

Human Papillomavirus—Lessons From History and Challenges for the Future

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Abstract

Human papillomavirus (HPV) is the most common sexually transmitted infection, and HPV-associated cervical cancer is a significant cause of morbidity and mortality worldwide. Recent advances in molecular biology have facilitated testing for HPV infection. Over the last decade, national and international cervical cancer screening programs have added HPV testing to their guidelines. The use of HPV prophylactic and therapeutic immunization may expand the need for systematic HPV testing to help define eligible subgroups for intervention. Given the worldwide variation in HPV subtype prevalence, basic Pap testing will continue to play an important role in cervical cancer screening, and methods to improve Pap smear sensitivity may help to improve screening in the future. This review focuses on the genetics and cellular biology of HPV infection, the natural history and prevalence of HPV infections, cervical cancer screening around the world and in Canada in particular, and evolving research to improve screening methods.

Résumé

Le virus du papillome humain (VPH) constitue l'infection transmissible sexuellement la plus courante et le cancer du col utérin associé au VPH constitue une cause importante de morbidité et de mortalité de par le monde. Des percées récentes en biologie moléculaire ont facilité le dépistage de l'infection au VPH. Au cours de la dernière décennie, certains programmes nationaux et internationaux de dépistage du cancer du col utérin ont ajouté le dépistage du VPH à leurs directives cliniques. L'utilisation de l'immunisation prophylactique et thérapeutique anti-VPH pourrait amplifier la nécessité du recours à un dépistage systématique du VPH en vue de contribuer à l'identification des sous-groupes admissibles à l'intervention. Compte tenu des variations mondiales en matière de prévalence des sous-types du VPH, le test de Pap de base continuera de jouer un rôle important dans le dépistage du cancer du col utérin; les méthodes permettant d'améliorer la sensibilité des frottis de Pap pourraient contribuer à l'amélioration du dépistage à l'avenir. Cette analyse est axée sur la génétique et la biologie cellulaire de l'infection au VPH, l'histoire naturelle et la prévalence des infections au VPH, le dépistage du cancer du col utérin de par le monde et au Canada en particulier, et l'évolution de la recherche visant l'amélioration des méthodes de dépistage.

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INTRODUCTION

Cancer of the cervix is the leading cause of gynaecologic-related death worldwide, but is almost completely preventable with regular Pap smear screening.¹ It is estimated that 1300 new cases and 380 deaths from cervical cancer occur annually in Canada.² Worldwide, women in developing countries account for about 85% of both the annual cases of cervical cancer (estimated at 493 000) and the annual deaths from cervical cancer (estimated at 273 500).¹

Our understanding of cervical cancer and its primary cause, HPV, began in the mid-nineteenth century. At that time, physicians began to observe that cervical cancer was common in prostitutes but extremely rare in nuns, with the exception of nuns who had previously been sexually active. They also observed that the rate of cervical cancer was very high among women married to men whose first wives had died of cervical cancer.³ From these initial observations, scientists deduced that cervical cancer was caused by a sexually transmitted agent. In 1976, Meisels and Fortin published evidence that HPV has pre-malignant and malignant potential,⁴ and one year later zur Hausen identified HPV in cervical cancer specimens.⁵

The role of papillomaviruses as cancer-causing agents is now well-established, and HPV infection has been implicated in over 90% of cervical cancers.⁶ Over 30 of the more than 70 known types of HPV cause genital infections, and 15 types of HPV are known to cause cervical cancer.⁷ All cases of external genital warts are caused by HPV infection; HPV 6 and HPV 11 are the cause in 90% of cases.⁸ Half of the population will acquire a genital HPV infection in their lifetime.⁹

In this review we focus on the genetics and cellular biology of HPV infection, the natural history and prevalence of cervical HPV infections, cervical cancer screening around the world and in Canada, and evolving research for improving screening methods.

