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Human Papillomavirus and Cancer Prevention: Gaps in Knowledge and Prospects for Research, Policy, and Advocacy

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Abstract

The recognition that human papillomavirus (HPV) infection is the central, necessary cause of cervical cancer paved the way to new fronts of prevention via improved screening methods and HPV vaccination. Much has been learned in all fronts, from the molecular basis of our understanding of how HPV causes disease to the health economics of preventive strategies at the individual and population levels. Progress in other areas of cancer control has yet to show the same multi- and trans-disciplinary gains seen in research on HPV-associated malignancies, which is one of the unequivocal success stories in disease prevention. Yet, as an embarrassment of riches, much more research is needed to fill the gaps in knowledge that remain before we are able to reap the benefits from the knowledge translation from all fronts. Public health research on setting-specific implementation of HPV-based preventive strategies and more concerted advocacy to counter barriers facing the adoption of these strategies are likely to yield major dividends in reducing the burden of HPV-associated diseases.

Keywords

Human papillomavirus; HPV; cancer prevention; policy; advocacy

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1. Introduction

In the past 25 years, basic, clinical, behavioral, and population health research on the prevention of cervical cancer have progressed at a very fast pace. The recognition that human papillomavirus (HPV) infection is the central, necessary cause of cervical cancer, paved the way to new fronts of prevention via improved screening methods and vaccination against HPV. Figure 1 shows the explosive growth in the field of HPV research, fuelled by the progress in our understanding of the role of this virus in disease and further accelerated with the advent of HPV vaccines.

Since this monograph series evaluated the domain of research on HPV and associated diseases in 2006 [1], the year when the first HPV vaccine was approved, there has been much progress in all areas. Recognition came in the form of a Nobel Prize in Physiology or Medicine to Dr. Harald zur Hausen, the first proponent of its causal role in cervical cancer (Fig. 1), in whose laboratory many of the distinct HPV types were first cloned and sequenced. The etiologic role of HPV infection in cancers in addition to that of the uterine cervix is now better understood. Much of the clinical research that aimed at demonstrating the efficacy of HPV vaccines is now completed, and many countries have incorporated prophylactic HPV vaccination in their immunization programs. Randomized controlled trials (RCT) of molecular HPV testing have now provided strong and coherent evidence that cervical cancer screening can be considerably improved with the adoption of this new technology. There is now also a concerted effort by policymakers at all levels to implement an ambitious evidence-based agenda for preventing HPV-associated diseases.

This chapter provides a summary of the promising areas in which we must invest research efforts to reap important dividends towards the cost-effective prevention and control of diseases caused by HPV infection. It was written as a consensus document that collected views expressed by international experts in all areas of cervical cancer control and prevention. The headings below, many of which are framed as a question, reflect key methodologic and content areas covered in the monograph. The few references are given to inform readers about main sources of reading material that contain pointers to other primary documents. The individual monograph chapters contain an extensive, yet selected, bibliography concerning the individual topics of relevance in cervical cancer control.

2. Burden of disease and epidemiology

Table 1 lists key gaps in our understanding of the epidemiology and natural history of HPV infection as it relates to different anatomical sites. It also shows research issues in addressing these questions. We also elaborate upon some of the specific areas of epidemiologic concern on which preventive interventions such as HPV vaccination and screening must rely.

2.1. Do we have sufficient population-based cancer registry coverage for the regions in greatest need of HPV-based preventive interventions?

The ongoing efforts of the International Agency for Research on Cancer (IARC) to compile incidence rates from all population-based cancer registries (PBCR) throughout the world represent the mainstay of our knowledge about international variations and time trends in

HPV-associated cancers. GLOBOCAN, the accompanying epidemiologic surveillance series maintained by IARC [2] provides global coverage via extrapolation of incidence data from mortality statistics for countries not properly served by PBCRs. Although volume IX of the IARC series Cancer Incidence in Five Continents [3] shows an increase in PBCR coverage this does not exceed 11% of the world population and extensive areas of the world still have considerable uncertainty with respect to their cancer burden. These include large populations in Asia and Africa as well as some populations in Eastern Europe and Latin America.

The IARC and its affiliated organizations, such as the International Association of Cancer Registries, can at most provide a guidance framework for collecting high-quality cancer incidence data and the means for bringing incidence statistics to the public domain. Funding for establishing new PBCRs and sustaining existing ones must be largely borne by local ministries of health or health departments. Obtaining complete population coverage and high-quality data on cancers ascertained via histopathology is a costly undertaking. Only high-resource countries have the means to sustain complete coverage for entire regions. Yet, it is the underserved regions of the world that bear the greatest burden of HPV-associated cancers. Strong political advocacy by the World Health Organization (WHO) and its affiliated agencies and by non-governmental organizations (NGO) is necessary to change the status quo of limited (and poor) coverage of cancer registration in developing countries. The benefits to stem from a concerted effort to establish more geographically equitable cancer surveillance will be obvious also in other areas of cancer control in which inequality of resources play a role in disease etiology (e.g., cancers of the liver, esophagus). Recently, the IARC and several partner organizations launched the Global Initiative for Cancer Registry Development in Low- and Middle-Income Countries (GICR). GICR's main goal is to establish regional "hubs" of resources and expertise toward the establishment of PBCRs. This initiative should eliminate some of the disparities in cancer surveillance affecting middle and low resource countries.

