ABSTRACT
Gardasil® is the first quadrivalent human papillomavirus (HPV)-types 6, 11, 16, 18 recombinant vaccine approved by the FDA on June 8, 2006. It induces genotype-specific virus-neutralizing antibodies and prevents infection with HPV. Various clinical trials demonstrated a reduction in the incidence of vaccine-type-specific persistent infections and of associated moderate- and high-grade cervical dysplasias and carcinomas in situ after its use. Gardasil is currently approved by FDA for prevention of genital warts, cancers and precancerous conditions of cervix and vulva in 9-26 years old females. Three doses of 0.5 ml of gardasil each at 0, 2 and 6 months are given intramuscularly. It is contraindicated in individuals who are hypersensitive to the active substances or to any of the excipients of the vaccine, patients with bleeding abnormalities or patients on anticoagulant therapy and during pregnancy. However, the vaccine, at an estimated $300-500 per course, is too expensive for many women in developing countries. Moreover, question regarding the longevity of the protection by vaccine is still unsolved. Hence, longer studies are required to establish its real status in cancer prevention.

KEY WORDS: Cervical cancers, quadrivalent human papillomavirus, recombinant vaccine

Microorganisms are responsible for 10-20% of all human tumors, and vaccines against viruses (hepatitis B virus, human papillomaviruses) that cause cancer are of the utmost importance in primary cancer prevention. Infection with oncogenic types of human papillomavirus (HPV) is the major risk factor for the development of malignancies in the uterine cervix. HPV is a double-stranded DNA tumor virus of the papovavirus family. The virus infects basal epithelial layers in the transformation zone of the cervix, where the most vulnerable (stem) cells are found. HPV types 16 and 18 account for nearly 70% of cases of cervical cancer, AIS (noninvasive cervical cancer), cervical intraepithelial neoplasia (CIN) 3, vulvar intraepithelial neoplasia (VIN) 2/3 and vaginal intraepithelial neoplasia (VaIN) 2/3 and for 50% of CIN 2 lesions. HPV 6 and 11 are responsible for approximately 90% of genital wart cases. These four types of HPV also cause approximately 35-50% of all low-grade cervical, vaginal and vulvar lesions (CIN 1, VIN I and VaIN I). As cervical cancer is associated with infection with high-risk types of HPV, anti-viral vaccination strategies have great potential in the prevention of cervical cancers.

INACTIVATED/LIVE ATTENUATED VACCINE VS. PURIFIED VIRUS-LIKE PARTICLES (VLPs) VACCINE
It is difficult to generate attenuated HPV virus for vaccine purposes, as HPV virus could not be propagated in culture. Virus replication and assembly is tightly linked to the differentiation program of epithelial cells. Infectious virions are produced only in the terminally differentiated cell and are shed as virus-laden squamous cells. This explains why HPV cannot grow in tissue culture. However, using recombinant DNA technology and novel cell-culture systems, papillomavirus L1 capsid protein, which contains the immunodominant neutralization epitopes, has been developed in vitro. These VLPs have potential to induce high levels of neutralizing antibodies.

Gardasil® by Merck is the first vaccine for cervical cancers approved by the FDA on June 8, 2006. Gardasil®, quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine was prepared from highly purified VLPs of the major capsid (L1) protein of HPV types 6, 11, 16, 18. The L1 proteins are produced by fermentation in recombinant Saccharomyces cerevisiae (in a media containing vitamins, amino acids, mineral salts and carbohydrates). After obtaining these from yeast cells by diffusion, the VLPs are further purified by a series of chemical and physical processes. Purified VLPs are adsorbed on preformed aluminum containing adjuvants (amorphous aluminum hydroxyl phosphate sulfate). Each 0.5 ml of gardasil contains 20 µg of HPV6L1 protein, 40 µg of HPV11L1 protein, 20 µg...
of HPV18L1 protein, 20 µg of HPV16L1 protein, 226 µg of aluminum, 9.56 mg of sodium chloride, 0.78 mg of L-histidine, 50 µg of polysorbate 80, 35 µg of sodium borate and water.

