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Perspective for Prophylaxis and Treatment of Cervical Cancer: An Immunological Approach

Marjorie Jenkins¹, Maurizio Chiriva-Internati², Leonardo Mirandola², Catherine Tonroy³, Sean S. Tedjarati⁴, Nicole Davis³, Nicholas D'Cunha³, Lukman Tijani³, Fred Hardwick³, Diane Nguyen³, W. Martin Kast⁵, and Everardo Cobos²

¹Division of Hematology & Oncology, Texas Tech University Health Sciences Center and Southwest Cancer Treatment and Research Center, Lubbock, Texas, USA; Departments of Internal Medicine and Obstetrics & Gynecology, Texas Tech University Health Sciences Center, Amarillo, Texas, USA; and Laura W. Bush Institute for Women's Health and Center for Women's Health and Gender-Based Medicine, Texas Tech University Health Sciences Center, Amarillo, Texas, USA

²Division of Hematology & Oncology, Texas Tech University Health Sciences Center and Southwest Cancer Treatment and Research Center, Lubbock, Texas, USA; and Laura W. Bush Institute for Women's Health and Center for Women's Health and Gender-Based Medicine, Texas Tech University Health Sciences Center, Amarillo, Texas, USA

³Division of Hematology & Oncology, Texas Tech University Health Sciences Center and Southwest Cancer Treatment and Research Center, Lubbock, Texas, USA

⁴Departments of Internal Medicine and Obstetrics & Gynecology, Texas Tech University Health Sciences Center, Amarillo, Texas, USA

⁵Departments of Molecular Microbiology & Immunology and Obstetrics & Gynecology and Urology, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, California, USA; and Cancer Research Center of Hawaii, University of Hawaii at Manoa, Honolulu, Hawaii, USA

Abstract

As the second most common cause of cancer-related death in women, human papilloma virus (HPV) vaccines have been a major step in decreasing the morbidity and mortality associated with cervical cancer. An estimated 490,000 women are diagnosed with cervical cancer each year. Increasing knowledge of the HPV role in the etiology of cervical cancer has led to the development and introduction of HPV-based vaccines for active immunotherapy of cervical cancer. Immunotherapies directed at preventing HPV-persistent infections. These vaccines are

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Address correspondence to Maurizio Chiriva-Internati, PhD, Director of Basic/Translational Research Program, Division of Hematology & Oncology, Texas Tech University Health Sciences Center, 3601 4th St., Mail Stop 9410, Lubbock, TX, 79430, USA
maurizio.chiriva@ttuhsc.edu.

Marjorie Jenkins and Maurizio Chiriva-Internati contributed equally to this article.

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already accessible for prophylaxis and in the near future, they will be available for the treatment of preexisting HPV-related neoplastic lesions.

Keywords

cancer vaccines; gynecologic oncology; HPV; immunotherapy; tumor prevention

Cervical cancer is the result of infection with the sexually transmitted virus human papilloma virus (HPV). In women worldwide, approximately 80% of cervical cancer cases are due to HPV subtypes 16 and 18 [1, 2]. Through complicated interactions with the cervical epithelium at the squamous–columnar junction, the high-risk HPV subtypes lead to epithelial changes of the cervix known as dysplasia. This dysplasia can be low or high grade and if untreated, may progress to invasive cervical cancer. With approximately 25% of U.S. women aged 14–19 years and 45% of women aged 20–25 years being HPV-positive, there are significant implications for the HPV vaccine in the potential treatment and prevention of cervical cancer [3–5]. We present here a review of cervical cancer and the HPV vaccine.

Epidemiology

Cervical cancer is one of the leading causes of gynecologic malignancy-related deaths. Approximately 80% of cervical cancer disease burden lies in developing nations, with the highest rates occurring in Central and South America, eastern and southern Africa, and the Caribbean. In nations where screening and intense treatment programs are not readily available and underreporting and inadequate data collection are commonplace, the actual mortality rate is likely to be higher [6].

While the United States has seen a decrease in cervical cancer rates since the initiation of cytological screening via the Pap smear, 2008 saw approximately 11,000 women diagnosed with cervical cancer and more than 3900 died of the disease [3]. Though the Pap smear is an excellent screening tool, it has a high false-negative rate, reported to be as high as 50% [4, 5, 7].

The age-specific HPV prevalence peaks between 25 and 35 years of age, followed by a sharp decline to lower levels in the fifth and sixth decades of life. Nearly 80% of women will be infected with HPV at some point in their lifetime. Most will be able to clear the virus within 2–4 years after infection; however, some data suggests HPV may remain dormant in the basal epithelial level and resurge later or there may be reinoculation [4, 5].

Aside from the morbidity and mortality associated with HPV infection and cervical cancer, there is a large financial burden as well. Cervical cancer screening in the United States alone is estimated to cost \$2.3 billion, annually. The overall estimated economic burden of HPV-related disease, both cervical and anogenital, reaches \$4 billion annually [8].

Basics: Etiology and Pathophysiology

HPV is a double-stranded DNA virus with over 130 identified genotypes. Approximately 30 genotypes are known to infect the genital mucosa with effects of infection, ranging from

benign genital warts, HPV types 6 and 11, to cervical cancer, HPV types 16, 18, 31, 33, and 45 [3, 9]. Of those oncogenic HPV genotypes, types 16 and 18 are the most common and account for approximately 70% of cervical cancers.

HPV, composed of the major capsid protein L1 and the minor capsid protein L2, acts by attaching itself to basal epithelial cells where it remains elusive to the reticuloendothelial system. There, infected cells divide, with some migrating toward the surface, allowing eventual infection of adjacent cells, while others remain in the basal layer and provide a medium for further cell replication. Once a cell is infected, oncogenic transformation of the cervical epithelial cell occurs primarily via two oncoproteins, E6 and E7. This transformation is achieved via binding to and inactivation of the p53 and retinoblastoma (RB) tumor suppressor genes, respectively [1], where E6 primarily targets p53 while E7 targets retinoblastoma suppressor genes [10]. Beyond tumor suppression, gene inactivation and genetic changes in DNA occur, including acquisition of chromosome 3q, DNA methylation of cytosine, and histone protein acetylation [10]. External co-factors that may potentiate the oncogenic processes and, therefore, the development of cervical intraepithelial neoplasia (CIN) include *Chlamydia trachomatis*, herpes simplex virus, HIV, immunosuppressed states, and tobacco smoke.