2.2. Are existing HPV surveillance mechanisms based on the IARC network of tumor registries and affiliated organizations adequate?

As a joint project, WHO, IARC, and the Catalan Institute of Oncology (ICO) have endeavoured to produce an ongoing web-based resource for countries interested in obtaining credible statistics on the surveillance of HPV infection and its associated cancers (WHO/ICO HPV Information Centre) [4]. These reports are regularly updated to link with cancer incidence and mortality data and reports on HPV prevalence from published studies. The data should aid policymakers in deciding about the impact of implementing HPV vaccination or of scaling up cervical cancer screening programs. Continuous updating and inclusion of national data within a country should be pursued.

2.3. Should registries start compiling incidence data for head and neck cancers based on causal link (tobacco and alcohol-related versus HPV-related)? What algorithms can be used to define likelihood of causal attribution?

Testing of tumor biopsies or surgical specimens for HPV DNA and its associated biomarkers (e.g., p16ink4a) is not yet recommended in most clinical guidelines for management of head-and-neck cancer, although knowing the likely etiology would probably

benefit the course of treatment since HPV-associated oral and oropharyngeal cancers have more a favourable prognosis. Without an objective indicator of the likely etiologic source of each disease case, epidemiologic surveillance of oropharyngeal cancers can at best rely on the valid annotation of the anatomic subsite and histologic type to infer cases likely to have originated from HPV infection. Using the International Classification of Diseases topography codes for oncology (ICD-O), base of tongue (C019), lingual tonsil (C024), tonsil (C090–C099), oropharynx (C100–C109), and Waldeyer ring (C142) are typically clustered as potentially HPV-related, whereas tongue (C020–C023, C025–C029), gum (C030–C039), floor of mouth (C040–C049), palate (C050–C059), and other and unspecified oral (C060–C069) are considered unrelated to HPV. The availability of morphological (histologic) coding offers more reliable restriction to squamous histology, e.g., ICD-O (3rd revision): 8050–8076, 8078, 8083–8084, 8094, for likely HPV origin. Research is needed on the validation of the above discrimination algorithm for likelihood of an HPV-related etiology for head and neck cancers.

2.4. Do we have good evidence-based estimates for the HPV-attributable fraction for all anatomic sites in which HPV infection plays a causal role?

Recent reviews have updated the estimates of HPV causal attribution for all cancer sites in which there is sufficient scientific evidence that this virus plays an etiologic role [5,6]. HPV infection is considered a necessary cause of cervical cancer; HPV DNA is identifiable in tumor biopsies or surgical specimens from virtually all cases of this disease, regardless of cell lineage, i.e., squamous or glandular. Large meta- and pooled analyses have updated the proportional contribution to the cervical cancer burden that originates from different HPV types. Invariably, HPV types 16 and 18 appear in these estimates as the most important in terms of etiologic fraction, being responsible on average for 70% of all cervical cancers. HPV16 is a more dominant type in cervical cancer and in other HPV-associated cancers, especially those of the other anogenital sites, the oral cavity, and the oropharynx. Unlike the role in cervical cancers in which HPV is a necessary cause, the latter cancers are not all linked to HPV as a sole causal agent. Why is HPV16 so much more dominant in non-cervical sites? The role of tobacco and alcohol in oral cancers apart, why is it that other anogenital cancers are not entirely linked to HPV? Research on the contribution of other risk factors, including smoking, is yet to show that carcinogenetic pathways can be traced to specific causes other than HPV infection. Obtaining credible and stable estimates of HPV-attribution, both overall and type-specific, in other non-cervical cancer sites will greatly assist prevention efforts and appropriate cost-effectiveness analyses of the impact of implementing gender-equitable HPV vaccination.

2.5. Is there a need for ongoing surveys of HPV infection prevalence? Do we know enough already?

With the advent of HPV vaccination, there has been a renewed interest in conducting HPV prevalence surveys. A typical justification is the need for obtaining baseline prevalence data prior to the rollout of HPV vaccination in a region with the implicit goal of comparing it with the type distribution to result post-vaccination. An often cited reason is the need to monitor for possible type replacement post vaccination, a phenomenon that occurred with pneumococcal vaccination. Unlike the latter, however, there is little biological plausibility or

empirical evidence that other HPV genotypes will emerge to occupy the ecological niche presently occupied by the vaccine-target types (HPVs 6, 11, 16, and 18). HPVs are less genetically dynamic microorganisms than *D. pneumoniae* bacteria and mutate with an extremely low frequency.

The decision by policymakers regarding vaccination implementation should be based more on existing evidence and the feasibility of implementing the intervention in the target population than on the availability of local HPV prevalence data. The balance between the need for locally-generated data against the potential benefits of faster intervention should thus be considered. Properly designing and conducting HPV surveys can be of interest; however, these surveys can also be very costly and complex, unduly burdening country resources with no meaningful impact on the health of the population.

