What is the rationale for using HPV tests as the primary screening option?

The most compelling rationale is the high negative predictive value of a negative HPV DNA test in women aged over 30 years. It is clear that primary screening using Pap smear cytology has been very effective in reducing cervical cancer incidence in some countries, despite the relatively low sensitivity of a single Pap test. These programs work because the test is applied frequently, thus meaning that a lesion missed in one visit is likely to be detected in the subsequent visit before it progresses to cervical cancer.

The problem is that the need for frequent screening is inefficient at best, and is a deal-breaker in settings with limited resources. Many studies have shown that the risk of cervical cancer and pre-cancer (cervical intraepithelial neoplasia of grade 3, CIN3) is very low up to 10 years after a negative high-risk (HR-HPV) test result and therefore the screening frequency could be reduced for the majority of women to once every three to six years without compromising patient safety.

The HPV DNA test, largely in the format of HR-HPV cocktails, has proven its worth in over 10 major clinical trials. What are its strengths and limitations?

HPV DNA tests have several strengths. For example, women who test HR-HPV DNA negative have been shown to have a low-risk of cervical cancer and pre-cancer for up to 10 years. In contrast, the risk of CIN3/cancer after a negative Pap test remains low for only about three years. Thus, a
PREVENTING HPV AND RELATED DISEASES GLOBALLY

Comprehensive control of HPV related diseases will require meeting the needs of the entire community: immunization for susceptible children, screening for older women who may have already been infected, treatment of pre-cancerous lesions and cancer, and palliative care for patients with disease too advanced to cure. We are extremely fortunate to have safe and effective HPV vaccines that are now becoming affordable to the developing world through GAVI, a global fund that buys vaccines for the poorest countries in the developing world, and a strong delivery infrastructure that reaches more than 85% of the world’s children with routine immunization. The delivery infrastructure will need to be expanded to reach pre-adolescents. This system of national immunization programs in every country in the world took many decades and many billions of dollars to develop. This occurred not only because of the global vision and hard work of many individuals and agencies, but also because of billions of dollars from bilateral, multilateral, and foundation-based donors. The donors responded because the underlying paradigm for global health during the 1980s was child survival, and immunization ranks high on the scale of interventions that can contribute to that end. When hepatitis B vaccine (which, like HPV vaccine, is given to children to prevent adult disease) became available and affordable the donor community also agreed to fund that vaccine. While cervical screening has been available and recommended for many decades, few developing countries screen more than a small proportion of women, mostly those from higher socioeconomic levels who can afford private care. Roll-out of new screening tools and methods will require very large investments in infrastructure, trained personnel, education and social mobilization at both national and global levels. Unlike immunization, national and global decision makers and funders do not have cervical screening high on their priority lists. As such, the HPV community must focus not only on research to develop effective tools and strategies, it must also enter the arena of political advocacy, educating and convincing decision makers and donors that cervical cancer prevention and treatment is indeed a national and global priority.

There are technical constraints as well. With many screening and treatment methods under study, the HPV community has not yet reached consensus on which tools are most appropriate in which settings. Until the community can present donors and decision makers with a clear, affordable, and evidence-based way forward it will have a hard time getting the support it needs.

The HPV community has many allies. Obstetrics and Gynecology, Reproductive Health, Cancer Control, Women’s (and Men’s) Health and Advocacy Groups, the Immunization Community, and other groups are excited about the possibility of HPV control and are willing to help if provided with information and a way forward. The global community is increasingly interested in women’s health in their roles as caregivers and as the primary support for so many families. It is critical that clear messages about the burden that HPV related diseases places on women and their families be developed and delivered to decision makers and funders.

Mark A. Kane
International Consultant on Vaccines and Immunization Policy.
Guest editor.
large proportion of women screened with HPV tests can avoid the harm resulting from over-screening by requiring less frequent screens. In addition, the standardization and objectivity of the HPV DNA test ensures more reproducible performance in multiple settings. In this sense, the increased reproducibility of the objective test may go a long way to reducing the geographic disparity in cervical cancer incidence that remains even in those countries that offer Pap screening programs.

The major limitation at present is the relatively low specificity of the HPV test compared with cervical cytology, and an uncertain management of screen-positive women when HPV DNA testing is used as a primary screening test. There is consensus that referring all HPV-positive women to immediate colposcopy is likely to result in an unfavorable compromise between benefit and harm. Thus, primary HPV testing will require a clear strategy to triage screen-positive women to further diagnostic follow-up. In addition, the costs of HPV DNA assays remain higher than conventional or liquid-based Pap cytology and this will limit application in regions with the highest need for alternative screening strategies.

**What is the potential afforded by HPV type-specific screening?**

HPV genotyping may offer a possible triage strategy to improve the specificity of HPV primary screening. The original HPV assays provided a single result indicating that the patient tested positive for one or more of 13-14 HR-HPV types. Because HPV-16 and -18 (particularly HPV-16) infections have a higher absolute risk of progression than the other HR-HPV genotypes, some commercial assays provide two results: HPV-16/-18 positive and "other HR-HPV" positive – these are commonly being called "genotyping" tests. HPV genotyping is being considered as a possible triage strategy in primary HPV screening programs, where the HPV-16/-18 positive women would be referred directly to colposcopy, and the remaining HR-HPV positive women would follow a more conservative management such as repeat testing at 6- or 12-months.

**Which other markers are showing promise for predicting disease and increasing specificity?**

Some of the other markers currently being considered to increase the specificity of primary HPV DNA screening target changes in the cell that are associated with malignant progression, rather than infection. This goes a step beyond oncogenic DNA and RNA detection because it reflects a functional consequence of infection. For example, p16INK4A and Ki67, which are markers of E7 oncogene expression and cellular proliferation, can be applied to a liquid-based cytology sample and are under evaluation as triage markers.