Once present, CIN is classified histologically by grade ranging from CIN I to CIN III. High-grade cervical dysplasia, or CIN III, is defined as histologically abnormal cells spanning the thickness of the cervical epithelium and is a prerequisite for invasive cancer [3]. If CIN remains undetected and is therefore allowed to progress, 80% of cases will develop squamous cell carcinoma. The eventual development of squamous cell carcinoma versus adenocarcinoma depends on whether squamous or glandular epithelial cells are infected, as well as the HPV type. Development of SCC occurs with infection of squamous epithelium and is associated with HPV 16. The second most common form of cervical cancer, adenocarcinoma, arises from glandular epithelium and is more commonly associated with HPV types 18 and 45 [9].

Risk Factors

Numerous risk factors have been associated with the development of HPV infection, the most significant being sexual activity. In a study of female college students, aged 18–22 years, the 12-month incidence of HPV infection after one reported sexual partner was 28.5%. This incidence increased each subsequent year with a 36-month incidence of 49.1%. Risk of HPV infection continues to increase with increased numbers of male sexual partners and directly correlates to the male partners' sexual history. Aside from the number of sexual partners, sexual activity with a new partner is also highly associated with new HPV infection [4, 5].

Another factor strongly correlated with development of HPV infection and subsequent development of dysplasia or invasive disease is age of onset of sexual activity. This may be accounted for by the longer period of repeated exposure to HPV and the longer duration of infection that occurs with the earlier age of sexual debut [4,5].

Parity also appears to play a role in the development of cervical cancer. Compared to nulliparous women, parous women are at higher risk of cervical dysplasia. There appears to be an increase in cervical cancer risk with the number of term births. This increase may be result of repeated trauma to the cervix during childbirth. Hormonal, immunologic, and nutritional factors have also been suggested as mechanisms possibly facilitating HPV infection [4, 5, 7].

Extended oral contraceptive (OCP) use has also been observed as a potential risk factor for cervical cancer with a more specific association with adenocarcinoma. OCP use of greater than 5 years duration is associated with cervical dysplasia. Evidence suggests sex steroids directly influence HPV gene activity [4, 5, 7].

Tobacco smoking as a co-factor for HPV infection has also been well documented. Among HPV-positive women, smoking has been associated with a threefold increase in the risk of high-grade CIN. There is also a dose–response relationship with regression of cervical dysplasia of almost 80% in women who quit tobacco smoking for over 6 months compared to 28% of those who did not [4, 5, 7].

Presentation

The usual presentation of cervical cancer is abnormal vaginal bleeding, postcoital bleeding, watery, purulent vaginal discharge, pelvic pain, and lower back pain with nerve root entrapment. Urinary frequency, hematuria, and hematochezia are uncommon complaints and may suggest more advanced disease [4].

Diagnosis and Staging

Cervical cancer is staged clinically, with physical/pelvic examinations being the key component. If the disease is microscopic, a colposcopic examination is the initial step. Cervical conization is used to detect the depth of invasion. If the disease is visible, multiple biopsies are mandatory for diagnosis. During bimanual examination, the size of the cervical mass is noted along with involvement of the vagina, extension into the parametrium, sidewall, urethra, or anus. Depending on the extent of local disease, cystoscopy and proctoscopy are performed to rule out involvement. Lymphatic survey is completed over the inguinal and supraclavicular areas. Unilateral leg edema is an infrequent but important sign, possibly indicating advanced disease. Radiographic studies include a chest radiograph to rule out metastasis that would indicate stage 4B, and intravenous pyelogram to rule out renal obstruction or nonfunctioning kidney, which would indicate advanced disease (stage 3B). A serum hemogram is used to detect anemia along with serum creatinine [4,5].

Treatment

There are treatment options for patients who desire fertility-sparing procedures such as a radical trachelectomy (removal of cervix and parametrial tissues) and pelvic lymphadenectomy, [19] although radiotherapy with concomitant platinum-based chemotherapy may also be offered in the early stages [11,12]. The effectiveness of either treatment is equal in early stages, but surgery is offered to preserve ovarian function and

preserve vaginal length. For stages II–IV, pelvic radiotherapy with concomitant platinum-based chemotherapy is the treatment of choice [13–15].

Differential diagnosis	Differentiating tests	Differentiating signs/symptoms
Endometrial cancer	If a patient with abnormal uterine bleeding is found to have a normal cervix, depending on the age and risk factors, an endometrial biopsy can assist in appropriate diagnosis.	Women over 50, obese, need evaluation of the endometrium through an endometrial biopsy.
Uterine fibroids or polyps	Same as above except that an transvaginal ultrasound will be an excellent tool in evaluating the uterine wall.	Almost 50% of women may have uterine leiomyomas and present with abnormal uterine bleeding and pain. Pelvic examination, endometrial biopsy, and ultrasound can help in diagnosis.
Sexually transmitted infections	Abnormal vaginal discharge and pain can be a presenting sign of an STD. It is important to obtain cervical cultures and evaluate the appearance of the cervix, which may be inflammatory and friable.	Many women who are sexually active can present with STDs with symptoms of abnormal vaginal discharge, bleeding. Treatment is based on history, physical examination that reveals an inflamed cervix with motion tenderness, leukocytosis. Cervical cultures assist greatly in diagnosis.