2.6. Do HPV infections acquired early in life become latent? Can we distinguish between new infections and latent infections in older women and men?

A longstanding, yet unanswered question in HPV epidemiology is whether or not infections clear completely or become latent, to reappear later in life. Clearance of HPV infections may simply reflect that viral load dipped below the threshold of detectability. HPV DNA testing in women enrolled in repeated-measurement cohort studies is done in exfoliated cell scrapes, which tend to sample predominantly the upper cellular layers of the ectocervix and thus may miss low viral load infections confined to the basal layer. Because of this dual methodologic limitation (test sensitivity and sampling inadequacy), it is impossible to verify whether or not an infection has fully cleared or stayed latent. If the latter, could it be reactivated later in life? If both phenomena (i.e., latency and reinfection) exist (which is likely) then quantifying the extent of each in explaining the often-observed second peak of HPV prevalence when women reach the peri-menopausal years remains a challenge for future research.

Understanding the nature of infection (latency vs. new infection) would have implications in evaluating age-related patterns of HPV prevalence and may affect the indication for HPV vaccination outside of the target age groups. It is tempting to conclude that HPV vaccination could be recommended to prevent reinfections and to minimize possible disease manifestations later in life. However, it must be borne in mind that the risk for these infections to result in cancer development is probably less than that observed from infections occurring at an earlier age.

3. Molecular biology and immunology

There are several gaps in our knowledge of HPV biology at the fundamental level. We need to determine and incorporate into the description of virus-host interactions the "deeper" metabolic and regulatory pathways necessary for support of viral persistence and completion of the reproductive cycle. Which pathways are involved in conditioning the host to tolerate the virus and support its reproduction? Which factors assist HPV to avoid host defenses? Host responses include innate and immunologic defenses, mechanisms that involve metabolic pathways, apoptosis control, DNA repair, cell cycle control, coordination of energy balance and mitochondrial function, and inter-cell communication. How does HPV

infection prevail over these mechanisms? What factors drive neoplastic transformation in an HPV-infected cell?

Overall, we need to identify those absolute requirements of the virus that are not essential for the well-being of the cell, tissue and host organism. Table 2 lists many of the lingering questions on the molecular biology and host-virus relationship. Some of the main relevant questions of clinical interest are discussed below.

3.1. Do we know enough about prognostic markers of cervical lesion progression? Which ones can be incorporated into clinical testing?

HPV typing information remains the most important predictor of the likelihood of lesion progression. Infection with HPV16 is by far the most important prognostic factor, and HPV18 also contributes substantial clinical impact. The role for viral load measurements remains elusive. On the other hand, semi-quantitative measurement of mRNA from the HPV E6 and E7 oncogenes provides high specificity for distinguishing benign productive infections from those that have initiated neoplastic progression and those that are already cancerous. Cytologic immunostaining for the cyclin-dependent kinase inhibitor p16^{INK4a} was the first use of a biomarker to attain clinical value because of its overexpression in HPV-transformed cervical cells. It remains the most used of all biomarkers to augment the value of cytology and histology (e.g., to assess grade 2 cervical intraepithelial neoplasia lesions). Other markers of cell proliferation, such as Ki-67, MCM2, and TOP2A, and of chromosome instability (e.g., 3q/TERC) have been evaluated in clinical studies with promising results.

Although there is great impetus for using the above immunocytochemical markers to improve the accuracy of cytology, such technologies are not yet viable for widespread use in primary screening, particularly in low-resource settings. The requirements for thin smears from liquid-based cytology preparations and expert interpretation (or computer-assisted imaging devices) are obstacles to wide-scale implementation in population-based screening programs, as are the costs of molecular testing. That said, these technologies may have a valuable role in the colposcopy triage of women found to be HPV DNA-positive for high-risk HPV types. Much research is needed in evaluating the added value of cytologic markers to improve the accuracy of cytology in the context of triage.

3.2. Role of intratypic molecular variants of high-risk HPVs: can it be used to improve understanding of HPV carcinogenicity or ultimately for clinical testing?

The qualitative information from genotyping of cervical samples collected during screening is gradually being incorporated into practice guidelines. Presence of HPV16 or HPV18 in a cervical sample indicates an elevated risk that a high-grade lesion is present and thus, colposcopy referral is warranted regardless of cytology result. Research during the last 15 years has also indicated that intratypic variation may also affect prognosis. Much of the earlier work focused on HPV16. Molecular variants that depart phylogenetically from the HPV16 prototype and belong mostly to non-European branches are associated with greater risk of developing high-grade cervical lesions than when the variants belonging to the European branch are present. More recent work indicates that intratypic polymorphism in

the LCR and E6 genes of other high-risk HPVs may also help predict which lesions may progress or regress and thus help clinicians to tailor clinical follow-up and surveillance.

