HPV virions,[53] but 1 or more L2 neutralising epitopes[54–56] are exposed when the HPV capsid undergoes a conformational change upon binding to its cellular receptors.

Although L2-specific neutralising antibodies are generated against a remarkably broad range of HPV types, neutralising antibody titres generated from recombinant L2 are considerably lower than for L1 VLP vaccines. Therefore current efforts are focused on enhancing L2 immunogenicity and developing L1 VLP L2 chimeras.[57,58]

A few additional viable HPV vaccine strategies

Viable adenovirus recombinants have been used to generate VLPs which are immunogenic in mice, but not yet in humans. Multiple L1 recombinant bacterial vaccines that have been tested in animal models include L1 recombinant bacille Calmette-Guerin,[59] recombinant *Lactobacillus casei*-based VLPs,[60] an attenuated *Shigella flexineri* strain expressing L1,[61] and L1 recombinant clones of attenuated *Salmonella enterica* serovar Typhimurium and Typhi strains.[62] The Salmonella systems included codon-modified L1 that induced strong neutralising antibody responses after a single intranasal or oral dose in mice. An attenuated Ty21 strain has been used in an oral vaccine to prevent typhoid fever and has shown an excellent safety profile. Experience with live recombinant vaccines is, however, limited and again regulatory and public concerns raised by genetically modified organisms may hinder their development.

Another promising candidate HPV vaccine has used a measles virus (MV) vector based on the Berna-commercial vaccine strain. The MV–HPV recombinant virus expressed the HPV16 L1 protein at high levels and induced humoral immune responses against both MV and L1 in genetically modified mice.[63] Advantages of this approach include: (1) the MV vaccine strain is already in use as a safe and efficacious vaccine,[64] (2) the production cost of MV vaccine is very low and (3) the current MV vaccine is well known to induce a strong and lifelong immunity.[64] The use of MV vector cocktails delivering simultaneously several additional antigens could be envisaged instead of the routine MV vaccination in early childhood.

Vaccines that could prevent new HPV infections and simultaneously induce regression of established infections would of course be the most desirable. Chimeric VLPs or L2 fusion proteins which incorporate polypeptides or peptides of HPV early gene products including E1, E2, E6 and E7 would represent obvious candidates.[65–69] In mice, VLP chimeras have induced both neutralising antibodies and T-cell responses to inserted polypeptides. Unfortunately, therapeutic HPV vaccine efficacy has not been adequately demonstrated to-date and therefore may need to be developed sufficiently and independently before rational combined prophylactic/therapeutic strategies can be undertaken.

Endpoints for evaluation of next-generation vaccines

A clear definition of endpoints for evaluation of HPV vaccine efficacy is essential for registration and labelling of new products. In the case of HPV-related disease, efficacy endpoints can be clinical, virological or immunological. Given the regressive nature of HPV infection and precancerous lesions, the higher the severity of the endpoint chosen, the higher its specificity as a predictor of cervical cancer will be. However, a requirement for high-grade outcomes (*i.e.*, CIN2+) implies large sample sizes and/or long-duration trials.

For interpretation of efficacy results for any type of prophylactic vaccine, a clear distinction is necessary between according to protocol (ATP) or per protocol analyses, which provide data on prophylactic vaccine efficacy against HPV vaccine types among individuals who are presumed naive to HPV vaccine type exposures before vaccination; and intention to treat (ITT) analyses, which provide data of greater relevance to program effectiveness and include all subjects vaccinated, representing a mixture of individuals who have been unexposed or exposed to HPV vaccine types before vaccination. For HPV vaccination, additional ITT analyses can include outcomes associated with vaccine and non-vaccine types, to estimate the potential vaccine impact on defined populations. It is important to note that even ITT analyses are not necessarily generalisable to real-world populations due to trial inclusion criteria (*e.g.*, women with limited numbers of sexual partners or women with no past history of anogenital abnormalities) and the high proportion of women receiving all 3 HPV vaccine doses within a clinical trial setting.