A rapid and potentially point-of-care test for the HPV-E6 oncoprotein from HPV-16/18/45 has shown promise as a very specific marker of CIN3/cancer, detecting very few infections with no or low-grade cytological abnormalities. This test is based on a simple lateral flow assay format (like a dip-stick pregnancy test), and may be of great use in resource-limited situations.

**What requirements in terms of proven quality would you demand in order for a triage test to be considered for widespread clinical programs?**

The triage markers must demonstrate improved specificity relative to HPV DNA testing. The programmatic application of triage markers in an HPV primary screening paradigm must also ensure that the proposed management algorithms will not ultimately sacrifice the high sensitivity of HPV DNA testing. This will require an evaluation of complex strategies, rather than direct test performance results, ideally in randomized controlled trials.

**Will HPV testing make a screening difference in middle-income countries?**

I think that HPV tests have great potential to improve screening in middle-income countries for a variety of reasons. The number of qualified cytopathologists is low in many of these countries. Regional laboratories with quality-controlled HPV DNA testing capabilities could easily be set up to ensure valid and reproducible screening results across a large geographical area. The requirement of frequent repeat screening cannot be met in many countries, both for resource and behavioral reasons. The use of HPV DNA testing could therefore effectively reduce the burden of cervical cancer while requiring only a few screens for most women in the course of their lifetime. HPV DNA screening from a self-collected cervicovaginal swab sample performs as well or better than Pap cytology and HPV testing from a directly collected cervical sample. As a consequence, HPV DNA testing of a self-collected primary sample offers a feasible strategy to increase screening coverage. It is important for developing economies to take a similar path to cervical cancer screening as that taken with the adoption of other technologies. Many Asian and South East Asian countries, for example, bypassed land-line based telecommunications and opted directly for mobile technologies, thus avoiding costly infrastructural development of obsolete technologies. Adopting molecular screening for cervical cancer should be viewed similarly.

Dr. Patti Gravitt designed the "PG" modifications to Michele Manos’ original MY09/11 consensus primers. The PGMY primers became among the most widely used early research tools and contributed significantly to understanding HPV type-specific natural history.
HIGH-THROUGHPUT E6, E7 mRNA QUANTIFICATION IN CERVICAL CANCER SCREENING USING FLOW CYTOMETRY INCREASES SPECIFICITY FOR CERVICAL INTRAEPITHELIAL NEOPLASIA OF GRADE 2 OR MORE LESIONS AND CAN DIFFERENTIATE PRE-SQUAMOUS CELL CARCINOMA FROM PRE-ADENOCARCINOMA

Background
Harald zur Hausen won the 2008 Nobel Prize for discovering that cervical cancer was caused by HPV. As part of his seminal work, Professor zur Hausen also helped elucidate the mechanism by which HPV causes cervical cancer. In a related article, Durst et al. described the overexpression of HPV oncoproteins E6 and E7 as being necessary for the development of cervical cancer. They also noted that the levels of E6 and E7 in cells are critical for high-grade lesions and found that the quantity of cells overexpressing E6 and E7 correlates with disease severity. HPV OncoTect® (IncellDx, Menlo Park, CA, USA) is an extension of this work that replaces radioactively labeled probes with their fluorescent counterparts to quantify both the levels of E6 and E7 mRNA per cell and the quantity of cells overexpressing E6 and E7 in a liquid-based cervical cytology sample.

HPV E6, E7 mRNA quantification using flow cytometry
The HPV OncoTect® assay uses flow cytometry, a powerful, high-throughput platform to analyze heterogeneous populations of intact cells. Other commercially available HPV E6, E7 tests, such as Pretect HPV Proofer® (NorChip AS, Klokkarstua, Norway), the Aptima HPV Assay® (Gen-Probe, San Diego, CA, USA), and NucliSENS EasyQ® (bioMérieux, Durham, NC, USA), genotype using the E6, E7 transcript, whereas HPV OncoTect® is currently the only assay that quantifies the overexpression of E6, E7 mRNA on a cell-by-cell basis.

Thus, the HPV OncoTect® assay quantifies the number of overexpressing cells in the squamous cell (ectocervical) or columnar cell (endocervical) component of the liquid-based cytology sample (Figure 1).

Post-assay analysis of the HPV OncoTect® assay begins with a step that identifies squamous cells of the ectocervix or anus and columnar cells of the endocervix. Ectocervical cells are then identified using morphometric measurements and electronically “chosen” or gated. Overexpression of E6, E7 mRNA is assessed in this cell population by ultrasensitive fluorescence in situ hybridization. Clinical studies are underway to examine the utility of HPV OncoTect® for the detection of pre-adenocarcinomas. This variation simply involves electronically gating the endocervical cells rather than the ectocervical cells and quantifying E6, E7 overexpression in this cell population separately, thus making HPV OncoTect® the only cervical cancer screening test that is able to distinguish pre-squamous cell lesions from pre-adenocarcinoma lesions (Figure 1).

HPV OncoTect® clinical performance
Clinical study data concerning the use of HPV OncoTect® in over 12,000 women enrolled in five major studies have been presented over the last two years (Table 1). In the first study, undertaken at Stanford, over 2000 women were screened using both the Hybrid Capture 2® test (HC2) (Qiagen Gaithersburg, Inc., MD, USA, previously Digene Corp.) and HPV OncoTect® and the results compared to biopsy in 260 cases. The sensitivity for cervical
Intraepithelial neoplasia of grade 2 or worse (CIN2+) was 92%, with a specificity of 85% (CIN2- including CIN1). HC2® had a sensitivity of 93% for CIN2+ and a specificity of 35% (CIN2- including CIN1). In addition, HC2® missed one out of eight squamous cell carcinomas, all of which were detected by HPV OncoTect®. In a second study presented at EUROGIN 2010, over 4000 women from a general screening population were screened for cervical cancer using cervical cytology and HPV OncoTect®. Similar to the Stanford study, the HPV DNA array missed a squamous cell carcinoma detected by HPV OncoTect®.