THE GENETICS OF HPV

Human papillomavirus is a non-enveloped DNA virus with a double-stranded circular DNA genome containing 7900 base pairs.¹⁰ The genome has three functional areas: (1) a non-coding upstream region responsible for the regulation of DNA replication and transcription of specific coding sequences or open reading frames, (2) an early open reading frame (ORF), and (3) a late ORF. The early ORF contains the early E1-E7 proteins that form the basis of malignant transformation; the late ORF contains L1 and L2 capsid proteins that form the viral shell.¹¹

Because HPV encodes for only 8 to 10 proteins, it must use the host cell factors to regulate viral transcription and replication. HPV replication begins with the interaction of host cell components and induces the transcription of the viral genes, which leads to loss of cellular control over G1 arrest, apoptosis, and DNA repair.¹² Further interaction of HPV proteins with host cell factors leads to the stimulation of DNA synthesis and cell proliferation.¹³

THE NATURAL HISTORY OF HPV

Initially, the natural history of HPV infection was thought to be a progression over time from mild or low-grade changes to high-grade dysplasia and eventually to invasive cancer (Figure 1). More recent evidence, however, supports a new paradigm in which most HPV infections resolve spontaneously through clearance by the immune system. In most cases, only when HPV is not cleared and the virus persists will infection lead to high-grade dysplasia (Figure 2).

HPV infections in younger women are mostly transient, with 70% to 90% regressing within three years.¹⁴ Of women with high risk HPV who have normal cytology initially, 15% to 30% will develop HSIL within four years.^{15,16} Of those who develop HSIL, 10% to 30% will progress to invasive cervical cancer.¹⁵

PREVALENCE

The prevalence of HPV varies greatly by age and geographical location. The prevalence in women 30 years of age and under ranges from 19% to 82%, but it decreases to less than 10% in women over age 30; failure to eradicate the virus after menopause is not uncommon, and explains a second (postmenopausal) peak in HPV prevalence that has been reported in a number of different populations.¹⁷⁻²⁰ The wide range of prevalence results from some studies enrolling women referred for colposcopy and others using random cohorts. In addition, the worldwide prevalence of HPV is variable; Spain has the lowest prevalence and Thailand the highest.²¹

HPV subtypes include 15 high-risk HPV types that cause cervical cancer (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82).⁷ The prevalence of these subtypes also varies internationally. In North America, approximately 50% of all HPV infections are caused by HPV 16.²² However, a recent study of 15 613 women aged 15 to 74 in 11 countries determined that women in sub-Saharan Africa were equally infected with HPV 35 and HPV 16 (both 8%).²¹ Recently there has been an increase in subtypes 33, 56, and 58 in Korea.²³ The variation in subtype prevalence has important implications for implementation of vaccination; with such variation in subtype prevalence, the current vaccines for 2 or 4 subtypes will not prevent all cervical cancer. For this reason, cervical cancer screening will continue to play a key role in prevention.

CERVICAL CANCER SCREENING

The Pap smear test, used to screen for precancerous lesions in asymptomatic women for the past 50 years, was developed by George Papanicolaou before HPV was implicated in cervical cancer.²⁴ The Pap smear is a screen for morphological changes in exfoliated cervical cells. The reporting system for Pap smears has evolved gradually; currently the Bethesda System, which was introduced in 1988 and updated in 2001, is used for reporting.²⁵ This system was recently updated at the American Society for Colposcopy and Cervical Pathology meeting in Bethesda, Maryland, in September 2006.²⁶ The CIN reporting system is based on tissue architecture, and was introduced in 1973.²⁷

The conventional Pap smear is cheap, easy to perform, and has a specificity as high as 99%.²⁸ Unfortunately, its sensitivity is only 58% to 80%,^{28,29} and false-negative results can occur, especially when the cells are not evenly spread on the microscope slide. In addition, contaminants such as bacteria or yeast may prevent the detection of abnormal cells in the specimens, and in slides exposed to air for too long before their fixation, the cervical cells can become distorted and unreadable. Human error is likely the main cause of false

ABBREVIATIONS

ALTS	ASCUS/LSIL Triage Study
CIN	cervical intraepithelial neoplasia
HCII	Hybrid Capture II
HPV	human papillomavirus
HSIL	high-grade squamous intraepithelial lesions
LBC	liquid based cytology
Pap	Papanicolaou
PCR	polymerase chain reaction
VLP	virus-like particle