Treatment of cervical cancer depends primarily on the stage of disease and the specific patient's overall health status. For microscopic disease with early stromal invasion of less than 3 mm (stage Ia1), a cervical conization either through loop electrical excision or cold knife technique is adequate. Simple hysterectomy may be performed if fertility is not a concern. There is no need for pelvic lymphadenectomy at this stage, as the incidence of lymphatic spread is <0.6% [16].

For stages Ia2–1b1 radical hysterectomy with pelvic lymphadenectomy is the surgical treatment of choice. Pelvic lymphadenectomy is critical in stage Ia2, as the overall rate of lymphatic spread is about 15% [17]. While an equal treatment option for this stage may include pelvic radiotherapy with platinum-based chemosensitization, in younger patients, surgery offers the advantage of maintaining a longer vaginal length and ovarian function [11]. Other options for stage Ia2 and small <2-cm Ib1 are a radical trachelectomy and pelvic lymphadenectomy, which may be the treatment of choice for those interested in maintaining fertility [18]. For microscopic disease with deeper stromal invasion greater than 3 mm or visible disease confined to cervix (stage Ib), a radical hysterectomy that includes dissection and resection of parametrial tissues, cardinal and uterosacral ligaments, and partial vaginectomy along with complete pelvic lymphadenectomy is performed [13,17,19].

In stage Ib2, the treatment options are controversial. Radical hysterectomy and lymphadenectomy is advocated by some and pelvic chemoradiation by others [13,19]. However, there is no consensus. For patients with stages Ib1 and Ib2 who undergo radical hysterectomy and lymphadenectomy, those with high-risk features, which include lymphovascular space invasion, depth of invasion, size, and parametrial or lymphatic invasion, may require postoperative chemotherapy and radiation [11]. This raises the question as to whether to offer primary radiotherapy instead of surgery followed by radiotherapy, as there are no clear data demonstrating better survival for one method over another [5]. Pelvic radiotherapy postradical hysterectomy for high-risk features does reduce

pelvic recurrence by almost 50% with an increase in the risk of complications. Data has shown that for stage 1b1 treated with pelvic radiotherapy, postradiation hysterectomy may decrease the rate of pelvic recurrence.

For stages IIa–IV, pelvic chemotherapy and radiotherapy are the treatments of choice [13–15]. In case of recurrence, the treatment depends on the pattern and prior treatment. If the recurrence is confined to the pelvis and the patient has not been treated with pelvic radiotherapy, then salvage radiotherapy is the treatment of choice. In the case of recurrent central pelvic disease after radiotherapy when no other metastatic disease is noted, then a pelvic exenteration may provide a 5-year survival of approximately 40%. If recurrence is multifocal, then systemic chemotherapy is the treatment of choice. The most active agent is cisplatin with response rates of 20–25%. Combining cisplatin with Hycamtin may offer a survival advantage in select patients [5,20,21].

Treatment Complications

Complications for cervical conization include bleeding, infection, cervical stenosis, predisposition to cervical incompetence, and preterm delivery. Radical hysterectomy along with pelvic lymphadenectomy can be complicated by infection, urinary or gastrointestinal fistulae, and bladder atony. Acute complications of pelvic radiotherapy include bladder hyperactivity, pelvic pain, and diarrhea. Long-term complications may include urinary or gastrointestinal fistulas, bowel obstruction, diarrhea, and vaginal stenosis.

Tumor Antigens and Screening Biomarkers

With cervical biopsies missing an estimated 33–50% of high-grade cervical lesions, there is an obvious need for more sensitive and specific screening tests, which may be available in the form of screening biomarkers and tumor antigens [22]. Using gene microarray analysis, it is possible to detect the presence of specific genes found to be overexpressed in the setting of high-grade CIN or cervical cancer. One such gene is p16^{INK4a}, or cyclin-dependent kinase inhibitor 2A, a tumor suppressor gene that has been found to be overexpressed in high-grade cervical lesions. The sensitivity of p16^{INK4a} for cervical lesions increases with grade, being nearly 100% sensitive for CIN3 [10,22].

While p16^{INK4a} has been shown to highly correlate with the presence of high-grade cervical lesions, it is important to note studies have reported the presence of SCC and CIN2 in p16^{INK4a}-negative specimens [23]. Therefore, while p16^{INK4a} expression is specific to high-grade cervical lesions, its poorer sensitivity for low-grade lesions, reportedly 42% for low-grade squamous intraepithelial lesions (LSIL) and 36% for atypical squamous cells of undetermined significance (ASC-US), make it a nonoptimal screening test [23]. Use of Hybrid Capture 2 (HC2) assay to detect high-risk HPV performed on cytological specimens when compared to p16^{INK4a} expression performed on the same specimens showed HC2 to be more sensitive for detection of cervical disease. HC2 positivity was reportedly 81% sensitive for LSIL lesions and 45% sensitive for ASC-US [23]. In a joint cohort study out of Europe, which combined cervical cytology and HPV testing as a screening tool for the development of CIN, results showed cytology plus HPV testing had a negative predictive value of 0.99 with the rate of CIN3 in this cytology-negative/HPV-negative group, being

only 0.28% at 6 years follow-up [24]. Beyond p16^{INK4a} other biomarkers have been identified that show association with cervical dysplasia. These include genes that exhibit overexpression in dysplasia; minichromosome maintenance protein-2 and -5(MCM), topoisomerase II α (TOP2A), E2F transcription factor 1, cyclin D1 prostaglandin E synthase, and telomerase; and those present in adenocarcinoma exhibiting hypermethylation: calcitonin (CALCA), adenomatous polyposis coli (APC), death-associated protein kinase (DAPK), and estrogen receptor 1 (ESR1) [10,22].

