The Immunological Basis for Immunization Series

Module 19: Human papillomavirus infection

Immunization, Vaccines and Biologicals

World Health Organization

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Contents

Abbreviations and acronyms

AAHS	aluminium hydroxyphosphate sulfate
AIDS	acquired immunodeficiency syndrome
APC	antigen-presenting cell
BP	base pairs
CDC	Centers for Disease Control and Prevention
CIN	cervical intraepithelial neoplasia
cLIA	competitive Luminex immunoassay
CTL	cytotoxic T lymphocyte
CVS	cervical vaginal secretion
CVS	cervical vaginal secretion
DC	dendritic cell
DNA	deoxyribonucleic acid
ECM	extracellular matrix
ELISA	enzyme-linked immunosorbent assay
GACVS	Global Advisory Committee on Vaccine Safety
GBS	Guillain Barré Syndrome
GMT	geometric mean titre
GSK	GlaxoSmithKline
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
HPV	human papillomavirus
HSIL	high-grade squamous intraepithelial lesion
HSV	herpes simplex virus
IARC	International Agency for Research on Cancer
Ig	immunoglobulin
IU	International Unit
LSIL	low-grade squamous intraepithelial lesion
Orf	open reading frames

PBNA	pseudovirion-based neutralization assay
PPE	per protocol for efficacy population
RRP	respiratory papillomatosis
SEAP-NA	secreted alkaline phosphatase neutralization assay
Th	T-helper (cells)
TLR	toll-like receptor
TM	transverse myelitis
Tregs	regulatory T cells
TVC-E	total vaccinated cohort for efficacy population
TZ	transformation zone
URR	upstream regulatory region
VIN	vulvar intraepithelial neoplasia
VLP	virus-like particle
WHO	World Health Organization

Preface

This module is part of the series *The Immunological Basis for Immunization*, which was initially developed in 1993 as a set of eight modules focusing on the vaccines included in the Expanded Programme on Immunization (EPI)[1]. In addition to a general immunology module, each of the seven other modules covered one of the vaccines recommended as part of the EPI programme — diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. The modules have become some of the most widely used documents in the field of immunization.

With the development of the Global Immunization Vision and Strategy (GIVS) (2005–2015) (*http://www.who.int/vaccines-documents/DocsPDF05/GIVS_Final_EN.pdf*) and the expansion of immunization programmes in general, as well as the large accumulation of new knowledge since 1993, the decision was taken to update and extend this series.

The main purpose of the modules — which are published as separate disease/vaccine-specific modules — is to give immunization managers and vaccination professionals a brief and easily-understood overview of the scientific basis of vaccination, and also of the immunological basis for the World Health Organization (WHO) recommendations on vaccine use that, since 1998, have been published in the *Vaccine Position Papers* (*http://www.who.int/immunization/documents/positionpapers_intro/en/index.html*).

WHO would like to thank all the people who were involved in the development of the initial *Immunological Basis for Immunization* series, as well as those involved in its updating, and the development of new modules.

[1] This programme was established in 1974 with the main aim of providing immunization for children in developing countries.

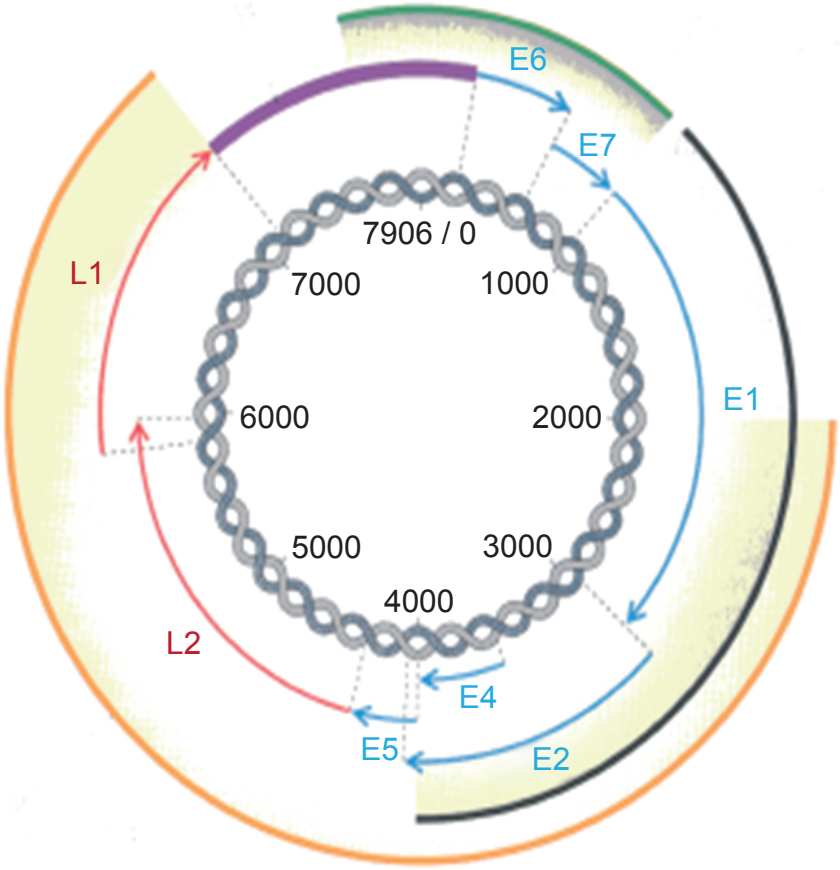
1. The organism and diseases

1.1 Human papillomavirus (HPV)

HPV is a non-enveloped, double-stranded circular deoxyribonucleic acid (DNA) virus that is enclosed by an icosahedral protein capsid. Its genome is about 8000 base pairs (BP) in length with its DNA usually in the supercoiled form (Figure 1). The viral open reading frames (*orf*) are on one strand of the HPV DNA and are described as being either in the early or late region of the genome based on the time of their protein expression within the infected host cells. The early region proteins, E1–E7, encode genes that are primarily involved in DNA replication, while the late region proteins, L1 and L2, code for the protein shell that encases the genome which can be recognized by the host's immune system. In the cervix, they are produced and assembled into highly immunogenic virions and released only in the terminally differentiated outer epithelial layer, away from the primary site of immune surveillance, the submucosa. Upstream from the early genes is an area of DNA that functions as the origin of viral replication (upstream regulatory region: URR)(*1*). HPV DNA can either remain in an episomal form, as is common in genital warts infection, or they can integrate into host DNA, as is seen in most, if not all, of cervical cancer cells (*2,3*).

HPV is the most common sexually-transmitted infection in the world, with a propensity to infect epithelial or mucosal surfaces. There are more than 100 known HPV genotypes numbered in order of their discovery. HPV genotypes that infect the genital mucosa are classified as high-risk or low-risk according to their oncogenic potential. At least 13 high-risk HPV genotypes can cause cervical cancer and are associated with other anogenital and oropharyngeal cancers: genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66. The two genotypes most strongly associated with cervical cancer are genotypes 16 and 18. Approximately 50% of all cervical cancer is attributable to HPV 16 *(4,5,6)* and an additional 15%–20% are attributable to HPV 18 *(7)*. Other more common types include HPV 45 (7%), and 31 (3%) *(4,8)*. Infection with low-risk genotypes very rarely causes cancer, but can cause benign or low-grade changes in cervical cells that may be indistinguishable from those caused by high-risk HPV genotypes *(7,9,10,11)*.

Figure 1: HPV genome



Blue regions are the early regions (E).
Purple region is the upstream regulatory region.
Red areas encode for the late regions (L)
Reproduced, by permission of the author, from Burk RD (*23*).

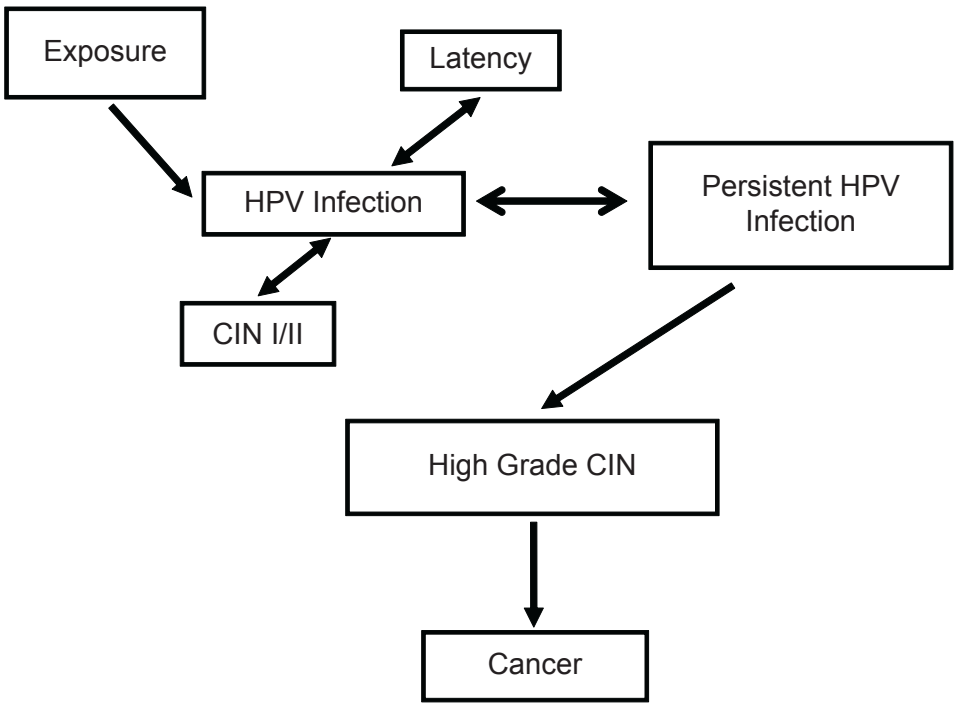
1.2 HPV infection and development of cervical cancer

Infection with HPV has been shown to be central and necessary for the development of all histologic types of cervical cancer, including squamous cell carcinoma, the histologic type in about 70%–80% of cervical cancers, and adenocarcinoma, the histologic type in approximately 10%–15% of cervical cancers (*4,7,12*). The vast majority of HPV infections are transient, and persistence of infection is necessary for the development of cervical precancerous lesions and cervical cancer (*13*). When the body fails to clear the viral infection and the virus replicates in the epithelial cells, precancerous lesions can develop. These precancerous cells can manifest themselves as a low-grade squamous intraepithelial lesion (LSIL) or equivocal lesion (atypical squamous cells), which is often a cytomorphologic manifestation of an acute HPV infection. When the HPV infection persists, lesions can undergo transformation to a higher grade lesion (high-grade squamous intraepithelial lesion, or HSIL). These high-grade lesions have prospectively been shown to be the immediate and necessary precursor to cervical cancer (*14*).

Local immune responses enable most women to clear their incident HPV infection. A lack of these appropriate immune responses allows for persistence of HPV and is likely to permit the development of cervical cancer (*15*). When a virus is undetectable by an HPV test, it is not known if it is present in a latent phase undetectable by current assays, or if the virus is no longer present in the tissue. It is currently unclear what percentage of viral clearance results in viral latency, and what percentage of the detected cancers result from latent infections (*13*). The proportion of spontaneous regression that is immune mediated is unknown. Nor is it known what fraction of newly detected cervical HPV infections in normal adult women, many of whom are likely to have been previously exposed, represent reactivation of potentially latent infections (*15*).

The clinical paradigm of cervical neoplasia focuses on persistence of HPV infection (see Figure 2), which is defined as having the same HPV type for 6–12 months, depending on the study. The longer a women has an HPV infection, the less likely she is to clear her infection (*16*). Women with persistent HPV infection of the same subtype have a 26-fold increased risk of developing a squamous intraepithelial lesion, with women infected by the high-risk subtypes having a 37-fold increase in risk (*17*). Risk factors for persistent HPV infection include: infection with specific oncogenic HPV types, more than eight lifetime sexual partners, and oral contraceptive use for more than 24 months (*18*). The vast majority of early cervical intraepithelial neoplasia (CIN) will regress spontaneously. It is only persistent CIN that has the potential for progression to a true cervical precancerous high-grade lesion which requires treatment.

Figure 2: The central factor for progression to cervical cancer is persistence of HPV infection



Most early HPV infections are self-limiting, with a woman's natural immune responses allowing for HPV regression. Similarly, most early HPV-associated cervical disease is an acute manifestation of newly prevalent HPV infection. It is when a woman has HPV persistence that it makes her more likely to progress to clinically-relevant CIN.

Adapted, by permission of the authors, from Einstein & Burk (176).

HPV 16 is the most common HPV infection in invasive cervical cancers. HPV 18 has been shown to play a more significant role in the development of cervical adenocarcinoma, with a prevalence of nearly 40% in these tumours (*19,20*). Although HPV 16 is still the most prevalent HPV infection in adenocarcinoma, infection with HPV 18 confers a higher risk of development of adenocarcinoma (*19*). In younger women, HPV 18 has been found in up to 34% of cervical adenocarcinoma and 35% of cervical adenosquamous carcinomas (*19,21*).

HPV 6 and 11 are HPV types that cause 90% of all anogenital warts and most cases of oropharyngeal papillomatoses (*22*). Most early HPV infections, especially low-risk types, are self-limiting and often do not result in clinical disease (*17*). HPV 16, 18, 6, and 11, the HPV types contained in the quadrivalent vaccine (discussed later in this module), are implicated in 30% of all CIN 1 disease. While uncommon in early CIN, HPV 16 and 18 are found in 50%–60% of CIN 2 and CIN 3 disease (*4,5,6,23,24*).

Genital HPV infections are primarily transmitted by sexual contact, predominantly, but not exclusively, through penetrative intercourse. HPV infections are highly transmissible, with most sexually active women and men acquiring an HPV infection at some time in their lives. Early studies demonstrate a prevalence of HPV infection in young women ranging from 20%–46% in various studies. The Centers for Disease Control and Prevention (CDC) estimates that by the age of 50 years, 80% of women in the United States of America will have acquired a genital HPV infection (*25*). Similar prevalence rates are consistent worldwide, although many of these epidemiologic studies are cross-sectional or focus on smaller cohorts.

1.3 Additional HPV-associated cancers

Numerous anogenital cancers are associated, to varying degrees, with HPV. Approximately 80%–90% of all anal cancers are caused by HPV 16 and 18 (*13,26,27*). Forty percent of vulvar cancers, which affect mostly older women, are associated with HPV (*28,29*). In addition, HPV is implicated in penile (*30*), vaginal (*31*), head and neck (*32,33,34*) and urethral cancers (*35*) in varying proportions, depending on the study and HPV detection method used.