Figure 1. Old Paradigm for HPV Infection

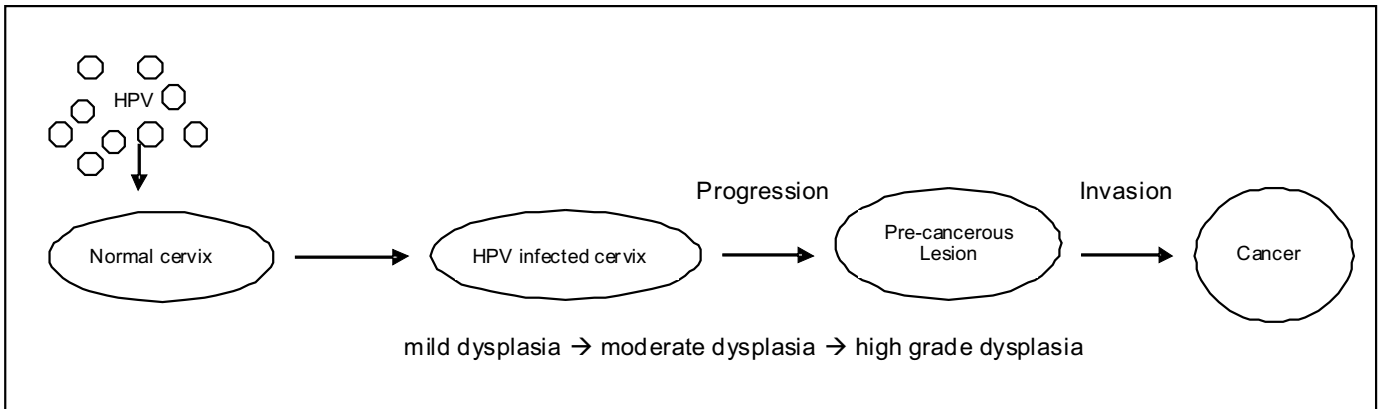
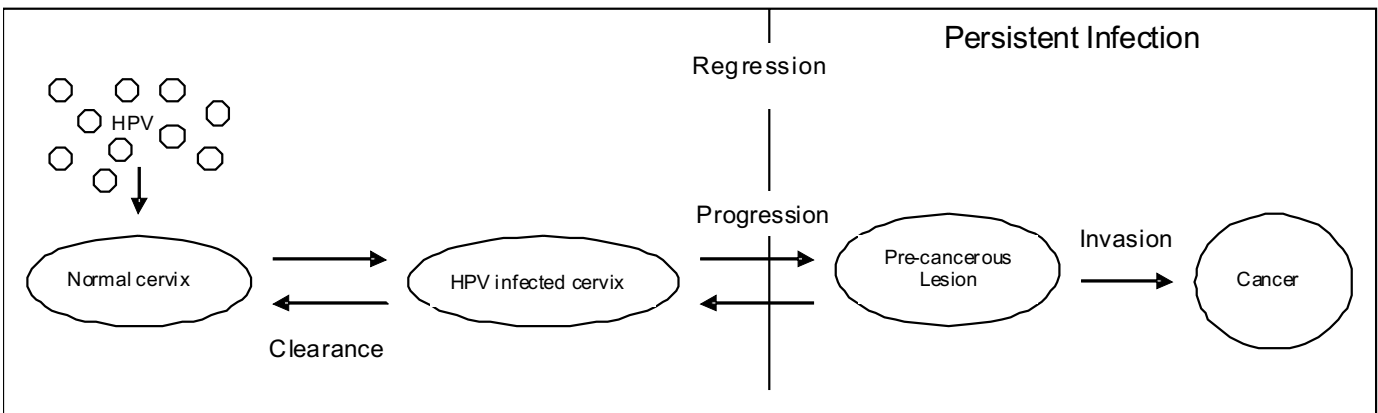


Figure 2. New Paradigm for HPV Infection



interpretation of a Pap smear. The average Pap smear slide contains anywhere from 50 to 300 000 cells to examine. Overworked or less experienced readers can miss abnormal cells if the sample contains only a few abnormal cells in a crowded background of normal cells.