**MECHANISM OF ACTION**

HPVs are small, non-enveloped viruses with about 8-kb circular genome encoding two structural proteins L1 and L2 and several nonstructural proteins. These proteins are important for the virus life cycle but are not incorporated into virions. HPVs infect cells of the basal epithelial layer after micro-trauma or erosion of the overlying epithelial layers. HPVs escape the immune system by limiting most of the viral gene expression and viral replication to supra-basal cell layers. Hence, HPV infections remain there for months or years. HPV oncogenes that are expressed in these cells are involved in their transformation, immortalization and progression towards malignancy. More than 100 genotypes (based on DNA sequences of the L1, E6 and E7 genes) of HPV have been described. The late L1 and L2 genes code for the viral capsid proteins, the early proteins E1 and E2 are responsible for viral replication and transcription, E4 aids virus release from infected cells and E6 and E7 encode the main transforming proteins. HPV E6 is able to interact with p53 (tumor suppressor genes), leading to its dysfunction. E6 also keeps the telomerase length above its critical point, thus inhibiting apoptosis of the cells. HPV E7 binds to retinoblastoma protein (pRb) and activates genes responsible for tissue proliferation. E5 has also been implicated in cellular transformation.

L1 protein of the vaccine has the intrinsic ability to self-assemble into VLPs. These VLPs can induce high levels of neutralizing antibodies IgG. Vaccine based on HPV virus-like particles induces genotype-specific virus-neutralizing antibody and prevents infection with HPV. Hence, gardasil acts by producing humoral immune response.

**CLINICAL TRIALS**

The interval between infection and the development of invasive cancer is more than 10 years.[6] Hence, FDA vaccine advisory panel recommended a reduction in the incidence of vaccine-type-specific persistent infections and of associated moderate- and high-grade cervical dysplasias and carcinomas in situ as end points for determining vaccine efficacy.[8]

In a randomized double-blind placebo-controlled phase II study, 277 women (mean age 20.2 years) were randomly assigned to quadrivalent HPV (20 microg type 6, 40 microg type 11, 40 microg type 16 and 20 microg type 18) L1 VLP vaccine; and 275 women (mean age 20.0 years) to one of the two placebo preparations.[13] They received placebo or vaccine at day 1, month 2 and month 6.[15] All participants underwent regular gynecological examinations, cervicovaginal sampling for HPV DNA, testing for serum antibodies to HPV and Pap testing for 36 months. Combined incidence of persistent infection with HPV 6, 11, 16 or 18 fell by 90% (95% CI, 71-97; P < 0.0001) in the vaccine group compared with the placebo group.[13]

In a double-blind study, 2,392 young women (16-23 years) were randomized to receive three doses of placebo or HPV-16 VLPs vaccine (40 µg per dose) at day 0, month 2 and month 6.[16] The women were followed for a median of 17.4 months after completing the vaccination regimen.[14] Genital samples to test for HPV-16 DNA were obtained at enrollment, 1 month after the third vaccination and every 6 months thereafter. After the third dose, the geometric mean titer (GMT) of HPV-16 antibodies was 1,510 mMU (milli Merck units) per milliliter among women who received HPV-16 vaccine; and less than 6 mMU/ml among women in the placebo group.[14] The incidence of persistent HPV-16 infection was 3.8 per 100 woman-years at risk in the placebo group and 0 per 100 woman-years at risk in the vaccine group (100% efficacy; 95% confidence interval, 90-100; P < 0.001).[14] However, 9 cases of HPV16-related CIN occurred in the placebo recipients.[14]

In extended randomized double-blind, placebo-controlled clinical trial, a total of 2,391 women (16-23 years) were given either 40 µg HPV16L1 VLP vaccine or placebo intramuscularly at day 1, month 2 and month 6.[15] Among 750 placebo recipients, 12 women developed HPV16-related CIN2-3; whereas no case was seen in 755 vaccine recipients.[15] There were 111 cases of persistent HPV-16 infection in placebo recipients and 7 cases in vaccine recipients (vaccine efficacy 94%; 95% CI, 88-98).[15] After immunization, HPV-16 serum antibody GMT peaked at month 7 (1,519 mMU/mL), declined through month 18 (202 mMU/mL) and remained stable from 30th to 48th month (128-150 mMU/mL).[15] Hence, the above study clearly indicated that vaccine provides high-level protection against persistent HPV-16 infection and HPV16-related CIN2-3 for at least 3.5 years after immunization.

