

Prevention of cervical, vaginal, and vulval cancers: role of the quadrivalent human papillomavirus (6, 11, 16, 18) recombinant vaccine

Maria Lina Diaz

Section of Ambulatory Gynecology
Cleveland Clinic Florida Weston,
Florida, USA

Abstract: The relationship between the human papillomavirus (HPV) and malignancies of the uterine cervix, vagina, and vulva has been established. The development of a quadrivalent HPV recombinant prophylactic vaccine represents the first time in history that primary prevention of these cancers is offered to girls and women. The prevalence of oncogenic HPV subtypes in cervical cancers has been the most studied, but prevalence has also been established for vaginal and vulvar cancers. Clinical trials demonstrate impressive efficacy in disease prevention as well as excellent safety and tolerability. The role the quadrivalent HPV recombinant vaccine promises to have in the reduction of gynecologic malignancies will depend on various factors, including acceptance and accessibility of the vaccine, duration of immunity, and cross-protection against other oncogenic HPV subtypes. The HPV vaccine's role in disease reduction will probably be viewed in the context of a strategy that involves continued secondary screening and lifestyle modification to reduce modifiable risk factors, along with widespread vaccination.

Keywords: human papillomavirus, quadrivalent vaccine, cervical cancer, vaginal cancer, vulvar cancer

Human papillomavirus (HPV)-associated gynecological cancers include cancers of the uterine cervix, the vagina, and the vulva. HPV-related non-genital cancers that can occur in either gender include anal cancer and oropharyngeal cancers. Non-malignant disease processes associated with HPV include genital warts and respiratory laryngeal papillomatosis. The understanding that many HPV-related cancers develop from precancerous states along with the knowledge that many of these cancers are mediated by similar types of high-risk oncogenic HPV has been revolutionary. The result of this knowledge has led to the development of prophylactic HPV vaccines. The quadrivalent HPV recombinant vaccine Gardasil® (Merck & Co., Inc., Whitehouse Station, New Jersey, USA) provides protection from 4 types of HPV: 6, 11, 16, and 18, and was licensed in 2006. The quadrivalent HPV recombinant vaccine was approved in the US by the Food and Drug Administration (FDA) for the prevention of anogenital warts, cervical intraepithelial neoplasia (CIN), adenocarcinoma in situ of the cervix (AIS), vulvar intraepithelial neoplasia (VIN), vaginal intraepithelial neoplasia (VaIN), and cervical cancer. In October 2008 the label was expanded to include vulvar and vaginal cancers. The bivalent prophylactic HPV Cervarix® (GlaxoSmithKline, Brentford, UK) vaccine provides protection from two types of HPV (16 and 18) and was licensed in 2007 but has yet to be approved by the FDA in the US. Prophylactic HPV vaccine development is an astounding accomplishment and represents the first time in

Correspondence: Maria Lina Diaz, MD
2950 Cleveland Clinic Blvd,
Weston, Florida USA
Tel +1 954 659 5559
Fax +1 954 659 5560
Email diazm2@ccf.org

history that a vaccine has been offered to girls and women for prevention of gynecological cancers. Despite its importance in medical history, the HPV vaccine is not the first vaccine to protect against a viral agent with a known association to malignancy, this being the hepatitis B vaccine for hepatocellular carcinoma.¹

There are over 100 types of HPV with approximately 35 types having affinity for the genital tract.² HPV viruses are further subdivided into two divisions – those with the ability to promote cancers and those that do not. The former division is also referred to as oncogenic or high-risk while the latter division is referred to as non-oncogenic or low-risk. The high-risk oncogenic HPV types include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 while the low-risk HPV types include types 2, 3, 6, 7, 10, 11, 13, 32, 40, 42, 43, 44, and 57. The types are related to each other phylogenetically, based on degree of genetic relatedness.³ In 1995 the International Agency for Research on Cancer established that out of the approximately 15 types of high-risk HPV, types 16 and 18 were responsible for about 70% of cervical cancers in 5 geographic regions of the world.⁴ It appears that HPV types 16 and 18 make up a larger fraction (72% to 77%) in developed countries compared to less-developed regions (65% to 72%).⁵ In a recent meta-analysis of HPV type-distribution in vulvar and vaginal cancers and their precursors, the HPV prevalence of vaginal cancers was found to be 65.5% while that of vulvar cancers was found to be 40.1%.⁶ In this meta-analysis, among cases of HPV-related vulvar and vaginal carcinoma, types 16 and 18 were the most common HPV types implicated. In another series that described prevalence and estimated attribution of HPV types in cervical, vaginal, and vulvar cancers it was observed that the proportion of any vulvar cancers testing positive for any HPV types was 65.3%, with HPV 16 contributing to about 50% of all cases overall.⁷ Although data for vaginal cancer were sparse in this report, HPV 16 contributed the largest proportion (63.2%) of HPV-positive vaginal cancers. It is interesting to note that HPV types 16 and 18 have also been implicated in penile cancer and in non-genital HPV-related malignancy of the anus⁸ as well as in the majority of cancers of the oropharyngeal cavity.⁹ The importance of this revelation is that HPV vaccines may also protect against these other malignancies. Low-risk HPV infections are implicated in genital warts and laryngeal respiratory papillomatosis. HPV types 6 and 11 are responsible for 90% of genital warts as well the majority of cases of laryngeal respiratory papillomatosis. The economic burden associated with non-oncogenic HPV disease is staggering. In an analysis, the

annual cost of conditions related to non-cervical HPV 6, 11, 16, and 18 (including genital warts and juvenile respiratory papillomatosis) in the US was estimated in 2003 to average US\$418 million.¹⁰

Cervical cancer

In women, the most studied HPV-related malignancy is cervical cancer. There is a dichotomy in the incidence of cervical cancer in developed versus developing countries of the world. For example, in the US, cervical cancer incidence had been in decline since the introduction of the Pap smear in the 1950s.¹¹ In the US cervical cancer incidence in 2008 was 11,070 with 3870 deaths.¹² However, in developing countries of the world, cervical cancer represents one of the top two causes of cancer deaths in women.¹³ Sub-Saharan Africa and South America are regions of the world where cervical cancer remains an insurmountable threat to women's health. Sadly the World Health Organization has predicted that by 2050 the annual incidence of new cervical cancer cases in the world will be one million with the vast majority of cases and deaths seen in developing countries. While organized secondary prevention programs of Pap smear screening with surveillance have been successful in significantly reducing cervical cancer incidence and death in developed regions, a large portion of women in less developed regions continued to die unnecessarily from a preventable disease. Although many reasons can be cited for the lack of introduction of cervical cancer screening programs in these regions, the underlying reason is lack of resources and infrastructure due to widespread poverty. It is important to realize that although HPV vaccines will hopefully reduce the incidence of cervical cancer, secondary screening programs cannot be abandoned.