In the last 10 years, LBC has been introduced as an alternative to the conventional Pap smear. Cells are collected in a liquid vial and a machine filters cells from this liquid, removes any extraneous matter, and transfers only cells to a slide. The cells are distributed as a single layer on the slide, making interpretation easier for cytopathologists. LBC has been reported to be more sensitive and has a lower rate of unsatisfactory specimens than conventional cytology (reduced from 10–15% to 1–2%), although results are mixed.^{30,31} In addition, LBC appears to be cost effective because fewer smears need to be repeated; in a recent large trial fewer than 1% of LBC smears were insufficient.³² The Ontario Cervical Cancer Screening Guideline recommends LBC as the optimal cervical screening tool, as do most international bodies.³³ However, results of a recent meta-analysis, which showed LBC was more likely to be interpreted as abnormal or equivocal but did not lead to

detection of more high-grade cervical intraepithelial neoplasia, may necessitate revision of current guidelines.³⁴

Since the discovery of HPV as the causal agent in cervical cancer, several methods of HPV testing have been developed for use in cervical cancer screening. In situ hybridization can be used to detect HPV DNA or RNA in biopsy tissue using DNA probes labelled with radioisotopes or chemi-fluorescence ligands.³⁵ The main advantage of in situ hybridization is that the HPV infection can be specifically localized. Unfortunately, this technique is time consuming and a large-scale commercial product is not available.

The HCII assay system (Digene USA) is the only HPV test approved by the Food and Drug Administration for the detection of HPV DNA in cervical specimens. In this assay, RNA primers for the 15 high-risk HPV subtypes are hybridized to DNA from cervical cells.³⁶ The hybrids are then recognized by an antibody that is visualized by chemiluminescence detection in order to measure the presence of HPV qualitatively and semi-quantitatively. This assay is widely used and has been robustly tested, but it cannot distinguish the specific subtype of HPV within the

high-risk group. Health Canada has approved both the HCII assay and Amplicor, a similar system made by Roche Diagnostics.

Type-specific PCR assays have also been developed. PCR technology uses the action of DNA polymerase on specific primers to selectively amplify target HPV DNA. The amplified DNA can be detected by a variety of methods, such as gel electrophoresis, dot blot, or line-strip hybridization. Because of the large number of variables in PCR methodology, the sensitivity and specificity of PCR results can vary depending on the primer sets, the size of the amplified PCR product, and the DNA polymerase used. Most protocols use consensus primers that target a very conserved region of L1. Type-specific assays that can potentially detect all HPV types target E6/E7. The consensus primers detect the presence of HPV, and hybridization with type-specific probes can be used to detect as many as 40 individual HPV types. The consensus primers GP5/6 (and the extended version GP5+/6+) ³⁷ and the degenerate primers MY09/11 (and the modified version PGMY09/11) ³⁸ are the most widely used. Unlike the HCII assay, PCR is not clinically used outside the research setting. Linear array HPV genotyping is a real-time PCR assay with promising results that may become more widely used in the future. ³⁹

A large trial to determine the role of HPV testing in cervical cancer screening began in the 1990s. The ALTS was a multicentre, randomized controlled trial conducted in the United States to compare the sensitivity and specificity of three management strategies to detect CIN3 in women with ASCUS and LSIL Pap smear results. ³² Women were randomized to undergo either immediate colposcopy, colposcopy based on HPV and cytology results, or colposcopy based on cytology alone. The study coordinators found that 82.9% of women with LSIL (532/642) were HR-HPV positive. Since previous studies had shown the majority of LSIL regress spontaneously, ⁴⁰ it was concluded that HPV testing is not a cost-effective screen for identifying those LSIL cases that will progress to CIN2/3. Interestingly, reports from smaller studies have described HPV rates closer to 50% to 60% in LSIL. ⁴⁰⁻⁴² This discrepancy is thought to be related to the time between the initial smear and the HPV test (2 months in the ALTS trial vs. approximately 6 months in other studies), the longer time period allowing for more spontaneous regression. Results from the ASCUS arm showed a sensitivity of 96.3% for CIN3 using HCII HPV testing, compared to 44% for cytology alone. The high negative predictive value of HCII HPV testing was also confirmed (99.5%).

