

Vaccines for human papillomavirus infection: A critical analysis

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ABSTRACT

This article takes a critical look at the pros and cons of human papillomavirus (HPV) vaccines. There is enough evidence to suggest that the prophylactic vaccines are efficacious in preventing various benign and malignant conditions (including cervical cancers) caused by HPV. Even though the vaccine is costly, hypothetical analysis has shown that HPV vaccination will be cost effective in the long run. Therapeutic HPV vaccines used to treat established disease are still undergoing evaluation in clinical studies, and results seem to be encouraging. Although several countries have started mandatory vaccination programs with the prophylactic HPV vaccines, conservatives have voiced concerns regarding the moral impact of such vaccination programs.

Key words: Cervarix, Gardasil, Human papillomavirus, Prophylactic, Therapeutic, Vaccine

INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection (STI) in the world,^[1] and the most important cause of cervical cancer, the second most common malignancy in the world.^[2] Cervical cancer is also the most frequent malignancy among rural Indian women, and India carries a fourth of world's cervical cancer burden.^[3]

Anogenital warts affect 1% of sexually active adults,^[4] and account for 15% to 20% of all STIs in many European countries, 70% in the United States, and 95% among high-risk populations in Africa.^[2] It is estimated that exposure to HPV would occur in 80% of sexually active women by the age of 50.^[2] More than 100 types of HPV have been identified,^[1] 40 of which are associated with anogenital diseases in men and women.^[2] On the basis of association with cervical cancers, 15 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82) were classified as high risk for cervical cancer, 3 as probable high risk (26, 53, 66) and 12 as low risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108).^[3]

HPV DNA is detected in 99% of cervical carcinomas^[5] – approximately 70% contain HPV types 16 or 18,^[6] of which HPV 16 is the single most common high-risk

HPV type causing anogenital cancer precursor lesions such as cervical intraepithelial neoplasia (CIN) and cervical cancers.^[2] HPV types 16 and 18 also cause 80% of anal cancer, and 30% of vaginal and vulvar cancers.^[7] In India, the predominant oncogenic HPV genotypes are HPV types 16 and 18.^[3] HPV types 6 or 11 are responsible for approximately 90% of genital warts.^[1,6] Coinfection with multiple HPV types does occur, especially in immunocompromised individuals.^[1]

Anogenital warts caused by HPV are associated with considerable physical and psychological morbidity, and, with currently available treatments options, have high recurrence rates, with numerous treatment-related adverse effects.^[1] The treatment of HPV-related genital warts and cervical diseases including cervical cancer screening program is associated with high cost, which was approximately \$5 billion in the United States in 2005, considerably higher than the combined cost for Hepatitis B virus (\$60 million), genital herpes (\$1.8 billion) and *Chlamydia* (\$2 billion).^[8] Cervical cancer affects 500,000 women worldwide every year, resulting in an annual mortality of about 200,000.^[2] Early detection by Papanicolaou (Pap) screening test has successfully decreased the incidence and mortality by cervical cancer.^[2] However, with 30% false-negative

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results inherent to a Pap test,^[2] new options for cervical cancers prevention are being explored.

Considering the prominent role played by HPV in the causation of cervical cancers, it is not surprising that HPV-based technologies are more enduringly pursued for cervical cancer prevention and control. Vaccination is a highly cost-effective approach to reduce the mortality related to HPV infection.^[9] It has been estimated that vaccine effective against 5 of the most prevalent high-risk HPV types would reduce the incidence of cervical cancer by 90%.^[1]

VIRUS-HOST INTERACTION

HPV is a nonenveloped DNA virus capable of infecting human epithelial tissues, including skin, anogenital epithelia and oral cavity mucosa.^[1] Genital HPV infection is transmitted directly by sexual contact, indirectly by contact with contaminated objects and rare vertical transmission.^[1] After gaining entry to the epithelium, the virus infects the young keratinocytes in or near the basal layer of the epithelium.^[3]

The virus does not synthesize any enzyme; hence, it is dependent on the host for its life cycle. The viral life cycle is tightly linked to the differentiation program of the infected epithelial cell.^[2] The viral encoded proteins are divided into early (E1-E8) and late (L1 and L2).^[2,10] E1 in association with E2 is believed to trigger the replication process.^[3] E4 is involved in cytoskeleton reorganization.^[9] E6 and E7 proteins of certain HPV types (“high risk” or “oncogenic” types) appear to have transforming function,^[10] and their presence is required to maintain the transformed state in cell lines.^[3] E5 may overlap with E2 and probably possess some oncogenic activity.^[10] L1 and L2 are structural capsid proteins of mature virion, where L1 makes up 80% of viral capsid.^[3,10] Upon infection, the early viral proteins are expressed in the infected basal cells and within the lower epithelial layers.^[2] As the infected cells reach the surface, the production of L1 and L2 proteins allow shedding of mature virions.^[2]