CIN3 is considered a high-risk cervical cancer precursor, and is probably an ideal trial endpoint in terms of its high positive predictive value, given that it is associated with HPV16 in the same proportion as it is associated with cervical cancer, is always treated and has good diagnostic reproducibility. HPV18 and 45, however, are under represented in CIN3 compared to cervical cancer.[70] CIN2, on the other hand is a clinically significant lesion, but represents a combination of true precursors and a low-grade lesions, with a mixture of HPV types and poor diagnostic reproducibility.[71] The composite trial outcome of CIN2+ is a compromise between diagnostic reproducibility, positive predictive value, clinical relevance and high incidence, which provides the necessary efficacy data with trials of a reasonable size. This endpoint was chosen by many regulatory authorities, industry and academic institutions for the trials that led to the initial licensing of HPV vaccines.

Secondary endpoints that have been used in clinical trials include 6- to 12-month HPV persistence for the evaluation of cross-protection against a range of HPV types[72] and therapeutic efficacy against prevalent infections[2] (Table I). Incident HPV infection is extremely common, is a very distant precursor of cancer, and in some cases may represent recent contamination and not true infection. It is therefore not usually considered a sufficiently valid endpoint of efficacy. Persistent infection has some advantages over the disease outcomes described earlier, because it has high reproducibility and is much more common than high-grade CIN. In addition, the attribution of causality to a particular HPV type in the context of multiple-type infections may be more accurate with a persistent infection endpoint. The duration of persistence that best predicts high-grade disease has not been fully defined, but recent data suggest that both 6- and 12-month HPV persistence may be equally predictive of CIN2+ at least in young women who were sampled at these frequent intervals.[17] In spite of some drawbacks (Table I), persistent HPV infection outcomes are expected to legitimately replace high-grade CIN for the evaluation of cross-protection[72] and for at least some next-generation vaccines targeting young women (*e.g.*, <21 years).

Immunogenicity bridging studies have been useful to demonstrate that the immune response of adolescents is at least as good as that of adult subjects,[73] and have facilitated licensure of the vaccine for females in age groups that were poorly represented, or absent, in the main clinical trials. The use of bridging studies to expand the use of the vaccine to even lower ages, together with the evaluation of duration of protection, may potentially lead to the incorporation of the HPV vaccine into worldwide highly successful vaccination programmes for infants or young children. However, immunogenicity alone is not sufficient proof of efficacy. In the case of males, for example, anatomic or physiologic differences could produce different efficacy at the local level despite similar systemic immune responses. However, very preliminary data presented at the

TABLE I – ENDPOINTS FOR ASSESSMENT OF HUMAN PAPILLOMAVIRUS (HPV) VACCINES

Endpoints	Advantages	Disadvantages
Antibody levels[1]	Very rapid ascertainment	No immunologic correlate of protection available
HPV infection/CIN1[2]	Very common	Consists of a large proportion of transient HPV infections Often not a precursor of cancer Associated with many HPV types thus attribution of disease not possible
Persistent infection	More common than high grade CIN Necessary precursor High reproducibility Reasonable endpoint when multiple infections present	Not treated currently Predictive value of different durations not defined for cancer risk Requires frequent follow-up visits
CIN2	Relatively common Clinically significant Usually treated	Mixture of lesions and HPV types Limited diagnostic reproducibility
CIN3	True cancer precursor High positive predictive value for cancer Improved diagnostic reproducibility HPV types similar as in cancer Always treated	Low frequency

CIN, cervical intraepithelial neoplasia.
[1]Antibody levels based on geometric mean titers.–[2]Persistent HPV infection measured through multiple consecutive detection of type-specific HPV DNA at pre-specified time intervals, *e.g.*, 6, 12, 18 months *etc*.

EUROGIN 2008 congress by Guiliano[74] lent support to the efficacy of the quadrivalent vaccine against penile HPV infections.