Short-term complications			
Complication	Description or complication	Treatment	
Bladder atony	Postradical hysterectomy. About 5% of patients suffer from this condition, which results from disruption of the nerve supply to the bladder during radical dissection.	Prolonged bladder drainage is the treatment of choice. Most patients will recover without sequela.	
Bladder and rectal hypermotility/ cystitis/proctitis	Results mostly in the acute phase of pelvic radiotherapy.	Antispasmodic agents are helpful in relieving bladder hyperactivity, ant motility agents are treatment of choice for diarrhea. Proctitis can be treated with local sitz baths and steroids suppositories.	
Vesicovaginal or ureterovaginal fistulae	Can occur in acute form after radical hysterectomy.	Most vesicovaginal fistulae can be managed by chronic drainage and resolve spontaneously. Ureterovaginal fistulae may be managed by percutaneous nephrostomy and stent placement. If these measures fail, surgical revision may be needed.	
Lymphocysts	May occur after pelvic lymphadenectomy.	Most will be asymptomatic and resolve spontaneously. If persistent or symptomatic then percutaneous drainage may be needed.	

Long-term complications			
Complication	Likelihood	Time frame	Detail
Fistula	Occurs in up to 8.8% of surgically treated patients. Rate is highest in patients with prior irradiation.	Variable	One-third to one-half will heal spontaneously. The remainder will require a reparative procedure such as ureteroneocystostomy.
Intestinal obstruction	5–15% in patients treated with radiation therapy.	Variable, up to years	Generally the result of fibrosis and ischemia secondary to the effect of radiation therapy on small blood vessels and connective tissue. Conservative management includes bowel rest, decompression, and diet modification. Refractory cases may require surgery.
Urinary urgency, incontinence, and frequency	26% incidence in patients treated with radiation alone.	Variable	Same dysfunction noted in about 10% of the general female population and incidence is higher in older women.
Sexual dysfunction	Not determined.	Variable	Surgery will shorten the functional length of the vagina, but pliability and lubrication are often preserved. Radiation therapy can reduce length, caliber, and lubrication. These symptoms can be alleviated in some patients by hormonal therapy, vaginal dilators, and lubricants.

Considering the HPV-related etiology of cervical cancer, clear benefits stem from the use of HPV DNA testing in case of equivocal smears, low-grade smears in older women and in

CIN follow-ups. However, because the presence of HPV DNA is frequently associated with transient infection, this approach can potentially afford a low specificity, which would be undesirable for large-scale screening purposes. To overcome this issue, the simultaneous HPV DNA typing and cytological screening for markers of proliferative lesions such as p16, mRNA coding for the viral E6 and/or E7 proteins have been studied [25].

Immunotherapy

Aside from the traditionally available cervical cancer treatments of cervical conization, trachelectomy or hysterectomy, and radiotherapy with or without chemotherapy, the potential of immunotherapy is emerging. Current immunotherapy for the prevention of cervical cancer via HPV vaccination is available while innovative immunologic strategies are being developed for the treatment of patients that display preexisting HPV infections.

Here we firstly review the rationale of preventive HPV vaccination and the latest results and benefits shown by HPV vaccination. Then, we discuss the novel strategies that have been recently proposed for the improvement and therapeutic use of HPV-based vaccines.

Up to now, 15 carcinogenic HPV genotypes have been shown to account for all cervical cancers worldwide [26]. The correlation between cervical cancer and HPV infections is so strong that physicians have developed a paradigm to describe cervical carcinogenesis: exposure to HPV, persistent infection, induction of precancer lesions, and invasion [27]. Considering this virtually complete etiological link between HPV infection and cervical cancer lesions, two distinct approaches are currently used for tumor prevention: administration of HPV vaccines to young women who have not been exposed yet to the virus (HPV-naïve) and detection of carcinogenic HPV DNA in cervical smears. So far, HPV vaccines have collectively shown more than 90% efficacy in preventing cervical cancer development for up to 8.5 years [28], while new tests for the detection of carcinogenic HPV DNA have been proven superior to traditional cytology analysis for routine screening [29]. However, available immunotherapy options cannot be used for the treatment of preexisting HPV infections [30].

Two preventive vaccines have been approved by the FDA for clinical use: Gardasil (Merck & Co, approximately \$125/dose) and Cervarix (GlaxoSmithKline, approximately \$100/dose). They are based on the virus capsid protein L1 that when mixed with appropriate adjuvants (aluminum hydroxyphosphate sulfate in Gardasil and AS04 in Cervarix formulations) trigger the production of neutralizing antibodies that likely block entry of HPV into cells. Gardasil is a quadrivalent vaccine consisting of recombinant L1 from genotypes 6,11,16, and 18, while Cervarix has a bivalent formulation with L1 from genotypes 16 and 18.

A number of clinical trials have been carried out in the last 2 years to evaluate the efficacy and the potential risks of HPV vaccines. Due to space restrictions, we present here only the most recent.

In the multicenter, open-labeled 48-month trial conducted by the group of Schwarz [31], the immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine (Cervarix) were

assessed. This phase III study consisted of two sequential steps: initially, the authors evaluated the vaccine-induced immune response to HPV-16 and -18, based on seroconversion rates, in women 26–45 and 46–55 years old compared with women 15–25 years of age; then they evaluated HPV-16 and -18 seropositivity rates and antibody levels. Seroconversion rates were 100% regardless of the age for up to 24 months after vaccine administration (the antibody's titer peak was observed at month 7 in all age groups). Mucosal HPV-specific antibody levels measured after 24 months were strongly correlated with serum antibody titers and did not differ between the age groups (correlation coefficients from 0.73 to 0.90 for HPV-16 and from 0.82 to 0.93 for HPV-18). The authors also reported excellent vaccine tolerability, with mild symptoms at the local injection site, unrelated to age of the subjects, and no adverse effect was observed on pregnancy. Overall, the study demonstrated that the HPV-16/18 AS04-adjuvanted vaccine triggers a long-lasting immune response in women older than 26 years of age and stimulates the production of antibodies in the serum and in the cervix epithelium that are most likely able to block viral entry into mucosal epithelial cells.