Based on the results of the ALTS and similar studies, ^{43,44} national and international cervical cancer screening guidelines have been updated to include HPV testing. In 2002 the

American Cancer Society published new guidelines for screening that include HPV testing for primary screening for all women over 30 years of age. ⁴⁵ The American College of Obstetricians and Gynecologists concurred with this recommendation. ⁴⁶ HPV testing is also recommended by the American Society for Colposcopy and Cervical Pathology for women 20 years and over with an ASCUS Pap smear result. ²⁶ The Canadian Guidelines recommend HPV testing for women over 30 years of age with an ASCUS result. ⁴⁷ This recommendation has been added to screening guidelines in Australia, France, Germany, and Ontario.

A recent study using mathematical modelling was performed to determine the most cost-effective method of cervical cancer screening. ⁴⁸ Costs associated with all types of screening and treatment, and lifetime risks of cervical dysplasia and cancer, were used to create the model. The study found that a program of annual conventional Pap smears costs US\$2457 over the lifetime of a woman and reduces the risk of cervical cancer by 89%, whereas triennial liquid-based cytology (using HPV DNA testing only for women with ASCUS) costs US\$1358 per woman over her lifetime and reduces the cervical cancer incidence by 90%.

SCREENING PROGRAMS IN CANADA

International studies have shown that in a well-conducted program, with expert cytology and high compliance from women offered screening, the risk of invasive cervical cancer developing in screened women can be reduced by more than 90%. In Canada, the reduction in age standardized death rates from cervical cancer, from 7.3 per 100 000 women in 1969 to 2.2 per 100 000 in 2000 is believed to be largely the result of cervical cancer screening. ⁴⁹

Eight provinces (all except Quebec and New Brunswick) have screening programs for cervical cancer. These programs vary considerably in scope. Only Saskatchewan issues initial personal invitations for screening. British Columbia uses a single, centralized laboratory service; the Ontario program does not receive laboratory results and a pilot project to create a linking information system in the province failed. ⁵⁰ Most programs do not have a system in place to recall women for routine re-screening—a key component of any screening program. In addition, screening guidelines vary from province to province; only Ontario includes HPV testing within the guidelines for management of ASCUS. It is interesting to note that incidence rates of cervical cancer are nearly the same for all provinces regardless of the presence (British Columbia) or absence (Quebec) of an organized screening program. ^{51,52} This likely reflects the fact that a consistent majority of women will seek out cervical screening and a consistent minority will avoid it

Table 1. Cell cycle components whose expression may be altered by HPV⁵⁶

Cell cycle component	Role	HPV effect
P16	Inhibits CKD4/6	Overexpression
Cyclin D1	Cellular oncogene	Overexpression
CDK4	Phosphorylates Rb	Overexpression
PRb/E2F	Mediates G1/S checkpoint	Unchanged
P21	CDK inhibitor	Overexpression
P27	Tumour suppressor	Underexpression
MDM2	Licensing protein	Overexpression

regardless of attempts to recruit them into screening programs.

The proportion of Canadian women who report having had a Pap smear within the previous three years has been estimated from data collected from the National Population Health Survey (NPHS)⁵³ and the Canadian Community Health Survey (CCHS).⁵⁴ Data from the 1994–1995 NPHS and 2000–2001 CCHS suggest little change over time, reflecting the high proportion of women who reported having had a recent Pap smear (73%) in both surveys. The proportion of women screened ranged from a low of 66% in Quebec (1994–1995) and Nunavut (2000–2001) to a high of over 80% in Alberta (1994–1995), Nova Scotia (2000–2001), and Yukon (2000–2001). The percentage of women tested varied by age, the lowest rates being observed for the youngest (20–24 years) and oldest (65–69 years) age groups. In both surveys, fewer than one half of women aged 18 to 19 reported ever having had a Pap smear. The prevalence of testing increased with successive age groups and peaked among women aged 25 to 34. After this, there was a gradual decline in the proportion of women being tested.