E2 functions as the principal regulator that regulates the expression of all the other viral genes, and particularly represses E6 and E7.^[9] During simple infection, HPV DNA is generally found in the cytoplasm of infected cells.^[2] However, the DNA of high-risk HPV types integrates into the host genome of the cervical tumor cells.^[2,9] Such integration commonly disrupts the

virus through the loss of virion production (L1 and L2 are not expressed),^[2] disruption of viral E2 gene^[9] with consequent overexpression of E6 and E7.^[2,3,9] E6 and E7 proteins interfere with the function of key tumor suppressor proteins p53 and retinoblastoma protein (pRb), respectively, thereby prolonging the cell cycle and suppressing apoptosis, contributing to the development of HPV-associated lesions ranging from “warty” epithelium with koilocytosis to overt malignancy.^[2,9]

IMMUNOLOGY OF HPV INFECTION

Most HPV infections are usually transient,^[2] and clinically significant genital warts develop in only about 1% of individuals infected with HPV types 6 and 11.^[1] Approximately 70% of new infections clear up within 1 year, and 91% within 2 years.^[2] Only 25% of all women infected with high-risk HPV develop CIN, and fewer than 1% will develop invasive cervical cancer.^[1] The nature of the immune response after HPV infection is not fully clear, and there is evidence that other factors such as age may also play a role.^[1]

Neutralizing antibodies, which are type specific with little or no cross-reactivity, prevent new infection of the keratinocytes,^[1] but they do not eradicate established infection because of intracellular location of the virus.^[9,11] Humoral immune response after HPV infection is relatively poor, probably because the virus is neither cytolytic, nor does it have blood-borne stage.^[1,10] HPV infections are mostly cleared by cell-mediated immunity (CMI), in which dendritic cells (DC), CD4+ T helper cells and CD8+ T cells play predominant roles.^[1,9]

Clearance of the infection with high-risk HPV types is very important, as the persistence of such an infection is required for the development of precancerous and cancerous lesions.^[1] Patients with cervical cancer have deficient CMI responses to both L1 and E7 peptides, and interleukin 2 production after stimulation with E6 and E7 peptides is deficient in women with high-grade CIN or cancer, suggesting that development of cancer is associated with failure of protective CMI responses to HPV.^[12] It has been found that the number of epithelial antigen-presenting cells (DC and Langerhans cells) is reduced in HPV-induced lesions, and Langerhans cells have impaired ability to present antigens and induce lymphocyte proliferation resulting in insufficient

CMI.^[1] There is also low HPV-specific cytolytic T lymphocyte (CTL) activity, which may be due to a certain degree of immunological tolerance or ignorance for the HPV-derived antigens.^[13]

Villada *et al.*^[14] showed that, in women with grade 3 vulvar intraepithelial neoplasia, there were neither any detectable anti-HPV T-cell responses in the blood nor any T-cell infiltrates in the lesional skin, and that the spontaneous clearance of the lesion in one patient was associated with high frequency of anti-E6 and anti-E7 effector blood T cells, as well as a marked dermal infiltrate containing a majority of CD4+ T lymphocytes and an epidermal infiltrate made up of both CD4+ and CD8+ T cells. Kadish *et al.*^[12] showed that lymphoproliferative responses to specific HPV-16 E6 and E7 peptides were associated with the clearance of HPV infection and the regression of CIN.