It has been established that persistence of the HPV virus is required for the development of cervical cancer.¹⁴ The natural history of cervical cancer starts with infection of the genital tract with the virus. This occurs through skin to skin contact of the genital region possibly at the site of micro-trauma. The life-cycle of the virus is integrally linked to maturation of the keratinocyte. Once this process has begun, several outcomes can occur including clearance of the virus or progression to precancerous states. If left unchecked, precancerous states can progress to cervical cancer. Risk factors in the development of cervical cancer include early age at onset of sexual activity, multiple sexual partners, having a high-risk sexual partner, and high parity. The HPV virus is cleared through competence of the immune system. It is not surprising therefore that the immunosuppressive states of HIV¹⁵ and transplant reciprocity¹⁶ are also risk factors

for the development of cervical cancer. In addition to the presence and persistence of the HPV virus, several cofactors are implicated in the development of cervical cancer. These cofactors include *Chlamydia trachomatis*,¹⁷ herpes simplex virus,¹⁸ cigarette smoking,¹⁹ and oral contraceptive pills.²⁰ Recently it has been postulated that certain genetic variations in women, specifically at two locations of the TAP gene, may reduce the rates of immune clearance and indirectly influence oncogenesis by promoting persistence of HPV.²¹

Vaginal and vulvar cancer

The natural history of vaginal and vulvar cancer is less studied and complicated by several factors. The study of vaginal cancer is limited due to the rarity of this malignancy. In 2008, there were fewer than 2210 new cases annually and fewer than 760 deaths in the US.²² Vaginal cancers represent 1% to 3% of gynecologic malignancies worldwide.²³ In addition to the rarity of vaginal cancer, the etiology of vaginal cancers is mixed. Although the majority of vaginal cancers are squamous cell carcinomas and caused by HPV infection, other types of vaginal cancers include melanoma and clear cell adenocarcinomas which are linked to in utero maternal diethylstilbestrol.²⁴ It is assumed that HPV-related vaginal cancer also follows a precursor state and it has been theorized that the rarity of vaginal cancers compared to cervical cancer may be related to the absence of a susceptible transformation zone and the protective nature of the keratinized vaginal mucosa. The precursor states of vaginal cancer are referred to as vaginal intraepithelial neoplasia (VaIN). Risk factors for the development of vaginal cancers include many of the same risk factors for the development of cervical cancers, including young age at first coitus, greater number of lifetime sexual partners, prior anogenital disease, and smoking. In a recent meta-analysis studying factors affecting risk of mortality from vaginal cancers, the mean age at diagnosis was 65.7 years and incidence rates of vaginal cancers were noted to increase with age.²⁵ Other findings of this meta-analysis include higher incidence rates with lower socioeconomic status, which is also observed in cervical cancer incidence rates.

Vulvar carcinoma is the fourth most common gynecologic malignancy in the US with an estimated 1100 new cases and 400 deaths annually.²⁶ In the UK, vulvar cancer is ranked 18th in incidence of all malignancies in women with just slightly over 1000 women diagnosed yearly and approximately 365 deaths per year.²⁷ In both of these developed countries, there is an increasing incidence of HPV-related vulvar cancers in the last 25 years particularly in younger women. Over 90% of vulvar carcinomas are

squamous cell carcinomas. There appears to be two types of vulvar squamous carcinoma. The first type, also referred to as Bowenoid type, is associated with HPV, particularly HPV 16, 18, and 33.²⁸ The other type of vulvar squamous cell carcinoma is associated with chronic vulvar dermatoses such as lichen sclerosus and lichen planus. In addition there are other non-HPV-related vulvar carcinomas including melanoma, basal cell carcinoma, sarcoma, extramammary Paget's disease, and Bartholin gland adenocarcinoma.²⁹ Although traditionally the precancerous states of vulvar carcinoma were referred to as VIN, current nomenclature designated by the International Society for the Study of Vulvovaginal Disease subdivides VIN into categories associated with HPV referred to as usual type (warty, baseloid, or mixoid) and into categories associated with vulvar dermatoses, referred to as well-differentiated type.³⁰ The increase of HPV-related vulvar cancers in young women highlights one of the differences between the natural history of cervical cancer and vulvar cancers – the interval between diagnoses of VIN usual type may be expressed in years versus decades as is often the case in the progression of CIN to cervical cancer.³¹

Development of the HPV vaccine

The human papillomavirus is a non-enveloped, double-stranded DNA virus. The circular HPV genome is about 8000 nucleotides in length and the genome is divided into two regions: the early region and the late region. The early region is expressed during early parts of viral replication cycles and contains the codes for genes important in viral replication. The late region is expressed during the later part of viral replication cycle and contains the codes for the two viral capsid proteins, L1 and L2. L1 is the major protein and is used to make the HPV vaccines. The vaccine is made by isolating the DNA of the naturally occurring HPV then cloning the gene or open reading frame encoding the L1 capsid protein into a plasmid. The plasmid containing the L1 gene is introduced into a eukaryotic cell. The L1 gene is transcribed into mRNA and the cell then translates the mRNA into L1 capsid proteins. The capsid proteins self assemble within the cells to form viral-like particles (VLPs). Because viral DNA (with the exception of the L1 gene) has not been introduced into the eukaryotic cell, viral genomes are not available to incorporate with the viral-like particles. This removes the danger of producing infectious virions or promoting cancer. After purification, the VLPs are injected into the host and elicit an immune response. During normal genital HPV infections, the primary immunologic response

to HPV-infected cells is a cellular one and few viral-specific antibodies are produced. Only 50% of naturally infected women make antibodies against HPV and the antibodies are not always neutralizing. The vaccine appears to provide higher antibody levels than that observed in naturally occurring exposure by a factor of 12 to 26 and immunity has been demonstrated through 3 years post vaccination.³² The observation of protection at a time when antibody levels have reached a plateau is encouraging for long-term protection.