1.4 Relationship of disease burden with HPV infection

While estimates of global incidence are not available, worldwide prevalence of HPV and cervical neoplasia is thought to be high, especially in HIV-infected individuals (*36*). The International Agency for Research on Cancer (IARC) has studied HPV prevalence in cervical neoplasia and invasive cancer in worldwide populations (Africa, North America, South America, Asia and Europe). In a meta-analysis, most cervical squamous cell carcinoma worldwide is caused by HPV 16 (46%–63%), HPV 18 (10%–14%), and HPV 45 (2%–8%). Cervical adenocarcinoma is caused by HPV 18 (37%), HPV 16 (26%–36%), and HPV 45 (5%–7%) (*19*). Similarly, 84% of all HSIL disease (CIN 2 and 3) is HPV positive (HPV 16-45%, HPV 18-7%, HPV 33-7%, HPV 45-2% and HPV 31-9%, other HPV types 14%) (*6*). Seventy to eighty percent of all LSIL pap smears are HPV positive (HPV 16-26%, HPV 31-12%, HPV 51-11% and HPV 53-10%) (*37*). However, HPV 16 is still the most common HPV type found in LSIL disease worldwide. Amongst women with normal cervical cytology, 9.2% have incident HPV infection (6.1% low-risk types and 2.5% high-risk types, and 0.5% with uncharacterized HPV types.) (*37*).

The disease and treatment morbidity and mortality due to cervical cancer is high worldwide. Countries with active cervical screening programmes also have a high economic burden due to screening and follow-up. Tremendous resources are required for the current screening methods, which predominantly focus on using the Pap test. While the Pap test is extremely useful for identifying disease in its earliest precancerous stage, access to screening and follow-up of equivocal results has led to challenges. An HPV-Pap algorithm has been suggested to be more clinically relevant to the vaccination era *(38)*, but this rationale is an area of continuing development pending mature datasets. Questions also arise with regard to the use of more advanced technologies, such as HPV genotyping, and their clinical significance, especially in the vaccine era. Conversely, vaccines have the potential to prevent cervical neoplasia at its earliest target, HPV infection. As such, there are clinical implications on burden of disease as well as screening and follow-up of early manifestations of HPV infection that may be transitory and not result in morbidity.. Such clinical implications may be more pronounced in countries that do not have active cervical cancer screening programmes, where the burden of disease is largest.

1.5 HPV effects in HIV-infected and immunosuppressed individuals

The impact of immune system defects on HPV persistence and the development of cervical cancer is clearly demonstrated in human immunodeficiency virus (HIV)-infected women. Women with acquired immunodeficiency syndrome (AIDS) are at increased risk for cervical cancer, which is considered an "AIDS-defining illness" (*39*). However, there is much debate in the literature, and inconsistency between many studies, partially due to the availability only of estimates of prevalent disease in regions where HIV-associated cervical cancer is most endemic; thus more research is still necessary (*40*). It remains unclear whether women coinfected with HPV and HIV have a higher risk of developing cervical cancer. It is possible that while these women may have an increased risk of in situ lesions, they may be able to clear these lesions. Additionally, HIV-infected women with high-grade dysplasia have a higher rate of failure to treatment than HIV-negative women with dysplasia (*41,42*). Similarly, HIV infection is also associated with an increased risk of HPV infection (*43,44,45*) and cervical lesions (*46,47*). Similar effects of HPV persistence and increased risk of cervical neoplasia is observed in other immunocompromised individuals, including transplant recipients (*48*). While HIV infection, CD4 count and viral load have been associated with HPV infection and in situ lesions, studies have failed to demonstrate an association between the level of immunosuppression and development of cervical cancer (*49,50*).

2. Immune response to HPV infection

2.1 Initial immune responses to HPV infection

HPV infection is restricted to epithelial cells. In the genital tract, specifically both keratinizing (skin) and non-keratinizing (mucosa) stratified squamous epithelia may be infected. The stratified epithelia are composed of undifferentiated basal and parabasal cells that have the ability to proliferate (*51*), and differentiating superficial layers. The cells in the most superficial layers are fully differentiated end- stage cells that have lost their ability to replicate and are shed into the environment (*29,52*). HPV multiplication is intimately linked with the differentiation of the stratified squamous epithelium and it is for this reason that HPV are not cultivatable by traditional viral culture methods.

In natural infection, HPVs cause infection following a minor abrasion or break of the squamous epithelium, firstly binding to the basement membrane. This complex interaction results in confirmational change of the L1 epitope, before HPV enters the keratinocyte by a novel endocytic pathway. The L1 portion of the HPV virion protein coat binds to heparan sulfate proteoglycans on the basal cells, which appear to be the primary attachment factor (*53,54,55*). However, the steps leading to virion internalization are not completely understood. In a murine challenge model, it appears the capsids undergo a conformational change while bound to the basement membrane that results in L2 cleavage, followed by the exposure of an N-terminal cross-neutralization L2 epitope and transfer of the capsids to the epithelial surface (*56*). Basal cells are also relatively accessible in transformation zones (TZ) where mutilayered squamous epithelia meet a simple glandular epithelia (*16*). This is the same region where squamous metaplasia occurs (a process whereby glandular epithelium is replaced by squamous). As a result of this junction, and the metaplastic process, immature basal cells are accessible in the TZ (*57,58.59*).

After infecting basal cells, HPV undergoes a low-level replication to about 100 copies of viral DNA per cell (*29*). As the cells undergo normal differentiation and migrate towards the epithelial surface, viral DNA replication is upregulated resulting in several thousands of copies of HPV DNA per cell (*52*). This high-level replication is dependent on host-cell replication enzymes but is mediated by HPV proteins E1/E2 as well as E6/E7 (*29,60*). The HPV E1 protein is a DNA helicase that binds to the viral orf and unwinds the double-stranded DNA (*61,62*). The E2 protein both regulates viral expression and binds to the E1 protein, increasing the binding affinity of the E1/E2 complex to the origin of viral replication (*63*). E6 and E7 prolong the lifespan of the host replication enzymes (*64*). Although incident infection may be entirely undetected, productive infections of the cervix results in lesions detected as low-grade squamous intraepithelial lesions (LSIL), or equivocal Pap tests are actually the viral cytopathic manifestations of incident HPV infection. On biopsy, these lesions are recognized as

CIN 1. The cyto-histologic feature of koilocytosis is characteristic, but not diagnostic, of productive HPV infection. These lesions are likely to be cleared as a result of cell-mediated immune responses directed to HPV proteins (*65*). These responses are eventually followed by antibody generation to HPV L1 in approximately half of those in whom an HPV DNA is detected. This is a slow and generally weak response to L1 and many women do not seroconvert (*66*).

2.2 Host immune response to HPV infection

HPV persistence, and the increased risk for neoplastic progression, is facilitated by an insufficient initial or sustained anti-HPV immune response and the capacity of HPV to evade natural host immune responses (*67,68*). Unlike bloodborne pathogens, such as hepatitis B virus, which induce systemic immune responses, HPV has no viremic phase, as infection is restricted to the epithelial compartment. Therefore, an immune response to HPV needs to be initiated at the site of infection in mucosa. As HPV infection is not cytolytic, the innate immune responses that would normally occur in response to cell death are limited and the virus is shielded from circulating immune cells as infection becomes established. Also, viral proteins are not expressed at high levels until the later stages of viral life-cycle when end-stage differentiated epithelial cells are shed from the epithelial surface. Together, the limited innate immune response, the low levels of viral gene expression in the lower layers of the epithelium, and the lack of cell death or necrosis, generally result in a delayed adaptive immune response to initial papillomavirus infection. By administering HPV vaccines parenterally, the L1 is taken up to regional lymph nodes and hence many of these HPV immune evasion mechanisms that naturally occur are overcome.

Natural humoral immune responses to HPV infection are weak, in part due to HPV evasion mechanisms and the lack of viremic phase during early infection. However, most early HPV infections will resolve spontaneously. Humoral immune responses begin with the growth and maturation of B cells which are dependent upon interaction with antigen presenting cells (APCs) and the cytokine profile secreted by T-helper cells. The resultant antibodies function to neutralize and opsonize foreign antigen for destruction, preventing infection of susceptible host cells.

Typically, protective immunity against HPV involves interplay between both innate and acquired immune responses. Innate responses are immediate and non-specific, whereas acquired immune response cells recognize specific viral antigens. Initially, virus-infected cells are lysed by the innate immune system and cytokines produced by these cells are able to further promote innate and acquired immune responses. Acquired immunity is mediated by the activity of B and T cells. The generation of memory T and B cells is dependent upon the initial presentation of antigen by the dendritic cell (DC) to the naive T lymphocyte. T cells then become activated upon recognizing viral proteins and produce additional cytokines that induce growth and maturation of B cells. Toll-like receptor (TLR) activation and signaling are key events in this process via pathogen-recognizing properties, and contribute to the activation of dendritic cells for antigen presentation and the primary activation of B cells (*69*). Both B and T cell responses are needed for sustained, effective protection against HPV, although a natural immune response to HPV may not be sufficient to clear infection, particularly when established as CIN 2/3.

2.3 Innate immunity against HPV infection

The intraepithelial life-cycle of HPV evades immune recognition, in part through its effects on Langerhans cells and dendritic cells. One of HPV's adaptive evasion mechanisms is its ability to replicate without inducing cell death and subsequent pro-inflammatory signals after apoptosis (*69*). In the non-inflammatory environment of an incident HPV infection, APCs such as macrophages and Langerhans cells are relatively ineffective (*65*). This probably results in partially-activated dendritic cells with a limited ability to migrate to the loco-regional lymph nodes. An antigen-specific immune response towards a pathogen may not be initiated by lymphocytes, due to immunologic tolerance which occurs when there is incomplete activation of APCs. Tolerized lymphocytes may not be able to respond to a subsequent antigen exposure, even in an activating environment. A second evasion mechanism is by suboptimal Langerhans cell responses as a result of low-level expression of HPV E6 and E7 and other viral proteins within the basal and immediate suprabasal layers of the stratified squamous epithelium, which are under active immune surveillance by Langerhans cells (*70*). Production of virions occurs only in the more immune-privileged differentiated apical layers of the epithelium. In the HPV-infected transformation zone (where the squamous epithelium and columnar epithelium meet) undergoing neoplastic transformation, especially in high grade CIN, there is both a reduction in numbers and a change in phenotype of Langerhans cells (*71–75*). Interactions between HPV and Langerhans cells are likely to be advantageous for viral persistence (*15*).

2.4 Adaptive immunity against HPV infection

Stimulating cells of the innate immune system leads to the proliferation and differentiation of cells involved in the adaptive immune response. This process is critical for $CD4^+$ T cells, since they are integral components for successful cellular and humoral host immune responses. Their activity has a direct influence on the function and predominance of both B and T cell effector responses. The local microenvironment cytokine milieu influences whether $CD4^+$ T cell responses will be of the T-helper (Th) 1 or Th2 phenotype. Th1 cells promote cell-mediated immune responses, while Th2 cells induce humoral effector immune responses. If $CD4^+$ T cells are not activated, or are skewed towards the suboptimal phenotype, then clearance of the offending pathogen will be hampered (*15*).

Cytotoxic T lymphocytes (CTLs) are a major effector component of Th1 immune responses and are capable of destroying virally-infected cells. Most HPV therapeutic vaccines, which are currently in development stages, as opposed to prophylactic vaccines, are targeted to the induction of CTLs to HPV E6 and E7 since these proteins play a key role in cellular transformation and are expressed during all stages of HPV infection and tumour progression. Thus, CTLs targeted against E6 and E7 should, in theory, be able to kill even HPV-infected basal cells in which only the early phase of viral replication takes place (*15*). Conversely, CTLs targeted against L1 and L2 would not be able to kill these cells, since viral proteins are only expressed later in the HPV life-cycle. In regressing warts associated with HPV and experimental HPV infections, CTLs are a prominent feature. In natural HPV infection, HPV E6 and E7-specific $CD8^+$ CTL responses have only been detected in a low percentage of patients with cervical cancer and even fewer responses are found in patients with high-grade CIN, suggesting that boosting these responses with a therapeutic vaccine or immunotherapy may be of benefit (*76,77,78*). To date, even with some positive trials (*79–83*), therapeutic vaccines are very much in their development infancy. These agents may produce the appropriate

immune response, but unfortunately have not resulted in disease prevention or immune responses correlating with clinical regression (*84*). A specific mechanism by which HPV may suppress CTL responses is via downregulation of the transporter associated with antigen protein-1 (TAP-1) (*85*).

Regulatory T cells (Tregs) control the immune system, being immunosuppressive when required, and are likely to play an important role in tolerance to viral antigens, supporting viral persistence, and in the development of cancer (*86*). In vivo imaging indicates that Treg cells form long-lasting interactions with DCs soon after they enter the lymph nodes. These interactions impair the ability of DCs to subsequently activate effector T cells, indicating that, in vivo, Tregs may inhibit T-cell responses indirectly by modulating the function of APCs (*87*). Several viruses have been shown to have the capacity to induce Tregs as a means of down-regulating host immune responses against infection. In addition to HPV (*88*), these includes HIV, hepatitis C virus and herpes simplex virus (HSV).

In respiratory papillomatosis (RRP), caused by HPV 6 and HPV 11, there is polarization of cell- mediated immune responses biased towards Th2-like T cell, cytokine and chemokine repertoires *(89–95*). The local mucosal immune response profile appears to be that of a T-suppressive state in RRP when compared to blood or normal respiratory mucosa (*88*). Thus, Tregs may have an important role in fostering persistent HPV infection and the development of RRP. These findings provide an intriguing glimpse of an inhibitory network of immunocytes that probably also prevent clearance of HPV in the cervix. Two studies based on peripheral blood specimens found higher Treg levels in women with cervical neoplasia (*96,97*), and another study, although limited in scope, suggested that there may be a difference in distribution and frequency of some Treg subgroups in CIN 3 and cervical cancer (*98*).

2.5 Mechanisms of protective immunity against HPV infection

B cells reside in the lymphoid tissue in the genital tract and are activated by interacting with T cells and binding of HPV antigens to their surface antibodies. An antibody response to HPV is initiated and neutralizing antibodies that specifically recognize or react with L1 or L2 are generated (*99*). These neutralizing antibodies are critical for inhibition of early infection before viral entry into cells.

Systemic serum antibodies have the capacity to neutralize HPV virions in the cervix, probably via transudation or exudation of neutralizing antibodies through the cervical epithelium. These transudated or exudated neutralizing antibodies from both the systemic and mucosal systems bind to virions and prevent their entry. Neutralizing L1 monoclonal antibodies have been found to have two different binding characteristics. Some prevent binding to the extracellular matrix (ECM) but not epithelial cells, and others prevent binding to epithelial cells but not to the ECM (*100*). The antibodies prevent internalization of the virions, perhaps by preventing a conformational change in the virion required for binding a secondary receptor (*53*). The in vivo cervicovaginal infection model of Roberts and colleagues has shown the sequence of events of natural internalization (*55*). The sequence appears to be: virus binds via L1 to the basement membrane; undergoes conformational change; binds via L2 to the keratinocyte and then via L1 to the cellular receptor for entry. The neutralizing antibodies that are present at the site of infection prevent initial binding to the basement membrane. This is thought to be the primary mechanism of action of prophylactic HPV L1 vaccines.