EMERGING HPV VACCINES

Vaccination aims at preventing infection by generating neutralizing antibodies to block HPV viral infection (prophylactic vaccines), or to eliminate infection by inducing a virus-specific T cell-mediated response (therapeutic vaccines).^[9]

Prophylactic HPV vaccines prevent incident infection by inducing antibodies against HPV capsid proteins L1 and L2.^[5] However, prophylactic HPV vaccines do not provide therapeutic efficacy against preexisting HPV infections and HPV-associated lesions.^[9] Cell-mediated immunity is critical for the control of established viral infections and malignant tumors.^[9] Therapeutic HPV vaccines aim to treat established HPV infections and HPV-associated malignancies by targeting nonstructural early viral antigens of HPV, such as E6 and E7,^[15] because these proteins are expressed throughout the life cycle of the virus as well as in HPV-transformed cells^[3,9] (L1 or L2 are not expressed by HPV-infected basal keratinocytes and HPV-transformed cells^[15]). The concept of “chimeric vaccine” (virus-like particles along with early viral proteins) is attractive because such a vaccine, at least in theory, has both therapeutic and protective functions.^[10]

PROPHYLACTIC VACCINES

Genetic diversity and inability to culture HPV *in vitro* have made development of therapeutic vaccines difficult.^[10] It has been observed that, when L1 and L2 proteins are expressed *in vitro*, they self-assemble

into a structure identical to the viral capsid known as “virus-like particles” (VLP).^[10,16,17] The VLP induces a humoral immune response similar to a live virion but does not produce infection in the recipient because it lacks viral nucleic acid.^[2]

Zhou *et al.* led the way to prophylactic vaccine in 1991 by demonstrating that the HPV-16 L1 capsid proteins self-assembled into conformational VLP that resembled native virions in a recombinant system.^[2] HPV L1 virus-like particles show great promise as prophylactic HPV vaccines in ongoing clinical trials, but L2-based preventative vaccines are yet to be tested in patients.^[5] The L2 protein is located more internally in the viral capsid; therefore, anti-L2-antibodies are less potent than anti-L1 antibodies.^[17] Virus-neutralizing anti-L1 antibodies are essentially type-specific, whereas anti-L2 antibodies appear to show some cross-reactivity to heterologous HPV types.^[17]

VLPs have shown to be highly immunogenic and also shown to elicit higher titers of neutralizing antibodies in many animal studies as well as early human studies.^[3] Villa *et al.*^[18] showed 12- to 26-fold stronger antibody response 2 months after vaccination with quadrivalent HPV types 6,11,16,18 L1 VLP vaccine in women with vaccine-type specific antibodies at baseline. A randomized control trial with VLP vaccine of HPV 16 L1 capsid protein showed 100% efficacy against persistent HPV 16 infection and HPV 16-related cervical intraepithelial neoplasia (CIN) in women who were HPV 16 negative.^[19] Polyvalent prophylactic vaccines are desirable, as there is little cross-reactivity among the HPV types.^[10] Several studies with bivalent HPV (types 16, 18) L1 VLP vaccine and quadrivalent HPV (types 6, 11, 16 and 18) L1 VLP vaccines have shown good efficacy in terms of inducing adequate antibody response, immunogenicity, safety, prevention of incident infection and protection against HPV-related squamous intraepithelial neoplasia (SIL) [Table 1].^[18,20-26] Duration of efficacy was shown to be maintained for up to 4.5 years^[26] to 5 years^[21] after a single course of immunization.

Pinto *et al.*^[27] demonstrated that, apart from eliciting antibody response, L1 VLPs vaccine induces L1-specific T cell response (both CD4 + and CD8 + T cells and *in vitro* production of both Th1- and Th2-type cytokines). Thus, prophylactic vaccines may have some role in clearing established HPV infections by mounting a CMI response. Several studies have demonstrated