Final results of the first randomized, double-blind clinical trial testing the quadrivalent Merck vaccine Gardasil was reported by the team of Muñoz [32]. Subject recruitment started in 2004 and lasted 1 year: 3819 women aged 24–45 years were enrolled from Colombia, France, Germany, Philippines, Spain, Thailand, and the United States into a randomized placebo-controlled double-blind study for the evaluation of safety, immunogenicity, and efficacy of the vaccine. Subjects were divided into two age groups (34 and 35 years): each group was randomly divided into two cohorts, one receiving the HPV vaccine ($n = 1911$), the other the adjuvant only (placebo, $n = 1908$). Serum antibody induction against all the four L1 genotypes (6, 11, 16, and 18) resulted similarly in the two age groups and was overall comparable to the seroconversion expected for younger women (from 16 to 23 years old). Regardless of the age, Gardasil displayed 90.5% efficacy, evaluated as protection against HPV infection and cervical and external genital disease, and 82% efficacy evaluated as prevention of HPV-6/11/16/18-related cervical intraepithelial neoplasias. No serious vaccine-related adverse events were observed, and the mild side effects were similar between the two age groups. This study clearly showed the high potential of the quadrivalent vaccine in preventing cervical cancer in sexually active women aged 24–45 years, provided that they are not infected with the HPV types included in the vaccine formulation.

A few months following the studies on the approved HPV vaccine formulations, the HPV PATRICIA Study Group reported the final analysis on Cervarix efficacy against HPV cervical infection and preneoplastic lesions in young women [33]. Between May 2004 and June 2005, healthy women aged 15–25 years from Asia, Europe, and Latin and North America were enrolled in the phase III randomized, double-blind, controlled Papilloma TRIal against Cancer In young Adults (PATRICIA), and randomized into two groups, one receiving Cervarix (HPV-16/18 AS04-adjuvanted vaccine, $n = 9319$), the other receiving hepatitis A vaccine as placebo control ($n = 9325$). The trial reported high vaccine efficacy against grade 2+ cervical intraepithelial neoplasia (92.9%, CI 79.9–98.3%), and lower efficacy against grade 3+ cervical intraepithelial neoplasia (33.4%, CI 9.1–51.5%). Cross-

protection against grade 2+ cervical intraepithelial neoplasia caused by 12 HPV oncogenic types not included in the vaccine formulation was observed (54.0%, CI 34.0–68.4%). The authors concluded that these results and the high tolerability of the vaccine represented the rationale for universal mass vaccination and catch-up strategies.

Along with multivalent HPV vaccines, the rationale for the use of monovalent formulations has been shown. Recently, the group of Koutsky [28] reported a long-term evaluation efficacy of HPV-16 monovalent prophylactic vaccination in an extended 8.5-year follow-up. The study recruited 500 women originally included in a cohort of 2391 subjects participating in a multicenter double-blind phase II-b randomized controlled trial of a monovalent HPV-16 vaccine in the United States that started in 1998 and ended in 1999. Over the 8.5-year follow-up period, none of the vaccinated subjects was infected by HPV-16 or had HPV-16-related cervical lesions, while in the placebo group, 6 women were infected by HPV and 3 displayed HPV-16-related preneoplastic lesions, accounting for a vaccine efficacy of 100% against both the primary events. This was the first time that the efficacy of HPV monovalent vaccines was proven over such an extended follow-up period.

In December 2009, the complete results of the Cervarix efficacy over an extended 6.4 year clinical trial were presented by the GlaxoSmithKline Vaccine HPV-007 Study Group [34]. In that study 1113 HPV-16/18 naïve women aged 15–25 years were enrolled in a double-blind, randomized, placebo-controlled clinical trial; of these subjects, 776 (393 in the vaccine group and 383 in the placebo group) participated in the long-term follow-up. Over the whole 6.5-year period, vaccine efficacy against HPV-16/18 infection was 95.3% (CI 87.4–98.7%), but the prophylaxis was 100% effective in preventing persistent infections (CI 81.8–100%). When considering vaccine protection against grade 2+ cervical intraepithelial neoplasia, efficacy was 100% (CI 51.3–100%) for HPV-16/18-related lesions. The vaccine also showed a cross-protective ability against HPV types different from 16 and 18 (71.9% efficacy, CI 20.6–91.9%). As stated, based on the currently available data, HPV vaccines are effective not only in adolescents, but also in adults. However, HPV immunization is generally recommended in adolescent girls, since they are expected to be HPV-naïve and infection-free [35,36]. Therefore, the need to perform HPV prophylaxis along with other adolescent and even preadolescent vaccination schedules is realistic. The recent work by the HPV Vaccine Adolescent Study Investigators Network, which includes research teams from Spain, Germany, France, Belgium, and the United States [37], has evaluated the immunogenicity and safety of HPV-16/18 vaccine (Cervarix) co-administered with the diphtheria–tetanus–acellular pertussis-inactivated poliovirus vaccine (dTpa-IPV) in an open, randomized, multi-center clinical trial. The study enrolled 751 women randomized in 3 cohorts: group 1 ($n = 248$) received standard HPV-16/18 AS04-adjuvated vaccine (Cervarix), group 2 ($n = 255$) received Cervarix and dTpa-IPV, while group 3 ($n = 248$) was given dTpa-IPV alone. Results showed that co-administration of the two vaccines was not inferior to single formulations in providing seroprotection against diphtheria, tetanus, poliovirus, and pertussis, or HPV types 16 and 18. The authors reported that dTpa-IPV caused lower anti-HPV-16/18 antibody titers, yet this difference had no clinically significant effect. Adverse effects of the combined vaccines were comparable to those of the single formulations. This study provides the rationale for a dTpa-IPV and HPV-16/18 AS04-

adjuvanted vaccine co-administration as part of the routine adolescent vaccination program that is likely to facilitate HPV vaccine use, acceptance, and compliance.