The overall sensitivity of HPV OncoTect® for CIN2+ in all these clinical studies was 90%, with a 93% sensitivity for CIN3+. The specificity based on CIN2- biopsies was 78% [94% based on >8000 cytologically negative Pap samples (negative for intraepithelial lesion or malignancy, NILM, cytology)]. The clinical utility of HPV OncoTect® extends to its use as a low squamous intraepithelial lesion (LSIL) progression assay and for the screening of anal intraepithelial neoplasia. In a prospective study from Spain, HPV OncoTect® was used on frozen ThinPrep® (Hologic, Inc. Bedford, MA, USA) samples collected from 60 women followed over the course of 24 months. Women with a positive HPV OncoTect® result progressed to more severe lesions in 95% of cases, whereas women with a negative HPV OncoTect® result did not progress in 88% of cases. In a screening study of over 300 anal cytology specimens from Thailand, HPV OncoTect® showed greater sensitivity and specificity for anal intraepithelial neoplasia (AIN 2,3) compared to HPV DNA (Linear Array® HPV Test, Roche Diagnostics, Mannheim, Germany).

Conclusions and future directions

With the cervical cancer screening field moving toward primary screening, HPV RNA tests such as HPV OncoTect® offer new hope for cervical cancer screening because of the promise of increased specificity, which is a necessary attribute for potential primary screening using HPV testing. Indeed, a distinction can now be made between HPV E6, E7 mRNA tests that genotype using amplified RNA (PreTect HPV Proofer®, Aptima® and NucliSSENS EasyQ®) and those that quantify the overexpression of HPV E6, E7 mRNA in cells (HPV OncoTect®). Quantification of E6, E7 mRNA on a cell-by-cell basis yields a performance profile similar to p16, which depends on E7 overexpression and retinoblastoma protein (Rb) degradation. The difference between HPV OncoTect® and p16 resides in the fact that OncoTect® is performed in suspension, high-throughput and highly automated. The recently presented HPV OncoTect® 3Dx technology, the next generation OncoTect® assay, allows for simultaneous E6, E7 mRNA quantification, cell cycle (ploidy analysis), and imaging of the cells while still in suspension (Figure 2). Using this technology, cytology specimens clearly fall into normal, LSIL or high squamous intraepithelial lesion (HSIL) diagnostic categories.

Recent Results and New Indications for the Quadrivalent HPV Vaccine (Gardasil®)

Both the currently available HPV vaccines have been designed for primary prevention of cervical cancer. In addition the quadrivalent HPV vaccine was also expected to prevent genital warts. Indeed, cervical intraepithelial neoplasia, adenocarcinoma in situ and genital warts caused by the four vaccine types (HPV-6, -11, -16, -18) have been prevented almost completely in those vaccinees who were not already infected with the relevant type at vaccination. As a result, the primary target cohorts for vaccination in the majority of vaccination programs are young girls and female adolescents, who are most likely to be HPV naïve. As our knowledge and understanding of HPV-related disease has grown rapidly since the launch of these vaccines, their indications have been extended. Thus, in addition to the prevention of cervical disease and genital warts in females, the quadrivalent HPV vaccine is highly efficacious in the prevention of high-grade vulvar (40% of the vulvar cancers, mainly in younger women, are HPV-related) and vaginal intraepithelial neoplasia (>70% of vaginal cancers are HPV related), both of which are known precursors of invasive cancer.

Although it has been demonstrated that HPV-naïve girls benefit most from the vaccine, women with a history of HPV infection (seropositivity) are also effectively protected by vaccination, with the efficacy against persistent HPV infection or disease caused by the four vaccine types in women aged 24 to 45 years reaching 90%. Vaccinating a general population including 27% of HPV-positive women leads to a substantial reduction of subsequent disease and related treatments.

Indeed, even after treatment for HPV-related disease of the cervix or vulva, vaccinated women have 35-46% less subsequent disease.

Figure 1 shows the time to progression/recurrence of HPV-related genital lesions in vaccinated and non-vaccinated women after treatment. Males both transmit HPV and carry a substantial burden of HPV-related disease. Indeed, 12,000 HPV-related cancers, roughly the same number as cervical cancers, are diagnosed in US males every year. In Europe, more than 14,000 HPV-related head and neck cancers, 1800 anal cancers and 1500 penis cancers are estimated to occur in males every year, as well as some 350,000 new cases of genital warts. Two key publications from the last year demonstrated the high efficacy of the quadrivalent HPV vaccine in men. The quadrivalent HPV vaccine demonstrated an efficacy against anal intraepithelial neoplasia associated with vaccine types of 50% in the general study population and 78% in the per-protocol efficacy population.

An increasing incidence of anal cancer has been observed in several developed countries, with women being more affected than men and more than 90% of anal cancers being HPV-related. Interestingly, the most significant HPV type in all extracervical locations is HPV-16. As a result, the US Food and Drug Administration (FDA) has licensed the quadrivalent HPV vaccine for protection against anal cancer in both women and men. The Center for Disease Control (CDC) recommends vaccination of boys and young men aged 11-21 years, and up to the age of 26 years in men having sex with men. Canada and Australia also established recommendations for males. Table 1 summarizes the current indications for Gardasil® (Merck & Co., Whitehouse Station, NJ USA) in the US, the EU and Australia.

An extensive vaccination program with the quadrivalent vaccine in Australia (>80% coverage of females aged 11-26 years) has led to a dramatic decrease in the incidence of
Infection with high-risk HPVs is recognized as one of the major causes of infection-related cancer worldwide, along with Helicobacter pylori and hepatitis B and C viruses. This figure shows the number of new cases attributable to the four main infectious agents worldwide in 2008, by development status, i.e. the number of cases that would have been prevented following the hypothetical intervention on infection exposure. High-risk HPVs (HR-HPVs) are responsible for 530,000 new cases of cancer of the cervix uteri worldwide, 85% of which occur in the developing regions. Another 78,000 cases attributable to HR-HPVs worldwide occur at other sites (vulva, vagina, penis, anus, and oropharynx).