Table 1: Outcomes of studies with prophylactic HPV vaccines

Ref.	Vaccine	Study group	Follow-up period	Outcome
18	Quadrivalent HPV-6/11/16/18 L1 VLP vaccine	1106 women (baseline HPV-naïve or HPV-infected) receiving vaccine or placebo	3 years	<ul style="list-style-type: none"> • 12- to 26-fold stronger antibody response at 2 months among women with vaccine-type antibodies at baseline • After a decline, anti-HPV responses plateaued and remained stable through 3 years • No vaccine-related serious adverse reactions
20	Quadrivalent HPV-6/11/16/18 L1 VLP vaccine	Vaccine - 277 women, and placebo - 275 women	36 months	Persistent infection or disease with HPV-6, 11, 16 or 18 decreased by 90% in the vaccine group compared to placebo
21	Quadrivalent HPV-6/11/16/18 L1 VLP vaccine	552 women (16-23 years)	3 years (5 years for a subset of 241 women)	<ul style="list-style-type: none"> • Persistent infection or disease reduced by 96% at 5 years • No cases of HPV 6/11/16/18-related precancerous cervical dysplasia or genital warts in vaccine recipients • Good anti-HPV response through 5 years
22	Quadrivalent HPV-6/11/16/18 L1 VLP vaccine	18,174 women irrespective of baseline HPV status (Vaccine = 9087), (Placebo = 9087)	Mean of 3 years (maximum 4 years)	<ul style="list-style-type: none"> • 100% efficacy against HPV16/18-related VIN2-3 or VaIN2-3 in HPV16/18-naïve women • 71% efficacy against HPV16/18-related VIN2-3 or VaIN2-3 in intention-to-treat women population • Overall, 49% efficacy against all VIN2-3 or VaIN2-3 irrespective of HPV types and HPV DNA status in the lesion
23	Quadrivalent HPV-6/11/16/18 vaccine	814 women receiving vaccine or placebo	–	<ul style="list-style-type: none"> • 100% efficacy against HPV 6/11/16/18-related CIN, VIN, VaIN and condyloma acuminata • High immunogenicity
24	Bivalent HPV-16/18 L1 VLP vaccine with AS04 adjuvant	1113 women (receiving vaccine or placebo)	27 months	<ul style="list-style-type: none"> • 91.6% efficacy against incident infection and 100% against persistent infection with HPV-16/18 • In intention-to-treat analyses, 95.1% efficacy against persistent cervical HPV-16/18 infection, and 92.9% against HPV-16/18-related cytological abnormalities • Vaccine was safe and highly immunogenic
25	Bivalent HPV-16/18 L1 VLP vaccine with AS04 adjuvant	Vaccine = 9258 women, Control = 9267 women	14.8 months	<ul style="list-style-type: none"> • 90.4% efficacy against HPV-16/18-related CIN2 • Similar safety profile in both the groups
26	Bivalent HPV-16/18 L1 VLP vaccine with AS04 adjuvant	Vaccine = 393 women, Placebo = 383 women	4.5 years	<ul style="list-style-type: none"> • More than 98% seropositivity was maintained • 96.9% vaccine efficacy against incident infection (HPV-16/18) • 94.3% efficacy against persistent infection after 6 months (HPV-16/18) • 100% efficacy against persistent infection after 12 months • 100% efficacy against HPV-16/18-related CIN • Cross-protection against incident infection with HPV-45 and HPV-31 • Good long-term safety profile
19	HPV-16 L1 VLP vaccine	2392 young women (received vaccine or placebo)	17.4 months	100% efficacy against persistent HPV-16 infection and HPV-16-related CIN

CIN - cervical intraepithelial neoplasia; VIN-vulval intraepithelial neoplasia; VaIN-vaginal intraepithelial neoplasia; All studies showed HPV-type specific protection; Cross-protection was noted in one study.^[26]; The age of women in these studies ranged from 15 to 26 years

clearance of persistent HPV infections after L1 VLPs vaccination^[19-21,24,26] [Table 1]. However, it is difficult to say whether such clearance of established infections was not due to spontaneous regression, which is known occur within 6 months to 2 years.^[11] Hildesheim *et al.*^[11] did not demonstrate accelerated clearance of preexisting

HPV infection after HPV types 16,18 L1 vaccination.

Cervarix (GlaxoSmithKline Biologics) and Gardasil (Merck and Co) are recombinant vaccines against HPV.^[6] Cervarix, a bivalent vaccine, targets HPV 16 and HPV 18, which are responsible for 70% of cervical cancers.^[28]

Gardasil, a quadrivalent vaccine, also targets HPV 16 and HPV 18, plus the HPV types 6 and 11 responsible for more than 80% of genital warts.^[28] Both vaccines have been effective in preventing persistent infection with targeted HPV types and in preventing cervical intraepithelial lesions, while Gardasil has also been effective in preventing vulvar and vaginal neoplasia and genital warts.^[28] Phase I, II and III studies have demonstrated that both vaccines are well tolerated and provide an excellent immunogenicity.^[6] Gardasil has been FDA-approved for cervical malignancy, whereas Cervarix has completed phase III clinical trials but is currently still under review by the FDA.^[29]