In December 2001, two of the largest randomized, double-blind, placebo-controlled trials for Gardasil HPV-6/11/16/18 vaccine (FUTURE I and II) were initiated, and enrolled 17,622 women (15–26 years old) in Australia, Austria, Brazil, Canada, Colombia, Czech Republic, Denmark, Finland, Germany, Hong Kong, Iceland, Italy, Mexico, New Zealand, Norway, Peru, Poland, Puerto Rico, Russia, Singapore, Sweden, Thailand, the United Kingdom, and the United States [38,39]. Both studies were carried out for 4 years and revealed high efficacy of the vaccination [40,41]. In March 2010, Muñoz et al. [42] reported the results of a 3.6-year follow-up. The authors showed that Gardasil was 100% effective in preventing HPV-16/18-related high-grade lesions in an HPV-negative group. In the population positive for at least one HPV type, the quadrivalent vaccine significantly reduced the risk of high-grade lesions (19% reduction) and of HPV-associated genital diseases and precursor lesions in the cervix, vulva, and vagina.

The following table outlines the cited clinical trials and the main results obtained in each investigation.

Recently Approved HPV Vaccines for the Treatment of Cervical Cancer

As stated above, at present two HPV vaccines are commercially available for cervical cancer prevention, namely Gardasil [43] and Cervarix [44]. They differ in their composition, but both are based on HPV-L1 structural proteins. L1 proteins are capable of self-assembling to form virus-like particles (VLPs) administered by intramuscular injection.

Both vaccines are produced as a sterile suspension in single-use glass vials or single-use prefilled syringes that should not to be frozen. The route of administration is intramuscular with a 0.5-mL dose. The quadrivalent vaccine is repeated at 2 and 6 months. The minimum interval between successive doses is 4 weeks between the first and second administration, and 12 weeks between the second and third dose [45]. The bivalent vaccine is repeated at 1 and 6 months. The minimum interval between successive doses is 1 month after the first dose. At present, a booster dose is not recommended for any HPV vaccine following completion of the primary vaccination series.

Unlike immunity naturally occurring after HPV infection, the vaccine induces a stronger and longer lasting protection [46]. The main mechanism of action of HPV vaccines seems to be the induction of both cellular immunity and neutralizing IgG [47]. Unfortunately, HPV vaccines are indicated for prophylaxis only, and there is no clear evidence that they are able to clear existing HPV infections [48]. FDA-approved indications for HPV vaccines are outlined in the following table.

Ref.	Trial number	Vaccine type	Outcome
[31]	105879/014	Bivalent anti HPV-16/18	Persistent immune response in women >26 years of age; antibodies detected in the cervical epithelium.
[42]	NCT00092521 and NCT00092534	Quadrivalent anti-HPV-6,11,16 and 18	90.5% efficacy against HPV infections; 82% efficacy in the prevention of HPV-6/11/16/18 related cervical intraepithelial neoplasias.
[33]	NCT00122681	Bivalent anti-HPV-16/18	92.9% efficacy against grade 2+ cervical intraepithelial neoplasias; 33.4% against grade 3+ cervical intraepithelial neoplasias. 54.0% cross-protection against grade 2+ cervical intraepithelial neoplasia caused by 12 HPV oncogenic types not included in the vaccine.
[28]	Not applicable	Monovalent HPV-16	Over the 8.5-year follow-up period, HPV-16 infection or HPV-16-related cervical lesions incidence was 0% in the vaccinated subjects. Compared with placebo, vaccine efficacy was 100.
[34]	NCT00120848	Bivalent anti-HPV-16/18	Over the whole 6.5 year period, vaccine efficacy against HPV-16/18 infection was 95.3% (CI 87.4–98.7%), but the prophylaxis was 100% effective in preventing persistent infections and grade 2+ cervical intraepithelial neoplasias. 71.9% cross-protection against HPV types other than 16 and 18.
[37]	108464/NCT00426361	Bivalent anti-HPV-16/18 co-administered with dTpa-IPV	Co-administration of the two vaccines was not inferior to single formulations in providing seroprotection against diphtheria, tetanus, poliovirus, and pertussis or HPV types 16 and 18. Adverse effects of the combine vaccines resulted comparable to those of the single formulations.
[39]	NCT00092534	Quadrivalent anti-HPV-6,11,16, and 18	98% efficacy for the prevention of cervical intraepithelial neoplasia grade 2 or 3, adenocarcinoma in situ, or cervical cancer related to HPV-16 or HPV-18.
[40]	NCT00092521, NCT00092534, and NCT00092482	Quadrivalent anti-HPV-6,11,16 and 18	17.7% reduced rate of HPV-31/33/45/52/58 infection by 17.7 and 18.8% of CIN 1–3 or adenocarcinoma in situ. 26.0–37.6% reduced rate of HPV-31/58/59-related CIN1–3/adenocarcinoma in situ.
[42]	NCT00092521 and NCT00092534	Quadrivalent anti-HPV-6,11,16, and 18	100% efficacy in preventing HPV-16/18-related high-grade lesions in a HPV-negative subjects. In the group positive for at least one HPV type, the vaccine significantly reduced the risk of high-grade lesions (19% reduction), of HPV-associated genital diseases and precursor lesions in the cervix, vulva, and vagina.

Since both vaccines are intended for prophylaxis, vaccination should be administered before the subject is exposed to HPV infection, that is, before the onset of sexual activity. Girls should be given HPV vaccine at 11–12 years. Those who have not completed/initiated the prophylaxis by that time should receive the vaccine between 13 and 26 years [49,50]. Currently available data indicate that young females naive to HPV infections develop specific antibodies after the third dose of vaccine [51]. Both vaccines have shown high efficacy in preventing condyloma, low- and high-grade CIN and AIS, as well as VaIN and

VIN associated with vaccine-related HPV types. However, while the efficacy in protection from of HPV-16/18-related CIN-II/III ranged from 90.4 to 98%, the overall HPV vaccine type-related CIN efficacy ranges from 89.2 to 100% [39,52]. The quadrivalent vaccine also displayed 91–100% efficacy against HPV-6/11/16/18-related VIN-2/VIN-3 or VaIN-2/VaIN-3 and 96–100% efficacy against HPV6/11/16/18-associated condyloma [38]. Since protection of both vaccines has been observed throughout their observation time course (5 years for Gardasil and 8.4 years for Cervarix) [53,54], it is estimated that antibody levels will remain detectable lifelong in 99% of subjects [47,55], yet further and longer observations are warranted. Overall, the current knowledge concerning the efficacy and the potential future applications (see following paragraph) of HPV vaccines clearly show that their development and introduction into the clinical practice is actually a milestone in the history of modern medicine.