Pivotal vaccine studies

The major quadrivalent HPV recombinant vaccine trials will be discussed below in considerable detail. It is noteworthy to mention that Gardasil® induces a sustained immune response among vaccinated subjects and that furthermore it is virtually 100% effective in preventing type-specific (HPV types 6, 11, 16 and 18) cervical, vulvar, and vaginal lesions provided there is no type-specific viral DNA present on the cervix at the time of vaccination and the patient is seronegative to these types at the time of vaccination. The studies are therefore presented in the context of the “perfect population” which includes subjects naïve to vaccine-specific HPV and analyzed in a type-specific manner with nearly 100% efficacious results. The other population studied is the “real world” which includes subjects naïve to HPV infections, those with current infections, as well as those previous infections. In this population the quadrivalent recombinant HPV vaccine significantly reduced the incidence of type-specific lesions including CIN/AIS and external genital warts.

The HPV vaccine trials utilized cervical intra-epithelial neoplasia 2 and 3 (CIN 2/3) and AIS as surrogate markers for prevention of cervical cancer as well as efficacy endpoints to assess prevention of cervical cancer. In addition, cases of vulvar and vaginal intraepithelial neoplasia 2 and 3 (VIN and VaIN 2/3) were efficacy endpoints to assess prevention of HPV-related vulvar and vaginal cancers. Observation for external genital warts was the efficacy endpoints for prevention of genital warts. Efficacy was assessed in 4 placebo-controlled, double-blind, randomized phase II and phase III clinical trials. The placebo administered was an amorphous aluminum hydroxyphosphate sulfate (AAHS) and was the adjuvant used in the quadrivalent vaccine. The first study in the development of HPV vaccine was the proof of principle trial.³³ This landmark study demonstrated that subjects receiving three vaccinations with a monovalent HPV 16 VLP on day 1, month 2 and month 6 demonstrated protection from persistent HPV 16 infection as well as from HPV 16-related CIN. In the study, 2392 women between the ages of 16 and

23 from 16 centers in the US were randomly assigned to receive either vaccine developed by Merck & Co. Research Laboratories consisting of 40 µg of highly purified VLP (empty capsids) of the L1 polypeptide of HPV 16 or placebo. Young women enrolled in the study were sexually active or planned to become sexually active in the near future. The subjects were not pregnant or planning to become pregnant in the near future and did not have a history of abnormal Pap test or had not had more than 5 male sexual partners during their lifetime. Out of the 2392 women enrolled, 1533 subjects were included in the primary analysis. The subjects were studied for a median of 17.4 months. After adjusting for lost to follow-up and eliminating women with evidence of prior HPV 16 infections, 768 women received all three doses of vaccine and 765 women received placebo. None of the vaccinated women demonstrated persistence of HPV 16 infection compared to 41 women in the placebo group. Persistence was defined as testing positive for HPV 16 on 2 consecutive visits. In this study 9 cases of CIN related to HPV 16 were detected in the placebo group while none were observed in the vaccine recipients. A follow-up study of the proof of principal monovalent HPV 16 phase II clinical trial extended the follow-up phase to 48 months.³⁴ This study compared 755 vaccinated women having completed all 3 vaccines to 750 women who had received placebo. There were 7 cases of persistent infection in the vaccinated women compared to 111 in the placebo group, representing 94% efficacy. This trial also studied the development of HPV 16-related CIN lesions in the same population and found no cases of CIN in the vaccinated women compared to 12 cases in the placebo group, representing 100% efficacy (Table 1).

The second phase II clinical trial evaluated all components of the quadrivalent vaccine, HPV 6, 11, 16, and 18 (Table 2).³⁵ The purpose of the study was to assess the efficacy of the vaccine via a composite primary endpoint of persistent infection associated with HPV types 6, 11, 16, 18, or cervical or external genital disease (ie, persistent HPV infection, HPV detection at the last recorded visit, CIN, cervical cancer, or external genital lesions caused by the HPV types in the vaccine).

This study enrolled 552 women ages 16 to 23 years recruited from US, Brazil, and Europe with the following characteristics: none were pregnant, all had a negative history of abnormal Pap smears, and all had a lifetime history of 4 or less male sexual partners. Women with previous HPV infection were not excluded from the study. Of the 552 women, 277 were assigned to vaccination and 275 were assigned to placebo. Women receiving the vaccine were

Table 1 Monovalent HPV 16 VLP vaccine trial

Population	HPV 16 LI VLP		AAHS	
	N	Cases	N	Cases
HPV 16 persistence				
17.4-month study	768	0	765	41
48-month study	755	7	750	111
HPV 16-related CIN				
17.4-month study	768	0	765	9*
48-month study	755	0	750	12

*Of the 41 patients in the 17.4-month study of the AAHS group, 9 developed HPV 16-related CIN.

Abbreviations: AAHS, amorphous aluminum hydroxyphosphate sulfate; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; N, number of patients vaccinated or receiving; AAHS, respectively; VLP, viral-like particles.

given 20 µg type 6, 40 µg type 11, 40 µg type 16, and 20 µg type 18, with 225 µg aluminum adjuvant via intramuscular injection at day 1 then at 2 and 6 months. This study showed significant protection from persistent infection by vaccinated types in subjects vaccinated per protocol compared to placebo as well as 100% protection from the development of CIN caused by HPV 6, 11, 16, and 18 in vaccinated subjects who were not infected with these types at first vaccination. Efficacy in prevention of genital warts caused by HPV 6 and 11 was reported as 100% in the vaccinated subjects (Table 3).

Villa et al³⁶ studied a subset of 241 patients from this population for a total of 5 years. The primary endpoint was the combined incidence of persistent infection or genital disease due to HPV 6, 11, 16, or 18, or HPV DNA detection at the last recorded visit, or detection of HPV DNA in biopsies of diagnosed cervical, vulvar, vaginal dysplasia, or genital warts. The combined incidence of HPV 6-, 11-, 16-, 18-related persistent infection or disease was reduced by 96% in the vaccinated population. There were 2 cases of persistent HPV infection in the vaccinated group versus 46 in the placebo group. There were no cases of CIN or genital

Table 2 Summary of phase II clinical trial

Vaccine type	HPV 6, 11, 16, and 18 VLP LI capsid component
Concentration	20 µg HPV 6 40 µg HPV 11 40 µg HPV 16 20 µg HPV 18
Dose	0.5 mL intramuscular
Sites	US, Brazil, Europe
Primary endpoint	Combined incidence of persistent HPV infection and genital disease due to vaccine type HPV
Trial size	552 women (277 vaccinated, 275 placebo)

Abbreviations: HPV, human papillomavirus; VLP, viral-like particles.

warts related to the vaccinated HPV types in the vaccinated group versus 6 cases in the placebo group. Immunity in the vaccinated group remained above what is observed with natural exposure during the 5 years.