Similarly, immunogenicity studies are not appropriate to extrapolate efficacy data to older women, given the fact that HPV vaccines are prophylactic and that HPV exposure usually occurs around initiation of sexual activity. Therefore, the group of older, sexually active women includes many who have been previously exposed, with a corresponding reduction of the potential impact of the vaccine in this group. ATP analyses in these age groups indicate efficacy among apparently unexposed women as shown by Muñoz et al.[15] but the proper evaluation of the potential utility of HPV vaccines at older ages would require efficacy and effectiveness analysis of large numbers of women (many thousands) to take into account the decreasing fraction of subjects in that age group who may still benefit from the prophylactic effect against CIN2+.

Endpoints specific to non-cervical conditions that have been reported include vulvar and vaginal intraepithelial neoplasia, genital warts (in the case of the quadrivalent vaccine)[75] and penile HPV infections.[74] The evaluation of efficacy against anal and oral infections is underway at present in relatively small studies.

In the future, as noted earlier, new vaccines with the potential to overcome some of the limitations of the current vaccines are expected to be developed. The evaluation of new products in the presence of licensed effective vaccines presents new challenges, such as ethical considerations related to the use of placebo, among others.

Placebo-controlled trials could not be justified or even feasible in a community where the vaccine has been introduced for the same population groups proposed for the clinical trial. However, it may be possible to identify populations where vaccination is not the standard of care (*e.g.*, areas where the vaccine is not licensed, populations not covered under vaccine label or by established programmes). In this context, trial participants should receive appropriate cervical cancer screening and treatment as needed, to assure their safety during the trial. In case the trials can be done using relatively short-term surrogate markers (*e.g.*, HPV persistence for 6–12 months), women could be offered the vaccine at the end of the trial.

An alternative to randomised controlled trials is the comparison of incidence rates of study endpoints in the group receiving the new product with the rates in properly selected historical controls deriving from cytologic, histologic or hospital registries. However, there are very few registries where the information is complete and reliable, and HPV data are unlikely to be available in these types of registries. Great benefit from linkage of HPV typing data will be realised in settings where population-based screening and vaccination registries have been established prior to, or early on in HPV vaccine implementation.[76]

The use of historical rates from the untreated group of previous clinical trials has the advantage of randomisation and probably the availability of HPV typing data and carefully documented risk factor and clinical outcome data. However, there are a limited number of such trials and they have most often been conducted in ill-defined populations (*e.g.*, volunteers), presenting challenges to reduce potential selection bias. In addition, ethical considerations have already resulted in the systematic provision of HPV vaccines to untreated trial participants.[17,18]

A new HPV vaccine could be evaluated in a randomised double-blind clinical trial using the licensed vaccine as a comparison group, with the intention of demonstrating equivalence or non-inferiority. Such trials, however, require large sample sizes and the use of endpoints less rare than CIN2+. This kind of trial may be used to evaluate vaccines including additional HPV types or with additional potential for cross-protection. In this context, the groups receiving the licensed vaccine can serve at least partially as true placebos, receiving at the same time the full benefit of the standard-of-care product.

Monitoring of HPV vaccination

Here, we briefly expand on the EUROGIN 2007 Roadmap and review the various uses of epidemiological monitoring and HPV laboratory approaches for assisting in vaccine implementation and surveillance of HPV infection as evaluation tools for HPV vaccination programmes. The biology of HPV infection places high demands on epidemiological monitoring and state-of-the-art laboratory services. HPV infections are asymptomatic and the incubation time between infection and major associated diseases is typically several decades. Furthermore, there exists a multitude of different HPV types requiring expert laboratory methodologies, and the epidemiology of HPV infections varies among different populations.[77]

Continuing follow-up of existing clinical trials would provide essential information regarding longer-term efficacy. Few real-world settings will allow however high levels of follow-up outside of the trials in Costa Rica and the Nordic countries, and in most settings placebo or control groups will have been offered HPV vaccination for ethical reasons.