Vaccine name	Indication	Subjects	Infections prevented
Gardasil	Prevention of vulvar cancer, vaginal cancer, cervical cancer, genital warts	9–26 y.o. females	HPV-6-11-16-18
Gardasil	Genital warts	9–26 y.o. males	HPV-6-11
Cervarix	Cervical cancer Grade I-II CIN	10–25 y.o. females	HPV-16-18

Epidemiologic models predict that vaccinating young adolescent females will dramatically reduce the incidence of cervical cancers, provided at least 70% coverage, and vaccine-induced protection lasts 10 or more years. Significant decrease in incidence is also likely to be observed for cancers of the vagina, vulva, anus, and head and neck. Considering that the vaccines afford protection if subjects are naive for the vaccine-related HPV types, the highest coverage possible of young adolescent girls before the onset of sexual activity will have the widest impact on public health [56]. Accordingly, WHO recommends that HPV vaccination be routinely performed in the context of national immunization programs [45]. Additionally, HPV vaccines will be significantly cost-effective. Epidemiologic and economics models indicate if high HPV vaccine coverage is achieved with nationwide programs, a significant reduction is expected in costs associated with cervical cancer screening, diagnosis, and treatment of precancerous and cancerous lesions [57].

The Future of Cervical Cancer Immunotherapy

At present, available HPV-vaccines do not have any therapeutic effect on preexisting HPV infections or related lesions. A great effort is now being spent for the development of a new generation of cervical cancer vaccines that are able to raise a therapeutic immune response against HPV-infected cells. After HPV infection, cells permanently express the viral antigens, E6 and E7, which are required for neoplastic transformation and therefore are not subject to negative regulation by immune tolerizing mechanisms. Based on this rationale, a number of E6- and E7-targeted vaccines are being tested, using different delivery systems [58]. Attenuated bacteria and virus vectors are powerful vaccine systems since they are intrinsically immunogenic and facilitate antigen spread by replicating into the host cells. Up to now, *Listeria monocytogenes* [59], *Lactobacillus lactis* [60], and *Lactobacillus plantarum*

[61] have been tested for the delivery of E6 and E7 genes. *Listeria* is promising because it can infect both monocytes and macrophages and secretes listeriolysin O. This allows for E7 presentation on MHC-I and -II, activating both CD4 and CD8 responses [62]. To boost the immunogenicity of E7 protein, a HPV-16 E7 fused with listeriolysin O has been reported to be well-tolerated by Maciag et al. [63] in a phase I clinical trial.

Besides bacteria, virus-based HPV vaccines have been tested. A vaccinia virus delivering HPV-16/18 E6 and E7 fusion gene induced humoral and cytotoxic responses in women with cervical cancer in a phase II clinical trial [64], while an adenovirus vector encoding the hepatitis B surface antigen fused with HPV-16 E7 was shown to induce anti-HPV antibodies and CD8⁺ T-cell response [65].

Given their unique ability to induce cellular-mediated immunity, DCs are used as natural vaccine adjuvants [66]. A clinical trial testing HPV-16/18 E7 loaded DC with IL-2 showed CD8⁺ response in 100% of treated cervical cancer patients [67].

Naked DNA-based vaccines represent an attractive alternative to the live vector or DC vaccines because they are considered safer, more stable, and capable of inducing longer protection than protein vaccinations. Importantly, DNA vaccines do not trigger the generation of neutralizing antibodies in the host, thus allowing for repeated administrations. However, DNA vaccines suffer from low immunogenicity and limited ability to spread *in vivo*. It is known that DC represent the main source of antigen presentation induced by DNA-based vaccines, and, consequently, strategies to enhance DC antigen presentation abilities have been developed [68,69]. A successful application of these strategies has been the use of demethylating agents: it has been shown that combining a DNA vaccine encoding HPV-16 E7 fused with the immunogenic protein calreticulin and the demethylating drug 5-aza-2'-deoxycytidine (DAC) resulted in the upregulation of antigen expression and anti-tumor response in mice bearing E7-expressing tumors [70]. To efficiently prime CD8⁺ T-cell responses, vaccine antigens must be presented in complexes with MHC-I molecules. Strategies to facilitate this process in DNA-based HPV-16/18 E7 vaccines have included the use of molecules that keep the DNA in the endoplasmic reticulum as beta-glucuronidase [71] or that enhance antigen degradation by the proteasome as plant virus coat proteins [72].

The major obstacle to the use of DC for antiviral vaccines is their short life due to the activation of naïve T cells that ultimately kill antigen-presenting DC themselves. Thus, anti-apoptotic agents have been tested to enhance and prolong DC-T lymphocyte interaction. In murine animal models, it has been shown that the co-administration of HPV-16 E7 DNA vaccines and XIAP, BCL-xL, and BCL-2 significantly improve CD8⁺ mediated responses against E7-expressing tumors [73]. The use of anti-apoptotic agents in oncology is always a highly risky approach. Therefore, the use of small interfering RNA (siRNA) to control the expression of pro-apoptotic proteins has been proposed. siRNA-mediated transient silencing of Bak and Bax in DC loaded with E7-encoding DNA prolonged DC life and improved CD8⁺ priming in mice [74].