The next studies were phase III clinical trials-Future I³⁷ and Future II.³⁸ Future I studied 5455 women aged 16 to 24 years to assess the efficacy of the quadrivalent vaccine to prevent HPV-related anogenital disease. The primary aim of this trial was to determine vaccine efficacy in reducing the combined incidence of anogenital warts, vulvar or vaginal intraepithelial neoplasia grades 1–3, or vulvar or vaginal cancers associated with HPV types 6, 11, 16, and 18. A secondary aim was to observe whether the administration of vaccine reduced the combined incidence of CIN grades 1–3, AIS, or cervical cancer associated by vaccine type HPV. The study randomized a total of 5455 women into 2 groups, 2723 receiving vaccine per protocol and 2732 receiving placebo. The results of this trial showed the vaccine was 100% effective in preventing external anogenital disease and demonstrated 100% efficacy in preventing CIN grades 1–3 or AIS with HPV types 6, 11, 16, and 18. In the intention to treat (ITT) population of this study which included subjects with prevalent infection or disease by vaccine type and non-vaccine types of HPV, vaccination reduced the incidence of vulvar, vaginal, or external anogenital disease regardless of causal HPV types by 34% and of cervical lesions regardless of causal HPV types by 20%. Future II was a multinational, prospective, double-blind, placebo-controlled trial with more than 12,000 subjects between the ages of 15 and 26 years who reported no more than 4 lifetime sexual partners. The subjects were randomized and assigned to receive 3 doses of vaccine or placebo. Sixty-five percent of participants were European and 26% were from Latin America. At baseline, cervical cytology was abnormal in 11% of both groups with 16% having evidence of infection with HPV 16 and 7% having evidence of infection with HPV 18. The mean duration of follow-up in the interim analysis used to support licensure was 3 years and the analysis of the completed 4-year trials will soon be published. The primary efficacy analysis was performed in subjects not having evidence of either HPV 16 or 18 infections through 1 month after the third dose of the vaccine. These subjects were referred to as “HPV susceptible” per protocol. The primary composite endpoint was CIN 2 or 3, AIS, or cervical cancer related to HPV types 16 or 18. The results of the trial demonstrated 98% efficacy in HPV-susceptible subjects (Table 4). However, the efficacy of the vaccine was lower (44%) for CIN 2 or 3 due to the vaccine-specific types in the overall population (also referred

Table 3 Phase II clinical trial

Population	Gardasil®	Placebo	Efficacy (95% CI)
Persistent infection			
HPV 6	0	13	100.0 (68.0, 100.0)
HPV 11	0	0	NS
HPV 16	3	21	86.0 (54.0, 97.0)
HPV 18	1	9	89.0 (21.0–100.0)
HPV 6-, 11-, 16-, or 18-related CIN (CIN 2, CIN 2/3) or AIS	0	3	100.0 (–138.4, 100.0)
HPV 6-, 11-, 16- or 18-related genital warts	0	3	100.0 (–139.5, 100.0)

Note: Populations were per protocol.

Abbreviations: AIS, adenocarcinoma in situ of the cervix; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; NS, not clinically significant.

to as the ITT population which included subjects naïve to HPV 16/18 and subjects infected with HPV 16/18 or other types at day 1). In this group there was an 18% reduction in CIN 2/3 or AIS due to any HPV type.

Additional studies have been undertaken to specifically evaluate VIN 2/3 and VaIN 2/3 which, as previously stated, are considered the immediate precursors of HPV-related vulvar and vaginal cancers. One study was a combined analysis of 3 randomized clinical trials and evaluated the effect of a prophylactic quadrivalent human papillomavirus L1 virus-like particle vaccine on the incidence of high-grade vulvar intraepithelial neoplasia (VIN 2/3) and high grade vaginal intraepithelial neoplasia (VaIN 2/3) associated with HPV types 16 and 18, as well as its effect on the overall rates of such lesions.³⁹ The combined analysis included 18,174 women between the ages of 16 and 26 years randomized to either vaccine or placebo at day 1, and months 2 and 6. Patients underwent comprehensive anogenital exams at day 1, month 7, and every 6 to 12 months for up to 48 months. The subjects were divided into 3 groups or populations for analysis of efficacy at an average of 3 years after administration of dose 1. The first group was the “per protocol susceptible group.” This group included 7811 vaccinated subjects and 7785 placebo subjects. This population was evaluated starting at month 7 and included those subjects who had received all 3 doses of vaccine or placebo within 12 months, and were seronegative and HPV DNA negative by polymerase chain reaction (PCR) for HPV 16 or HPV 18 at day 1. The subjects in this population also remained negative by PCR for the same HPV types through month 7 and had no major protocol violations. Subjects were included even if results of cervical cytological exam at day 1 were abnormal. In this population, the recombinant quadrivalent vaccine was 100% effective in preventing VIN 2/3 or VaIN 2/3 by the

vaccine-specific types. In the placebo groups, 15 cases of these precancerous conditions were observed. The second group evaluated was referred to as the “unrestricted susceptible population”. They were evaluated starting after day 1 with consideration of variable vaccine dose intervals and included subjects who were in the per protocol susceptible analysis as well as women with protocol violations. In this group, the vaccine had 97% efficacy in prevention of VIN 2/3 or VaIN 2/3 by vaccine specific types. Only 1 case was noted in the vaccine group versus 29 in the placebo group. All vaccinated subjects were included in an analysis referred to as an ITT general study analysis. The analysis started after day 1 and included all women, including those with evidence of infection and genital disease associated with HPV 16 and/or HPV 18 prior to vaccination. In this analysis the incidence of VIN 2/3 or VaIN 2/3 by vaccine specific types in the vaccinated group was reduced by 71%. Nine cases were observed in the vaccinated subjects versus 31 cases in the placebo groups. In the ITT group, vaccine efficacy was reported as 49%, irrespective of causal HPV type as well irrespective of whether or not HPV DNA was detected in the lesion. In the ITT group there was 1 case of squamous cell perianal carcinoma in a vaccinated subject 18 months after completion of the vaccine series.