Table 1. Selected indications for Gardasil® in the US, the EU and Australia.

<table>
<thead>
<tr>
<th>Disease</th>
<th>US (FDA)</th>
<th>EU (EMA)</th>
<th>Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN</td>
<td>✔</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>AIS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vulvar cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIN</td>
<td>✔</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>Vaginal cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VaIN</td>
<td>✔</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>Anal cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIN</td>
<td>✔</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>Genital warts</td>
<td>✔</td>
<td>✔</td>
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High-risk HPV is a common genital infection that has the potential to evolve to cervical cancer. Fortunately, the large majority of infected women clear the infection within a few months as a consequence of the cellular immune response. It is now well known that persistent infection is necessary for the development of cervical precancerous lesions.

HPV displays a full intraepithelial cycle and, as such, has developed specific mechanisms to reduce immune surveillance and control in order to establish such a persistent infection (Figure 1A). The virus infects basal keratinocytes by taking advantage of a microabrasion of the mucosal epithelium. The expression of early viral proteins, which are poor immunogens in basal keratinocytes, subsequently allows amplification of the viral genome. Completion of the full viral cycle, which is dependent on differentiation of the epithelial cells, allows the expression of late proteins in the upper layers of the epithelium and gives rise to virion assembly and shedding of highly immunogenic virions away from the major cellular components of the immune system.

As a consequence of this specific viral cycle, HPV infection does not induce viraemia (no contact with circulating immune cells), does not induce cytolysis (no induction of immune response after cell death), and does not cause inflammation that can recruit cells involved in the innate immune response.1

The HPV immune evasion from innate responses can be explained by the fact that HPV early proteins E6 and E7 expressed in infected primary keratinocytes induce the reduction of alpha-interferon expression, negatively regulate transcription factors involved in the interferon cellular response, and also negatively regulate expression of pro-inflammatory chemokines. These soluble mediators are crucial to initiate the response and as an ultimate step in the activation of cytotoxic T lymphocytes involved in clearance. E6 and E7 have also been shown to down-regulate the expression of Toll-like receptors, such as TLR9, that recognize viral DNA.2 Human Toll-like receptors are found at the surface or in the cytoplasm of a variety of cell types, including keratinocytes, and can recognize various components of microorganisms, subsequently initiating signalling pathways that are important for generation of the cytokines and chemokines involved in innate and acquired immune responses. Moreover, clinical studies have indicated that TLR expression is reduced during persistent infection whereas expression is enhanced in tissues of women who have cleared infection.3 Together, or individually as a causal factor, reduced TLR expression and interferon synthesis can explain the absence of HPV-induced inflammation. Dendritic (DC) and Langherans cells (LC) are dedicated antigen-presenting cells that form part of the innate immune system and play a pivotal bridging role in the induction of adaptive immune responses against immunogens or infections. DCs reside in the dermis in cervical tissue, whereas LCs are mainly found in the epidermis and are theoretically in contact with viral antigens. A reduction in LC density in the HPV-infected cervix has been evidenced and linked to down-regulation of E-cadherin by E6, a molecule involved in LC-keratinocyte adhesion.4 In vitro experiments using virus-like particles (VLPs) have indicated that LCs bind, but are not activated, and therefore do not migrate to draining lymph nodes, when exposed to VLPs, whereas DCs are fully activated by VLPs.5

HPV early proteins also affect the efficacy of the adaptive immune response, particularly by interfering with

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**Figure 1:** HPV life cycle and interaction with the immune system during natural infection (A) and following prophylactic immunization (B). LC: Langerhans cells. Adapted from Kahn JA, NEJM 2009.

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[Diagram highlighting the HPV life cycle and immune interactions]
CD8+-induced cytotoxicity against HPV-infected keratinocytes that present low levels of viral peptide-loaded major histocompatibility complex class I (MHC-I) molecules at the surface. E7 has been shown to down-regulate expression of the antigen peptide transporter-1 (TAP-1), which is involved in the transport of processed peptides to the endoplasmic reticulum before association with MHC-I molecules. Expression of E5 protein in the ER allows HPV to interfere with peptide/MHC-I association and translocation to the cell surface, where the complex can be recognized by the T cell receptor of CD8+ cytotoxic lymphocytes. In persistently infected tissue, the reduced cytotoxicity can also be correlated with the increased presence of a specific subset of CD4+ suppressor lymphocytes known as regulator T cells or TReg. All the elements mentioned above contribute to the immune evasion of HPV and are responsible for the low immune response, particularly the humoral immune response. If persistent, seroconversion to the major capsid protein L1 occurs 8 to 10 months after infection. However, only low titers of antibodies are generated. Furthermore, although they are neutralizing in vitro assays, these antibodies are unable to prevent re-infection. The development of HPV DNA-free VLPs composed of L1 or L1 and L2 protein in the early 1990s was a major achievement in the development of prophylactic HPV vaccines. Immunization experiments in HPV animal models indicated that VLPs are strongly immunogenic and induce type-specific conformation-dependent neutralizing antibodies which are responsible for protection against viral challenge. These encouraging results accelerated the launch of clinical trials in which strong immunogenicity was confirmed in humans through antibody titers that were four to five orders of magnitude above the response obtained after natural infection. This difference is mainly due to the fact that VLPs are injected intramuscularly in the presence of adjuvant and bypass the immune evasion mechanisms.