Cross-neutralizing antibodies are able to target multiple antigens by cross-reaction or recognition of non-identical, but closely-related capsid proteins (phylogenetically related HPVs e.g. HPV 16 and 31). Some B cells differentiate into memory cells and have the capacity to specifically recognize viral capsid proteins upon reinfection or secondary exposure. The memory cells are both shortlived as well as long-lived, residing primarily in the spleen or bone marrow. These two compartments are independent of one another: those from the bone marrow give long-term antibody persistence, and those from the spleen are responsible for the anamnestic response. Memory cells have a higher affinity for HPV antigens and are able to respond more rapidly and effectively to subsequent infection (*101,102*).

Inadequate HPV-specific T-cell responses may allow persistent HPV infection, which can lead to the development of high-grade CIN lesions. However, evidence for natural T-cell responses to HPV has been based on ex-vivo assays assessing cell-mediated responses to E6 and E7 proteins and E2. Many of these studies show that infection with oncogenic HPV types can skew the host T-cell response to favour HPV survival in the epithelium of the cervix (*76,77,103,104,105*). It is important to note that, in these studies, immune cells have been taken from different patients who may have multiple confounding factors, and that it is difficult to obtain reproducibility with ex vivo assays. However, added biologic plausibility is found in patients with T-cell deficiencies, such as HIV-infected individuals, and transplant recipients who have been observed to have more frequent and severe HPV-induced diseases, and which are more recalcitrant to surgical or ablative treatments (*48,106,107,108*). Patients with antibody immunodeficiencies, however, are no more susceptible to reinfection with HPV than antibody immunocompetent women, which suggests that while antibody may be sufficient for protection, it is not effective once disease is present (*69*).

3. Prophylactic HPV vaccines

3.1 Available prophylactic HPV vaccines and mechanism of action

There are two vaccines currently licensed that are sold internationally. Both are prepared from purified L1 protein, the major capsid protein, that self-assembles to form type-specific HPV virus-like particles (VLPs). These VLPs closely resemble the outer surface of HPV virions. VLPs contain no viral DNA and are therefore non-infectious. The vaccines are designed for prophylactic (preventative) use and have not been found to effectively clear existing HPV infections or treat HPV-related diseases (*109,110*).

The quadrivalent vaccine was first licensed in the United States in 2006. The L1 proteins for each type are expressed via a recombinant *Saccharomyces pombe* (type of yeast) vector. Each 0.5 ml dose contains 20 µg of HPV-6 L1 protein, 40 µg of HPV-11 L1 protein, 40 µg of HPV-16 L1 protein and 20 µg of HPV-18 L1 protein adsorbed onto 225 µg of the adjuvant, amorphous aluminium hydroxyphosphate sulfate (AAHS). This vaccine has been licensed for use in girls as young as age nine, to prevent cervical precancers, cervical cancers, vulvar precancers and vaginal precancers, as well as anogenital warts. In some countries, the vaccine is also licensed for the prevention of anogenital warts in males.

The bivalent vaccine was first licensed in 2007. The L1 proteins for each type are expressed via a recombinant baculovirus (type of insect cell) vector. Each 0.5 ml dose contains 20 µg of HPV-16 L1 protein and 20 µg of HPV-18 L1 protein adsorbed onto a proprietary AS04 adjuvant system containing 500 µg of aluminium hydroxide and 50 µg of 3-O-desacyl-4'-monophosphoryl lipid A, a novel adjuvant. This vaccine has been licensed for use in girls as young as age 10 to prevent cervical precancers and cervical cancers. Registration for indications in males has not been sought.

The mechanisms of action of the HPV L1 vaccines are not known. Current hypotheses are based on data from experiments in rabbits (*111*) and dogs (*112*) demonstrating that naive animals passively immunized with purified serum IgG from either VLP immunized or naturally-infected animals were completely protected against high dose of viral challenge. Briefly, it is thought that VLPs are rapidly bound by myeloid DCs and B lymphocytes and signal via TLR-dependant pathways essential for B-cell activation and antibody generation (*65,113,114*). The protection is thought to be due to direct action of serum antibodies transudating and exudating to the site of infection at the cervix. However, the level of antibody required for protection is not known.

3.2 Adjuvants in HPV vaccines and clinical relevance

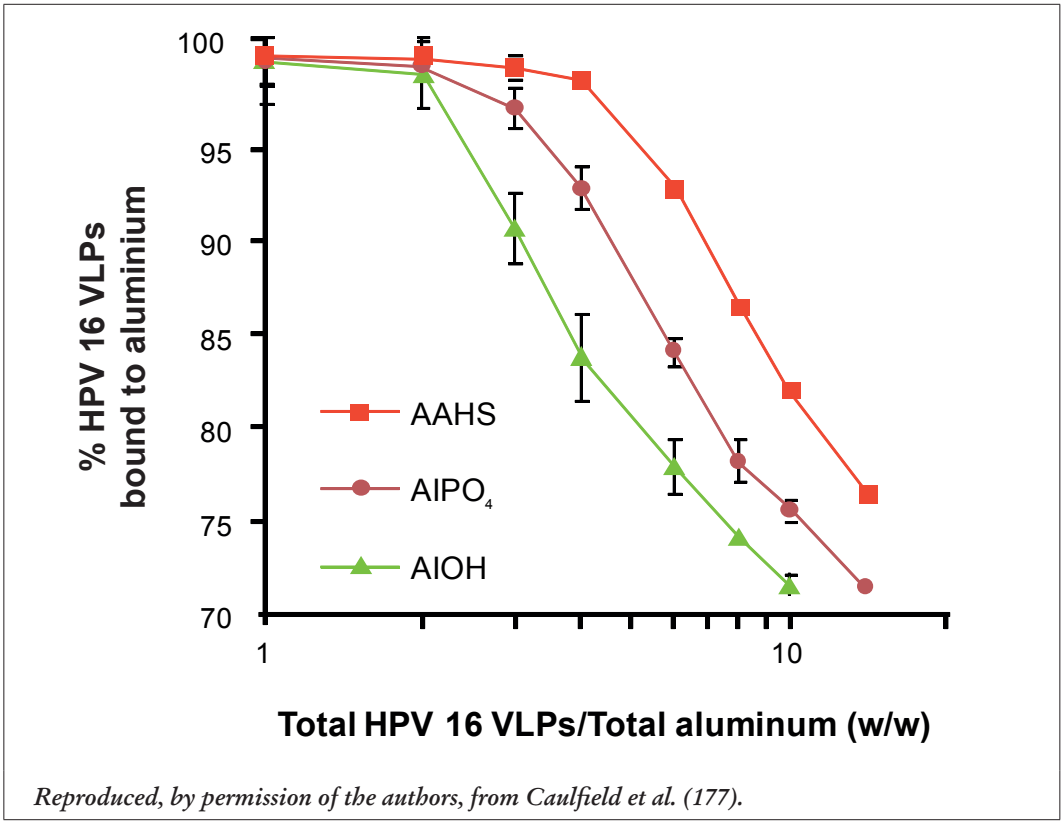
HPV L1 VLPs are themselves highly immunogenic. However, adjuvants in HPV L1 vaccines increase VLP immunogenicity by inducing cytokines or chemokines, which then act directly or indirectly on helper lymphocytes to modulate immune responses. Adjuvants also increase both antigen uptake by, and maturation of, antigen-presenting cells (*115*). It is believed that the adjuvants in HPV L1 vaccines: 1) accelerate the generation of robust immune responses; 2) induce local mucosal immune responses; 3) generate antibodies with enhanced avidity and neutralizing capacity; 4) elicit the response of cytotoxic T cells; 5) increase the response rate in low-responder individuals; 6) reduce the required amount of antigen necessary to generate the desired immune response (*116*).

The quadrivalent and bivalent vaccines use different alum-containing adjuvants. The quadrivalent vaccine contains AAHS as the adjuvant, while the bivalent vaccine uses a purified lipopolysaccharide modified endotoxin from the wall of bacteria (*Salmonella minnesota*), monophosphoryl lipid A, which is adsorbed on AlOH forming a novel adjuvant known as AS04.

3.2.1 Quadrivalent vaccine adjuvant

AAHS was first licensed for use in the late 1980s with the approval of PedvaxHIB® [Haemophilus b conjugate vaccine (meningococcal protein conjugate)]. Since that time AAHS has been used in millions of children and adults, and has an extensive, proven and predictable safety profile. The AAHS dose in the quadrivalent vaccine is approximately 225µg. AAHS has a higher binding capacity and induces a higher antibody response than the other two aluminium salts used in vaccines, AlOH and $AlPO_4$ (Figure 3). Compared to VLPs alone, AAHS improves neutralizing antibody titres in a monkey model (*117*). With this adjuvant, the dose of 20/40/40/20 µg of HPV 6, 11, 16 and 18 L1 VLPs was chosen, since it was the lowest dose formulation which elicited similar immune responses to the higher dose formulations in prospective clinical trials (*118*). At these doses, no evidence of interference was observed among the multiple types contained within the quadrivalent vaccine, compared to the monovalent HPV16 vaccine (*119*).

Figure CC: AAHS binding capacity to HPV 16 VLPs, compared to $AlPO_4$ and AlOH in mice



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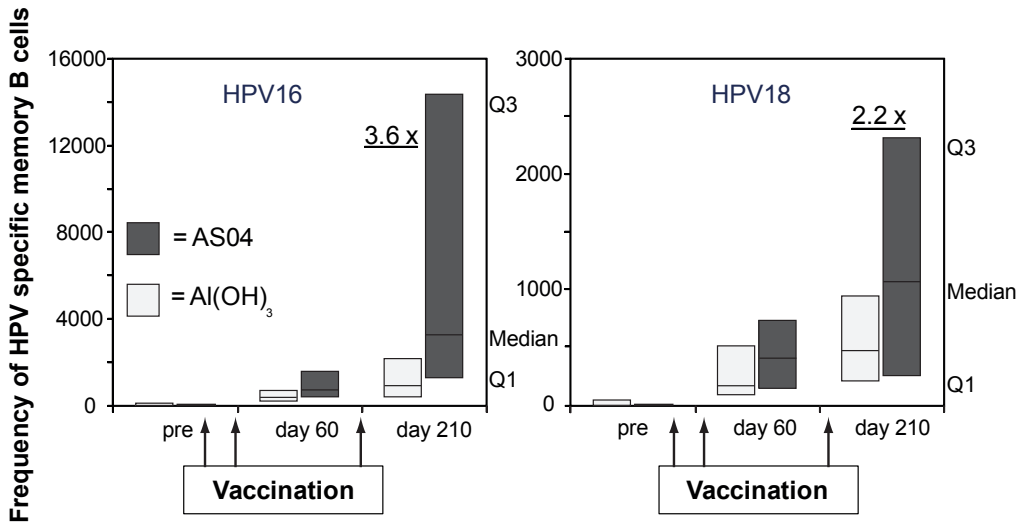
3.2.2 Bivalent vaccine adjuvant

AS04 is an agonist of TLR4 (*120*). AS04 has been used in other vaccines manufactured by GlaxoSmithKline (GSK) including HBV (Fendrix™) and a herpes simplex virus candidate vaccine. Clinical trial experience with AS04 has shown an excellent safety profile in over 68 000 administered doses (*121*). The AlOH dose in the bivalent vaccine is 500 µg with 50 µg of monophosphoryl lipid A. Compared to HPV 16 and 18 VLPs adjuvanted with aluminium salts alone, the addition of AS04 induces a higher antibody response at multiple dose timepoints (*122*). Compared with a candidate vaccine comprised of the aluminium salt component of AS04 alone (AlOH), AS04 also induces a higher frequency of memory B cells (*122*) (Figure 4).

Figure 4: Bivalent vaccine induces higher frequency of memory B cells as compared to the same vaccine formulated with AlOH ($p<0.05$ using Wilcoxon's test)



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4. Immunologic response to prophylactic HPV vaccines

4.1 Neutralizing antibody response assays and measurements in prophylactic HPV vaccine trials

Currently, there is no 'standard' assay format, or standard commercial reagents that are available for the assessment of antibody responses to HPV. Currently-available assay methods are briefly described in Table 1. The direct VLP-based enzyme-linked immunosorbent assay (ELISA) was the principal assay used in measuring the immunogenicity in the bivalent vaccine clinical trials (*123,124*). This measures total antibodies of IgG class which may include neutralizing as well as non-neutralizing antibodies. The assay used in the quadrivalent vaccine studies is the competitive Luminex immunoassay (cLIA) (*125*), which utilizes monoclonal antibodies to one epitope and measures only a subset of neutralizing antibodies, although of all immunoglobulin classes. The pseudovirion-based neutralization assay (PBNA) or secreted alkaline phosphatase neutralization assay (SEAP-NA) is a reproducible quantitative neutralization assay that presumably measures all neutralizing antibodies of all immunoglobulin subclasses (*126*). Consequently, it must be appreciated that it is not possible to compare titres from one assay output to another, let alone from one vaccine to another given that different serological assays with controls from different populations have been used in clinical trials. However, application of international standards will improve the comparability of data generated from different assay formats and allow antibody measurement traceable to an International Unit (IU).

Table 1: Summary of seroassays used to detect antibody responses to HPV infection on HPV L1 vaccine clinical trials

Assay	Methodology	Antibodies Detected
Pseudovirion-Based Neutralization Assay (PBNA)	A specific and sensitive assay that is based upon the binding of neutralizing antibodies to pseudovirions, which become neutralized from infecting a reporter cell line.	Measure most neutralizing antibodies and is based upon predefined epitopes considered to be important for neutralizing HPV.
Competitive Luminex Immunoassay (cLIA)	This technique relies on the binding of the neutralizing antibody in place of a specific fluourescent antibody.	Measures a limited number of neutralizing antibodies and is based upon predefined epitopes considered to be important for neutralizing HPV.
Enzyme Linked Immunosorbent Assay (ELISA)	A sensitive technique that detects antibodies bound to VLPs coated on microwell plates. These antibodies are quantitated by optical density.	Measures total antibody levels and does not distinguish between neutralizing or non-neutralizing types.

Reproduced, by permission of the authors, from Einstein et al. (15).

Based on their respective assays (to be described in the next section), both the quadrivalent and bivalent vaccines were shown to generate high levels of antibodies one month following the recommended three- dose vaccination course in nearly all female vaccinees who were initially naive to vaccine-related HPV types (*127–131*). Prospectively-followed studied cohorts 5–8.5 years after vaccination have shown that antibody titres peak after the third dose, decline gradually, then level off by 24 months after the first dose. In general, geometric mean titres (GMTs) have been found to be higher in 10–15 year olds than in females 16–23 for the quadrivalent vaccine, and 15–25 for the bivalent vaccine.