Are the research findings from our understanding of intratypic variation of HPVs sufficient to permit formulating clinical decisions based on more complex testing for HPV DNA? Although the findings concerning HPV16 seem robust enough in the sense that non-European variants (mostly from African and Asian branches) are correlated with increased risk, there is no standard or common testing protocol that would permit translating these results into practice. Furthermore, while infections with non-European variants of HPV16 seem more likely to develop into high-grade lesions, little is known with respect to risk of invasive cancer [7].

Testing for molecular variants is still the domain of research laboratories. Standardization of a common molecular signature that would collectively indicate the variation in nucleotide positions that is predictive of increased risk is required for validation studies. Pooled analyses of existing specimen banks from cohort studies should be able to obtain more precise estimates of effect and provide validation of candidate DNA polymorphism signatures. Such pooled analyses are indeed essential for studying the role of intratypic variation of less common high-risk HPV types.

3.3. Role of epigenetic changes in HPV viral and host genes: are epigenetic modifications important in HPV carcinogenicity?

Important research has emerged in recent years concerning epigenetic phenomena related to gene expression silencing via methylation. Research on the role of epigenetic events in carcinogenesis has grown exponentially in recent years. Understanding whether the degree of methylation in candidate genes affects the natural history of cervical neoplasia can add new tools to our ability to predict lesion development beyond what can be deduced from cytologic and HPV DNA testing. Several candidate genes have been studied but results have not been entirely consistent. A few genetic markers (DAPK1, CADM1, and RARB) seem to be valid enough for further investigation because they show consistently high methylation levels in cervical lesions [8].

In addition to the role of methylation in host genes, studies of HPV genome sequence methylation have also been promising. HPV16 genome methylation levels at CpG sites in L1, L2, and E2/E4 genes are strongly indicative of the presence of high-grade lesions and predictive of subsequent risk during follow-up [9]. Methylation of HPV DNA genes may be a host response to a foreign intracellular agent, or may be a signaling event that indicates viral integration into the host genome. Differences in methylation patterns may also be indicative of the likelihood of infections to persist or to clear, a feature that would also have clinical predictive value. Infection persistence is mediated by the immune system. Thus, it is also tempting to speculate that high methylation levels may help the virus to evade immune recognition. DNA methylation-mediated silencing of HPV oncogenes is also observed post integration of the viral DNA into host chromosomes. Although viral sequences are amplified and translocated into multiple sites in the host genome, only one copy of the viral genome is transcriptionally active; the remaining copies of integrated HPV DNA are methylated to silence their expression. Preventing or reversing methylation turns on the expression of E6

and E7 and (from intact integrated copies, E2) and this leads to cell apoptosis [10]. Although seemingly counter-intuitive (permitting multiple copies of the viral genome to be expressed), this observation could form the basis for epigenetic interference as therapy for high-grade lesions.

Understanding the role of epigenetic events in host and viral genes is an important and promising area of investigation that can be expected to translate into new tools in risk stratification and prognosis to be added to clinical management. Likewise, developing tools that would permit selective interference with viral DNA methylation may lead to therapeutic interventions to reverse lesion progression [10]. Validation studies are needed for specific host and viral genes using pooled specimen repositories. Mechanistic studies should also help explore the pathways whereby risk is modulated by epigenetic events.

4. Primary prevention via immunization

The efficacy and safety of the two available HPV vaccines have been described in several publications that are summarized elsewhere in this monograph. The wealth of data that has been generated by the two vaccine manufacturers and the United States National Cancer Institute through several RCTs has also left many questions unanswered. Table 3 lists these questions and associated research issues and underlying hypotheses that would benefit from further research and epidemiologic surveillance. Some of these questions could be addressed by post-hoc exploration of completed phase 3 RCTs. Partnership with industry should be sought to provide valuable insights that would aid our understanding of the role of cross-protection, the extent of protection with incomplete vaccination regimens, pan-mucosal protection against vaccine-targeted types, and anamnestic response from subsequent exposure to HPV. Likewise, the establishment of surveillance systems linking HPV vaccination registries with data from periodic HPV surveys, screening registries, and PBCRs could provide valuable information concerning duration of protection, possible type replacement, protection against other cancers, and safety.

5. Secondary prevention via screening

Successfully deployed in high-resource countries, cervical cancer screening with Pap cytology is credited with having achieved substantial reductions in morbidity and mortality from this disease over the last 50 years. Yet, Pap cytology is seen as inefficient because it requires complex and costly infrastructure to ensure consistent quality, coverage, and treatment of precancerous lesions. Because of the high false-negative rate of cytology, medical guidelines have historically required annual or (at most) triennial intervals between screen visits, which over-burdens the healthcare system of most countries. The emergence of liquid-based thin smear cytology has mitigated some of these problems and improved the efficiency for laboratories in processing cytology caseloads; in essence, however, the problems of low sensitivity, subjective interpretation of cellular morphology, and sampling errors have not been resolved.

The advent of molecular testing for HPV DNA has opened a new era of secondary prevention for cervical cancer. Over the last 15 years, a wealth of evidence from RCTs has pointed to this test's much greater sensitivity and somewhat lower specificity than cytology,

which would permit lengthening of screening intervals with adequate safety. HPV testing has high reproducibility, a lower requirement for personnel training, and is amenable to using self-collected cervical samples, all attributes that would permit its deployment in large scale screening and in remote areas. On the other hand, although several commercially available assays exist, HPV testing is still costly relative to cytology. However, lengthening of screening intervals and economies of scale post-implementation in screening programs would largely alleviate the costs associated with HPV testing.