**CONCLUSION**

HPV evades the immune system by targeting crucial steps of innate and adaptive immunity, although the natural immune response is nevertheless able to clear most infections. As such, therapeutic strategies (immunomodulators and therapeutic vaccines) have to be explored continuously. The high immunogenicity and clinical efficacy of prophylactic VLP-based vaccines have to be fully exploited by universal HPV vaccination.

Conflicts of interest: Antoine Touzé has received travel/congress grants from GlaxoSmithKline and Sanofi-Pasteur-MSD, served as a consultant for GlaxoSmithKline, Sanofi-Pasteur-MSD and AuraBiosciences, and has spoken at GSK symposia.

**References:**

In spring of 2008, the Finnish National Institute for Health and Welfare (THL) appointed an Expert Group to survey HPV disease burden and to propose the best possible means to further reduce cervical cancer and the disease burden caused by HPV. This Expert Group was particularly expected to include in its proposal a statement on the most effective screening method for the organized programme and the inclusion of an HPV vaccine in the national vaccination programme.

To estimate the total HPV disease burden, we linked all the health care registries available in Finland, including Mass Screening, Cancer, Cause of Death, and Population registries as well as the National Hospital Discharge Registry, the KELA health insurance reimbursement registry, the KELA pharmaceuticals reimbursement registry, the HUSLAB pathology laboratory data system and the Finnish Student Health Service (YTHS) registry, for the first time.

After linking and collecting the data the Expert Group was able to state that approximately 150 cervical cancer cases and 50 deaths caused by this cancer are diagnosed in Finland (population 5.3 million) annually. Furthermore, almost 500,000 Pap tests are performed annually for screening purposes, of which approximately two-thirds take place outside the organised screening programme and are opportunistic smears (Figure 1). A total of 16,800 colposcopies are performed annually. Of these, 2800 pre-cancer cases are found each year, of which about 2000 are cervical intraepithelial neoplasia of grade 2 and 3 (CIN2 and CIN3). As a result, roughly 80% of cervical cancer cases are presently prevented with screening in Finland.

The annual cost of all testing with a screening intention is €23 million. The healthcare costs resulting from the treatment and management of cervical cancer and its precursors are currently around €17 million per year.

A new dynamic mathematical model was developed by the Group to allow simulation of HPV infection transmission and cancer development in a population, using a variety of vaccination and screening scenarios. Based on the extensive evaluations and mathematical modeling performed, the Expert Group concluded that, compared to current practice, more cervical cancer cases could be prevented, with fewer adverse outcomes and considerably lower costs, by reducing Pap testing outside the organized screening program and by optimizing the screening program.

This can be achieved by discouraging any kind of screening in women under 25 years of age, thus reducing the current excessive testing and excessive treatments amongst young women, simultaneously starting the organised screening programme at 25 years of age instead of the present 30 years, continuing to screen HPV-positive women who are beyond the current screening age (60–65 years), and performing screening in a controlled and planned manner with the help of primary HPV testing instead of Pap testing.

The different screening scenarios are shown in Figure 2 and Table 1.

The Expert Group gave a new recommendation for organised screening to start at age 25 and to continue until age 65. At ages 25 and 30, women are screened with Pap tests, whereas from age 35 onwards, primary HPV testing...
with cytology triage for HPV-positive women will be used. In addition, if a positive result is obtained at age 65 (3% of this age cohort), screening should be continued until the age of 85 or until a negative HPV test result is obtained in the next screening round.

Organized screening is currently complemented by vast opportunistic screening performed, for example, in the private sector, primary care and student health care. The costs of these extra screening tests are about triple those of organized screening.

Screening Pap tests performed on young women outside the screening program tend to result in unnecessary treatments since the majority of the lesions detected would resolve without treatment. The Expert Group therefore proposes practical measures to reduce heavy Pap screening, for example by providing guidelines for primary care professionals and eliminating health insurance reimbursement for opportunistic Pap tests.

The proposal of the Expert Group is presently being considered by the Ministry of Social Affairs and Health for possible implementation into routine practice.

Table 1: Present recommendations (blue line), present practice (red line) and three different screening scenarios based on the simulation performed using a dynamic mathematical model.

Columns indicate the predicted number of annual CIN and Ca cases (2-5), the quality adjusted life years lost (QALY) (6) and the total costs of the program or modeled alternatives (7). Cost changes and incremental costs per QALY gained (8 and 9) are compared with present practice (red line), which combines the costs of the organized and the opportunistic screening programs.

**Table 1**

<table>
<thead>
<tr>
<th>Screening scenario</th>
<th>CIN1 cases</th>
<th>CIN2 cases</th>
<th>CIN3 AIS cases</th>
<th>CxCa cases</th>
<th>QALY loss</th>
<th>Cost million euro</th>
<th>Δ cost million euro</th>
<th>ICE in euros/ QALY gain</th>
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<tbody>
<tr>
<td>Organized throughout (Current recommendation) 30 to 60 (5 years)</td>
<td>260</td>
<td>417</td>
<td>885</td>
<td>187</td>
<td>1,507</td>
<td>14.4</td>
<td>baseline</td>
<td>baseline</td>
</tr>
<tr>
<td>Organized throughout 25 to 60 (5 years)</td>
<td>367</td>
<td>552</td>
<td>959</td>
<td>157</td>
<td>1,367</td>
<td>15.8</td>
<td>+1.4</td>
<td>10,000</td>
</tr>
<tr>
<td>Organized throughout 30 to 70 (5 years)</td>
<td>278</td>
<td>445</td>
<td>946</td>
<td>155</td>
<td>1,294</td>
<td>16.2</td>
<td>+1.8</td>
<td>8,451</td>
</tr>
<tr>
<td>Organized throughout Cytos: 25-34 (5 years) HPV: 35 to 65 (5 years) and HPV exit test at 65 (New recommendation)</td>
<td>459</td>
<td>675</td>
<td>1,035</td>
<td>98</td>
<td>985</td>
<td>17.9</td>
<td>+3.5</td>
<td>6,705</td>
</tr>
<tr>
<td>Current organised and non-organised (Present practice)</td>
<td>621</td>
<td>775</td>
<td>901</td>
<td>137</td>
<td>1,375</td>
<td>34.0</td>
<td>+19.6</td>
<td>148,485</td>
</tr>
</tbody>
</table>

ICE: incremental cost effectiveness. CIN: cervical intraepithelial neoplasia. CxCa: cervical cancer. QALY: quality adjusted life years lost
The EUROGIN 2011 roadmap represents a continuing effort to provide and interpret updated information on cervical cancer screening and vaccines. The EUROGIN event took place in Lisbon on March 2011.