The current serology- and nucleic acid-based assays used to define HPV susceptible populations impair progress in HPV vacci-

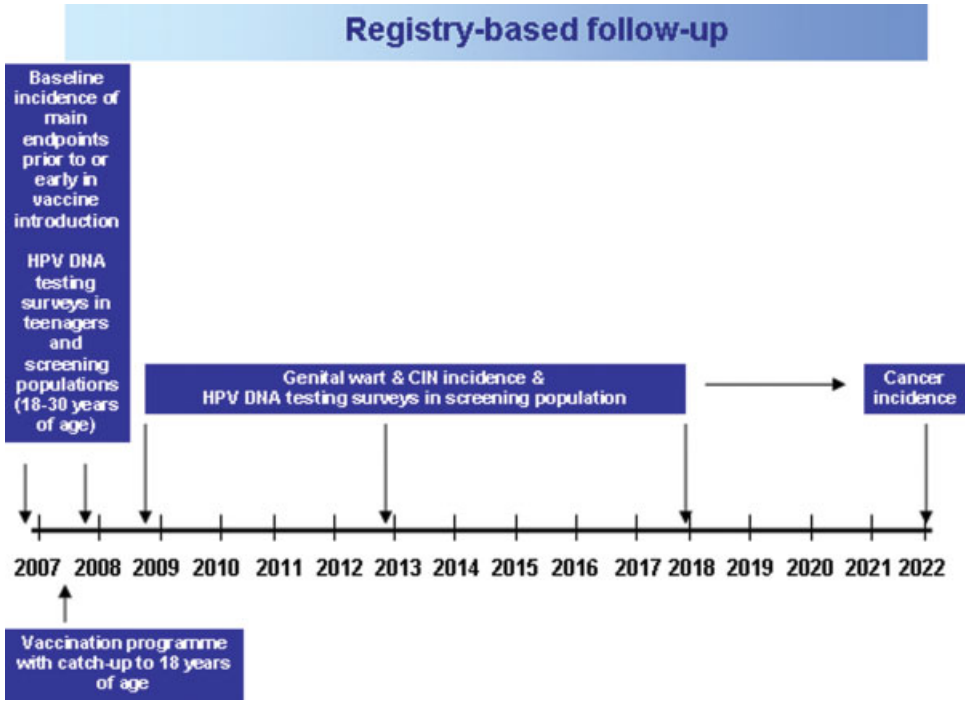

FIGURE 4 – Timeline and options for monitoring human papillomavirus (HPV) vaccine impact and potential effectiveness. For a hypothetical HPV vaccination programme starting in 2007 and including limited catch-up vaccination up to age 18 years. For programmes with only baseline vaccination at 12 years of age, the effectiveness and monitoring options will all be delayed by at least 6 years. CIN, cervical intraepithelial neoplasia.

nology as trials are difficult to compare. These problems may increase with an increasing number of trials performed in many sites throughout the world, and using many different laboratories and assays.[77]

As noted earlier in this EUROGIN 2008 Roadmap, future trials will most likely be based on early endpoints such as HPV infection or vaccine immunogenicity. This approach will also require, however, the use of standardised tests for the presence of HPV DNA or anti-HPV antibodies.[78,79] HPV DNA testing is particularly important for estimating global disease burden studies, and enables reasonably accurate estimations of the gains from HPV vaccination.

In post-vaccination surveillance, there are 3 different levels that could be implemented depending upon the amount of data and resources available in different countries. The basic surveillance level, which every country delivering HPV vaccinations should consider, is to monitor population coverage and major vaccine-related safety using passive vaccine safety surveillance systems. An intermediate level of surveillance would include monitoring of at least some aspects of the clinical impact of the vaccination programme on incidence of HPV infections or precancerous lesions. Whenever possible, monitoring activities should try to generate outcomes within reasonable timeframes in order to inform program design or modifications, thus early evaluation possibilities are essential. For example, if the quadrivalent HPV vaccine is implemented, incidence of genital warts is of interest as a clinically evident disease that occurs with a relatively short incubation time after HPV infection (Fig. 4).

Monitoring reductions in the prevalence of a broad spectrum of high-risk HPV types will require comprehensive and standardised HPV DNA testing strategies. Random population-based samples or cervical sampling at selected sentinel clinics providing health services to teenagers are conceivable options. Anonymised HPV testing of Chlamydia screening samples and/or cervical cancer screening samples where they can be obtained from mass screening programs of asymptomatic persons, are also good options. Medium and long-term evaluation of HPV vaccination programmes should include HPV DNA typing of low- and high-grade CIN, cervical cancer and other HPV-associated cancers (Fig. 3).