Another combined analysis of the quadrivalent HPV L1 virus-like particle recombinant vaccine versus placebo evaluated the rates of external genital disease including genital warts, VIN, VaIN, vulvar cancer, and vaginal cancer in 3 trials including Future I and Future II.⁴⁰ In the per protocol analysis the quadrivalent HPV recombinant vaccine was found to be 99% effective in preventing HPV 6-, 11-, 16-, or 18-related genital warts, and VIN or VaIN of any grade. In summary, when reviewing these pivotal quadrivalent HPV recombinant vaccine trials as well as when reviewing

meta-analyses of vaccine trials conducted,⁴¹ a significant reduction in high grade lower genital tract disease caused by vaccine type HPV types is observed particularly in HPV-susceptible subjects. An interesting and attractive finding in some vaccine trials has been cross-protection. Since HPV types have varying degrees of genetic relatedness, it had been theorized that cross-protection to phylogenetically similar non-vaccine HPV types may be observed in vaccinated women. The additional protection against non-vaccine types HPV types may prevent even more HPV-associated malignancies than initially predicted, especially against HPV 45 which is the third most common HPV type implicated in cervical carcinoma and cervical adenocarcinoma.⁴² Cross-protection remains a theoretical benefit at this time and remains under investigation. Support of vaccination with the quadrivalent HPV recombinant vaccine in early adolescence was arrived at based on bridging studies than proved that vaccine-induced neutralizing antibody responses in 10- to 15-year-old girls and boys were non-inferior and in fact higher than those observed in 16- to 23-year-old females.⁴³ Based on this immunogenicity bridging, the efficacy of Gardasil® in 9- to 15-year-old girls is inferred.

Safety data

Safety data from the quadrivalent HPV vaccine trials have been evaluated and appear extremely favorable. In the 4 placebo-controlled trials local and systemic events were monitored. Approximately 83% of vaccine recipients reported local site reactions within 5 days after any dose compared to 77% of subjects receiving aluminum adjuvant placebo. The most common local reactions reported included pain, swelling, or redness at the injection site and the majority of these reactions were mild to moderate in severity. Systemic reactions were also monitored in the 4 placebo-controlled trials. Both vaccinated and placebo recipients had comparable systemic adverse events within 15 days after any dose (59% versus 60%). The most common systemic events in both Gardasil® and placebo groups included headache and nausea.

Serious adverse events were comparable in number between Gardasil® recipients and placebo groups (136 versus 125). There were 10 deaths in subjects receiving Gardasil® and 7 among placebo recipients. The most common cause of death was motor vehicle accident (4 in Gardasil® group versus 3 in placebo group) followed by suicide/overdose (1 in Gardasil® group versus 2 in placebo group) and thromboembolism (1 case in each group). Rare events included 2 cases of sepsis in the Gardasil® group, 1 case occurring 395 days post dose

3 and another occurring in a subject 625 days post dose 3. Additionally, 1 case of pancreatic cancer was reported in the Gardasil® group 578 days following dose 3, and 1 case of arrhythmia 27 days post dose 1 in a young male with a family history of arrhythmia. In the placebo group 1 case of asphyxia was reported.

The pregnancy outcomes in pregnant subjects receiving either Gardasil® or placebo were comparable. Gardasil® recipients had similar rates of live births (62%) compared to placebo recipients (60%). The rate of spontaneous abortion was also similar between the 2 groups and approximated 25%. The rate of adverse events and occurrences in pregnant subjects is similar between the 2 groups and included conditions leading to cesarean section, premature labor, and pre-eclampsia. Congenital anomalies were also monitored and the incidence was similar in both vaccinated and placebo groups. Data on infants of nursing mothers have also been evaluated and there was a higher proportion of cases of respiratory illnesses and gastroenteritis among infants of mothers administered Gardasil® during the time they were breast-feeding: 12 cases of respiratory illnesses in the Gardasil® group compared to 6 cases in the placebo group, and 5 cases of gastroenteritis in the Gardasil® group compared to 2 cases in the placebo group. All cases of respiratory events occurred in the Latin American region and the majority of the subjects received further vaccine without additional observed respiratory events in their infants. Due to the small number of events and the variable times between vaccination and events, a definitive association could not be made. The post-market recommendations noted in the package insert advise that the vaccine is not recommended for use in women known to be pregnant and urges caution with administration to nursing mothers. Merck & Co. has established a pregnancy registry in the US to prospectively collect data on spontaneously reported exposures to Gardasil® during pregnancy.

Since introduction of the quadrivalent HPV recombinant vaccine on June 8, 2006 in the US, the FDA and the Vaccine Adverse Event Reporting System (VAERS) have been monitoring the safety of the vaccine.⁴⁴ VAERS receives unconfirmed reports of possible side effects. The FDA analyzes adverse events and possible side effects associated with individual lots or batches of vaccine to identify any unusual patterns. To date no unusual patterns have been observed in the FDA's review of the quadrivalent HPV recombinant vaccine.

Over 16 million doses of Gardasil® have been given in the US to date. Data received by VAERS on post-vaccination events may or may not be caused by vaccination. As of June 30, 2008 there have been 9749 VAERS reports of adverse events following Gardasil® vaccination. Ninety-four percent of these

Table 4 Analysis of efficacy of Gardasil® in the PPE^a population of 16- through 26-year-old women for vaccine HPV types

Population	Gardasil®		AAHS control		% Efficacy (95% CI)
	N	Number of cases	N	Number of cases	
HPV 16- or 18-related CIN 2/3 or AIS					
Future I	2201	0	2222	36	100.0 (65.1, 100.0)
Future II	5306	2	5262	63	96.9 (88.2, 99.6)
HPV 6-, 11-, 16-, or 18-related CIN (CIN 1, 2/3) or AIS					
Future I	2241	0	2258	77	100.0 (95.1, 100.0)
Future II	5388	9	5374	145	93.8 (88.0, 97.2)
HPV 16- or 18-related VIN 2/3					
Future I	2219	0	2239	6	100.0 (14.4, 100.0)
Future II	5322	0	5275	4	100.0 (-50.3, 100.0)
HPV 16- or 18-related VaIN 2/3					
Future I	2219	0	2239	5	100.0 (-10.1, 100.0)
Future II	5322	0	5275	4	100.0 (-50.3, 100.0)
HPV 6-, 11-, 16-, or 18-related genital warts					
Future I	2261	0	2279	58	100.0 (93.5, 100.0)
Future II	5404	2	5390	132	98.5 (94.5, 99.8)

^aPPE population consisted of individuals who received all 3 vaccinations within 1 year of enrollment, did not have major deviations from the study protocol, and were naïve (PCR negative and seronegative) to the relevant HPV type(s) prior to dose 1 and through 1 month post dose 3.