The EUOGIN roadmap represents a continuing effort to provide and interpret updated information on cervical cancer screening and vaccination against, and management of, HPV-related disease. This year the roadmap is focused on the role and impact of cervical HPV infection and beyond, including the potential public health benefits of implementing screening strategies and vaccine programs.

More than 5% of all cancers worldwide or 600,000 cases, are caused by HPV. HPV causes all cervical cancers and a large proportion of other anogenital cancers, including cancers of the vagina, vulva, penis and anus, and some head and neck cancers of the oropharynx, for which the proportion differs by geographical region. Whereas the incidence of cervical cancer has been decreasing over time, the incidence of anal and oropharyngeal carcinoma, for which there are no effective screening programs, has been increasing over the last couple of decades.

Despite screening programs, cervical cancer is still an important public health concern and non-cervical HPV-associated cancers, which individually are relatively rare, now collectively parallel the burden of cervical cancer in many countries, thus highlighting the need to continue our efforts as regards early detection and immunization programs. Cervical cancer screening can certainly be improved by replacing frequent cytology with HPV screening. The use of HPV screening will increase and the role of HPV testing, either alone or combined, will vary by country and resources. A single HPV test is much more sensitive for cervical intraepithelial neoplasia of grade 3 or worse (CIN3+) and more highly protective (negative predictive value, NPV) in the long term than cytology, thus allowing an increase in the screening interval to 5-8 years. The best management strategy for high-risk HPV positive (HR HPV+) women is under evaluation. Specificity can be improved by using mRNA testing, genotyping, p16 stains and other biomarkers. In a recent randomised controlled trial (RCT) in cytology-negative women, HPV-16/18 genotyping alone had a sensitivity and a positive predictive values (PPV) similar to abnormal squamous cells of undetermined significance (ASC-US) for CIN3+. Thus, justifying immediate colposcopy. New cost-effective strategies, including the adequate management of cytology-negative HPV+ women, will dismantle the barriers of the conservative medical community and their resistance to change due to the relatively low point of specificity of HPV DNA testing compared to cytology.

Indeed, HPV testing followed by cytology triage of HPV+ women will probably replace cytology as the preferred cervical cancer screening paradigm in the near future in several European countries.

This is particularly likely given that a substantial proportion of cervical cancers (70%), and an even greater proportion of HPV-associated non-cervical cancers (85-95%), are caused by HPV-16 and -18. Current HPV vaccines may also hold great promise for reducing the burden of HPV-associated non-cervical cancers. The increasing incidence of HPV-related anal pre-cancers and cancers and oropharyngeal carcinoma has an implication for public health. Currently female-only HPV vaccination programs may affect the incidence of HPV-related anal cancer and oropharyngeal carcinoma. Various modeling studies on the effect of HPV vaccination in boys have shown that it cannot be justified on health economics grounds. However, the high incidence of HPV-related anal pre-cancers and cancers in men who have sex with men (MSM) and HPV-related oropharyngeal carcinoma will have a significant impact on the cost-effectiveness of vaccinating boys.

With the new generation of multivalent vaccine, the potential perspective to eradicate cervical and other cancers, mainly in the poorest countries where screening is not available, may become a goal for the coming decades.
HIGH LEVEL EVIDENCE DEMONSTRATES MALE CIRCUMCISION REDUCES HPV INFECTION

Male circumcision (MC) is a simple and safe surgical procedure with multiple medical benefits, including the prevention of balanitis, phimosis (and paraphimosis), urinary tract infections, and penile cancer. More recent evidence demonstrates its protective effects regarding the acquisition of sexually transmitted infections (STIs), including human immunodeficiency virus (HIV) and HPV.

Indeed, if brought to scale, MC will benefit public health by reducing the transmission of HIV and HPV and thereby the attendant population-level morbidity, mortality, and related healthcare costs.

Randomized controlled trials (RCTs) are strength of the intervention in preventing HPV infection.

The epidemiologic mechanism for the protective effects of MC on HPV infection was recently elucidated in a meta-analysis of HPV prevalence and incidence by Albero and colleagues, which included a review of 23 observational and experimental studies from 1971-2010. Their work illustrated an overall robust inverse association between MC and genital HPV prevalence in men, decreased duration of HPV infection, as well as a decreased rate of HPV acquisition. That study, along with prior epidemiologic evidence demonstrating the protective effect of MC on cervical cancer in women, provides a strong rationale for supportive public health policy enabling male circumcision.

A summary of the observational, experimental, and biological evidence available strongly supports the protective effect of MC on HIV and HPV infection. Indeed, some scholars have suggested that MC be considered as a vaccine against STIs. There is currently, however, an effective vaccine against genital warts and cervical cancer. The combined implementation of male circumcision and HPV vaccination could therefore have a profound effect on both genital cancers and HPV-related morbidity.

It is now time for us to put personal opinions aside and allow evidence to guide rational public health policy for HIV and HPV prevention.


disease in countries where HIV might not be common but HPV infection is. Of course, in countries where both HIV and HPV infections are common, a public health policy supporting widespread male circumcision is imperative.