In a clinical trial to assess the immunogenicity of the vaccine, 1,106 young women were randomized to receive a quadrivalent HPV (types 6/11/16/18) L1 VLP vaccine or placebo formulation.[16] At month 2 (post-dose 1), among women with vaccine-type antibodies at baseline, vaccine-induced anti-HPV responses were approximately 12- to 26-fold higher than those observed in baseline-naïve women.[14] Following an initial similar-sized decline, anti-HPV responses plateaued and remained stable through end of study (3.0 years).[16] No vaccine-related serious adverse experiences were reported.

In another recent randomized double-blinded controlled trial, 1,113 women (15-25 years) were randomized to receive three dosages of either the vaccine, formulated with 500 µg aluminum hydroxide and 50 µg 3-deacylated monophosphoryl lipid (AS04) adjuvant, or placebo at 0, 1 and 6 months.[13] Women were followed for 27 months. Vaccine efficacy was found to be 91.6% against incident infection.
and 100% against persistent infection with HPV-16/18. In the intent-to-treat analyses, vaccine efficacy was found to be 95.1% against persistent cervical infection with HPV-16/18 and 92.9% against cytological abnormalities associated with HPV-16/18 infection.[] TIl date, the clinical studies available are of short duration, and hence long-term trials are required to evaluate the duration of protection provided by the vaccine and the need for revaccination.

**Therapeutic uses**
The FDA approved gardasil for the prevention of cervical cancer, cervical pre-cancers (CIN2/3 and AIS), vulvar pre-cancers (VIN2/3) and vaginal pre-cancers (VaIN 2/3) caused by HPV types 16 and 18. It is also approved for the prevention of genital warts and low-grade cervical lesions (CIN 1) caused by HPV types 6, 11, 16 and 18. It is approved for use in 9-26 years old girls and women.[10] Gardasil is also being investigated for its use in preventing HPV16-/HPV18-caused anal cancer.[11] A 2005 study in San Francisco found that 95% of HIV-infected gay men also had anal HPV infection, of which 50% had precancerous HPV-caused lesions.[12]

**Dose and route of administration**[6]
Three doses of 0.5 ml of gardasil each at 0, 2 and 6 months are given intramuscularly.

**ADVERSE DRUG REACTIONS**[6,10,14]

Vaccine-related adverse experiences that were observed in clinical trials at a frequency of at least 1.0% were pain, swelling, erythema, fever and pruritus. Systemic side effects (>1%) like nausea, nasopharyngitis, dizziness, diarrhea, vomiting, myalgia, cough, toothache, upper respiratory tract infection, malaise, arthralgia, insomnia and nasal congestion were also seen. Serious side effects like appendicitis, gastroenteritis and pelvic inflammatory disease were also reported (0.2-0.3%). One case of bronchospasm and two cases of asthma drug interactions were reported in clinical trials. Autoimmune diseases like juvenile arthritis, rheumatoid arthritis, reactive arthritis, etc., can also occur with its use. However, Koutsky et al., demonstrated similar incidence of adverse events in the women treated with HPV-16 vaccine and placebo group.

During clinical trials including 2,266 women (1,115 vaccine vs. placebo 1,151), 5 cases of congenital anomaly (pyloric stenosis, congenital megacolon, congenital hydropneumosis, hip dysplasia, club foot) were reported in infants of women in the vaccine group, who got pregnant within 30 days of vaccination. In women who got pregnant after 30 days of vaccination, 10 cases of congenital anomaly were reported in the vaccine group as against 16 in the placebo group. In clinical trials including lactating mothers (vaccine group 500 vs. 495 placebo group), 6 infants whose mothers received vaccine within 30 days experienced acute respiratory illness against 2 infants from the placebo group.

**CONTRAINDICATIONS**[6]
This vaccine is contraindicated in individuals who are hypersensitive to the active substances or to any of the excipients of the vaccine. Although animal studies on female rats have demonstrated no effect of this vaccine on mating performance, fertility, lactation and embryonic/fetal survival, it is contraindicated in pregnancy and should be used with caution during lactation. It should not be injected or injected (if required) with extreme caution in patients with bleeding abnormalities or patients on anticoagulant therapy.