GARDASIL

Gardasil is the first quadrivalent HPV types 6,11,16,18 recombinant vaccine to be approved by the FDA on June 8, 2006.^[16] It was prepared from the highly purified VLPs of the major capsid (L1) protein of HPV types 6,11,16,18 adsorbed on adjuvants.^[16] Advisory Committee on Immunization Practices (ACIP) has recommended Gardasil for females aged 11 to 12 years, but it may be administered in females aged 9 to 26 years.^[29] It is most effective when administered before initiation of sexual activity. Immunization schedule is completed by three intramuscular injections (preferably deltoid muscle) with 0.5 mL of Gardasil at 0, 2 and 6 months.^[30] History of abnormal findings on Papanicolaou (Pap) test, a positive HPV test result, or genital warts do not influence the decision to carry out immunization with Gardasil,^[1] and it may also be given with other vaccines.^[29,30] No HPV testing or a Pap smear is necessary before vaccination.^[1] Vaccine cost is \$360 for 3 shots, plus the cost of administration.^[8]

It has been estimated that, if the vaccine is between 70% and 100% effective, the lifetime risk of cervical cancer would be reduced to 47% and 30%, respectively, compared to an 86% risk with current screening programs.^[8] The exact duration of efficacy of the vaccine is not yet known,^[10] but studies show good efficacy for at least 5 years.^[2,21] It has not yet been determined whether booster doses are required and, if yes, how often. FDA approved Gardasil for the prevention of cervical cancers, cervical precancers (CIN 2/3 and noninvasive cervical cancers), vulvar precancers (VIN 2/3) and vaginal precancers (VaIN 2/3) caused by HPV types 16,18.^[16] It is also approved for the prevention of genital warts and low-grade cervical lesions (CIN 1) caused by HPV types 6,11,16,18.^[16]

Several states in the United States have introduced compulsory HPV vaccines for school-aged children (mandatory vaccination prior to admission to the sixth grade) into legislation.^[8] On September 22, 2006, Gardasil was approved by the European Commission for use in girls and boys aged 9 to 15 years and in females aged 16 to 26 years.^[1] Vaccination of men will decrease genital warts and penile anal cancers in men as well as provide herd immunity that will eventually reduce cervical cancer in unvaccinated women.^[1] A recent study showed good immunogenicity of HPV vaccines in males and recommended a gender-neutral HPV vaccination programs.^[31]

Gardasil has been tested in thousands of women and found to be nearly 100% effective in protecting against diseases caused by the four HPV types.^[29] Side effects are uncommon occurrence (1%).^[16] The most common adverse event was pain at the injection site, followed by swelling, erythema, fever and pruritus.^[1] Systemic side effects such as nausea, nasopharyngitis, dizziness, diarrhea, vomiting, myalgia, cough, toothache, upper respiratory tract infections, malaise, arthralgia and insomnia may be seen.^[16] Serious adverse events may occur in <0.1% individuals, reportedly bronchospasm, gastroenteritis, hypertension and vaginal hemorrhage.^[31] No definitive conclusion can be drawn from the available data regarding the risk of teratogenicity of the vaccine; however, acute respiratory illness in breastfeeding infants whose mother received the vaccine within 30 days has been reported.^[16] Contraindications to the vaccine include pregnancy, severe acute illness or hypersensitivity to the vaccine components or to yeast.^[29]

COST-EFFECTIVENESS OF GARDASIL

A study of cost-effectiveness analysis from Stanford University revealed that, if a cohort of all 12-year-old girls currently living in the United States received an effective vaccine against HPV, 1340 deaths from cervical cancers could be averted over the cohort's lifetime, and that the HPV vaccine would be cost effective, even assuming vaccine efficacy as low as 40% or that booster shots would be required every 3 years.^[32]

Another analysis reported that adding a quadrivalent HPV types 6,11,16,18 vaccine (with 98% vaccine efficacy, a lifetime duration and 85% coverage) to current cervical screening program in the United

Kingdom would be potentially cost-effective.^[33] The study also revealed that vaccination of 100,000 girls could avoid more than 400 cases of cervical cancer, 6700 cases of cervical intraepithelial neoplasia and 4750 cases of genital warts.^[33]

VIEWS OF THE OPPONENTS REGARDING MANDATORY GARDASIL VACCINATION

Prophylactic HPV vaccines have demonstrated good efficacy in the prevention of HPV-related benign and malignant conditions. There are still many voices of concerns regarding the rationale or the cost-effectiveness of these vaccines under different circumstances, and conservatives have questioned the mandatory vaccination policy for school-aged children prevailing in several states in the United States.