The protective effect of foreskin removal is biologically plausible and clear. In uncircumcised men, the foreskin is susceptible to microtrauma and viral infection may result during sexual intercourse with an infected partner. Secondly, the inner mucosal surface of the foreskin is not keratinized and the unkeratinized tissue is more susceptible to infection than the keratinized epithelium of a circumcised penis. Finally, the foreskin is densely populated with the dendritic Langerhans cells, or target cells, that readily attract viral particles.

Table 1: Studies from three randomized controlled trials of the effect of male circumcision on HPV and HIV infections.

<table>
<thead>
<tr>
<th>Outcome Year</th>
<th>Journal</th>
<th>Trial site</th>
<th>N</th>
<th>Age</th>
<th>MC type</th>
<th>Follow-up time</th>
<th>Sample type</th>
<th>Effect estimate (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>HIV</td>
<td>South Africa</td>
<td>2229</td>
<td>18-24</td>
<td>foresceps</td>
<td>21 months</td>
<td>blood</td>
<td>RR 0.40 (0.24-0.68)</td>
</tr>
<tr>
<td>2007</td>
<td>HIV</td>
<td>Kenya</td>
<td>1501</td>
<td>18-24</td>
<td>foresceps</td>
<td>24 months</td>
<td>blood</td>
<td>RR 0.47 (0.28-0.78)</td>
</tr>
<tr>
<td>2007</td>
<td>HIV</td>
<td>Uganda</td>
<td>1973</td>
<td>15-49</td>
<td>sleeve</td>
<td>24 months</td>
<td>blood</td>
<td>IRR 0.49 (0.28-0.84)</td>
</tr>
<tr>
<td>2009</td>
<td>HIV</td>
<td>Uganda</td>
<td>520</td>
<td>15-49</td>
<td>sleeve</td>
<td>24 months</td>
<td>penile swab</td>
<td>RR 0.65 (0.45-0.94)</td>
</tr>
<tr>
<td>2009</td>
<td>HPV</td>
<td>South Africa</td>
<td>1264</td>
<td>18-24</td>
<td>foresceps</td>
<td>21 months</td>
<td>urethral swab</td>
<td>PRR 0.86 (0.51-0.86)</td>
</tr>
</tbody>
</table>

IRR: Incidence Rate Ratio; PRR: Prevalence Rate Ratio; RR: Relative risk; * bold: statistically significant risk reduction

Outcomes of interest in the field of epidemiology: to prevent HPV-related infection and disease in countries where HIV might not be common but HPV infection is. Of course, in countries where both HIV and HPV infections are common, a public health policy supporting widespread male circumcision is imperative.

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The PapillomaVirus Episteme (PaVE) provides sequence information and bioinformatic resources to the papillomavirus research community. Papillomavirus sequences provide a treasure trove of information for comparative genomics, and as such the PaVE resource can greatly assist in our understanding of papillomavirus-associated disease and could ultimately aid in treatment. There has been no central resource for papillomavirus sequences since the Los Alamos HPV database ceased functioning in 1997. Although the National Center for Biotechnology Information (NCBI) and the European Molecular Biology Laboratory (EMBL) public sequence databases contain thousands of papillomavirus-related sequences, many contain errors and it is difficult to identify reference HPV types. A major goal of the PaVE is to host uniformly annotated and corrected reference sequences for each papillomavirus.

The PaVE consists of a relational database and web applications that facilitate sequence retrieval and analysis. The PaVE uses Open Source software and existing tools. It has a powerful search engine to find specific nucleotide, protein and reference genome sequences belonging to different hosts and genera. It also hosts a Basic Local Alignment Search Tool (BLAST) tool that will specifically search for papillomavirus nucleic acid and protein sequences in the PaVE databases. To date, the PaVE database contains only sequences of Reference Genomes (named papillomavirus types). Variant genome sequences can be accessed from PaVE but they have not yet been incorporated into the databases. The actual sequences will be integrated into these databases in the near future so that they can be analyzed with PaVE tools.

As the annotation of papillomavirus genomes in the public databases is quite variable, the reference genomes have been uniformly annotated in PaVE. This annotation will be more finely tuned as we learn more about viral proteins and cis-elements. Efforts are underway to include proteins encoded by spliced messages in the database. Maps of characterized viral messenger RNA transcripts are hosted in the PaVE.

The structures of many papillomavirus proteins have been solved and these are stored in the PaVE and can be visualized in Protein Explorer. A novel feature of this tool is that proteins from different viral types can be aligned with those whose structure has been solved. The position of amino acid differences can be readily viewed on the protein structure.

Another tool under development is the ability to visualize multiple alignments of selected papillomavirus protein sequences. Other goals include providing tools to analyze papillomavirus-related protein-protein interactions and images of biological lesions.

PaVE contains information about viral classification, submission of new HPV types, and links to other resources. Additionally, experts have been recruited to write review chapters on various aspects of PV genomics and proteomics.

PaVE was spearheaded by Alison McBride in the National Institute of Allergy and Infectious Diseases (NIAID) Division of Intramural Research in collaboration with the NIAID Bioinformatics and Computational Biosciences Branch (BCBB), headed by Yentram Huyen. The scientific content and bioinformatic tools currently hosted on PaVE are the work of Koenraad van Doorslaer and Alison McBride and the BCBB group (Qina Tan, Sandhya Xirasagar, Sandhya Bandarus, Vivek Gopalan and Yasmin Mohamoud). The development and direction of the PaVE is guided by a panel of advisors consisting of Hans-Ulrich Bernard, Thomas Brettin, Thomas Broker, Chris Buck, Robert Burk, John Doorbar, Ethel-Michele de Villiers and Marc van Ranst. However, the PaVE team greatly welcomes additional input from the international scientific community.