4.2 Neutralizing antibody responses in prophylactic HPV vaccine clinical trials

Serum levels of type-specific anti-L1 antibodies have been used as the primary immune endpoint in all HPV vaccine trials. However, since the methodologies used for the quadrivalent and bivalent vaccine registration trials are different, the immune response data cannot be directly compared. Additionally, immune response data published from the vaccine efficacy trials is generally restricted to the subset of enrolled subjects who were HPV naive, DNA negative, and HPV seronegative prior to vaccination e.g. per protocol for efficacy population (PPE), or total vaccinated cohort for efficacy population (TVC-E). This is because HPV naive women are the target population for prophylactic HPV vaccination. The immune response data from those who previously have been HPV-exposed or have a detectable HPV DNA infection (or both) are limited in the published data. While it is recognized that vaccine efficacy is less in those with prior or current HPV exposure, it should also be noted that the correlative immune data generated from mostly HPV naive individuals, is not necessarily generalizable to HPV-exposed individuals (*132*).

4.2.1 Competitive Luminex immunoassay

Serum antibodies for the quadrivalent vaccine have primarily been tested during clinical trials using a competitive Luminex immunoassay (cLIA) or a competitive radioimmunoassay (See Table 1 for a brief explanation of cLIA). The assay uses Luminex microspheres (Invitrogen) coated with the VLP types. The serum samples from study subjects are evaluated on their ability to prevent VLP binding by a type-specific neutralizing monoclonal antibody that has a fluorescent tag. The strength of the antibody response is inversely proportional to the detection of the monoclonal antibody signal. The major advantages of this assay are that it is highly type-specific, provides quantitative levels of antibodies to all four vaccine types in a single sample, and is automated. Its disadvantage is that it only measures serum antibodies that bind, or compete with, the single neutralizing epitope chosen for the assay. They are not a measurement of total serum anti-L1 VLP antibodies (*69*). Since there are vaccine responses that may occur as a result of antibodies that do not compete with the monoclonal antibody, this assay may under-measure the potentially protective antibody response induced by the quadrivalent vaccine (*126*).

Standard curves were generated for each individual HPV type (*125*). Since the binding affinities are different for each type, direct comparisons of antibody titres generated by any of the VLP types tested cannot be performed. Also, direct comparisons of the relative immunogenicities for the VLP components cannot be made from the absolute titres since the titres for the reference sera and the cLIA-recognized epitopes for the individual HPV types are not identical (*118*).

4.2.2 ELISA

Serum antibodies for the bivalent vaccine have been tested using an ELISA (see Table 1 for a brief explanation of ELISA). The ELISA measures antibodies that bind to a VLP antigen on a solid wall surface. Bound antibodies are then detected by a secondary antibody that recognizes the constant region of the specific class of human antibody, which in the case of VLP-induced antibodies are immunoglobulin (Ig) G. These assays measure the total serum anti-VLP IgG to tested HPV types, and direct comparisons can be made between responses to individual VLPs. The measured responses are non-specific, often representing a cross-neutralizing epitope response in addition to the response generated by functional epitopes. The major advantages of the ELISA are that it is sensitive, rapid, reproducible, and can be automated (*126*). However, the reproducibility is dependent on VLP quality and can be difficult for laboratories to prepare, which can in turn affect quality control. The disadvantages of the ELISA are: 1) the fraction of neutralizing IgG antibodies cannot be quantified; 2) it requires titration to determine titre, so that even antibody levels, let alone neutralizing antibody levels, may be difficult to determine. Also, IgA is not detectable with the ELISA assays used in the vaccine clinical trials. However, it has been shown that neutralizing antibodies and ELISA titres are directly correlative, with the ELISA over-measuring neutralizing antibody titres (*69,133,134*).

4.2.3 Pseudovirion-based or secreted alkaline phosphatase neutralization assays (PBNA or SEAP-NA)

The pseudovirion-based neutralization assay (PBNA) or secreted alkaline phosphatase neutralization assay (SEAP-NA) is an in vitro neutralization assay not limited by the availability of infectious capsids. It involves the cell-culture production of high-titre infectious L1/L2 pseudovirions that have encapsulated a gene whose activity can be easily measured as a marker for infection (*126,135*). Specifically, pseudovirions are produced by co-transfecting human embryonic kidney cells expressing SV40 T antigen (293TT) with plasmids coding for prototype HPV 16 or 18 L1 and L2 genes (*135*). The pseudovirus carries the reporter gene for SEAP, which enables the infection to be quantified by measuring the ability of culture supernatants to cleave a colorigenic substrate (*126*). The PBNA detects functional neutralizing antibodies regardless of their source; i.e. those generated from natural infection as well as from either the quadrivalent or bivalent vaccines. The results of this assay correlate with protection, because it presumably measures all neutralizing antibodies regardless of immunoglobin class. This assay is more type-specific than standard ELISA, but may be less type-specific than cLIA (*136*). Cross-reactivity detected in the neutralization assay appears to correlate with biologically significant cross-protective responses (*126*). Disadvantages of neutralization assays are that they are labour-intensive and titres measured in the neutralization assays cannot be directly compared.

4.2.4 Cervical vaginal secretion (CVS)

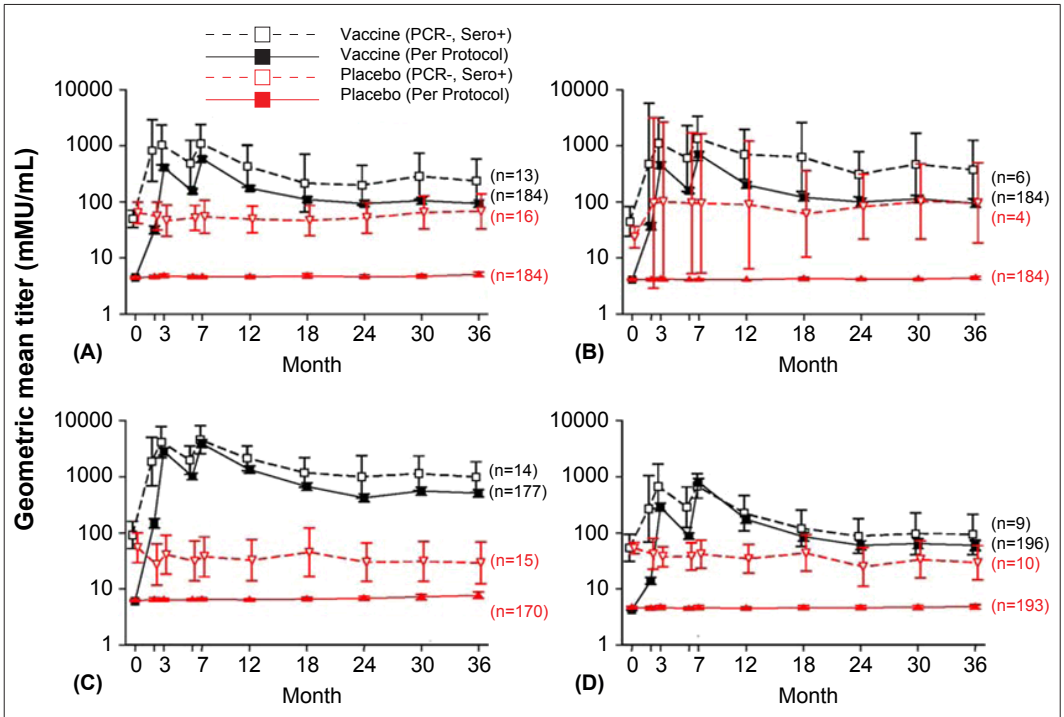
Studies have shown correlation between serum antibody levels and neutralizing antibody levels in CVS. This is one of the primary reasons why it is believed that prophylactic HPV vaccines give protection as a result of transudation of neutralizing antibodies from the serum. Moreover, exudation at sites of trauma allows for neutralizing antibodies to clear virus before it binds to basement membrane and infects keratinocytes.

(For detailed information regarding immune responses in vaccine trials, see section C.1 in *http://www.who.int/biologicals/publications/trs/areas/vaccines/human_papillomavirus/HPVg%20Final%20BS%202050%20.pdf*).

4.2.5 Quadrivalent vaccine neutralizing antibody responses

In a study of 1106 young women who were randomized to receive one of three formulations of the quadrivalent vaccine (differing in VLP dose) or placebo, women were followed prospectively over a three-year period to assess immunogenicity using a cLIA assay for HPV 6, 11, 16, and 18. All subjects who were initially HPV DNA and HPV seronegative at baseline, seroconverted for all four HPV types in the quadrivalent vaccine. Baseline vaccine-type HPV naive subjects who received the formulation found in the current quadrivalent vaccine, mounted robust immune responses that, as expected, waned over time (Figure 5a). A revaccination study was performed with the quadrivalent vaccine in which a subset of vaccinated women were given a challenge dose five years after enrolment. This vaccine boost elicited a rapid rise in antibody titre consistent with an anamnestic response, suggestive of immune memory (Figure 5b) (128). This rechallenge response is encouraging because it mirrors what happens in animal models where, despite low levels of circulating antibody, there is long-lasting protection (137).

Figure 5a: Anti-HPV cLIA GMTs (mMU/mL with 95% confidence intervals) for quadrivalent vaccine and placebo recipients (pooled placebo arms)

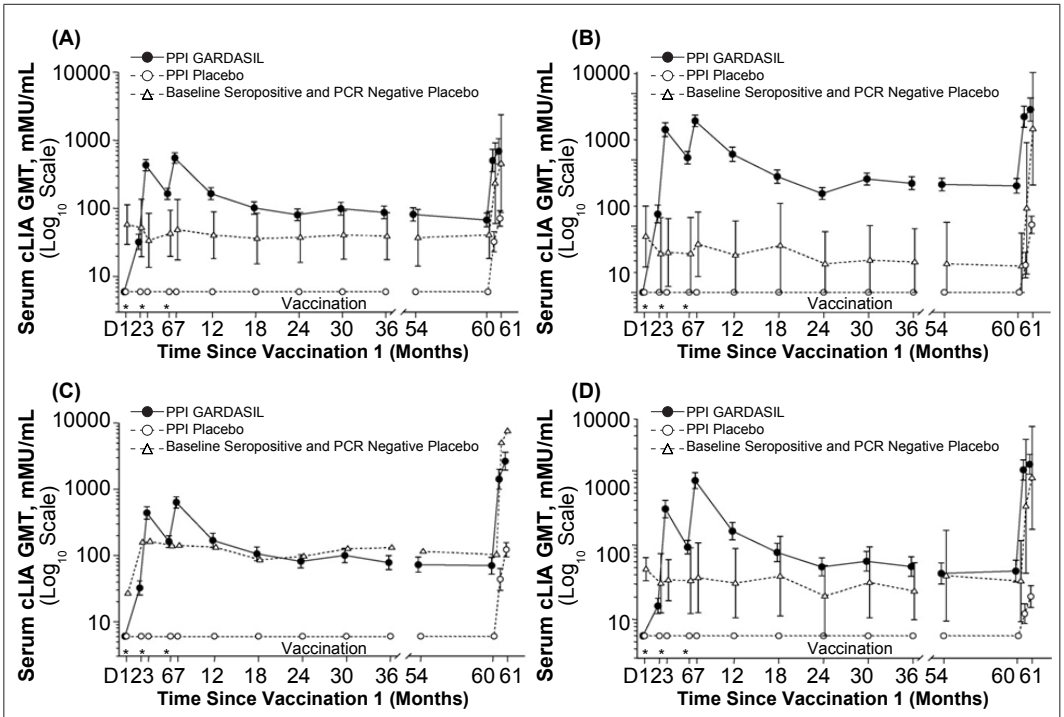


Longitudinal plots are shown by HPV type for the PPI population and for subjects who were baseline seropositive and PCR negative: (A) HPV 6; (B) HPV 11; (C) HPV 16; and (D) HPV 18. Because the titres of the reference sera are not identical, one cannot draw conclusions from the absolute titres with regard to the relative immunogenicity of the four VLP components in the vaccine.

n = number of subjects contributing to the month 36 timepoint.

Reproduced, by permission of the authors, from Villa et al. (118).

Figure 5b: Anti-HPV responses following a three-dose regimen of quadrivalent HPV vaccine or placebo



Data are shown for subjects in the extension per-protocol immunogenicity population who received quadrivalent HPV vaccine in the original study, subjects in the extension per-protocol immunogenicity population who received placebo in the original study, and subjects who were seropositive and PCR negative to vaccine HPV types who received placebo in the original study. A fourth booster dose was administered at month 60.

Anti-HPV 6, 11, 16 and 18 cLIA results are in panels (A–D), respectively.

Error bars represent 95% confidence intervals.

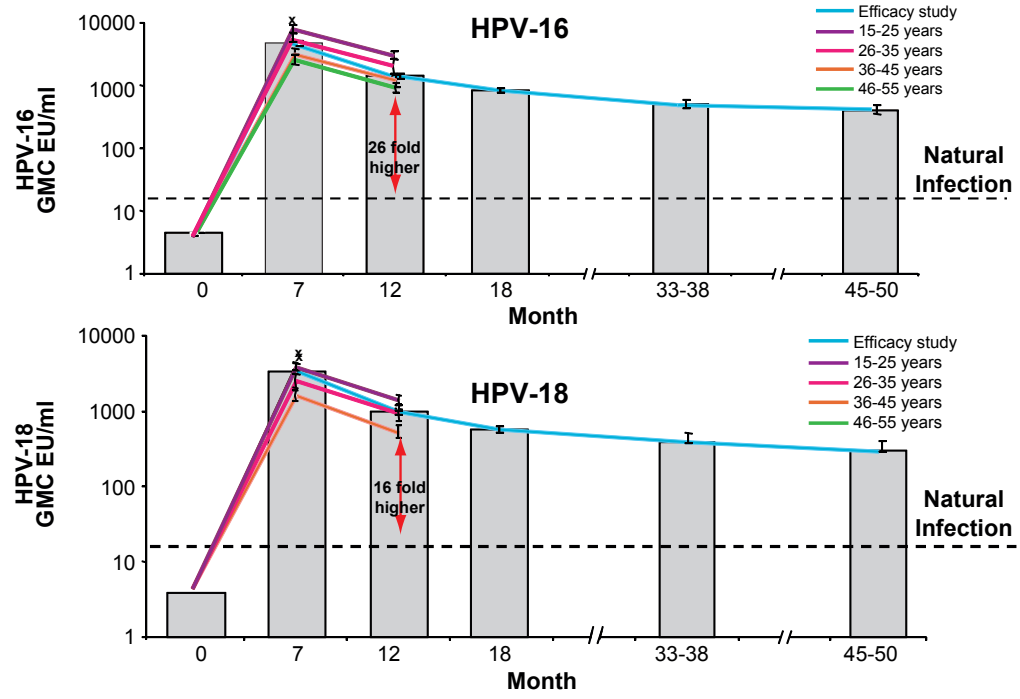
Reproduced, by permission of the authors, from Olsson et al. (128).

The quadrivalent vaccine has been shown to develop higher immune responses as detected by cLIA in 10–15 year old girls and boys when compared to 16–23 year old women. In a study of 506 girls and 510 boys, >99% of the subjects seroconverted. Additionally, the titres were noninferior and 1.7–2.7 times higher when compared to 16–23 year old subjects using cLIA assays for measurement (*138*).