The lack of specialized personnel and resource infrastructure in low-resource settings has prompted the emergence of low-technology screening methods, such as visual inspection with acetic acid (VIA). The latter has been extensively field-tested in low-resource regions in Africa, Asia, and Latin America, with variable results in terms of screening accuracy. VIA may be a suitable starting point to assist the establishment of screening programs in low-resource countries in connection with immediate treatment, such as cryotherapy. In such settings, it may also be used to triage women testing positive with a low-cost HPV DNA assay (WHO is currently evaluating in the field an HPV assay system that does not require running water and relies on battery power). Research on how to combine HPV testing and VIA in screen-and-treat conditions in low-resource countries is urgently needed.

In addition to HPV testing, with its different modalities for high- and low-resource settings, and VIA, other techniques show promise as screening enhancements and are the subject of intensive research. These screening enhancements include the use of immunocytochemical markers to improve the accuracy of cytology and computer-assisted pre-selection of abnormal areas in liquid-based smears. Because of their complexity, cost, and requirement for trained personnel and laboratory infrastructure, these technological enhancements can be implemented exclusively in high-resource areas. Table 4 summarizes the existing gaps in knowledge and areas of research interest related to the secondary prevention strategies, as well as the issues pertaining to their implementation in different settings.

6. Public health strategies and advocacy

As soon as HPV vaccination became incorporated into immunization practices of a few countries in late 2006, there was considerable mobilization of stakeholders tasked with planning the delivery and financing of this new form of preventive strategy. Policy decisions must rely on sound judgment of the available evidence, as well as on the cost-to-benefit ratio for large scale interventions. As of this writing, health technology assessments have invariably endorsed the notion that there will be a substantial impact of HPV vaccination delivered as pre-exposure prophylaxis of young women in eventually reducing the incidence and mortality from cervical cancer (and other HPV-associated diseases). Likewise, mathematical models of the cost-effectiveness of HPV vaccination based on Markov models and micro-simulation modeling with various degrees of complexity have also indicated that vaccination will be cost effective for all high-resource settings, as well as for low-resource countries, provided that for the latter, the cost of unit doses of the vaccines follow a strict tiered pricing system. WHO, NGOs, and the GAVI Alliance consortium of multiple stakeholders, funders, and industry provide guidance to assist low- and middle-resource

countries in deciding whether to implement HPV vaccination and, if so doing, on how to deploy the intervention and seek financial assistance for implementing it.

Not surprisingly, decisions are highly complex because of the problem of harmonizing an HPV immunization agenda with pre-existing cancer control policies and mechanisms for cervical cancer screening. Up until recently, countries seeking to establish a cervical cancer control agenda had only to devote resources to implement a cytology-based program that met WHO guidelines for quality and coverage. The core method itself, conventional Pap cytology, is a public domain technology; thus, there were no commercial interests involved. Nowadays, cervical cancer screening is an area of great complexity, in which there are many different technologies that can be used in a bewildering array of competing algorithms. Many of these technologies were developed by biotechnology companies and, thus, commercial interests tend to infiltrate the messages aimed at influencing policy. Choices that countries have to consider include screening methods and their combinations, target screening ages, frequency of screening, use of triage and diagnostic steps, and the opportunity for implementing screen-and-treat approaches.

Table 5 lists some of the key questions that public health practitioners and policymakers face in most countries and the research and policy questions that need further insight. An important requirement for successful policymaking and implementation of vaccination or screening programs is buy-in from all stakeholders, particularly with respect to vaccination. One of the most important obstacles that countries face in implementing wide-scale HPV vaccination is the need to fight anti-vaccine activism, a phenomenon that has grown in influence in recent years because of the penetration of the internet as a potent conduit for ill-conceived health-related messages. A cursory inspection of internet websites that portray themselves as source of authoritative information about vaccines shows that many are grounded on misperceptions and erroneous interpretation of the importance and safety of vaccines or on myths that are hard to dispel. Properly educating the public is essential in allaying the fears that anti-vaccination activism seeks to spread. Likewise, it is imperative that policymakers and healthcare providers be provided with the simple facts about the lack of validity in the arguments used by the anti-vaccine movement [11].

In conclusion, a wealth of evidence discussed elsewhere in this monograph indicates that there is opportunity for the adoption of improved strategies for cervical cancer control via improved screening and HPV vaccination. Use of VIA testing, field-adapted HPV assays, and screen-and-treat approaches can bring substantial reductions in cancer prevention and control in developing countries. In high-resource settings, there is now a clear impetus for science-driven changes in screening using molecular technologies. Although cost is still an issue, implementation of economies of scale will quickly offset these concerns. Studies of technology acceptability and provider education can help with the implementation of new guidelines for HPV-based preventive strategies. To this end, it is imperative that international agencies, professional societies, and NGOs establish more active, advocacy-oriented outreach. There is great opportunity for such action via concerted alliances with HIV prevention organizations and networks focusing on mother/child health, e.g., those funded by the Gates Foundation. Although the challenges may seem daunting, there are

substantial gains in disease control and prevention that may be realized in the immediate future.