- The incidences of cervical cancer and related mortality have dramatically decreased in the United States in the past 30 years, mostly due to the aggressive screening measures. Consequently, the populations that would benefit the most from a vaccine are the ones in the developing countries.^[1] On the other hand, high cost associated with HPV vaccine (Gardasil) precludes its widespread use in the developing countries.
- Advisory Committee on Immunization Practices (ACIP) did not recommend vaccination in males (the European Committee recommended vaccination of boys as well). Theoretically, males should also be vaccinated against HPV to provide herd immunity. Vaccination in males will also reduce incidence of genital warts and penile and anal cancers in males. However, there are no available data on the efficacy of HPV vaccination in males. Therefore, the benefit or cost-effectiveness of vaccinating males is unknown.^[1]
- Vaccination of girls as young as 12 years for an infection that spreads almost exclusively by sexual route is likely to spark off strong argument regarding the moral impact of such a practice. Twelve years is not the appropriate age to begin sex education, and parents have voiced concerns that vaccination might increase risky sexual behavior in their children by providing a false sense of protection.^[8]
- Some parents were unwilling to give too many vaccines to their children and wanted to limit

immunizations to those that are “strictly necessary.” They rather preferred screening their children for cancer or changing their children’s lifestyles.^[8]

- There could be adverse health hazards in women who stop routine cervical cancer screening because of a false belief that the vaccine would eradicate their chance of getting cervical cancer. Continued screening is necessary because the vaccine does not prevent other cancer-causing strands of HPV or other causes of cervical cancer.
- It may be too early to presume long-term efficacy and cost-effectiveness of Gardasil. Merck spent a fortune (almost \$400,000 between 2003 and 2006) on lobbying for Gardasil to influence representatives, and the CDC has suggested that vaccine manufacturers (such as Merck) have unduly influenced vaccine policy.^[8]

CERVARIX

It is a bivalent vaccine containing HPV 16 and 18 VLPs, with an aluminum salt plus monophosphoryl lipid A (AS04) adjuvant.^[2] It is administered as three 0.5-mL intramuscular injections at 0, 1 and 6 months.^[2] This vaccine has been approved for use in Australia (for girls and women aged 10 to 45 years),^[2] but it is still under review by the US Food and Drug Administration for approval in the United States.^[11] Several studies with bivalent HPV types 16,18 VLP L1 vaccine showed more than 90% efficacy against incident infection,^[24,26] 100% efficacy against persistent infection^[24,26] and 90.4% to 100% efficacy against HPV types 16,18-related CIN.^[25,26] Vaccine is highly immunogenic,^[24] with more than 98% seropositivity after 4.5 years,^[26] and good long-term safety profile.^[26] Cross-protection against incident infection with HPV types 45 and 31 has also been seen with the bivalent HPV types 16,18 vaccine.^[26]

THERAPEUTIC VACCINES

These vaccines are used to treat established HPV infections, HPV-related cervical precancers and cancers by targeting E6 or E7 oncoproteins.^[3,5] They are especially useful to prevent recurrences after primary excision or destruction of the precancer/cancer. Although other early viral antigens show promise for vaccination against papillomas, therapeutic vaccines targeting E6 and E7 may provide the best opportunity to control HPV-associated malignancies.^[5] Various candidate therapeutic HPV vaccines are currently

being tested whereby E6 and E7 are administered in live vectors, as peptides or proteins, in nucleic acid form, as components of chimeric VLPs or in cell-based vaccines.^[5] Fusion protein vaccines are also safe, but cytotoxic T cell responses appear to be inconsistent, which may be due to the adjuvant used.^[10]

Among different forms of therapeutic HPV vaccines, DNA vaccines appear to be the most promising ones, as naked DNA is relatively safe, stable, easy to produce, able to sustain high levels of antigen expression in cells and can be repeatedly administered without the risk of antibody production.^[9] However, DNA vaccines have limited potency due to the lack of amplifying and spreading abilities and due to the suboptimal intracellular processing/presentation of tumor antigens.^[34]