The highest level of post-vaccination surveillance could include cervical cytology, histology, serology or cancer registry-based follow-up systems. Registry-based follow-up will be done over decades, but the endpoints measured can change and will require repeat assessments with the passing of time (Fig. 4). In Nordic countries, for instance, all individuals are assigned, at birth, a unique Personal Identification Number (PIN), which is used to establish identity wherever required (tax collection, conscription, receipt of social benefits, admission to hospitals *etc*.) and in all health data registries. Population-based biobanking systems, storing for instance all cervical smears and all serum samples from serological screening during maternity care with PIN-based biobank registries also exist.[77,79] In addition, in Sweden an HPV vaccination registry based on enclosing a patient's information and a registration form in the package with each vaccine dose has been implemented. Currently, about 90,000 HPV vaccine doses have been registered and >95% of subjects have provided an informed consent for PIN-based monitoring studies. The major aims of the registry-based follow-up are to study long-term duration of protection, long-term and large-scale safety and cost savings (*e.g*., reductions in the use of Pap smears, biopsies, colposcopies and cancer treatment). The persistence over time of the level and functional activity of vaccine-induced HPV antibodies will also be studied in the search for laboratory-based correlates of immunity.

Once an HPV vaccination monitoring programme has been designed, it needs to be sustained and checked for completeness and quality as has been done for organised screening programmes in some areas.[80] Quality assurance does not only apply to the laboratory assays used, but also must apply to every step of the monitoring process such as sampling strategies, sample handling, testing, data reporting, data analysis and dissemination of results.[78]

The WHO Global HPV Laboratory Network aims to contribute to improving the quality of HPV vaccination surveillance through enhanced, state-of-the-art laboratory support not otherwise avail-

TABLE II – PERCENT OF CERVICAL CANCERS ULTIMATELY AVOIDABLE IN DIFFERENT COUNTRIES BY COMBINATIONS OF SCREENING AND HUMAN PAPILLOMAVIRUS (HPV) 16 AND 18 VACCINATION[1]

Already avoided by screening[2] (%)	Avoidable by screening + vaccine HPV vaccine coverage (%)		
	85	50	10
85	95	91	86
50	82	69	54
10	67	44	17
0	64	38	8

[1]Assumes HPV16 and 18 vaccine prevents 75% of cervical cancer.–[2]Depending not only upon coverage, but also the quality of the entire screening process.

able in some countries, notably developing countries. The work has so far resulted in the establishment of a WHO Reference Reagent for definition of HPV16 antibody levels (available for ordering at www.nibsc.ac.uk) that could be used for serologic surveillance and an international proficiency panel for HPV DNA testing that could be applied to surveillance for HPV infection (available for annual subscription at www.who.int). International Biological Standards for definition of an International Unit of amount of HPV16 or 18 DNA were established in 2008 and a WHO laboratory manual describing the work required for internationally comparable quality is expected during 2009.[79]

Final remarks

Many different combinations of screening programmes and immunisation campaigns can be conceived according to the resource levels available in different parts of the world.[19] Immunisation programmes have shown, within and between countries, a greater potential to reach underprivileged populations than most other medical interventions. If high coverage of adolescents (*e.g.*, through school-based programmes) can be achieved, HPV vaccination may especially benefit population subsets who are underserved by screening programmes. Table II shows the ultimate percent of cervical cancers which are not prevented by screening that may be avoided by HPV16 and 18 vaccination. With 85% coverage, HPV16 and 18 vaccination may, for instance, raise the fraction of cancers avoided by a fairly good, though not perfect, screening programme from 50 to 82% (Table II). The worst-case scenario in all combinations of screening and vaccination shown in Table II would be that HPV vaccinations fail to reach unscreened women. On a worldwide scale, this corresponds to lack of HPV vaccination in the poorest populations most likely not to undergo adequate cervical cancer screening in their lifetime.

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