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MC-D: No relationship with industry

SI: No relationship with industry

MHS: Has received free CareHPV equipment and reagents for an independent analysis of low-cost HPV testing in Africa. He has also received free specimen testing from Roche for epidemiologic projects. He was the NCI co-Project Officer and co-Medical Monitor of an independent evaluation of GSK HPV vaccine, for which GSK donated vaccine and financed the regulatory components.

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Highlights

- There are several gaps in our knowledge of HPV biology at the fundamental level.
- Cross-protection of vaccination and protection by incomplete regimens are not clear.
- Research on how to combine HPV testing and VIA in low-resource countries is needed.
- It is imperative to establish more active, advocacy-oriented outreach.

Figure 1. Number of research articles on papillomavirus in Medline's PubMed database by year of publication. Data for 2012 is a projection for a whole year based on article counts until the end of April. IARC: International Agency for Research on Cancer.

Table 1

Gaps in knowledge and pertinent research issues and hypotheses regarding the epidemiology and natural history of HPV infection

Key questions	Research issues and ancillary hypotheses
Why do age-specific cervical HPV prevalence curves vary internationally?	In particular, prevalence of HPV at older ages can rise secondarily or never fall after the peak among young women. The varying contributions of re-activation from latency versus new infections are not clear.
Is the variation in duration of infection among different HPV types random?	Is infection duration affected by anatomic location, the differentiation stage of the infected cells, and/or whether a lesion has developed? There is conflicting evidence regarding whether different hrHPV types persist longer than others in the absence of CIN development. Does long-term persistence of HPV16 or of another hrHPV always result in high-grade lesions? Does viral persistence precede malignant transformation or does the latter favor a persistent infection?
What is the role of host immunity?	Despite much research we still have limited understanding of the role of HLA polymorphisms and other immune markers in carcinogenesis. What is the role of natural immunity? Is it protective against reinfections and in preventing reappearance of latent infections? What are the contributions of local microflora and host inflammatory responses?
What is the role of condom use in protection?	Although there seems to be some degree of protection, there are conflicting data on the role of condoms in preventing infection and in clearing existing HPV-associated lesions.
What is the role of cofactors in cervical carcinogenesis?	Although etiologic cofactors such as smoking, long-term oral contraceptive use, and multiparity are well established, their role in affecting the risk of viral persistence is still unclear. Is chlamydial infection an important risk factor? If so, how does it mediate risk of disease?
What is the meaning of CIN2 lesions?	Is CIN2 a true intermediate state in the precancer lesion spectrum or is it an artificial histological entity used to classify indeterminate CIN1 and CIN3 lesions? Can guidelines be brought into line with epidemiologic understanding?
How does HPV infection lead to cancer in other sites?	The natural history of penile intraepithelial neoplasia is completely unknown and that of anal HPV infection has been studied nearly exclusively in men who have sex with men.
What is the role of HPV in head-and-neck cancers?	Evidence for the role of HPV16 in a subset of oropharyngeal cancers is unequivocal but data beyond DNA detection and E6/E7 are lacking or insufficient for other hrHPV types or for a carcinogenic role in other upper aero-digestive sites. Is the association between HPV and some laryngeal cancers truly causal, or does it represent associations for misclassified oropharyngeal primary cancers?
Does HIV infection alter the course of infection with different HPV types and risk of invasion?	Are infections with non-16/18 HPV types more likely to progress to cancer in HIV-infected persons? Is the differential in risk of lesion progression among different oncogenic HPV types the same in HIV-positive and HIV-negative women?

CIN: Cervical intraepithelial neoplasia; HIV: Human immunodeficiency virus; HLA: Human leukocyte antigen; hrHPV: High-risk human papillomavirus.

Table 2

Gaps in knowledge and pertinent research issues and hypotheses regarding the biology of HPV infection and the host-virus relationship