As our understanding of the molecular biology of HPV infection evolves, new methods to improve screening are being developed. To initiate the neoplastic processes, the HPV genome must be integrated into replication-competent cervical squamous cells where it may induce chromosomal instability and thus initiate carcinogenesis.⁵⁵ Therefore, a marker that differentiates HPV integration from simple infection would overcome the current screening limitations and help detect lesions that need to be treated and closely followed. Cell cycle components whose expression may be altered by HPV are shown in Table 1.⁵⁶

The interpretation of cervical biopsies and smears relies on two distinct tasks: (1) the locator function (finding suspicious cells in an abundance of various cell types in a smear), and (2) the interpreter function (the cytopathologist's assessment of previously identified, potentially abnormal cells). The locator function may be improved by a sensitive

biomarker that allows the observer to focus on the analysis of cells highlighted by the biomarker, thus improving the interpreter function. Ongoing research may help determine if any of the cell cycle components shown in Table 1 could be such a biomarker.

HPV VACCINES

The number of HPV subtypes to be included as immunogens for vaccine development is an important issue. As described, the prevalence of HPV subtypes varies from country to country. Epidemiologic evidence permits ranking of the different oncogenic subtypes of HPV with respect to the expected proportion of cervical cancers they would cause. Data from a recent overview of several case-control studies suggest a pentavalent vaccine against HPV subtypes 16, 18, 45, 31, and 33 would potentially prevent 83% of all cervical carcinomas.⁵⁷

At present, one HPV vaccine is available to the Canadian public, with a second in development. Cervarix (GlaxoSmithKline) is a bivalent vaccine against HPV 16 and 18 and is currently in phase III clinical trials. Gardasil (Merck) is a quadrivalent vaccine targeting HPV 6, 11, 16 and 18; it was approved by the FDA in June 2006 for the prevention of cervical cancer, genital warts, and certain precancerous lesions in girls and women aged 9 to 26 years. Health Canada approved Gardasil in July 2006. It is currently available in Canada, and the 2007 federal budget included \$300 million in funding for the vaccine. Provinces and territories will have the flexibility to draw down funding, as they require, over the next three years.⁵⁸ Exactly how each province will implement a vaccination protocol has yet to be determined. A Health Canada advisory committee has recommended the vaccination of all girls between the ages of nine and 13.⁵⁹ The vaccine costs approximately \$150 per injection, with three injections recommended for full benefit. Table 2 outlines the immunization programs across the provinces.^{60–69}

Table 2. Provincial vaccination programs

Province	Grade offered (girls only)	Program status
British Columbia ⁶⁰	Grade 6 and 9	Starting September 2008
Alberta ⁶¹	Grade 5	Starting September 2008, available for girls in grade 9 from September 2009 to June 2012
Saskatchewan ⁶²	Grade 6	Starting September 2008, also available for Grade 7 girls in 2008-2009 school year only
Manitoba ⁶³	Grade 6	Starting September 2008
Ontario ⁶⁴	Grade 8	Currently offered
Quebec ⁶⁵	Grade 4 to 8	Starting September 2008, available at no charge for girls under 18 who are not in school cohort
New Brunswick ⁶⁶	Grade 7	Starting September 2008, also available for Grade 8 girls in 2008-2009 school year only
Nova Scotia ⁶⁷	Grade 7	Currently offered
Newfoundland and Labrador ⁶⁸	Grade 6	Currently offered
PEI ⁶⁹	Grade 6	Currently offered

Many countries are in the process of developing HPV vaccination guidelines. The Advisory Council on Immunization Practices in the United States recommends HPV vaccination for women up to age 26.⁷⁰ To date, only Virginia has made HPV vaccination compulsory for girls entering the sixth grade, but other states are considering such legislation.⁷¹ Australia has a government funded program offering vaccination to girls and women aged 12 to 26.⁷²