4.2.6 Bivalent vaccine neutralizing antibody responses

In a follow-up study of a subset of 393 vaccinated women and 383 who received placebo, all of whom were in the according-to-protocol analysis (i.e. initially HPV DNA negative and HPV seronegative using an ELISA at baseline to the respective vaccine-related HPV DNA), 98% of the subjects seroconverted for HPV 16 and HPV 18. Baseline vaccine-type HPV naive subjects who received the formulation found in the current bivalent vaccine, mounted robust immune responses that, as expected, plateau after the initial peak and fall (Figure 6). It should be noted that typical natural titres were determined by use of blood samples obtained from women in a preceding epidemiology study who were found to be seropositive for HPV 16 and HPV 18 (*124*). A revaccination challenge study has not, to date, been performed with the bivalent vaccine.

Figure 6: Anti-HPV ELISA GMTs (Eu/ml with 95% confidence intervals) for bivalent vaccine



Dashed line represents estimated titre induced from natural infection alone.
Different lines represent different age cohorts.

Reproduced, by permission of the authors, from Harper et al. (123).

The bivalent vaccine has been shown to develop higher immune responses using an ELISA assay in 10–14 year old girls when compared to 15–25 year old women. In a study of 158 girls, 100% of the subjects seroconverted. Also, the titres were noninferior and approximately twice as high as the 15–25 year old subjects using ELISA for measurement (*131*). This factor of achieving a better response in younger individuals is shown for both vaccines, and is a basic principle of vaccinology.

CVS collection and extraction has been performed for the bivalent vaccine according to previously described methods (*139*). Total IgG and specific HPV-16 and 18 IgG were measured in both serum and CVS by ELISA. To control for the fluctuation in IgG levels during the menstrual cycle, serum and CVS anti-HPV-16 and anti-HPV-18 IgG were normalized against the total serum and CVS IgG levels. A high correlation between antibody levels between serum and CVS was noted for all age ranges tested, including 15–25 and 25–55 year olds (*140*).

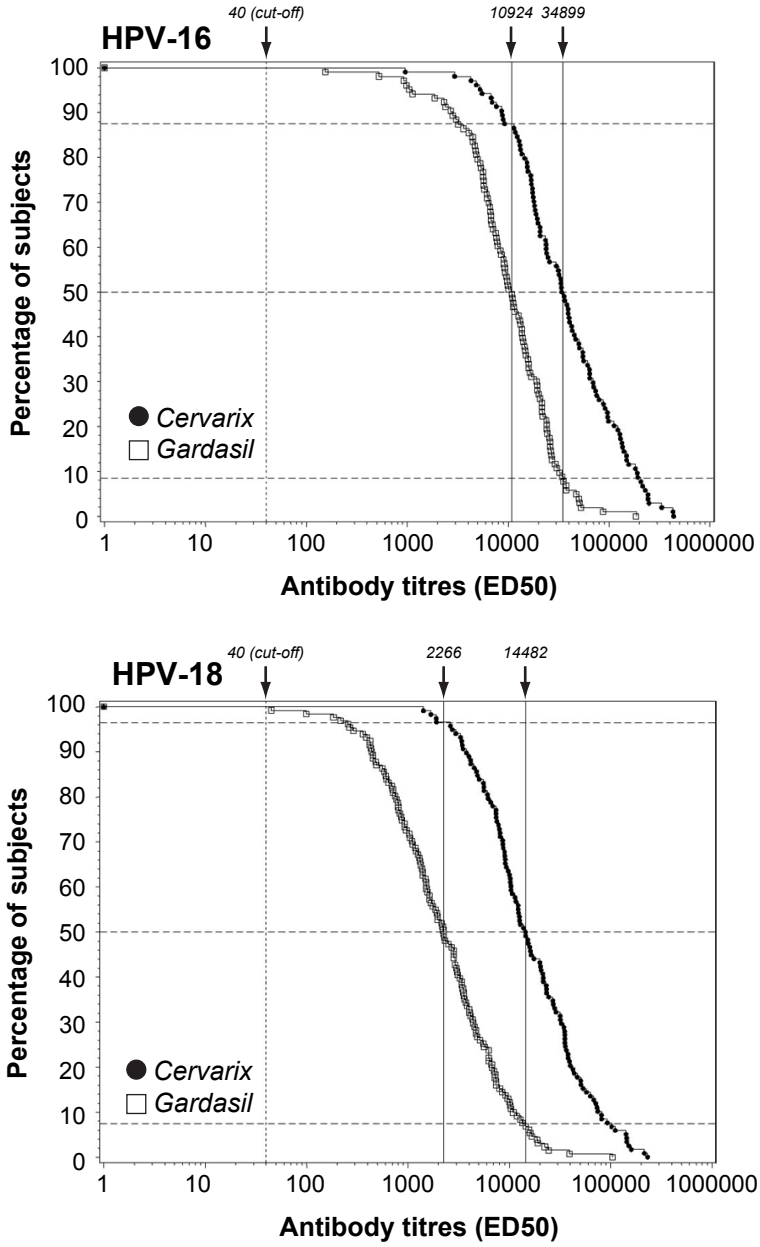
4.2.7 Head-to-head immunogenicity trial

Due to numerous differences between the efficacy trials for the quadrivalent and bivalent vaccines, direct comparison of immune responses observed between the two vaccines cannot be inferred. These differences included: choice of placebo; accrual regions; end-point measures; inclusion criteria; exclusion criteria and, as previously mentioned, differences in the assays used to measure vaccine-induced antibody production, as well as HPV DNA measures. The manufacturer of the bivalent vaccine sponsored a Phase IIIb, observer-blind, randomized, multicentre study to compare the immunogenicity of the bivalent vaccine to the quadrivalent vaccine in females 18–45 years old (*141,142*). Powering a study to compare efficacy would be impossible given the high efficacy of both vaccines. The PBNA assay (see description of PBNA earlier in this module) was adopted for use in this head-to-head trial as the "neutral' assay since, as previously mentioned, it measures functional antibodies regardless of immunoglobulin class.

In the head-to-head immunogenicity trial, the quadrivalent and bivalent vaccines were administered as per their regular schedule. In order to maintain blinding, a dose of AlOH was used at month one or month two depending on which vaccine the subject received. Blood was collected at months 0, 6, and 7 for immunologic testing. The primary end-point was measurement of both neutralizing and functional antibodies. Secondary end-points included total IgG and L1 VLP measurement. A subset of the subjects had immune response measurements for T cells, memory B cells, and immune responses in CVS. There were no measurements for disease outcomes.

At month seven after first vaccination, all women in the according-to-protocol cohort who were seronegative/DNA negative before vaccination for the HPV type analysed, had seroconverted for HPV-16 and HPV-18 serum neutralizing antibodies, as measured by PBNA, except for two women aged 27–35 years in the *Gardasil®* group who did not seroconvert for HPV-18 (98%). Geometric mean titres of serum neutralizing antibodies ranged from 2.3–4.8-fold higher for HPV-16 and 6.8–9.1-fold higher for HPV-18 after vaccination with *Cervarix™* compared with *Gardasil®* across all age strata. In the total vaccinated cohort (all women who received at least one vaccine dose, regardless of their serological and DNA status prior to vaccination), *Cervarix™* induced significantly higher serum neutralizing antibody titres in all age strata ($p<0.0001$) (see Figure 7). Positivity rates for anti-HPV-16 and -18 neutralizing antibodies in cervicovaginal secretions and circulating HPV-16 and -18 specific memory B-cell frequencies were also higher after vaccination with *Cervarix™* compared with *Gardasil®* (*141,142*).

Figure 7: Reverse cumulative distribution curves of HPV-16 and HPV-18 serum neutralizing antibodies measured by pseudovirion-based neutralization assay at month 7 (ATP cohort for immunogenicity, seronegative and DNA negative prior to vaccination) 18-26 years



Solid vertical lines represent median values. For example, for women aged 18–26 years (a), the median titre of serum anti-HPV-16 neutralizing antibodies was 34899 ED50 in the Cervarix™ group versus 10924 ED50 in the Gardasil® group. More than 85% of women (see upper horizontal dashed line) aged 18–26 years who received Cervarix™ had anti-HPV-16 antibody titres above the median titre for Gardasil®. Less than 10% of women (see lower horizontal dashed line) aged 18–26 years vaccinated with Gardasil® had anti-HPV-16 antibody titres above the median titre for Cervarix™. For HPV-18, the median antibody titre was 14482 ED50 in the Cervarix™ group versus 2266 ED50 in the Gardasil® group. More than 95% of women who received Cervarix™ had HPV-18 antibody titres above the median titre for Gardasil®. Less than 10% of women vaccinated with Gardasil® had HPV-18 antibody titres above the median titre for Cervarix™. Similar trends were observed in women aged 27–35 years and 36–45 years (data not shown).

Reproduced, by permission of the authors, from Einstein et al. (141).

What this means clinically is yet to be determined. Currently there is no immune correlate of protection since in the efficacy trials there were no breakthrough cases. Moreover, it has been shown that, for the quadrivalent vaccine, despite a lack of seropositivity for HPV 18 at five years follow-up, there was no disease breakthrough (*143*). It may be that it is more important to have immune memory, as has been shown for hepatitis B vaccination. Hence, once an immune response was achieved, in most cases that then became seronegative, on challenge with a newly-incident HPV infection, there was an anamnestic response. This has been shown in an experimental situation for the quadrivalent vaccine, whereby women given a fourth dose of vaccine showed a rapid antibody response that was higher than originally achieved after a third dose consistent with evidence of an anamnestic response and immune memory (Figure 5b) (*128*). Whether this difference in anti-VLP titres may be related to the duration of protection against HPV-16/18, and may reflect different requirements for a booster, if a booster is needed at all, will need to be ascertained by following populations of vaccinated cohorts using standardized assays for immunogenicity and correlation with HPV and cervical disease status.

4.3 Immunogenicity of HPV vaccines across different covariates and in different regions/countries

The large registration trials for both the quadrivalent and bivalent vaccines were performed in multiple, mostly developed regions of the world. Immunogenicity was found to be high across all groups, with little difference in immunogenicity for any one group. However, trials for both vaccines were limited in non-Caucasian populations. For the quadrivalent vaccine, immunogenicity was comparable among subjects with differing baseline characteristics. Age at vaccination initiation was inversely proportional to the vaccine-induced anti-HPV response (*144*). A higher immune response in younger subjects has been a consistent finding in all immune-bridging studies (see Table 2). For the bivalent vaccine, separate trials in India (*145*) and China, Hong Kong Special Administrative Region (*146*) revealed comparable high levels of immunogenicity to the Phase III registration trials. There is limited published data on the efficacy of HPV vaccines in other high-prevalence cervical cancer regions, such as sub-Saharan Africa and many areas in south-east Asia, as well as aboriginal and rural populations outside urban areas in higher income countries.

Table 2: Immunogenicity bridging studies for quadrivalent and bivalent vaccines

Study	Vac.	Groups/Age (N)	Sero-assay	% Sero-conversion*	Conclusions
Reisinger (181)	6/11/ 16/18	Boys 9–16 (567) Girls 9–15 (617)	cLIA	≥99.5 ≥99.6	Boy's GMT noninferior to girl's (1.1–1.5X)
Block (138)	6/11/ 16/18	Boys 10–15 (510) Girls 10–15 (506) Women 16–23 (513)	cLIA	≥99.7 100 ≥99.1	Boy's & girl's GMTs noninferior to women's (1.8–2.7X; 1.7–2.0X)
Pedersen (131)	16/18	Girls 10–14 (158) Women 15–25 (458)	ELISA	100 100	Girl's GMTs noninferior to women's (2.1–2.4X)
Petaja (176)	16/18	Boys (10–18)	ELISA	100	Noninferior to 15–26 yr women (2.1–3.1X)
Schwarz (174)	16/18	Women 15–25 (224) Women 26–35 (226) Women 46–55 (211)	ELISA	100 100 100	GMTs in older women lower, but higher than natural infection GMTs

Titres measured after third dose in seronegative, HPV DNA negative (ATP) cohorts.

Adapted, by permission of the authors, from Schiller et al. (178), Schiller (179) and Reisinger et al. (180).

4.4 Use of seroassays for vaccine programmes

While the assays used for the quadrivalent and bivalent vaccine trials are appropriate for high-throughput testing, serology is not recommended routinely after vaccination, as seroconversion is the rule and there is no immune correlate of protection. The high efficacy of both vaccines has resulted in very few breakthrough type-specific cases in HPV-naive subjects, making determinants of protection impossible. Hence, given the extremely low prevalence of breakthrough cases, determining serologic correlates of protection will only be possible in well-followed vaccinated populations. Prospective tracking of fully vaccinated individuals for breakthrough type-specific disease, with correlative serologic data, will be crucial to guide future recommendations regarding need for a booster (*133*).

4.5 Immune response in HIV-infected individuals

There is limited information on the immune response after HPV vaccination in HIV-infected individuals or those immunocompromised due to medications that induce immunosuppression. Although the immunogenicity and efficacy of HPV vaccines may be reduced in HIV-infected females, the potential benefit of vaccination in this group is high due to their increased risk of HPV-associated diseases, including cervical cancer. Safety and efficacy trials in HIV-infected adults have only recently begun. There are separate ongoing safety and tolerability trials for the quadrivalent vaccine for both males and females in the USA, as well as an efficacy trial in South African HIV-infected individuals. In a study of 126 HIV-infected children in the USA aged 7–12 years, with some on highly active antiretroviral therapy (HAART), >99.5% developed antibodies against HPV 6, 11, 16 and 18 after being vaccinated with the quadrivalent vaccine (*36,147*). Compared with age-matched historical controls, GMTs for all four HPV types were lower in HIV-infected children, with differences only being statistically significant for HPV 6 and HPV 18. Adult safety and efficacy trials in HIV-infected adult men and women are ongoing. There is no data available in HIV-infected individuals with the bivalent vaccine. Many policy-making organizations in countries who have licensed HPV vaccines, based on expert opinion, have not restricted HPV vaccination in HIV-infected individuals (*148*); however, continued study of HPV vaccination in HIV is clearly needed. Unfortunately, vaccination of adults may be compromised by the fact that many will have already been infected with HPV, this being exceedingly common in this group.

4.6 Reactogenicity and safety of prophylactic HPV vaccines

Local reactions at the injection site (erythema, pain, swelling) that were mild and transient were 10%–20% more frequent among those who received an HPV vaccine than in their respective controls. In the head-to-head trial sponsored by the bivalent vaccine company, both vaccines were generally well tolerated (*141,142*). The incidence of unsolicited adverse events was also comparable between vaccinated groups. The incidence of solicited symptoms was generally higher after *Cervarix*™, injection site reactions being the most common. However, compliance rates with the three-dose schedules were similarly high (≥84%) for both vaccines (*141,142*). No systemic adverse reactions thought to be causally related to HPV immunization have been reported. While the data are limited, no serious adverse outcomes with the quadrivalent vaccine (*147*) have been reported in HIV-infected children, or when either HPV vaccine was inadvertently administered to pregnant women (*36*).