Abbreviations: AAHS, control, amorphous aluminum hydroxyphosphate sulfate; AIS, adenocarcinoma in situ of the cervix; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; N, number of subjects with at least 1 follow-up visit after month 7; VaIN, vaginal intraepithelial neoplasia; VIN, vulvar intraepithelial neoplasia.

events have been classified as non-serious events and 6% have been classified as serious events. The non-serious events have included syncope, pain at injection site, headache, nausea, and fever. Falls related to syncope have the potential for injury; therefore recommendations are to keep patients seated for up to 15 minutes post vaccination for observation. Twenty deaths have been reported to VAERS. A common pattern to the deaths was not identified, suggesting they were not caused by the vaccine. In cases where autopsy, death certificate, or medical records were available, none of the deaths were attributable to the vaccine. Another serious report has been post-vaccination cases of Guillain-Barré syndrome (GBS). GBS is a rare neurological disorder causing muscle weakness. The FDA and VAERS have reviewed reports of GBS submitted to VAERS and have concluded that there is no evidence that Gardasil® has increased the rate of GBS above that expected in the population. In a recent review of GBS following Gardasil® vaccination, investigators found 36 cases of GBS reported in girls and women ages 13 to 50.⁴⁵ Gardasil® was the only vaccine administered in 60% of patients while 40% of patients had received Gardasil® in combination with other vaccines. The incidence of GBS was calculated as 7.0 per million in the post-Gardasil® population compared to 4.0–10.0 per million in the general population. Another serious safety concern about

the quadrivalent HPV vaccine is thromboembolic events. It appears that individuals experiencing these disorders post vaccination had risk factors for blood clots such as the use of oral contraceptive pills. This issue continues to be studied through the Vaccine Safety Datalink (VSD) project, which is a collaborative effort between the Centers for Disease Control and managed care organizations. Anaphylaxis was not reported in any of the vaccine trials. However since the 2007 Australian implementation of the national human papillomavirus vaccination program, which provides free HPV vaccinations to females ages 12 to 26 years, a small rate of anaphylaxis has been observed.⁴⁶ There have been 269,680 HPV vaccines administered in schools with an anaphylaxis incident rate of 2.6 per 100,000 doses. Possible explanations for the difference in vaccine study groups and the Australian experience may be due to sample size. Some authorities believe that the anaphylaxis reaction in the Gardasil® vaccine may be related to the presence of polysorbate 80 which is a stabilizer with a known history of causing anaphylaxis or generalized hypersensitivity reactions.

Other pressing issues

Thus far we have reviewed the excellent efficacy of the recombinant quadrivalent HPV vaccination in the prevention

of the cervical, vulvar and vaginal precancerous states and cancers noting that protection seems superior in the sexually naïve and HPV susceptible recipient. The impressive safety data during vaccine trials as well as in the post-marketing era has also been reviewed. Remaining issues of significant importance are many and include duration of immunity and the possibility of the need for “booster” immunization with attendant adjustment of cost-effective formulas for this added expense. Studies have shown quadrivalent HPV recombinant vaccine immunity through 5 years⁴⁷ and Merck & Co. have committed to continue monitoring immunogenicity through the Nordic Long-term Follow-up Study. Therefore the optimal age for vaccination as well as cut-off age for vaccination must be wisely determined with priority for the sexually uninitiated adolescent. Gardasil® has been approved in over 80 countries, most recommending vaccination in early adolescence. In the US, the Advisory Committee on Immunization Policies recommends vaccinating 11- to 12-year-olds but allows vaccination as early as 9 years of age with “catch-up” vaccines until age 26. It is of utmost importance to realize that secondary prevention of cervical cancer through continued Pap smear screening cannot be abandoned in vaccinated populations. As previously mentioned, HPV types 16 and 18 appear responsible for 70% of cervical cancers but the remaining 30% of cervical cancers are due to other HPV types. There are reported cases of cervical cancers⁴⁸ and also of vulvar carcinoma in vaccinated women, highlighting the importance of continued surveillance with cytology screening and routine gynecological exams to monitor for cervical and genital disease regardless of cause. The exact nature of cross-protection against other non-vaccine HPV types must be investigated as this may provide wider protection than previously thought. The goal of vaccination ideally should be to provide lifelong protection but others view the role of the vaccine in the context of reducing disease and maintaining effectiveness during ages of peak oncogenic HPV exposure. Male vaccination is also a controversial topic. Vaccination for HPV types 16/18 in combination with annual Pap smear screening commencing at age 18 was estimated in a Markov model to offer the largest overall reduction in cancer incidence and mortality at a cost of US\$236,250 per life-year gained for female-only vaccination.⁴⁹ The addition of male vaccination has been projected to further reduce cervical cancer incidence by only 2.2% at an incremental cost-effectiveness ratio of US\$442,039/quality-adjusted life-year (QALY). Some countries such as Australia have elected to vaccinate males while others have not. However, no country has included male vaccination in its publicly financed

immunization programs. Several arguments have been posed to support male vaccination, including prevention of non-genital HPV-related disease, prevention of genital warts, and reduction of disease through herd immunity. In men, about 50% of penile cancers are linked to oncogenic HPV.⁵⁰ In December 2008 Merck & Co. presented the FDA with data on male vaccination with Gardasil® evaluating penile intraepithelial neoplasia, and the FDA may update recommendations for males based on these data. Another area of discussion has been the concern that although prophylactic HPV vaccines show protection in genital premalignant disease, they have not been studied long enough to definitely demonstrate prevention of genital malignancy, particularly cervical cancer. A limitation to definitive proof is the lengthy period of time between HPV exposure and development of cervical cancer. Phase III clinical studies are underway enrolling sufficient numbers of subjects to guarantee enough power to enable evaluation of data on efficacy of vaccination against carcinoma in situ of the cervix and higher by the year 2020.⁵¹ Another area of concern is niche selection which is the possibility that as HPV 16 and 18 are eliminated, other currently less common oncogenic types will become more pervasive and lead to vaccine modification in order to address disease from those types. Acceptance of the vaccine represents an obstacle. Resistance to the HPV vaccine in some cases is based on religious objections from groups that believe the availability of a vaccine that prevents a sexually transmitted disease is a license to engage in premarital sex. In the United States groups with this philosophy as well as groups with the concern that the HPV vaccines undermine abstinence-only programs and promote promiscuity have strongly objected to mandated vaccination programs despite the proposal of “opt-out” options. Unfortunately, wide acceptance and availability of vaccines may not come to fruition unless there are government guidelines and programs in place to not only require the vaccine but also to make it available to those who cannot afford it. Another important role of government which is linked to acceptance is public education in matters related to disease prevention. It has been shown that in terms of vaccine acceptance, developing public health messages that focus on HPV infection and its link to cervical cancer to educate parents may have the greatest impact on improving the uptake of the vaccine.⁵² By far the greatest challenge pertaining to the quadrivalent HPV recombinant vaccine is its affordability particularly for developing nations where burden of disease is the highest, secondary screening programs are suboptimal and large gaps in education exist. Hopefully, organizations such as