**DRUG INTERACTIONS**[6]

Efficacy of the vaccine decreases in patients on immunosuppressant drugs or in immunodeficiency states (HIV infection). It can be used successfully with hepatitis B vaccine. However, its concomitant use with other vaccines is not studied.

**LIMITATIONS AND ISSUES RELATED TO GARDASIL**[5,8,11,17]

The introduction of the HPV vaccine has great potential to assist prevention campaigns against cervical cancers. However, the vaccine does not address all high-risk HPVs, although it possesses some cross-reaction to other HPVs such as types 31 and 45. Secondly, it requires refrigeration, must be injected and consists of an initial and two booster doses, adding logistical and economic challenges to any widespread vaccination program. Another issue related to vaccine is its usefulness in women already exposed to HPV, as a large population of adolescent girls and women are sexually active and are at high risk of getting HPV infection within the first few years after sexual debut. Moreover, in older women protection by the vaccines is low, since most of them have already been exposed to the viruses. However, women infected with one type of HPV are still at risk for infection with other types; hence, there is substantial benefit in vaccinating women who are already exposed to HPV. Moreover, no commercial serum assay for type-specific HPV antibodies is available currently. There is a continued need for screening in vaccinated women for CIN and cervical cancer because there is uncertainty about the duration of its efficacy, and the oncogenic types covered by gardasil account for only 70% of cervical cancers. However, screening intervals may be increased and the age of onset of screening may be safely delayed, with subsequent cost savings.

Other issue that remains unanswered is the efficacy of the vaccine in men. Earlier, a subunit vaccine for type 2 herpes simplex virus (HSV gD vaccine), another sexually transmitted viral infection, was found to be effective in women but not in men. This could be because of the fact that HSV infection is more likely to be mucosal in women and cutaneous in men (higher antibody titers in mucosa than in skin). However, efficacy trials of the HPV vaccine in men will surely resolve this issue.
IMPACT OF VACCINATION ON THE NATURAL HISTORY OF PREVALENT HPV INFECTION[18]

Impact of vaccination on the natural history of prevalent HPV infection is another issue in question. However, the vaccine has the potential to reduce the efficiency of transmission of an early infection from one genital site to other genital sites, presumably via specific antibodies in the genital tract. Keeping in view the current infection rates, predicted treatment-regimen compliance and population demographics, gardasil has been predicted to produce a 39.0% reduction in HPV16- or HPV18-related CIN 2/3 or AIS (98.8% prophylactic efficacy); a 69.1% reduction in HPV16- or HPV18-related VIN 2/3 and VaIN 2/3 (100.0% prophylactic efficacy); a 46.4% reduction in HPV6-, HPV11-, HPV16-, HPV18-related CIN (CIN 1, CIN 2/3) or AIS (93.7% prophylactic efficacy); and a 68.5% reduction in HPV6-, HPV11-, HPV16-, HPV18-related genital warts (93.4% prophylactic efficacy) in the general population.

PRESENT STATUS OF GARDASIL

On June 1, gardasil (FDA-approved) was approved in Mexico and is under consideration for approval in Argentina, Australia, Brazil, the European Union, New Zealand, Singapore and Taiwan.[4] In December 2005, Merck announced a partnership with India’s Council of Medical Research to study it.[4] On June 29, 2006, a panel of experts, the Advisory Committee on Immunization Practices, gave their approval for the vaccination of gardasil in children as young as 9 years old.[3] They also recommended free vaccination to children under the age of 18, who are uninsured. Unfortunately, the vaccine, at an estimated $300-500 per course, is too expensive for many women in the developing countries.[49] Benefits of gardasil vaccine can be passed on to women in developing counties by advocating policies that will ensure maximum access and coverage, including school-entry requirements, catch-up vaccination and universal insurance coverage.

CONCLUSION

Gardasil is currently approved by FDA for prevention of genital warts, cancers and precancerous conditions of cervix and vulva in 9-26 years old females. However, question regarding the longevity of the protection by vaccine is still unsolved. Hence, longer studies are required to establish its real status in cancer prevention.

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