Key questions	Research issues and ancillary hypotheses
What drives selection toward speciation of the HPV genotypes?	Are these based upon cell entry pathways, immunologic barriers, transcription factors? For a virus so small and with only 8 genes for most PV types, the number of encoded proteins is remarkable, resulting in many regulatory and metabolic pathways being affected.
Is HPV differentially regulated depending on the infection site?	Is the viral life cycle comparable in all squamous epithelia of the cervix, anogenital sites, and oropharynx? What is different about viral life cycle and gene expression in columnar (glandular) epithelium?
Why does the probability of HPV-associated cancer vary by site?	Why is the cervix so remarkably more vulnerable to malignancy stemming from HPV infection relative to other sites that can be infected by HPV?
Is there an analogy in diversity of PVs for other primate or mammalian hosts?	Selective forces have led to a very large number of HPV genotypes. Is there a comparable diversity among PVs specific to other host species? Is there something unique about human behavior or immune function that leads to excessive speciation?
What is the role of Beta and Gamma PVs in disease causation?	Little is known about the epidemiology and natural history of cutaneous HPVs. How much analogy is there to the course of infections by mucosotropic HPVs and to host immunologic responses?
Do we know enough about the viral lifecycle?	What are the stages of attachment, internalization, uncoating, transport of the genome to the cell nucleus, replication, activation, amplification, virion morphogenesis, shedding, survival in the external environment, and reinfection of susceptible tissues?
How important are epigenetic events?	How do DNA methylation and chromatin acetylation and deacetylation affect HPV DNA replication and RNA transcription? What is their role in programming viral replication, neoplastic progression, tumor invasion and metastasis?
What is the role of micro-RNAs?	Are micro-RNAs critical in coordinating and balancing virus-host interactions in benign infections and during neoplastic progression?
Do early life cycle events confer advantage in persistence, replication, and shedding?	Oncogenesis seems to be an anomaly and is an unintended consequence of other essential virus-host interactions. Per se, viral DNA integration and the ultimate death of the host cannot be a positive selective force.
What triggers HPV DNA integration?	Is it a property only of the high-risk types? Is it possible to minimize the risk of integration into host chromosomes or, at least, to have an early indicator of when that irreversible step has happened? What are the epigenetic responses and distinctions among different integrated copies?
What is the nature of chromosome instability triggered by HPV?	Is this a property of the oncogenes E6, E7 and even E5, the replication genes E1 and E2, or a result from the integration of foreign viral DNA sequences?
What is the role of E2 in pathogenesis?	Why do HPV tumors invariably exhibit silencing of E2 expression? Is this because E2 is able to repress E6-E7 transcription, or is it due to its role in HPV DNA segregation during cell division, or both?
Why is HPV16 the most successful mucosotropic HPV type (based on prevalence)?	How does it differ from its closely related alpha-9 types? Why is it the most oncogenic type in its species? The same can be asked of HPV18 among the members of the alpha-7 species.

PVs: Papillomaviruses.

Table 3

Gaps in knowledge and pertinent research issues and hypotheses regarding the role of HPV vaccination as a primary prevention strategy

Key questions	Research issues and ancillary hypotheses
What is the extent of cross-type protection by the existing L1 VLP-based vaccines: are benefits to be expected at the population level?	Cross-protection for types that are phylogenetically close to the vaccine types seems real but limited in efficacy and duration of protection. Differences between the vaccines, if real, could suggest adjuvant effects or be a result of how VLPs are produced.
Can correlates of immune protection be identified?	Serologic antibody titers post-vaccination or other immune markers do not predict protection at an individual level. Long-term follow-up of vaccinated populations may shed light on determinants of protection. Research is needed on different definitions of viral or lesion outcomes.
How many vaccine doses are needed? Could fewer doses provide protection? Could different injection intervals achieve equal protection?	Regulatory RCTs were designed to address the efficacy of three-dose regimens. Simplified regimens with fewer doses or different scheduling could enhance coverage and decrease costs of deploying vaccination. Can fewer doses elicit long-lasting protection?
Anamnestic response by sexual exposure post-HPV vaccination: is it expected?	Natural boosting of the immune response post-vaccination via sexual exposure to HPV infection could be examined in surveillance studies augmented by behavioral questionnaires. Is antigenic exposure high enough to heighten serological titers? Would response times be sufficient to prevent infection?
Is protection expected to be pan-mucosal?	Plausibly, vaccination exerts a prophylactic effect in all mucosal sites that serve as port of entry for HPV infections. However, there is scant data to document protection against new infections or lesions in non-cervical sites.
Does vaccination prevent recurrent infection in the same, adjacent, or distant mucosal sites?	Vaccination will not clear existing infections but may have a protective effect in adjacent areas, thus potentially having a benefit in preventing multi-focal infections and recurrent lesions in the cervix, vagina, and oral sites. More research is needed on mucosal immunity.
Is type replacement to be expected post-vaccination? Can vaccination be detrimental for the natural history of non-vaccine-target HPV types? What are the methodologic caveats in investigating this possibility?	HPVs are highly stable DNA viruses; thus, selective pressures from vaccination may not elicit the emergence of new types but may vacate existing ecological niches currently taken by HPVs16/18. Long-term follow-up of vaccinated populations will provide answers but analyses of existing cohorts can provide valuable insights as to whether or not some types are presently out-competed by HPVs 16 and 18 and could thus increase in prevalence later.
Should boys be vaccinated?	As one of the currently most pressing questions, it remains one of affordability for most countries. The benefits are the protection against HPV-associated diseases in men and the enhanced herd immunity with consequent reduction in HPV transmission in populations (ultimately benefiting both genders). Can countries attain sufficiently high male vaccination coverage rates?

RCTs: Randomized, controlled trials; VLP: Virus-like particle.