the Global Alliance for Vaccines and Immunization (GAVI Alliance), which provides technical assistance and financial support for vaccines in countries with gross national income of less than US\$1,000 per capita as well as China, India, and Indonesia, will make an impact in the most distressed areas of the globe. Currently the GAVI Alliance has made a commitment to provide girls in struggling nations with HPV vaccines and is trying to overcome funding shortages to do so. It is important to view the prophylactic HPV vaccines as an important part of the multifaceted strategy to prevent cancers of the cervix, vagina, and vulva. Other important parts of the strategy must include continued secondary prevention programs and education on lifestyle modifications to reduce risks and disease.⁵³ Despite unanswered questions and differing opinions as to how to best utilize the quadrivalent HPV recombinant vaccines, we must recognize that we have an invaluable tool in our hands – a tool that could eventually lead to disease eradication. Time will tell if we are wise and visionary stewards of this medical legacy.

Acknowledgments and disclosures

Merck & Co., Inc. for providing technical information. American Society for Cervical Pathology and Colposcopy for Educate the Educators: HPV and the New HPV Vaccines Program.

The author reports no conflicts of interest related to the writing of this article.

References

- Chang MH. Cancer prevention by vaccination against Hepatitis B. Recent results. *Cancer Res.* 2009;181:85–95.
- De Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of human papillomaviruses. *Virology.* 2004;324(1):17–27.
- Chan SY, Delius H, Halpern AL, Bernard HU. Analysis of genomic sequences of 95 papillomavirus types: uniting typing, phylogeny and taxonomy. *J Virol.* 1995;69(5):3074–3083.
- IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Human papillomaviruses. Vol 64. Lyon (France): International Agency for Research on Cancer; 1995.
- Clifford G, Franceschi S, Diaz M, Munoz N, Villa LL. Chapter 3: HPV type-distribution in women with and without cervical neoplastic disease. *Vaccine.* 2006;24(Suppl 3):S26–S34.
- Smith J, Backes D, Hoots R, Kurman R, Pimenta J. Human papillomavirus type-distribution in vulvar and vaginal cancers and their associated precursors. *Obstet Gynecol.* 2009;113(4):917–924.
- Insinga R, Liaw KL, Johnson L, Madeleine M. A systemic review of the prevalence and attribution of human papillomavirus types among cervical, vaginal, and vulvar precancers and cancers in the United States. *Cancer Epidemiol Biomarkers Prev.* 2008;17(7):1611–1622.
- Hoots BE, Palefsky JM, Pimenta M, Smith JS. Human Papillomavirus type distribution in anal cancer and anal intraepithelial lesions. *Int J Cancer.* 2009;124(10):2375–2383.
- D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med.* 2007;356(19):1944–1956.
- Myers E. The economic impact of HPV vaccines; not just cervical cancer. *Am J Obstet Gynecol.* 2008;198(5):487–488.
- Centers for Disease Control. The national breast and cancer cervical cancer early detection program-reducing mortality through screening. http://www.cdc.gov/cancer/nbcccdep/bccpdfs/BCC-FS2003_Update.pdf. 2006. Accessed Aug 24, 2009.
- American Cancer Society. Cancer facts and figures 2008. <http://www.americancancersociety.org>. Accessed Aug 24, 2009.
- Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics. *CA Cancer J Clin.* 2005;55(2):74–108.
- Wallin KL, Wiklund F, Angstrom T, Bergman F, Stendahl U, Wadell G, et al. Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. *N Eng J Med.* 1999;341(22):1633–1638.
- Levi JE, Kleter B, Quint WG, Fink MC, Canto CL, Matsubara R, et al. High prevalence of human papillomavirus (HPV) infection and high frequency of multiple HPV genotypes in human immunodeficiency virus-infected women in Brazil. *J Clin Microbiol.* 2002;40(9):3341–3345.
- Penn I. Cancers of the anogenital region in renal transplant patients. *Cancer.* 1986;58(3):611–616.
- Herrington CS. Human papillomavirus and cervical neoplasia. II. Interaction of HPV with other factors. *J Clin Path.* 1995;48(1):1–6.
- Smith JS, Herrero R, Bosetti C, Munoz N, Bosch FX, Eluf-Neto J, et al. Herpes simplex virus-2 as a human Papillomavirus cofactor in the etiology of invasive cervical cancer. *J Natl Cancer Inst.* 2002;94(21):1604–1613.
- Plummer M, Herrero R, Franceschi S, Meijer CJ, Snijders P, Bosch FX, et al. Smoking and cervical cancer: pooled analysis of the IARC multicentric case-control study. *Cancer Causes Control.* 2003;14(9):805–814.
- Moreno V, Bosch FX, Munoz N, Meijer CJ, Shah KV, Walboomers JM, et al. International agency for research on cancer. Multicentric cervical cancer study group. Effect of oral contraceptives on risk of cervical cancer in women with human Papillomavirus infection; the IARC multicentric case-control study. *Lancet.* 2002;359(9312):1085–1092.
- Einstein MH, Lanza S, Chiu LG, Schlecht NF, Goldberg GL, Steinberg BM, et al. Genetic variants in TAP are associated with high-grade cervical neoplasia. *Clin Cancer Res.* 2009;15(3):1019–1023.
- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. Cancer Statistics 2008. *CA Cancer J Clin.* 2008;58:71–96.
- Creasman WY. Vaginal cancers. *Curr Opin Obstet Gynecol.* 2005;17(1):71–76.
- Merino MJ. Vaginal cancer: the role of infectious and environmental factors. *Am J Obstet Gynecol.* 1991;165:1255–1262.
- Shah CA, Goff BA, Lowe K, Peters WA. Factors affecting risk of mortality in women with vaginal cancer. *Obstet Gynecol.* 2009;113(5):1038–1045.
- U.S. Cancer Statistics Working Group. United States cancer statistics; 1999–2002 incidence and mortality Web based report. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute; 2005. <http://www.cdc.gov/cancer/npcr/uscs>. Accessed Aug 24, 2009.
- [Info.cancerresearchuk.org/cancerstats/types/vulva](http://info.cancerresearchuk.org/cancerstats/types/vulva).
- Iwasawa A, Nieminen P, Lehtinen M, Paavonen J. Human Papillomavirus in squamous cell carcinoma of the vulva by polymerase chain reaction. *Obstet Gynecol.* 1997;89(1):81–84.
- Berek JS, Hacker NF, eds. *Practical Gynecologic Oncology*. 3rd edition. Philadelphia: Lippincott Williams & Wilkins; 2000.
- Sideri M, Jones RW, Wilkinson EJ, Preti M, Heller DS, Scurry J, et al. Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. *J Reprod Med.* 2005;50(11):807–810.
- Jones RW, Rowan DM, Stewart AW. Vulvar intraepithelial neoplasia: aspects of the natural history and outcome in 405 women. *Obstet Gynecol.* 2005;106(6):1319–1326.
- Villa LL, Ault KA, Giuliano AR, Cosota RL, Petta CA, Andrade RP, et al. Immunologic responses following administration of vaccine targeting human Papillomavirus types 6, 11, 16, 18. *Vaccine.* 2006;24(27–28):557–583.