Close post-marketing surveillance of safety, including autoimmune diseases, have been ongoing in countries where HPV vaccines are approved. Common acute and self-limiting side-effects in passive reporting predominantly include local reactogenicity, headache and pyrexia (*149*). Close tracking of serious autoimmune-related events and diseases such as anaphylaxis, Guillain Barré Syndrome (GBS), or transverse myelitis (TM) show no causal relationship with HPV vaccination. There is only temporal association of rare case events of GBS and TM in the immediate post-vaccine window (*150*). No confirmed reports of anaphylaxis have occurred from vaccine safety data (*150*). Numerous regulatory bodies are continuing to track this data.

In June 2007, WHO's Global Advisory Committee on Vaccine Safety (GACVS) concluded that both vaccines had good safety profiles. In December 2008 and June 2009, GACVS reviewed data on early post-marketing surveillance. No reports raised concern sufficient to change previous advice given by GACVS (*151*).

4.7 Prophylactic HPV vaccine immune response with co-administration of other vaccines

Studies of co-administration of both HPV vaccines with other currently used vaccines are ongoing. Co-administration of the quadrivalent vaccine with a recombinant hepatitis B vaccine in females aged 16–23 was well-tolerated and did not interfere with the immune response generated by the HPV vaccine alone. High month seven anti-HBs GMTs were also observed following concomitant vaccination. These GMTs were lower, but non-inferior, when compared to those induced by the HBV vaccine alone (*152*). Co-administration of the quadrivalent vaccine with a combined diphtheria–tetanus–pertussis–inactivated poliomyelitis vaccine in females and males aged 11–17 years resulted in immune responses non-inferior to non-concomitant administration (*153*). In a separate study, co-administration of the quadrivalent vaccine with both a meningococcal vaccine and a tetanus-diphtheria-pertussis vaccine was well-tolerated and did not interfere with the immune response of the respective vaccines (*154*). Co-administration of the bivalent vaccine with a combined diphtheria–tetanus–pertussis–inactivated poliomyelitis vaccine (in females aged 10–18 years) also did not significantly impair the immune response to any of the involved antigens (*154,155,156*).

5. Future prospects

5.1 Questions regarding natural HPV immune response evaluation

The natural history of HPV is a continuum of exposures to infections, clearance of virus, or potential latency. Reinfection and/or re-emergence are possible in women of all ages, but may vary with age or other cofactors. Difficulties remain in assessing the complexities of the antibody response to incident infection or reactivation from latency. As a result, it is unclear how well natural immune responses have induced true "clearance" of genital oncogenic HPV infection and protection from subsequent reinfection, re-expression of oncogenic HPV, or neoplasia (*15*).

It is also uncertain whether the immune effector mechanism(s) that induce HPV clearance and prevent reinfection after natural infection are the same as those that prevent HPV infection after VLP vaccination. Because the increased risk of HPV-induced neoplasia seen in HIV-infected individuals is not seen in those with B-cell deficiencies, a dominant role of T cell-mediated mechanisms in controlling HPV-induced neoplasia is suspected. The mechanism of HPV L1 vaccine-induced protection appears to be higher than that from natural infection. However, the serum levels of antibodies needed to confer protection from infection are unknown, so the duration of protection afforded by the vaccines remains to be determined. Additionally, there are ongoing cohorts and analyses of non-inferiority of immune responses with alternative vaccine-dosing schedules. All of these questions will probably not be effectively answered by extended cohorts in randomized trials, due to a lack of sufficient power, but will need to be determined prospectively in population-based registries and close study by regulatory and policy-making bodies. While HPV serology has been helpful in clinical-trial analysis, it is not clear if it is the most appropriate immune correlate of protection or which immunoassay most correlates with clinical protection.

5.2 Future vaccines from an immunologic perspective

While current HPV L1 vaccines elicit strong immune responses and have been shown to protect against persistent HPV infection and HPV-associated diseases, looking to the future there is still more to consider. Some features of current HPV L1 vaccines hinder adoption in low-resource countries, in addition to their high cost. A three dose schedule which is not given in the existing infant vaccine schedule requires establishment of a new vaccine delivery platform, leading to new and higher costs for vaccine delivery. The current formulations require a complex infrastructure to maintain cold-chain storage of the vaccine, provide for sterile intramuscular injection and follow-up methods to assure all three doses are received. Also, current vaccines only protect against two oncogenic HPV types. While these two types account for approximately 70% of cervical cancers worldwide, there is a clear need for more type coverage. Next-generation increased valency of HPV vaccines are currently in clinical trials.

However, it is important to weigh the benefit of increased valency of future vaccines against the potential risk that adding antigens might interfere with the extremely high efficacy against HPV 16 and HPV 18-related disease. Such interference did not occur with the addition of three additional VLP types in the quadrivalent vaccine, as non-inferiority of the HPV 16 antibody response was proven (*119*). This will need to be investigated closely in the clinical trials.

Next-generation HPV vaccines should ideally be thermostable, require less than 3 doses, able to be delivered with existing health visits (rather than require new health visits), and delivered through non-parenteral routes. Some investigators have looked at delivery of HPV vaccines via mucosal or nasal delivery (*157,158,159*). High levels of inexpensively produced VLPs can be achieved in plants or yeast (*65,160*) so oral candidate vaccines may be studied. These routes would improve the ease of administration. It will be important to determine the bioavailability of alternate routes and to verify that appropriate non-inferior antibody responses are generated for any second-generation vaccine

The goals for future HPV vaccines include protection against more HPV types as well as the introduction of a therapeutic benefit. To increase protection against more types, L2 vaccines are very promising, since they have been shown to neutralize a broad range of cutaneous and mucosal HPV types (*161,162*). However, L2 vaccines do not induce robust responses, especially in B cells; thus they will require further optimizing, probably with adjuvants and protein constructs altering availability of the L2 epitopes. To induce therapeutic as well as prophylactic responses, early HPV proteins have been added to L1 proteins to form chimeric virus-like particles. Animal models have shown robust immune responses to chimeric HPV vaccines, including rejection of E7-positive syngeneic tumour cells *(163–166*). In women with CIN 2/3 who received a chimeric vaccine, L1 and E7 antibodies were boosted, although E7 antibodies were found in fewer subjects (*167*).

Many other therapeutic vaccines are currently being investigated in early-phase clinical trials — for reviews see (*82,83*). Most include HPV 16 E7 or E6 constructs in various vectors, or as plasmids or fusion peptides. Proof of principal trials for many HPV-targeted therapeutics have been performed in a Phase II setting in high-grade CIN, vulvar intraepithelial neoplasia (VIN) or advanced cancer, all of which include disease and viral end-points (*79*, Einstein, 2007 #614, *81,168,169,170*). While the current prophylactic vaccines are likely to change clinical paradigms of cervical cancer prevention worldwide, there is a pressing clinical need for continued development of effective therapeutic vaccines and immunotherapies that have the promise to help the millions of women who suffer from cervical neoplasia and other HPV-associated neoplasias worldwide.

5.3 Areas needed for future research regarding HPV vaccine immune responses

While the promise for eradication of cervical disease has the potential to exist in the era of prophylactic vaccination against HPV, there are still many challenges that lie ahead, especially with regard to the immune responses generated as a result of HPV vaccination. The first challenge is research on establishing immune correlates of protection from HPV-associated disease. The second major challenge is to correlate these immune responses with vaccine efficacy using an appropriate, reproducible serologic immunoassay. While the numerous assays that have been used in the clinical trials have been helpful in elucidating efficacy, it is unclear whether higher serologic responses and correlating higher secretion of anti-HPV L1 antibody at the site of infection is truly the immunologic mechanism of action of HPV vaccine efficacy. If so, are waning titres suggestive of waning efficacy? Or is there a strong local anamnestic response that protects the subject during an HPV re-challenge? Also, are the immune responses that are detectable in peripheral blood correlative and indicative of what is happening locally at the cervix? Plus, how does immune persistence affect response to new infection?

There are also questions regarding the number of vaccine doses needed. Early reports suggest two doses compared with three doses of quadrivalent vaccine result in non-inferior antibody responses (*171*). Also, evaluation of alternative vaccine dose scheduling, such as variations in timing of the third dose are ongoing.

Policy-making organizations that have adopted routine use of HPV vaccines have focused on HPV-naive young females. However, more clinical data is being generated by the companies in sexually- active, mature women (*172,173*) and also in men (*174,175*). Similar immune response correlations with efficacy need to be generated with any new potential indication for HPV vaccine use. Current safety data suggest HPV vaccines do not appear to result in significant teratogenic or pregnancy events; however, more prospectively collected data is needed. Also, there is currently no available data regarding trans-placental antibody transmission, or transmission through breastmilk. Immune responses are clearly higher in young adolescents and children. As proven data accumulates about safety and longevity of vaccine protection, there may be the potential for moving vaccination to younger populations.

As the vaccines become more readily available worldwide, continued tracking of vaccine immunogenicity with efficacy in different populations, regions, dosing schedules, and age at administration is important , to track the potential need for a booster and to determine long-term correlates of protection. Also, when possible, study of the immune status of breakthrough cases will be important for cervical cancer screening policies and in the development of next-generation vaccine (though establishing a definition of a “break through case” will be difficult given the inability to ascertain baseline HPV status or initial immune response to the vaccine.) Adoption of standardized assays for tracking immunogenicity over time in populations or in registries will facilitate long term efficacy data for the vaccine. Development of international standards for, and standard operating procedures of HPV serology assays, e.g. VLP-ELISA have been addressed by the WHO HPV LabNet(*http://www.who.int/biologicals/vaccines/hpv/en/index.html*).

References

1. Chong T et al. The enhancer of human papillomavirus type 16: binding sites for the ubiquitous transcription factors oct-1, NFA, TEF-2, NF1, and AP-1 participate in epithelial cell-specific transcription. *Journal of Virology*, 1991, 65:5933–5943.
2. Dell G, Gaston K. Human papillomaviruses and their role in cervical cancer. *Cellular and Molecular Life Sciences : CMLS*, 2001, 58:1923–1942.
3. Einstein MH et al. Utilization of the human genome sequence localizes human papillomavirus type 16 DNA integrated into the TNFAIP2 gene in a fatal cervical cancer from a 39-year-old woman. *Clinical Cancer Research : an official journal of the American Association for Cancer Research*, 2002, 8:549–554.
4. Bosch FX, de Sanjose S. Chapter 1: Human papillomavirus and cervical cancer—burden and assessment of causality. *Journal of the National Cancer Institute. Monographs*, 2003, 31:3–13.
5. Clifford GM et al. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *British Journal of Cancer*, 2003, 88:63–73.
6. Clifford GM et al. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. *British Journal of Cancer*, 2003, 89:101–105.
7. Munoz N et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *The New England Journal of Medicine*, 2003, 348:518–527.
8. Herrero R et al. Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. *Journal of the National Cancer Institute*, 2000, 92:464–474.
9. *IARC Monographs on the evaluation of carcinogenic risks to humans: volume 90*. In: IARC, ed. Lyon, 2005.
10. Clifford G et al. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. *Vaccine*, 2006, 24:(Suppl. 3): S3/26–34.
11. de Villiers EM. Papillomavirus and HPV typing. *Clinics in Dermatology*, 1997, 15:199–206.
12. Schiffman M et al. Human papillomavirus and cervical cancer. *Lancet*, 2007, 370:890–907.
13. Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. *Journal of the National Cancer Institute. Monographs*, 2003, 31:14–19.

14. McCredie MR et al. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. *The Lancet Oncology*, 2008, 9:425–434.

15. Einstein M et al. Clinician's guide to human papillomavirus immunology: knowns and unknowns. *The Lancet Infectious Diseases*, 2009 [in press].

16. Moscicki AB et al. Chapter 5: Updating the natural history of HPV and anogenital cancer. *Vaccine*, 2006, 24(Suppl. 3):S42–S51.

17. Ho GY et al. Natural history of cervicovaginal papillomavirus infection in young women. *The New England Journal of Medicine*, 1998, 338:423–428.

18. Brisson J et al. Determinants of persistent detection of human papillomavirus DNA in the uterine cervix. *The Journal of Infectious Diseases*, 1996, 173:794–799.

19. Castellsague X et al. Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: implications for screening and prevention. *Journal of the National Cancer Institute*, 2006, 98:303–315.

20. Altekruse SF et al. Comparison of human papillomavirus genotypes, sexual, and reproductive risk factors of cervical adenocarcinoma and squamous cell carcinoma: Northeastern United States. *American Journal of Obstetrics and Gynecology*, 2003, 188:657–663.

21. An HJ et al. Prevalence of human papillomavirus DNA in various histological subtypes of cervical adenocarcinoma: a population-based study. *Modern Pathology : an official journal of the United States and Canadian Academy of Pathology, Inc.*, 2005, 18:528–534.

22. Clifford GM et al. Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. *Cancer Epidemiology, Biomarkers & Prevention : a publication of the American Association for Cancer Research, co-sponsored by the American Society of Preventive Oncology*, 2005, 14:1157–1164.

23. Burk RD. Human papillomavirus and the risk of cervical cancer. *Hospital Practice (1995)*, 1999, 34:103–111; quiz 12.

24. Schiffman MH, Brinton LA. The epidemiology of cervical carcinogenesis. *Cancer*, 1995, 76:1888–1901.

25. Genital HPV infection fact sheet. *Rockville, MD*, 2004.

26. Daling JR et al. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. *Cancer*, 2004, 101:270–280.

27. Saslow D et al. American Cancer Society guideline for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. *CA: a Cancer Journal for Clinicians*, 2007, 57:7–28.

28. Trimble CL et al. Heterogeneous etiology of squamous carcinoma of the vulva. *Obstetrics and Gynecology*, 1996, 87:59–64.

29. Munoz N et al. Chapter 1: HPV in the etiology of human cancer. *Vaccine*, 2006, 24(Suppl. 3):S1–S10.

30. Rubin MA et al. Detection and typing of human papillomavirus DNA in penile carcinoma: evidence for multiple independent pathways of penile carcinogenesis. *The American Journal of Pathology*, 2001, 159:1211–1218.

31. Daling JR et al. A population-based study of squamous cell vaginal cancer: HPV and cofactors. *Gynecologic Oncology*, 2002, 84:263–270.

32. Forastiere A et al. Head and neck cancer. *The New England Journal of Medicine*, 2001, 345:1890–1900.

33. Herrero R et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *Journal of the National Cancer Institute*, 2003, 95:1772–1783.

34. Kreimer AR et al. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiology, Biomarkers & Prevention : a publication of the American Association for Cancer Research, co-sponsored by the American Society of Preventive Oncology*, 2005, 14:467–475.

35. Cupp MR et al. Detection of human papillomavirus DNA in primary squamous cell carcinoma of the male urethra. *Urology*, 1996, 48:551–555.

36. *Human papillomavirus vaccines. WHO Position Paper.* Geneva, World Health Organization, 2009.

37. Clifford GM et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *Lancet*, 2005, 366:991–998.

38. Franco EL et al. Chapter 20: Issues in planning cervical cancer screening in the era of HPV vaccination. *Vaccine*, 2006, 24(Suppl. 3):S3/171–177.