Table 4

Gaps in knowledge and pertinent research issues and hypotheses regarding the role of screening technologies in secondary cervical cancer prevention

Key questions	Research and implementation issues
What answers are still needed from the studies of HPV testing in screening?	Is there sufficient buy-in for wide-scale implementation in high-resource settings? Can HPV DNA or RNA testing be implemented cost-effectively in middle- and low-resource settings?
Cotesting versus serial testing: what is the best option for high-resource settings?	The USA is the only country to have formally included cotesting (parallel use of HPV plus Pap cytology) in practice guidelines. Can serial testing (HPV followed by Pap triage of HPV positives) attain the same level of safety for guidelines?
If HPV testing is adopted for women ages 30 and older, what screening options should be recommended for younger women?	The technology “neglected” age range of 21–29 years continues to rely on cytology. What types of evidence will be required for increasing the age of screening initiation? Could a compromise solution exist via a single policy of serial testing (HPV followed by Pap triage) beginning at age 25?
Is VIA a solution for low-resource settings, either alone or as triage for low-cost HPV primary screening?	VIA is not as accurate as HPV testing but is easier to deploy. Is it a method that should only be combined with screen-and-treat strategies? What is the value of VIA for the triage of HPV-positive women to improve the effectiveness of screen-and-treat strategies?
Is self-sampling a solution to expand the coverage and bring equity to screening?	HPV testing of self-collected samples could permit reaching remote areas, urban women who are missed by invitations to screen, and women who refuse provider-assisted sampling. Is the balance between lower accuracy and higher coverage acceptable?
Algorithm management versus risk stratification: what is most suitable for guidelines?	Can healthcare providers learn and apply risk stratification via multiple biomarker testing as part of practice guidelines? Does it confer a more personalized level for screening and management? Is it cost-effective?
What is the role of HPV viral load as a clinical tool?	Should HPV testing be based on higher thresholds of viral load for improved specificity? Is the greater complexity of quantitative HPV assays worth the extra cost to be borne in screening?
Is there a role for genotyping in screening or triage?	Genotyping for HPV16, 18, and other priority hrHPVs improves the positive predictive value of screening and permits more rational colposcopy referral. Can genotyping become affordable in the near future to be implemented in screening, triage, and surveillance?
What is the role of cytology-based staining for prognostic markers of lesion progression?	Is the accrued accuracy of enhancements in cytology based on the identification of these markers worth the added cost to cytologic triage of HPV positive women? Can it compete with genotyping as a cost-effective strategy?
How should we educate healthcare providers and patients concerning HPV testing results?	Gradual introduction of HPV testing leads to patient anxiety and confusion related to the diversity of guidelines. The change from an oncologic to an STI-detection paradigm in cervical cancer screening requires research on sound educational approaches to demystify the implications of HPV positive results.
What will be the impact of HPV vaccination on screening performance?	As vaccinated young women reach the age of screening, a gradual decrease in lesion prevalence will adversely impact test performance. Which tests will be less likely to be affected? Can guidelines be safely relaxed in HPV-vaccinated populations? Can HPV-based screening be integrated with HPV vaccination strategies for shared resources and improved surveillance?

hrHPV: High-risk human papillomavirus; STI: Sexually-transmitted infection; USA: United States of America; VIA: Visual inspection with acetic acid.

Table 5

Key public health and policy questions and related research issues in implementing cancer control mechanisms based on HPV prevention

Question	Public health and policy issues and research directions
Are cost-effectiveness models coherent? Are they being used for policy decisions?	HPV vaccination and cervical cancer screening are not intended to be competing approaches to disease prevention but may be perceived as such in some settings. Decisions are highly complex and are influenced by commercial interests.
What is the role of WHO and NGOs in financing interventions?	WHO and NGOs provide guidance and assist with planning and implementation research, whereas financing of large-scale deployment must be borne by the countries (some of which will receive assistance from GAVI). Centralized procurement of vaccines and HPV tests by WHO may lower costs and enhance coverage.
How to address cross-cultural characteristics in delivering HPV-based interventions?	A one-size-fits-all approach to deploying HPV vaccination and new screen-and-treat strategies will not work well in low-resource settings. Culturally sensitive programs must take into account deeply seated beliefs stemming from religion, culture, and tradition.
How can preventive strategies be coordinated?	Integrating reproductive health programs (e.g., maternal & child health, family planning) with screening and vaccination activities may help to save resources. However, sound policies must establish priorities so as not to overload existing systems.
What does success look like?	What are the benchmarks for successful primary and secondary preventive interventions? Should they be different between high- and low-resource settings? What are the realistic goals in assessing disease prevention?
How to deal with the issue of privacy?	Proper surveillance and control require measures such as partner notification, specimen storage, linkage between vaccination records and screening, and referrals across different healthcare providers. More research is needed on the allowable ethical boundaries in the delivery of effective control programs.
How can advocacy deal with anti-vaccine activism?	Fear of undue influence by pharmaceutical companies, myths and misperceptions about the value and safety of HPV vaccination are amplified in the internet and in social media. Simple scientific reasoning is not sufficient to counter anti-vaccine activism. More research is needed on sociocultural, behavioral, and psychosocial strategies toward more effective advocacy.

NGO: Non-governmental organization; WHO: World Health Organization.