HPV-Vaccine  Against Cervical Cancer: An Overview

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Abstract:
The main risk factor for invasive cervical carcinoma is persistent infection by the high-risk Human Papilloma Virus (HPV). HPV is the most prevalent sexually Transmitted Infection (STI) and has been linked to 15 different cancers. Cervical cancer is one of the most frequent cancers among women, particularly in resource-limited countries. Cervical cancer is an HPV- disease with the highest worldwide burden in resource-limited nations. Human papillomavirus (HPV) vaccines, which were introduced in many countries in the past decade, have shown promising results in decreasing HPV infection and related diseases, such as warts and precancerous lesions. In this review, we present the updated information about current HPV vaccines, focusing on vaccine coverage and efficacy. In addition, pan-gender vaccination and current clinical trials are also discussed. Currently, more efforts should be put into increasing the vaccine’s coverages. Vaccinations against all HPV subtypes, namely, bivalent, quadrivalent, and nonavalent, are available. Approximately 20% of all cancers are associated with infectious agents such among them, human papillomaviruses (HPVs) are very common and are now recognized as the etiological agent of cervical cancer, the second most common cancer in women worldwide, and they are increasingly linked with other forms of dysplasia. Carcinogenesis is a complex and multistep process requiring the acquisition of several genetic and/or epigenetic alterations. HPV-induced neoplasia, however, is in part mediated by the intrinsic functions of the viral proteins. In order to replicate its genome, HPV modulates the cell cycle, while deploying mechanisms to escape the host immune response, cellular senescence and apoptosis. As such, HPV infection leads directly and indirectly to genomic instability, further favouring transforming genetic events and progression to malignancy. The molecular mechanisms exploited by HPV to induce neoplasia, with an emphasis on the role of the 2 viral oncoproteins E6 and E7. Greater understanding of the role of HPV proteins in these processes will ultimately aid in the development of antiviral therapies, as well as unravel general mechanisms of oncogenesis. One of the key events of HPV-induced carcinogenesis is the integration of the HPV genome into a host chromosome. HPV genome integration often occurs near common fragile sites of the human genome , but there are no apparent hot spots for integration and no evidence for insertional mutagenesis, Integration follows a more specific pattern with respect to the HPV genome. Human papillomavirus (HPV) vaccines are among the most effective vaccines available, the first to prevent infection by a mucosatropic sexually transmitted infectious agent and to do so without specific induction of mucosal immunity.
Keywords:(HPV- Human Papilloma virus), HPV- Vaccine, Cervical cancer , Immunoprevention.

Introduction:
Cervical Cancer begins on the surface of the cervix. It happens when the cells on your cervix being to change to precancerous cells. The human papillomavirus (HPV) belongs to the Papillomaviridiae family and is a DNA virus most commonly implicated in causing sexually transmitted diseases. Until now, more than 40 HPV subtypes have been isolated that have the potential to cause infection in the genital areas of both genders. Different subtypes of the human papillomavirus affect different areas of our body. But among all the subtypes, a total of only 15 human papillomavirus subtypes are related to the development of cervical cancer. Among the subtypes causing cervical cancer, the HPV 16- genotype accounts for the causative organism in 70% of cases.

Infection by human papillomavirus (HPV) is extremely common and associated with the development of benign warts or malignant lesions of the skin and mucosa. Infection by a high-risk (oncogenic) anogenital HPV type, most often through sexual contacts, is the starting point of virtually all cases of cervical cancers and the majority of anal cancers. The same viral types are also increasingly being linked with a subset of head-and-neck and non-melanoma skin cancers. Although prophylactic vaccines are now available to protect against the four types most commonly found in cervical and anal cancers (HPV16 and HPV18) and anogenital warts (HPV6 and HPV11), these neither protect against all genital HPV's nor are of therapeutic utility for already infected patients. Thus, the need for antiviral agents to treat HPV-associated diseases remains great, but none currently exist. This article reviews the recent progress made towards the development of antiviral agents to treat HPV infections, from target identification and validation to the discovery of lead compounds with therapeutic potential.