Hung *et al.*^[9] elucidated various ways of enhancing the potency of DNA vaccines as summarized below: (1) delivering the DNA vaccines directly into the DC *in vivo* via gene gun; (2) promoting the spread of vaccine antigen from transfected cells to DCs by linking the antigen to proteins capable of intercellular transport (such as herpes simplex virus type 1 tegument protein VP22, bovine herpes virus VP22, Marek's disease virus VP22); (3) increasing the number of antigen-expressing or antigen-loaded DCs by linking the vaccine antigen to molecules (such as heat shock protein 70, Fms-like tyrosine kinase 3-ligand) that bind to the antigens on the surface of DCs; (4) coadministration of DNA vaccines with chemotherapeutic agents such as epigallocatechin-3-Gallate, which promote the release of antigen from apoptotic tumor cells; (5) replacement of rarely recognized codons in the vaccine gene sequences with more commonly recognized codons; (6) linking the vaccine antigen to proteins (such as *Mycobacterium tuberculosis* heat shock protein 70, γ -tubulin, calreticulin, or the translocation domain of *Pseudomonas aeruginosa* exotoxin A) that target the antigen for proteasomal degradation or entry into the endoplasmic reticulum (ER), thereby facilitating major histocompatibility complex (MHC) class I presentation of the antigen; (7) facilitating MHC II presentation for enhanced CD4+ response by linking the vaccine antigen to lysosomal-associated membrane protein type 1; (8) bypassing the stage of antigen processing in DCs by linking the vaccine gene sequence to β 2-microglobulin and an MHC class I heavy chain; (9) prolonging the life span of DCs by coadministering the vaccine peptide with the DNA encoding any of the antiapoptotic factors

(Bcl-xL, Bcl-2, X-linked inhibitor of apoptosis protein, and dominant negative mutants of caspases such as caspase-9 and caspase-8) and (10) boosting the CD4+ T cell responses as a strategy for augmenting CD8+ T cell responses. Massa *et al.*^[34] showed that fusing mutated HPV-16 E7 gene to the potato virus X coat protein sequence (PVX-CP) resulted in better immune response than mutated HPV-16 E7 vaccine alone.

Most of the candidate therapeutic vaccines are in experimental stage or in early clinical trials. In a phase I trial of HPV-16 E7 vaccine, 18 women with CIN/VIN-II/III were administered 4 immunizations of increasing doses of the vaccine each at 3-week intervals.^[35] Three weeks after the fourth immunization, complete regression of CIN was noted in 3 patients, and partial regression in 6 patients. There was also an increase in virus-specific cytolytic T cell activity and S100 + DC infiltrate. However, all biopsy samples were still positive by *in situ* RNA hybridization after vaccination.^[35] Welters *et al.*^[36] showed that HPV type 16 E6 and E7 synthetic long peptides vaccine induced broad immune response involving both CD4+ and CD8+ T cells, which could be detected up to 12 months after the last vaccination. Vaccinations with the HPV 16-derived 35 amino acid long peptide E7 (43-77), containing both a CTL epitope and a Th epitope, resulted in the induction of far more robust E7-specific CD4+ Th and CD8+ T cell responses than vaccinations with the minimal CTL epitope only.^[37] The efficacy of the therapeutic vaccines in several studies are summarized in Table 2 and results appear to be encouraging.^[38-41]

POSSIBLE ROLE OF HPV VACCINES FOR OTHER DISEASES

Anal cancer is associated with HPV infection^[2] and may be preceded by high-grade anal intraepithelial neoplasia (HGAIN), especially in certain at-risk groups, such as men who have sex with men (MSM), and immunosuppressed individuals, including those with HIV infection.^[42] However, the effect of prophylactic HPV vaccines to prevent anal HPV infection and anal cancer is yet to be determined. HPV type 16 is associated with up to 70% of lower tongue and pharyngeal cancers,^[43] and although adequate data is yet to be generated, vaccination may be preventive against these malignancies. A majority of cases of recurrent respiratory papillomatosis are caused by HPV types 6 and 11, but current data regarding the efficacy of the vaccines for this indication is insufficient.^[44] HPV