DNA-based vaccines for cervical cancer have been already translated into clinical trials. DNA encoding HLA-A2-restricted epitopes of HPV-16 E7 was successfully used in women

with HPV-16⁺ grade II and III cervical intraepithelial neoplasias, showing histological responses in 25% and anti-E7 cellular responses in 73% of patients [75]. Recently, Trimble et al. [76] reported the results of a phase I clinical trial using Rb binding site deleted, HPV-16 E7 encoding DNA linked to *M. tuberculosis* heat-shock protein 70 (hsp70) to enhance MHC-I presentation: complete histological regression was detected in 33% of patients, without any serious adverse effects.

The tumor microenvironment plays a fundamental role in the efficacy of cancer vaccines. Regulatory T cells secrete TGF- β and IL-10, preventing HPV-infected cells killing [77,78], while the tumor itself induces immunosuppression through the expression of B7-H1, IDO, and galectin-1 [79–81]. All these have been proposed as potential targets for immunomodulation of future HPV therapeutic vaccines [82]. Interestingly a recent article demonstrated that modulating the tumor microenvironment through the introduction of a co-stimulatory molecule could increase the efficacy of T-cell-inducing vaccines very significantly [83]. Furthermore, there is promising new immunotherapy development ongoing that addresses the infection by HPV itself before it induces cancer [84].

The Next-generation HPV Vaccines

Immediately after the FDA approval for the use of VLPs-based vaccines in cervical cancer patients, a currently ongoing effort was put in the development of next-generation HPV vaccines. The new experimental vaccine requires lower production costs compared with current HPV vaccines and it is expected to protect from more than 100 HPV strains. HPV impact on health and life quality is greater for women who do not have access to effective prevention. Therefore, more than 80% of cervical cancer-related deaths are registered in developing countries. Since current vaccines are likely to be too expensive for extensive use in global vaccination programs, next-generation vaccines could save hundreds of thousands of women worldwide. Noteworthy, currently available vaccines target only two of the 15 oncogenic HPV types, which account for only 30% of cervical cancers, despite some evidence of cross-protection from related HPV types. A cost-effective solution to all these issues could come from the development of L2-based vaccines [85]. Minor capsid protein L2-based vaccines induce antibodies that cross-bind to many different HPV types. Because specific antibody titers against L2 type contained in the vaccine is considerably higher than those against other types, concatenated multitype L2 fusion proteins derived from known cross-protective epitopes of several divergent human papillomavirus (HPV) types might have been developed to enhance immunity against relevant HPV types. Preclinical studies in animal models showed that multitype L2 fusion proteins (adjuvated with GPI-0100, alum, or 1018 ISS adjuvants) were able to induce high neutralizing antibody titers against all of the HPV type tested [86].

In conclusion, compared with L1 vaccines, L2-based formulations are less expensive and afford a broader protective immunity. However, L2 vaccine development was started only recently compared with L1-VLP, and therefore it still requires several technical and biological validation studies.

Very recently, a completely innovative vaccine formulation has been developed for the treatment, rather than prophylaxis, of HPV-related cervical cancers [87]. This new vaccine strategy is based on subcutaneous injections of long synthetic peptides (from 23 to 45 amino acids) combined with Freund adjuvant (also known as Montanide ISA-51). Preclinical studies showed that these vaccines elicited a more effective MHC class I-restricted T-cell responses compared with short peptides. A single long peptide is made of 13 overlapping peptides, overall entirely covering the two HPV-16 oncogenic proteins E6 and E7. It was demonstrated that long peptide vaccines induced complete regression of lesions and virus eradication in 9 of 20 women with high-grade vulvar intraepithelial neoplasia [87]. This novel approach is likely to prove effective not only in HPV-naïve individuals, but also in patients with malignant tumors caused by this virus, such as HPV16⁺ cervical cancer, anal cancer, and head and neck cancer.

HPV Infections and Related Diseases: Not Just a Woman's Concern

Although the most frequent clinical outcome of HPV infection is cervical cancer, penile, oral, and anal cancers are also common, and in developed countries HPV displays the same mortality rates in men as cervical cancer in women [88,89]. Further, HPV infection in men is highly correlated with HPV-related morbidity in women and increases in the risk of HIV infection [90].

The high relevance of male HPV infection and HPV-related diseases in women, including cervical cancer, has been shown by a number of recent studies [91–94]. Accordingly, women with CIN frequently have HPV-positive sexual partners [95].

A phase III clinical trial has investigated the efficacy of the HPV-6/11/16/18 quadrivalent vaccine to prevent anogenital HPV infections and related diseases in heterosexual boys and men aged 16–23 years and homosexual men aged 16–26 years [96]. The study revealed 86% vaccine efficacy in reducing persistent infections in the external genital area in all subjects, regardless of age or sexual habits. The vaccine also showed a 90% efficacy in protection from external genital lesions. Based on these encouraging clinical results, the use of the quadrivalent vaccine in 9- to 26-year-old males for the prevention of genital warts was FDA-approved in October 2009. Male vaccination not only protects males from HPV-related diseases, but it is also expected to reduce HPV morbidity and mortality among women and homosexual men by preventing virus transmission between partners.

Conclusion

Despite the introduction of Gardasil and Cervarix in the clinical practice, representing a breakthrough in the prevention of HPV and related neoplasias, cervical cancer is still a deadly disease because of the lack of effective therapies. High production costs of the vaccine and logistic difficulties with vaccine preservation and transportation still prevents its use in developing countries, where the highest prevalence of HPV infection is recorded. Recent studies have revealed the molecular bases of HPV-induced tumorigenesis and interaction with the host immune system. Innovative vaccine formulations are being developed for cheaper production and easier storage and administration. These innovations

represent the best hope for the prevention of HPV-related disease in millions of men and women in the world. Simultaneously, there is a considerable effort ongoing for the development of effective therapeutic immunotherapy of HPV infections and HPV-induced cancers.

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