33. Koutsky LA, Ault KA, Wheeler CM, Brown DR, Alvarez FF, Chiacchierini LM, et al. A controlled trial of human papillomavirus type 16 vaccine. *N Engl J Med.* 2002;347(21):1645–1651.
34. Mao C, Koutsky LA, Ault KA, Wheeler CM, Brown DR, Wiley DJ, et al. Efficacy of human papillomavirus-6 vaccine to prevent cervical intraepithelial neoplasia. *Obstet Gynecol.* 2006;107(1):18–27.
35. Villa LL, Costa RLR, Petta CA, et al. Prophylactic quadrivalent human Papillomavirus (types 6, 11, 16, 18) L1 virus-like particles vaccine in young women: A randomized double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol.* 2005;6(5):271–278.
36. Villa LL, Costa RLR, Petta CA, Andrade RP, Paavonen J, Iversen OE, et al. Efficacy of prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through up to 5 years of follow-up. *Br J Cancer.* 2006;95(11):1459–1466.
37. Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N Eng J Med.* 2007;356(19):1928–1943.
38. Future II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med.* 2007;356(19):1915–1927.
39. Joura EA, Leodolter S, Hernandez-Avila M, Wheeler CM, Perez G, Koutsky LA, et al. Efficacy of a quadrivalent prophylactic human papillomavirus (types 6, 11, 16, 18) L1 virus-like particle vaccines against high-grade vulval and vaginal lesions: a combined analysis of three randomized clinical trials. *Lancet.* 2007;369(9574):1693–1702.
40. Villa L. Efficacy of a quadrivalent HPV (types 6,11,16, 18) L1 VLP vaccine against external genital disease: a combined analysis. 6th European Research Organization on Genital Infection and Neoplasia (EUROGIN), Paris, France. April 23–26, 2006:4–24.
41. Rambout L, Hopkin L, Hotton B, Fergusson D. Prophylactic vaccination against human papillomavirus infections and disease in women: a systemic review of randomized control trials. *CMAJ.* 2007;177(5):469–479.
42. Brown DR, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, Wheeler CM, et al. The impact of quadrivalent human papillomavirus (HPV types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic non-vaccine HPV types in generally HPV-naïve women aged 16–26 years. *J Infect Dis.* 2009;199(7):926–935.
43. Block SL, Nolan T, Sattler C, Barr E, Giacoletti KE, Marchant CD, 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 adults. *Pediatrics.* 2006;118(5):2135–2145.
44. Information from CDC and FDA on the Safety of Gardasil Vaccine. <http://www.fda.gov/cber/safety/gardasil071408.htm>. Accessed Aug 24, 2009.
45. Press release. <http://www.aan.com/press/index.cfm?fuseaction=release.view&release=692>. Accessed Aug 24, 2009.
46. Brotherton HM, Gold MS, Kemp AS, McIntyre PB, Burgess MA, Campbell-Llyod S, et al. Anaphylaxis following quadrivalent human papillomavirus vaccination. *CMAJ.* 2008;179(6):525–533.
47. Olsson Se, Villa LL, Costa RI, Petta Ca, Andrarde RP, Malm C, et al. Induction of immune memory following administration of prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine. *Vaccine.* 2007;25(26):4931–4939.
48. Beller U, Abu-Rustum NR. Cervical cancers after human papilloma vaccination. *Obstet Gynecol.* 2009;113(2 Pt 2):550–552.
49. Kulasingam SL, Myers ER. Potential health and economic impacts of adding a Human Papillomavirus vaccine to screening programs. *JAMA.* 2003;290(6):781–789.
50. Krustup D, Jensen HL, van den Brule AJ, Frisch M. Histological characteristics of human Papillomavirus-positive and negative invasive and in situ squamous cell tumours of the penis. *Int J Exp Pathol.* 2009;90(2):182–189.
51. Lehtinen M, Apter D, Dubin G, Kosunen E, Isaksson R, Korpivaara E, et al. Enrolment of 22,000 adolescent women to cancer registry follow-up for long-term human papillomavirus vaccine efficacy: guarding against guessing. *Int J STD AIDS.* 2006;17(8):517–521.
52. Wong LP. Preventing cervical cancer through human papillomavirus vaccination: perspective from focus groups. *J Low Genital Tract Dis.* 2009;13(2):85–93.
53. Diaz ML. Human papillomavirus – prevention and treatment. *Obstet Gynecol Clin North Am.* 2008;35(2):199–217, vii–viii.

International Journal of Women's Health

Publish your work in this journal

The International Journal of Women's Health is an international, peer-reviewed open-access journal publishing original research, reports, reviews and commentaries on all aspects of women's healthcare including gynaecology, obstetrics, and breast cancer. Subject areas include: Chronic conditions (migraine headaches, arthritis, osteoporosis);

Submit your manuscript here: <http://www.dovepress.com/international-journal-of-womens-health-journal>

Dovepress

Endocrine and autoimmune syndromes; Sexual and reproductive health; Psychological and psychosocial conditions. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.