Human papillomavirus (HPV) vaccines are among the most effective prophylactic vaccines available and have established several important landmarks in human vaccinology. They are also the first subunit vaccines to consistently induce long-term (more than a decade) stable serum antibody responses. HPV vaccines appear to induce sterilizing immunity from initial infection for a least a decade without additional booster vaccination. The high efficacy found in prelicensure clinical trials has been confirmed by dramatic impact and effectiveness observed in national immunization programs over the past decade. There are now ambitious goals for reduction of HPV-associated disease. In contrast to prophylactic vaccines, vaccines to treat HPV infections or induced neoplasia have had limited clinical success to utilize. All HPV vaccines protect against at least HPV types 16 and 18, which cause the greatest risk of cervical cancer. It is estimated that HPV vaccines may prevent 70% of cervical cancer, 80% of anal cancer, 60% of vaginal cancer, 40% of vulvar cancer, and show more than 90% efficacy in preventing HPV-positive oropharyngeal cancers. They
additionally prevent some genital warts, with the quadrivalent and nonavalent vaccines that protect against HPV types HPV-6 and HPV-11 providing greater protection.

Cervical cancer is a cancer arising from the cervix. It is due to the abnormal growth of cells that have the ability to invade or spread to other parts of the body.

**Two Types of Cervical Cancer:**
1. Squamous Cell Carcinomas
2. Adenocarcinoma.

**Etiology:** (Cervical Cancer)

Etiologic factors include genetic influences, HPV- Human Papilloma virus, skin-to-skin or skin-to-mucosa contact, Vaginal, Anal, Oral- sex, Sharing sex toys, tumor, Sexually Transmitted Infection. DNA-mutations(Cervical-cells- Cancerous cells).

As the primary etiological agents of cervical cancer, human papillomaviruses (HPVs) must deliver their genetic material into the nucleus of the target cell. The viral capsid has evolved to fulfil various roles that are critical to establish viral infection. The particle interacts with the cell surface via interaction of the major capsid protein, L1, with heparan sulfate proteoglycans. You can pass HPV to your baby if you have genital warts when you’re pregnant.

**Hormonal Influences:**
Hormones may play a role in developing Cervical Cancer in some individual patients. The exact mechanism is high range of Estrogen concentration in women sufficient to Cervical cancer development with high risk of HPV-infection. Hormonal Contraceptives, Hormonal Imbalance. GHR- Growth Hormone Receptor- (Growth Hormone signals may act in Proliferation of utrine and cervical neoplasms.

Vaginal amebiasis (mimic symptoms and imaging of cervical cancer). Immunotolerance for the hosts immune system infection of HPV for cervical cancer.

**Cell Proliferation:**
Multiple cell proliferation- DNA-cell is multiple out-of-control-cells Accumulate in growth called tumors.

**Symptoms:** Early on, typically no symptoms are seen. Later symptoms may include abnormal vaginal bleeding, pelvic pain or pain during sexual intercourse.

**Signs and Symptoms:**
- Vaginal Discharge- foul odor, Difficult /painful urination, Abdominal pain.

**Stages of Cervical Cancer:**
- Cervical Cancer stage from I to IV
  - Stage- 1A,1B( Early stage)
  - Stage- 2B( Locally Advance Stage)
  - Stage- 3 &4A( Spread to other part)

**Risk factors for HPV:** Human Papilloma Virus

The contribution of risk factors like alcohol consumption, cigarette smoking, exposure to the sun, and other radiation for a prolonged period to the causation of cancer is well-documented. But the general public is less aware that a significant amount of the world's cancer burden is linked to infectious diseases. Epidemiological studies suggest that socioeconomic factors like education and income, sexual and reproductive factors, and other lifestyle factors and specific health behaviours mentioned above contribute to the development of cervical cancer HPV -16, which belongs to the high-risk HPVs group, is a major risk factor in developing invasive cervical cancer. Therefore, there could be a significant decrease in the incidence of cervical cancer by preventing persistent HPV infection.

**Transmission:** (HPV)
Sexually transmitted HPV is divided into two categories: low-risk and high-risk. Low-risk HPVs cause warts on or around the genitals. Type 6 and 11 cause 90% of all genital warts and recurrent respiratory papillomatosis that causes benign tumors in the air passages. High-risk HPVs cause cancer and consist of about a dozen identified types. Types 16 and 18 are responsible for causing most of HPV-caused cancers. These high-risk HPVs cause 5% of the cancers in the world. In the United States, high-risk HPVs cause 3% of all cancer cases in women and 2% in men.