39. Mbulaiteye SM et al. Immune deficiency and risk for malignancy among persons with AIDS. *Journal of Acquired Immune Deficiency Syndromes*, 2003, 32:527–533.

40. Einstein MH, Kadish AS. Anogenital neoplasia in AIDS. *Current Opinion in Oncology*, 2004, 16:455–462.

41. Massad LS et al. Outcomes after treatment of cervical intraepithelial neoplasia among women with HIV. *Journal of Lower Genital Tract Disease*, 2007, 11:90–97.

42. Reimers LL et al. Are extirpative procedures for CIN effective in HIV-positive women? In: *Proceedings of the international papillomavirus meeting 2009*. Malmo, 2009.

43. Sun XW et al. Human papillomavirus infection in human immunodeficiency virus-seropositive women. *Obstetrics and Gynecology*, 1995, 85:680–686.

44. Moscicki AB et al. Prevalence of and risks for cervical human papillomavirus infection and squamous intraepithelial lesions in adolescent girls: impact of infection with human immunodeficiency virus. *Archives of Pediatrics & Adolescent Medicine*, 2000, 154:127–134.

45. Jamieson DJ et al. Characterization of genital human papillomavirus infection in women who have or who are at risk of having HIV infection. *American Journal of Obstetrics and Gynecology*, 2002, 186:21–27.

46. Duerr A et al. Human papillomavirus-associated cervical cytologic abnormalities among women with or at risk of infection with human immunodeficiency virus. *American Journal of Obstetrics and Gynecology*, 2001, 184:584–590.

47. Ellerbrock TV et al. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. *JAMA : the journal of the American Medical Association*, 2000, 283:1031–1037.

48. Paternoster DM et al. Human papilloma virus infection and cervical intraepithelial neoplasia in transplanted patients. *Transplantation Proceedings*, 2008, 40:1877–1880.

49. Frisch M, Biggar RJ, Goedert JJ. Human papillomavirus-associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. *Journal of the National Cancer Institute*, 2000, 92:1500–1510.

50. Goedert JJ et al. Spectrum of AIDS-associated malignant disorders. *Lancet*, 1998, 351:1833–1839.

51. Doorbar J. The biology of human papillomaviruses. In: Sjat SK, ed. *Human papillomaviruses — clinical and scientific advances*. London, Arnold Publishers, 2001:9–23.

52. Hoffmann R et al. Different modes of human papillomavirus DNA replication during maintenance. *Journal of Virology*, 2006, 80:4431–4439.

53. Day PM et al. Mechanisms of human papillomavirus type 16 neutralization by l2 cross-neutralizing and 11 type-specific antibodies. *Journal of Virology*, 2008, 82:4638–4646.

54. Culp TD, Budgeon LR, Christensen ND. Human papillomaviruses bind a basal extracellular matrix component secreted by keratinocytes which is distinct from a membrane-associated receptor. *Virology*, 2006, 347:147–159.

55. Roberts JN et al. Genital transmission of HPV in a mouse model is potentiated by nonoxynol-9 and inhibited by carrageenan. *Nature Medicine*, 2007, 13:857–861.

56. Kines RC et al. The initial steps leading to papillomavirus infection occur on the basement membrane prior to cell surface binding. *Proceedings of the National Academy of Sciences of the United States of America*, 2009, 106:20458–20463.

57. Joyce JG et al. The L1 major capsid protein of human papillomavirus type 11 recombinant virus-like particles interacts with heparin and cell-surface glycosaminoglycans on human keratinocytes. *The Journal of Biological Chemistry*, 1999, 274:5810–5822.

58. Giroglou T et al. Human papillomavirus infection requires cell surface heparan sulfate. *Journal of Virology*, 2001, 75:1565–1570.

59. Shafti-Keramat S et al. Different heparan sulfate proteoglycans serve as cellular receptors for human papillomaviruses. *Journal of Virology*, 2003, 77:13125–13135.

60. DiMaio D, Liao JB. Human papillomaviruses and cervical cancer. *Advances in Virus Research*, 2006, 66:125–159.

61. Seo YS et al. Bovine papillomavirus (BPV)-encoded E1 protein contains multiple activities required for BPV DNA replication. *Proceedings of the National Academy of Sciences of the United States of America*, 1993, 90:702–706.

62. Yang L et al. The E1 protein of bovine papillomavirus 1 is an ATP-dependent DNA helicase. *Proceedings of the National Academy of Sciences of the United States of America*, 1993, 90:5086–5090.

63. Frattini MG, Laimins LA. Binding of the human papillomavirus E1 origin-recognition protein is regulated through complex formation with the E2 enhancer-binding protein. *Proceedings of the National Academy of Sciences of the United States of America*, 1994, 91:12398–12402.

64. Middleton K et al. Organization of human papillomavirus productive cycle during neoplastic progression provides a basis for selection of diagnostic markers. *Journal of Virology*, 2003, 77:10186–10201.

65. Stanley M. Immunobiology of HPV and HPV vaccines. *Gynecologic Oncology*, 2008, 109:S15–S21.

66. Carter JJ et al. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *The Journal of Infectious Diseases*, 2000, 181:1911–1919.

67. Ho GY et al. Natural history of human papillomavirus type 16 virus-like particle antibodies in young women. *Cancer Epidemiology, Biomarkers & Prevention : a publication of the American Association for Cancer Research, co-sponsored by the American Society of Preventive Oncology*, 2004, 13:110–116.

68. Stern PL. Immune control of human papillomavirus (HPV) associated anogenital disease and potential for vaccination. *Journal of Clinical Virology : the official publication of the Pan American Society for Clinical Virology*, 2005, 32(Suppl. 1):S72–S81.

69. Stanley M. Immune responses to human papillomavirus. *Vaccine*, 2006, 24(Suppl. 1):S16–S22.

70. Doorbar J. The papillomavirus life cycle. *Journal of Clinical Virology : the official publication of the Pan American Society for Clinical Virology*, 2005, 32(Suppl. 1):S7–S15.

71. Giannini SL et al. Influence of the mucosal epithelium microenvironment on Langerhans cells: implications for the development of squamous intraepithelial lesions of the cervix. *International Journal of Cancer. Journal International du Cancer*, 2002, 97:654–659.

72. Tay SK et al. Subpopulations of Langerhans cells in cervical neoplasia. *British Journal of Obstetrics and Gynaecology*, 1987, 94:10–15.

73. Connor JP et al. Evaluation of Langerhans cells in the cervical epithelium of women with cervical intraepithelial neoplasia. *Gynecologic Oncology*, 1999, 75:130–135.

74. Hubert P et al. E-cadherin-dependent adhesion of dendritic and Langerhans cells to keratinocytes is defective in cervical human papillomavirus-associated (pre) neoplastic lesions. *The Journal of Pathology*, 2005, 206:346–355.

75. Viac J et al. Langerhans cells and epithelial cell modifications in cervical intraepithelial neoplasia: correlation with human papillomavirus infection. *Immunobiology*, 1990, 180:328–338.

76. de Jong A et al. Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6. *Cancer Research*, 2004, 64:5449–5455.

77. Steele JC et al. T-cell responses to human papillomavirus type 16 among women with different grades of cervical neoplasia. *British Journal of Cancer*, 2005, 93:248–259.

78. de Gruijl TD et al. T-cell proliferative responses against human papillomavirus type 16 E7 oncoprotein are most prominent in cervical intraepithelial neoplasia patients with a persistent viral infection. *The Journal of General Virology*, 1996, 77(Pt. 9):2183–2191.

79. Kenter GG et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *The New England Journal of Medicine*, 2009, 361:1838–1847.

80. Einstein MH et al. Heat shock fusion protein-based immunotherapy for treatment of cervical intraepithelial neoplasia III. *Gynecologic Oncology*, 2007, 106:453–460.

81. Roman LD et al. A Phase II study of Hsp-7 (SGN-00101) in women with high-grade cervical intraepithelial neoplasia. *Gynecologic Oncology*, 2007, 106:558–566.

82. Kadish AS, Einstein MH. Vaccine strategies for human papillomavirus-associated cancers. *Current Opinion in Oncology*, 2005, 17:456–461.

83. Trimble CL, Frazer IH. Development of therapeutic HPV vaccines. *The Lancet Oncology*, 2009, 10:975–980.

84. Trimble CL et al. Naturally occurring systemic immune responses to HPV antigens do not predict regression of CIN2/3. *Cancer Immunology, Immunotherapy : CII*, 2009.

85. Einstein MH et al. Genetic variants in TAP are associated with high-grade cervical neoplasia. *Clinical Cancer Research : an official journal of the American Association for Cancer Research*, 2009, 15:1019–1023.

86. Belkaid Y, Tarbell K. Regulatory T cells in the control of host-microorganism interactions. *Annual Review of Immunology*, 2009, 27:551–589.

87. Tang Q et al. Visualizing regulatory T-cell control of autoimmune responses in nonobese diabetic mice. *Nature Immunology*, 2006, 7:83–92.

88. Hatam LJ et al. $CD4^{+}Foxp3^{+}CD127^{+low}$ T-regulatory cells are increased in HPV infected papillomas in patients with recurrent respiratory papillomatosis (RRP). *The Journal of Allergy and Clinical Immunology*, 2008, 121:S211.

89. Bonagura VR et al. Recurrent respiratory papillomatosis: altered CD8(+) T-cell subsets and T(H)1/T(H)2 cytokine imbalance. *Clinical Immunology (Orlando, Fla.)*, 1999, 93:302–311.

90. Bonagura VR et al. HLA alleles, IFN-gamma responses to HPV-11 E6, and disease severity in patients with recurrent respiratory papillomatosis. *Human Immunology*, 2004, 65:773–782.

91. DeVoti JA et al. Immune dysregulation and tumor-associated gene changes in recurrent respiratory papillomatosis: a paired microarray analysis. *Molecular Medicine (Cambridge, Mass.)*, 2008, 14:608–617.

92. DeVoti JA et al. Failure of gamma interferon but not interleukin-10 expression in response to human papillomavirus type 11 E6 protein in respiratory papillomatosis. *Clinical and Diagnostic Laboratory Immunology*, 2004, 11:538–547.

93. Rosenthal DW et al. Human papillomavirus causes a T_H2-like chemokine predominance in recurrent respiratory papillomatotosis (RRP). *The Journal of Allergy and Clinical Immunology*, 2008, 121:S15.

94. Rosenthal DW et al. Recurrent respiratory papillomatosis (RRP): increased T_H2-like chemokine expression. *The Journal of Allergy and Clinical Immunology*, 2006, 117:S104.

95. Rosenthal DW et al. Recurrent respiratory papillomatosis (RRP): disease severity associates with enhanced T_H2-like dendritic cell chemokine (DC-CK1) plasma expression. *The Journal of Allergy and Clinical Immunology*, 2005, 115:S81.

96. Molling JW et al. CD4(+)CD25hi regulatory T-cell frequency correlates with persistence of human papillomavirus type 16 and T-helper cell responses in patients with cervical intraepithelial neoplasia. *International Journal of Cancer. Journal International du Cancer*, 2007, 121:1749–1755.

97. Visser J et al. Frequencies and role of regulatory T cells in patients with (pre)malignant cervical neoplasia. *Clinical and Experimental Immunology*, 2007, 150:199–209.

98. Adurthi S et al. Regulatory T cells in a spectrum of HPV-induced cervical lesions: cervicitis, cervical intraepithelial neoplasia and squamous cell carcinoma. *American Journal of Reproductive Immunology (New York, N.Y. : 1989)*, 2008, 60:55–65.

99. Gambhira R et al. Vaccination of healthy volunteers with human papillomavirus type 16 L2E7E6 fusion protein induces serum antibody that neutralizes across papillomavirus species. *Cancer Research*, 2006, 66:11120–11124.

100. Day PM et al. Neutralization of human papillomavirus with monoclonal antibodies reveals different mechanisms of inhibition. *Journal of Virology*, 2007, 81:8784–8792.

101. McHeyzer-Williams LJ, McHeyzer-Williams MG. Antigen-specific memory B-cell development. *Annual Review of Immunology*, 2005, 23:487–513.

102. Vajdy M. Generation and maintenance of mucosal memory B-cell responses? *Current Medicinal Chemistry*, 2006, 13:3023–3037.

103. Bais AG et al. A shift to a peripheral Th2-type cytokine pattern during the carcinogenesis of cervical cancer becomes manifest in CIN III lesions. *Journal of Clinical Pathology*, 2005, 58:1096–1100.

104. Clerici M et al. Cytokine production patterns in cervical intraepithelial neoplasia: association with human papillomavirus infection. *Journal of the National Cancer Institute*, 1997, 89:245–250.

105. Sheu BC et al. Predominant Th2/Tc2 polarity of tumor-infiltrating lymphocytes in human cervical cancer. *Journal of Immunology (Baltimore, Md. : 1950)*, 2001, 167:2972–2978.

106. Ahmed AM, Madkan V, Tyring SK. Human papillomaviruses and genital disease. *Dermatologic Clinics*, 2006, 24:157–165, vi.

107. Kobayashi A et al. Functional attributes of mucosal immunity in cervical intraepithelial neoplasia and effects of HIV infection. *Cancer Research*, 2004, 64:6766–6774.

108. Petry KU et al. Cellular immunodeficiency enhances the progression of human papillomavirus-associated cervical lesions. *International Journal of Cancer. Journal International du Cancer*, 1994, 57:836–840.

109. Ault KA. Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3, and adenocarcinoma in situ: a combined analysis of four randomized clinical trials. *Lancet*, 2007, 369:1861–1868.

110. Hildesheim A et al. Effect of human papillomavirus 16/18 L1 virus-like particle vaccine among young women with preexisting infection: a randomized trial. *JAMA : the journal of the American Medical Association*, 2007, 298:743–753.

111. Breitburd F et al. Immunization with virus-like particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. *Journal of Virology*, 1995, 69:3959–3963.

112. Ghim S et al. Spontaneously regressing oral papillomas induce systemic antibodies that neutralize canine oral papillomavirus. *Experimental and Molecular Pathology*, 2000, 68:147–151.

113. Yang BH et al. Cervical cancer as a priority for prevention in different world regions: an evaluation using years of life lost. *International Journal of Cancer. Journal International du Cancer*, 2004, 109:418–424.

114. Yan M et al. Activation of dendritic cells by human papillomavirus-like particles through TLR4 and NF-kappaB-mediated signalling, moderated by TGF-beta. *Immunology and Cell Biology*, 2005, 83:83–91.

115. Vogel F. Immunologic adjuvants. In: Plotkin S, Orenstein W, Offit P, eds. *Vaccines*. Philadelphia, WB Saunders, 2004.

116. Pashine A, Valiante NM, Ulmer JB. Targeting the innate immune response with improved vaccine adjuvants. *Nature Medicine*, 2005, 11:S63–S68.

117. Ruiz W et al. Kinetics and isotype profile of antibody responses in rhesus macaques induced following vaccination with HPV 6, 11, 16 and 18 L1-virus-like particles formulated with or without Merck aluminium adjuvant. *Journal of Immune Based Therapies and Vaccines*, 2005, 3:2.