Table 2: Therapeutic vaccines in HPV-related cervical precancers and cancers

Ref.	Vaccine	Study group	Immunization schedule	Outcome
38	Mutated HPV-16 E7 linked to <i>Haemophilus influenzae</i> protein D (PD-E7), and adjuvant AS02B	7 women with HPV-positive CIN (5 with CIN 3 and CIN 2 with a CIN 1)	Three intramuscular injections at 2-week intervals	<ul style="list-style-type: none"> 5 showed significantly increased IFN-γ CD8+ cell responses 2 generated long-term T-cell immunity All patients made significant E7- and PD-specific IgG
39	TA-HPV, a recombinant vaccinia virus encoding modified HPV-16 and HPV-18 E6 and E7	18 women with HPV-16-positive high-grade VIN	Single injection into the skin by scarification over deltoid region	<ul style="list-style-type: none"> 13 women showed an increased HPV-16-specific immune response Reduction in lesion diameter of at least 50% in 8 women (4 of which also had significant symptom relief) Viral load decreased in 6 of 8 lesion-responders, and in 6 of 10 nonresponders Increase in lesional CD4 and CD8 T cells in all patients
40	TA-HPV, a recombinant vaccinia virus encoding modified HPV-16 and HPV-18 E6 and E7	12 women with high-grade HPV-positive VIN or VaIN of up to 15 years duration	Single injection into the skin by scarification over deltoid region	<ul style="list-style-type: none"> 5 (42%) patients showed at least a 50% reduction in total lesion diameter over 24 weeks Complete regression in 1 patient Overall, 83% of women showed some improvement with an average decrease in lesion size of 40% Increased specific T cell response in 6 of the 10 cases
41	TA-HPV, a recombinant vaccinia virus encoding modified HPV-16 and HPV-18 E6 and E7	29 patients with stage Ib or IIa cervical cancer	2 doses at least 4 weeks apart	<ul style="list-style-type: none"> Antibodies to HPV-16 E7 were detected in 11 of 29 patients, and overall antibodies to HPV-18 E7 were detected in 15 of 29 patients 4 patients developed a specific CTL response as a result of vaccination The vaccine was well tolerated in all patients with only mild to moderate local toxicity, and no serious adverse events

causes approximately 40% to 50% of penile cancers,^[45] but there are inadequate data regarding the usefulness of HPV vaccines in preventing penile cancers.

CONCLUSION

HPV vaccines hold great promise in the prevention and treatment/control of HPV-related diseases. Given the burden of HPV-related diseases in the general population, the immediate need and role of such a vaccine need not be overemphasized. From scientific perspectives, the long-term beneficial effects of HPV vaccines are probably understated, as our intuitive perceptions of such benefits are yet to be substantiated by adequate clinical studies. With the success of the prophylactic HPV vaccines, cervical cancer may soon become one of the vaccine-preventable malignancies. However, to be more effective, rare types of HPV causing cervical cancers should also be included in the vaccines. As more scientific evidences are gathered, it may be possible to prevent and treat a number of other HPV-related conditions by these vaccines.

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Multiple Choice Questions

1. Which is the most common HPV type causing cervical cancers:
 - a. HPV-16
 - b. HPV-18
 - c. HPV-31
 - d. HPV-33
2. Which HPV-encoded protein is 'oncogenic':
 - a. E1
 - b. E2
 - c. E4
 - d. E6
3. Which is the principal regulator of HPV genes:
 - a. E1
 - b. E2
 - c. E4
 - d. E7
4. Which of the following is true:
 - a. Humoral immune response is strong after HPV infection
 - b. Anti-HPV antibodies are type-specific with little or no cross-reactivity
 - c. Antibodies eradicate established infection
 - d. Persistence of the high-risk HPV types is not required for the development of cervical cancers
5. What is "VLP":
 - a. Very Large Particle
 - b. Very Long Peptide
 - c. Virus Like Particle
 - d. Virus Linked Protein
6. Which of the following is not true:
 - a. Both L1 VLP and L2 VLP are being used for prophylactic HPV vaccine
 - b. L2 is more internally located in the viral capsid
 - c. L1 antibodies are type specific
 - d. L2 antibodies have some cross-reactivity
7. Maximum recorded duration of efficacy after prophylactic HPV vaccine:
 - a. 2 years
 - b. 5 years
 - c. 10 years
 - d. Life long
8. Which of the following is not true of Gardasil:
 - a. It is HPV-6,11,16,18 VLP vaccine
 - b. ACIP recommends it for males and females aged 9 to 26 years
 - c. Pap test result does not influence the decision to vaccinate
 - d. Three doses are given for vaccination
9. Not true regarding Cervarix:
 - a. It is a bivalent HPV vaccine containing HPV-16,18 VLPs
 - b. Three doses are required for vaccination
 - c. It has been approved by FDA
 - d. Cross-protection has been seen with HPV-45 and HPV-31
10. True regarding therapeutic HPV vaccine:
 - a. They target E2 or E4 oncoprotein
 - b. They are best used for first line treatment of cervical cancers
 - c. DNA vaccines are the most promising
 - d. Linking the DNA vaccine protein to γ -tubulin will enhance binding to the dendritic cells

1 - a, 2 - d, 3 - b, 4 - b, 5 - c, 6 - a, 7 - b, 8 - b, 9 - c, 10 - c

Answers