PaVE: THE PAPILLOMAVIRUS EPISTEME

Alison A. McBride
Koenraad van Doorslaer
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The Papillomavirus Episteme can be accessed at http://PaVE.niaid.nih.gov (last accessed August 2012). Comments should be sent to Alison McBride at amcbride@nih.gov.
The patient was a 60-year-old female who had undergone vulvar biopsy and had subsequently been diagnosed with stage IV B vulvar squamous cell carcinoma with positive pelvic lymph nodes eight months prior to admission. The bilateral inguinal lymph nodes were enlarged at that time. Palliative radiation therapy had been planned; however the patient had been noncompliant with therapy. On her final admission, she presented to the emergency room with pain and breakdown of the skin overlying the left inguinal lymph node, with serosanguinous drainage, vulvar cellulitis, and left lower extremity lymphadema. She had been unable to walk due to pain. She was admitted and treated with intravenous antibiotics, pain medication, and comfort care. A fine needle aspiration of the left inguinal lymph node confirmed malignancy, with extensive necrosis. After stabilization, she was transferred to hospice.

Squamous cell carcinoma of the vulva may or may not be HPV-related. HPV-unrelated lesions are usually seen in older patients, and may be preceded or associated with differentiated vulvar intraepithelial neoplasia (VIN), which is considered to be the precursor lesion. Lichen sclerosus is also thought to be associated with HPV-unrelated squamous cell carcinoma of the vulva, and a possible risk factor. HPV-related vulvar squamous cell carcinomas occur in younger women and are generally preceded by, and associated with, HPV-related (usual) VIN.

Fine needle aspiration of the inguinal lymph node confirmed the clinical diagnosis of metastatic disease in our patient, and has also been utilized, with some success, to evaluate suspicious groin nodes in patients with cervical cancer (Figure 1). In conjunction with ultrasound, fine needle aspiration of groin nodes has been shown to have potential utility in the preoperative assessment of vulvar squamous cell carcinoma. P16 is a cyclin-dependent kinase 4 inhibitor, and positive immunohistochemical staining for p16 is used as a surrogate for high-risk HPV infection. P16 has been shown to correlate with high-risk HPV E6 and E7 transcripts and with RNA in situ hybridization for HPV in a study of oropharyngeal squamous cell carcinoma, with a 96.4% concordance between p16 and HPV E6/E7 mRNA. Immunohistochemistry for p16 can be used to help determine whether a (primary or metastatic) carcinoma is HPV-related. This has been established in both the head and neck literature, where it has assisted in identifying metastatic squamous cell carcinoma in cervical lymph nodes as likely being of oropharyngeal origin, as well as in vulvar carcinomas and precursor lesions, where it can help to distinguish HPV-related squamous cell carcinoma and VIN from non-HPV-related lesions. It should be noted, however, that the p16 profile in the metastatic lymph node may not match that of the primary vulvar tumor.

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International Agenda

Rome, Italy
7th-12th October 2012
International Federation of Gynecology and Obstetrics, (FIGO) 2012
Venue: Fiera di Roma
E-mail: figo2012.secretariat@triumphgroup.it
Web: www.figoint.com

Lyon, France
16th-18th October 2012
World Vaccine Congress 2012
Venue: Lyon Convention Centre
E-mail: enquiry.uk@terrapinn.com
Web: www.terrapinn.com/2012/world-vaccine-congress-lyon/index.htm

Berlin, Germany
18th-20th October 2012
6th International Conference of HPV, Polyomavirus and Ultraviolet (UV) Radiation in Skin Cancer
Venue: Kaiserin-Friedrich-Haus
E-mail: hpv2012@porstmann-kongresse.de
Web: www.hpv2012.de

Lisbon, Portugal
8th-11th November 2012
17th World Congress on Controversies in Obstetrics, Gynecology & Infertility (COGII)
Venue: Lisboa Congress Center
E-mail: cogii@congressmed.com
Web: www.congressmed.com/cogii10/

San Juan, Puerto Rico
30th Nov 2012 - 6th Dec 2012
28th International Papillomavirus Conference & Clinical and Public Health Workshop
Venue: Puerto Rico Convention Centre
E-mail: events@hpv2012pr.org
Web: www.hpv2012pr.org

Munich, Germany
14th-17th February 2013
2nd Conference on Controversies in Vaccination in Adults (CoVAC)
Venue: Leonardo Royal Hotel Munich
E-mail: info@contemed.de
Web: www.contemed.de/covac/2013

Paris, France
26th-30th May 2013
18th International Congress of Cytology
Venue: Palais des Congrès
E-mail: exhi@cytologyparis2013.com
Web: www.cytologyparis2013.com

Milan, Italy
22nd-27th August 2013
15th International Congress of Immunology
Venue: Milano Congress
E-mail: sci@triumphgroup.it
Web: www.sci2013.org

Prague, Czech Republic
5th-7th September 2013
6th European Congress of the European Federation for Colposcopy
Venue: Prague Congress Centre
E-mail: skrivanek@g-centrum.cz
Web: www.e-f-c.org

Rome, Italy
11th-14th September 2013
5th European Congress of Virology
Venue: Cité Centre des Congrès
E-mail: rehott@viro.med.uni-erlangen.de
Web: www.eurowirology2013.eu

Brussels, Belgium
17th-21th September 2013
10th Congress of the European Society of Gynecology (ESG)
Venue: Square Meeting Centre
E-mail: seg2013@btcongress.com
Web: www.seg2013.com

Cape Town, South Africa
19th-22nd November 2013
8th World Congress on Pediatric Infectious Diseases – WSPID 2013 Congress
Venue: Cape Town International Convention Centre (CTICC)
E-mail: wspid@kenes.com
Web: http://www2.kenes.com/wspid/Pages/Home.aspx

Seattle, Washington, USA
20th-25th August 2014
29th International Papillomavirus Conference & Clinical and Public Health Workshop
Venue: To confirm
E-mail: hpv2014@conferenceSolutionsInc.com
Web: www.hpv2014.org

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