118. Villa LL et al. Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. *Vaccine*, 2006, 24:5571–5583.

119. Garland SM et al. Noninferiority of antibody response to human papillomavirus type 16 in subjects vaccinated with monovalent and quadrivalent L1 virus-like particle vaccines. *Clinical and Vaccine Immunology : CVI*, 2007, 14:792–795.

120. Green SJ. Clinical development of TLR agonists as adjuvants: "post-alum adjuvant" candidates may reach beyond their intended purpose. *Clinical Pharmacology and Therapeutics*, 2008, 83:813–814 [author reply 4].

121. Verstraeten T et al. Analysis of adverse events of potential autoimmune aetiology in a large integrated safety database of AS04 adjuvanted vaccines. *Vaccine*, 2008, 26:6630–6638.

122. Giannini SL et al. Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only. *Vaccine*, 2006, 24:5937–5949.

123. Harper DM et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomized control trial. *Lancet*, 2006, 367:1247–1255.

124. Harper DM. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomized controlled trial. *Lancet*, 2004, 364:1757–1765.

125. Opalka D et al. Simultaneous quantitation of antibodies to neutralizing epitopes on virus-like particles for human papillomavirus types 6, 11, 16, and 18 by a multiplexed luminex assay. *Clinical and Diagnostic Laboratory Immunology*, 2003, 10:108–115.

126. Schiller JT, Lowy DR. Immunogenicity testing in human papillomavirus virus-like-particle vaccine trials. *The Journal of Infectious Diseases*, 2009, 200:166–171.

127. Harper DM. Sustained immunogenicity and high efficacy against HPV-16/18 related cervical neoplasia: long-term follow-up through 6.4 years in women vaccinated with Cervarix™. In: *Society of Gynecologic Oncologists annual meeting 2008*. Tampa, FL, Society of Gynecologic Oncologists, 2008.

128. Olsson SE et al. Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine. *Vaccine*, 2007, 25:4931–4939.

129. Paavonen J et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a Phase III double-blind, randomized controlled trial. *Lancet*, 2007, 369:2161–2170.

130. Villa LL. Overview of the clinical development and results of a quadrivalent HPV (types 6, 11, 16, 18) vaccine. *International Journal of Infectious Diseases : IJID : official publication of the International Society for Infectious Diseases*, 2007, 11(Suppl. 2):S17–S25.

131. Pedersen C et al. Immunization of early adolescent females with human papillomavirus type 16 and 18 L1 virus-like particle vaccine containing AS04 adjuvant. *The Journal of Adolescent Health : official publication of the Society for Adolescent Medicine*, 2007, 40:564–571.

132. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *The New England Journal of Medicine*, 2007, 356:1915–1927.

133. Ferguson M et al. Results of the first WHO international collaborative study on the standardization of the detection of antibodies to human papillomaviruses. *International Journal of Cancer. Journal International du Cancer*, 2006, 118:1508–1514.

134. Harro CD et al. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. *Journal of the National Cancer Institute*, 2001, 93:284–292.

135. Pastrana DV et al. Reactivity of human sera in a sensitive, high-throughput pseudovirus-based papillomavirus neutralization assay for HPV16 and HPV18. *Virology*, 2004, 321:205–216.

136. Smith JF et al. Antibodies from women immunized with Gardasil™ cross-neutralize HPV 45 pseudovirions. *Human Vaccines*, 2007, 3:109–115.

137. Christensen ND et al. Immunization with virus-like particles induces long-term protection of rabbits against challenge with cottontail rabbit papillomavirus. *Journal of Virology*, 1996, 70:960–965.

138. Block SL et al. Comparison of the immunogenicity and reactogenicity of a prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in male and female adolescents and young adult women. *Pediatrics*, 2006, 118:2135–2145.

139. Castle PE et al. Comparison of ophthalmic sponges for measurements of immune markers from cervical secretions. *Clinical and Diagnostic Laboratory Immunology*, 2004, 11:399–405.

140. Poncelet S et al. Induction of cervical mucosal HPV IgG in women 15–55 years old following systemic vaccination with GSK cervical cancer candidate vaccine. In: *Annual meeting of the European Society for Paediatric Infectious Diseases (ESPID) 2007*. Porto, European Society for Paediatric Infectious Diseases, 2007.

141. Einstein MH et al. Comparison of the immunogenicity and safety of Cervarix™ and Gardasil™ human papillomavirus (HPV) cervical cancer vaccines in healthy women aged 18–45 years. *Human Vaccines*, 2009, 5:705–719.

142. Einstein MH et al. Comparative evaluation of immunogenicity of two prophylactic HPV vaccines. In: *Proceedings of the international papillomavirus meeting 2009*. Malmo, 2009.

143. Joura EA et al. HPV antibody levels and clinical efficacy following administration of a prophylactic quadrivalent HPV vaccine. *Vaccine*, 2008, 26:6844–6851.

144. Giuliano AR et al. Impact of baseline covariates on the immunogenicity of a quadrivalent (types 6, 11, 16, and 18) human papillomavirus virus-like-particle vaccine. *The Journal of Infectious Diseases*, 2007, 196:1153–1162.

145. Bhatla N et al. Immunogenicity and safety of GlaxoSmithKline's HPV 16/18 L1 VLP AS04 adjuvanted vaccine in Indian females aged 18–35 years. In: *Proceedings of the International Gynecologic Cancer Society meeting 2008*. Bangkok, International Gynecologic Cancer Society, 2008.

146. Ngan HY et al. Immunogenicity and safety of Cervarix™ in healthy women in Hong Kong, SAR. In: *Proceedings of the International Gynecologic Cancer Society meeting 2008*. Bangkok, International Gynecologic Cancer Society, 2008.

147. Moscicki AB et al. Safety and immunogenicity of a quadrivalent vaccine to prevent HPV infection in HIV-infected children: IMPAACT P1047. In: *Proceedings of the international papillomavirus meeting, 2009*. Malmo, 2009.

148. Markowitz LE et al. Quadrivalent human papillomavirus vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR. Recommendations and Reports : Morbidity and Mortality Weekly Report. Recommendations and Reports / Centers for Disease Control*, 2007, 56:1–24.

149. Reisinger KS. Updated safety profile of prophylactic human papillomavirus (types 6,11,16, and 18) L1 virus-like particle vaccine. In: *Proceedings of the meeting of the European Society for Paediatric Infectious Diseases (ESPID), 2008*. Graz, European Society for Paediatric Infectious Diseases, 2008.

150. Slade BA et al. Postlicensure safety surveillance for quadrivalent human papillomavirus recombinant vaccine. *JAMA : the journal of the American Medical Association*, 2009, 302:750–757.

151. *WHO Global Advisory Committee on Vaccine Safety: report of meeting 17–18 June 2009*. Geneva, World Health Organization, 2009:325–332.

152. Wheeler CM et al. Safety and immunogenicity of co-administered quadrivalent human papillomavirus (HPV)-6/11/16/18 L1 virus-like particle (VLP) and hepatitis B (HBV) vaccines. *Vaccine*, 2008, 26:686–696.

153. An open-label, randomized, multicentre study of the safety, tolerability, and immunogenicity of a quadrivalent HPV 6/11/16/18 vaccine (Gardasil™) given concomitantly with Repevax™ in healthy adolescents 11–17 years of age — Protocol 024. In: *Proceedings of the European Conference of Infectious Diseases in Obstetrics and Gynecology, 2008*. Leuven, 2008.

154. Reisinger KS et al. An open-label, randomized, multicenter study of safety, tolerability and immunogenicity of GARDASIL® given concomitantly with Menactra® and ADACEL® in healthy adolescents 11–17 years of age. In: *Proceedings of the annual meeting of the Pediatric Academic Society, 2008*. Baltimore, MD, Pediatric Academic Society, 2008.

155. Schwarz TF et al. Co-administration of AS04-adjuvanted HPV 16/18 cervical cancer vaccine with dTpa-IPV in 10–18 year-old girls: month 7 results from a randomized trial. In: *Proceedings of the meeting of the European Society for Paediatric Infectious Diseases (ESPID), 2009*. Brussels, European Society for Paediatric Infectious Diseases, 2009.

156. Wheeler CM. HPV-16/18 AS04-adjuvanted vaccine co-administered with other vaccines in female adolescents. In: *Proceedings of the Infectious Diseases Society of America, 29 October–1 November 2009*. Philadelphia, PA, Infectious Diseases Society of America, 2009.

157. Nardelli-Haefliger D et al. Immune responses induced by lower airway mucosal immunization with a human papillomavirus type 16 virus-like particle vaccine. *Vaccine*, 2005, 23:3634–3641.

158. Revaz V et al. Humoral and cellular immune responses to airway immunization of mice with human papillomavirus type 16 virus-like particles and mucosal adjuvants. *Antiviral Research*, 2007, 76:75–85.

159. Glueck R. Pre-clinical and clinical investigation of the safety of a novel adjuvant for intranasal immunization. *Vaccine*, 2001, 20(Suppl. 1):S42–S44.

160. Maclean J et al. Optimization of human papillomavirus type 16 (HPV-16) L1 expression in plants: comparison of the suitability of different HPV-16 L1 gene variants and different cell-compartment localization. *The Journal of General Virology*, 2007, 88:1460–1469.

161. Gaukroger JM et al. Vaccination of cattle with bovine papillomavirus type 4 L2 elicits the production of virus-neutralizing antibodies. *The Journal of General Virology*, 1996, 77(Pt. 7):1577–1583.

162. Roden RB et al. Minor capsid protein of human genital papillomaviruses contains subdominant, cross-neutralizing epitopes. *Virology*, 2000, 270:254–257.

163. Jochmus I et al. Chimeric virus-like particles of the human papillomavirus type 16 (HPV 16) as a prophylactic and therapeutic vaccine. *Archives of Medical Research*, 1999, 30:269–274.

164. Garcea RL, Gissmann L. Virus-like particles as vaccines and vessels for the delivery of small molecules. *Current Opinion in Biotechnology*, 2004, 15:513–517.

165. Muller M et al. Chimeric papillomavirus-like particles. *Virology*, 1997, 234:93–111.

166. Schafer K et al. Immune response to human papillomavirus 16 L1E7 chimeric virus-like particles: induction of cytotoxic T cells and specific tumour protection. *International Journal of Cancer. Journal International du Cancer*, 1999, 81:881–888.

167. Kaufmann AM et al. Vaccination trial with HPV16 L1E7 chimeric virus-like particles in women suffering from high grade cervical intraepithelial neoplasia (CIN 2/3). *International Journal of Cancer. Journal International du Cancer*, 2007, 121:2794–2800.

168. Maciag PC, Radulovic S, Rothman J. The first clinical use of a live-attenuated Listeria monocytogenes vaccine: A Phase I safety study of Lm-LLO-E7 in patients with advanced carcinoma of the cervix. *Vaccine*, 2009, 27:3975–3983.

169. Garcia F et al. ZYC101a for treatment of high-grade cervical intraepithelial neoplasia: a randomized controlled trial. *Obstetrics and Gynecology*, 2004, 103:317–326.

170. Melief CJM et al. Eradication of VIN 3 lesions by vaccination with long peptides. In: *Proceedings of the international papillomavirus meeting 2009*. Malmo, 2009.

171. Dobson S et al. Are two doses of HPV vaccine adequate in girls? In: *Proceedings of the international papillomavirus meeting 2009*. Malmo, 2009.

172. Munoz N et al. Safety, immunogenicity and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24–45 years: a randomized, double-blind trial. *Lancet*, 2009, 373:1949–1957.

173. Schwarz TF et al. Immunogenicity and tolerability of an HPV-16/18 AS04-adjuvanted prophylactic cervical cancer vaccine in women aged 15–55 years. *Vaccine*, 2009, 27:581–587.

174. Guris D. Immunogenicity of quadrivalent HPV (types 6/11/16/18) vaccine in young men. In: *Proceedings of the international papillomavirus meeting 2009*. Malmo, 2009.

175. Petaja T et al. Immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine in healthy boys aged 10–18 years. *The Journal of Adolescent Health : official publication of the Society for Adolescent Medicine*, 2009, 44:33–40.

176. Einstein M, Burk R. Persistent human papillomavirus infection: definitions and clinical implications. *Papillomavirus Report*, 2001, 12:119–123.

177. Caulfield MJ et al. Effect of alternative aluminium adjuvants on the absorption and immunogenicity of HPV16 L1 VLPs in mice. *Human Vaccines*, 2007, 3:139–145.

178. Schiller JT et al. An update of prophylactic human papillomavirus L1 virus-like particle vaccine clinical trial results. Vaccine, 2008, 26(S 10):K53–K61.

179. Schiller JT. HPV Vaccines: trials and results. In: *Proceedings of the international papillomavirus meeting 2009*. Malmo, 2009.

180. Reisinger KS et al. Safety and persistent immunogenicity of a quadrivalent human papillomavirus types 6, 11, 16, 18 L1 virus-like particle vaccine in preadolescents and adolescents: a randomized controlled trial. *The Pediatric Infectious Disease Journal*, 2007, 26:201–209.

The World Health Organization has provided technical support to its Member States in the field of vaccine-preventable diseases since 1975. The office carrying out this function at WHO headquarters is the Department of Immunization,Vaccines and Biologicals (IVB).

IVB's mission is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases. The Department covers a range of activities including research and development, standard-setting, vaccine regulation and quality, vaccine supply and immunization financing, and immunization system strengthening.

These activities are carried out by three technical units: the Initiative for Vaccine Research; the Quality, Safety and Standards team; and the Expanded Programme on Immunization.

The Initiative for Vaccine Research guides, facilitates and provides a vision for worldwide vaccine and immunization technology research and development efforts. It focuses on current and emerging diseases of global public health importance, including pandemic influenza. Its main activities cover: i) research and development of key candidate vaccines; ii) implementation research to promote evidence-based decision-making on the early introduction of new vaccines; and iii) promotion of the development, evaluation and future availability of HIV, tuberculosis and malaria vaccines.

The Quality, Safety and Standards team focuses on supporting the use of vaccines, other biological products and immunization-related equipment that meet current international norms and standards of quality and safety. Activities cover: i) setting norms and standards and establishing reference preparation materials; ii) ensuring the use of quality vaccines and immunization equipment through prequalification activities and strengthening national regulatory authorities; and iii) monitoring, assessing and responding to immunization safety issues of global concern.

The Expanded Programme on Immunization focuses on maximizing access to high quality immunization services, accelerating disease control and linking to other health interventions that can be delivered during immunization contacts. Activities cover: i) immunization systems strengthening, including expansion of immunization services beyond the infant age group; ii) accelerated control of measles and maternal and neonatal tetanus; iii) introduction of new and underutilized vaccines; iv) vaccine supply and immunization financing; and v) disease surveillance and immunization coverage monitoring for tracking global progress.

The Director's Office directs the work of these units through oversight of immunization programme policy, planning, coordination and management. It also mobilizes resources and carries out communication, advocacy and media-related work.

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