A survey initiated in January 2007 by the Vaccine European New Integrated Collaboration Effort has assisted with policy making in Europe.⁷³ By March 2007 four countries had already made policy decisions. The vaccine recommendations were for pre-adolescent girls in Austria; for girls aged 12 to 17 in Germany; for girls age 14, with catch-up vaccinations offered to girls and women up to 23 years old in France; and for girls age 12 in Italy. "Catch-up vaccination" involves the inclusion at the start of the vaccination program of some older birth cohorts than those targeted for routine vaccination. A guideline published in January 2008 further outlines strategies for implementing vaccination programs across the European Union.⁷⁴

Both Cervarix and Gardasil are VLP-based vaccines. The outer capsid of HPV is primarily composed of the viral protein L1. Purified recombinant L1 protein self-assembles into VLPs. These VLPs are empty icosahedral shells that are nearly indistinguishable from native HPV virions when viewed by electron microscopy.⁷⁵⁻⁷⁷ Because VLPs contain no HPV DNA, they are not infectious, but they have been shown to cross-react with antibodies to native HPV.⁷⁸ The VLPs used in Cervarix and Gardasil are assembled from recombinant HPV 16 and 18 L1 produced in baculovirus or insect cell and yeast expression systems respectively.^{79,80}

The vaccines also differ in the contained adjuvant. Gardasil contains amorphous aluminum hydroxyphosphate, while Cervarix uses AS04, a novel adjuvant reported to produce higher persistent antibody titres, which may result in an enhanced immune response.^{80,81}

Cervarix was tested in nearly 18 000 women aged 15 to 25 in the United States, Europe, South America, and Asia.⁸¹ In a recent 4.5-year follow-up study, 98% of women remained seropositive and the vaccine was 100% effective against CIN with cross-protection against types 45 and 31 (in 94% and 55%, respectively).⁸² In addition, the National Cancer Institute is sponsoring a second eight-year trial in approximately 15 000 women aged 18 to 25 in Costa Rica.⁸³ Gardasil was tested in more than 25 000 people throughout the world.⁸¹ Interim data from one of these trials confirmed the efficacy of 100% reported in phase II trials; this large double-blind, randomized placebo-controlled trial enrolled 12 167 women aged 16 to 23 years to receive Gardasil or placebo at 0, 2, and 6 months and included an average of two years of follow-up.⁸⁴

CURRENT STATUS

While we are fortunate to be on the cusp of using prophylactic vaccines, we are decades away from observing benefits from HPV vaccination. In addition, many developing countries may not benefit from vaccines that do not cover their most prevalent HPV subtype. Cervical cancer in these countries is mainly a disease of poor women with high fertility rates, where lifetime risks can exceed 10%.⁸⁵ For example, much of the global disease burden lies in Africa and Latin America, where cervical cancer screening is available only for the small proportion of women who can afford private health care in more urban centres. The Global Alliance

for Vaccines and Immunization may provide financial help in implementing immunization programs in these countries, but this has not been confirmed.⁸⁶ Indeed, even in Canada the details of vaccination implementation and cost coverage have yet to be worked out in the North West Territories. For prophylaxis, vaccines must be given at an age that is young enough to ensure that exposure to HPV has not occurred. However, what about young women who are already sexually active with a completely normal Pap smear history, or older women who are recently widowed or divorced and are now starting a new relationship? It is in these populations that HPV testing^{87,88} and methods to improve Pap smear sensitivity will be important and will help to improve screening in the future.

CONCLUSION

HPV-associated cervical cancer is a significant cause of morbidity and mortality. Recent advances in molecular biology have facilitated testing methods for HPV infection. Over the last decade, national and international cervical cancer screening programs have added HPV testing to their guidelines. The development of HPV therapeutic and prophylactic immunization may expand the need for systematic HPV testing to help define eligible subgroups for intervention. Given the worldwide variation in HPV subtype prevalence, and the financial impossibility of vaccinating all women of reproductive age, it is vital that primary prevention by HPV vaccination is integrated with established secondary prevention programs via screening. Primary high risk HPV testing and improvements in Pap smear sensitivity will help to strengthen the quality of screening in the future.

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