**Epidemiology and Clinical Aspects:**
Mucosal HPV types can infect epithelia of the anogenital and upper aerodigestive tract. Although HPV infections are very common, most remain asymptomatic and are cleared within 6–12 months through an effective immune response. Nonetheless, a small subset of infections ultimately results in neoplasia. The link between HPV infections and cervical cancer is now strongly established, such that HPV is detected in 99.7% of cases and in the vast majority of high-grade neoplasia. HPV infections have also been associated with anal carcinogenesis and HPV is found in 80% of anal cancers, with HPV16 accounting for the majority of cases. The occurrence of anal cancers has steadily increased since the 1980s, particularly in the HIV-positive population of men who have sex with men. This has led to the suggestion that screening programs should be implemented within this population and other ones at a higher risk of developing anal dysplasia. Aside from their high susceptibility to anogenital cancers, HIV-positive and other immunosuppressed patients are at high risk of developing non-melanoma skin cancers when infected with HPV, especially at sun-exposed sites. Susceptibility to HPV-induced non-melanoma skin cancers upon UV exposure is also observed in the rare inherited disorder epidermodyplasia verruciformis. Furthermore, HPV has been linked to other types of genital neoplasia, including vulvovaginal and penile cancers, and accumulating evidence suggests that HPV is associated with a subset of head and neck cancers, and in particular those of the tonsils. With the use of more sensitive viral DNA detection technologies, recent case-control studies proposed that HPV infection was causally associated with these cancers and was a risk factor independent from those previously reported, such as cigarette and alcohol consumption.

**HISTORY OF Human Papilloma Virus:** (HPV)-
Human Papilloma viruses are small non-enveloped double-stranded DNA viruses with genomes containing 8 kb of DNA Sequences. Papillomaviruses with 55-nm-diameter icosahedral capsids that contain double-stranded DNA genomes of approximately 8,000 bp. They are widely distributed throughout the animal kingdom, specifically infect squamous epithelia, and cause the generation of warts. An infectious etiology of warts was long suspected and eventually proven in the 19th century. One of the first recorded experimental wart transmission cases in humans appears to have been accidental and was reported in 1845 by a certain Chandler, who “when removing a large acicular condyloma with his instrument injured his assistance beneath the thumbnail. On the injured place there appeared after a short time a wart, which was repeatedly destroyed, but reappeared, until the nail of the injured thumb was removed”. Ullmann also noted a similar accidental transmission of laryngeal papillomas and performed self-inoculation experiments with laryngeal papilloma extracts applied to scabified sites on his forearm, and these experiments yielded warts after a lengthy latency period of 9 months. Similar inoculation experiments had also been performed with extracts derived from common hand warts, and serial inoculation experiments with human subjects were performed.

Genital warts and cervical cancer were long regarded as manifestations of then-common venereal diseases such as syphilis and gonorrhea. Extracts of a penile condyloma that was harvested from a young medical student who did not exhibit other overt symptoms of venereal diseases were used to inoculate sites on the forearms of the author and his assistant as well as the genital mucosa of a “virgo intacta.” After a period of 2.5 months, the unfortunate female subject developed genital condyloma, and flat warts appeared on the forearms of two male probands. These and other experiments led to the realization that genital warts represent distinct disease entities that are caused by a transmissible agent.

The concept that some warts have an inherent propensity for malignant progression was established from studies by Shope, Rous, and others who studied experimental transmission of warts that occur naturally in cottontail rabbits. These investigators discovered that lesions that formed in domestic rabbits after inoculation with cottontail rabbit wart extracts were particularly susceptible to malignant progression. Careful transmission studies demonstrated that such extracts caused the emergence of warts only in rabbits and not in other animals, thus illustrating the exquisite species specificity of papillomaviruses.

Harald zur Hausen’s laboratory was the first to demonstrate that genital warts contain human papillomavirus (HPV) genomes. Subsequent low-stringency hybridization experiments with HPV sequences isolated from genital warts performed in his laboratory led to the discovery of related HPV sequences in cervical cancer tissues.

**HPV Gene Expression in Cervical Cancers:**

One of the key events of HPV-induced carcinogenesis is the integration of the HPV genome into a host chromosome. HPV genome integration often occurs near common fragile sites of the human genome, but there are no apparent hot spots for integration and no evidence for insertional mutagenesis. Integration follows a more specific pattern with respect to the HPV genome. Expression of the viral E6 and E7 genes is consistently maintained, whereas other portions of the viral DNA are deleted or their expression is disturbed. Loss of expression of the HPV E2 transcriptional repressor is significant, as it may result in deregulated HPV E6 and E7 expression. There is also evidence for increased HPV-16 E6/E7 mRNA stability after integration, and specific alterations of host cellular gene expression have been detected upon HPV genome integration. Cells that express E6/E7 from integrated HPV sequences have a selective growth advantage over cells with episomal HPV genomes. The concept that loss of E2 repressor function may be critical for malignant progression is supported by experiments showing that reexpression of E2 in cervical cancer cell lines causes growth suppression. These experiments clearly demonstrate that continued E6/E7 expression in cervical cancers is necessary for the maintenance of the transformed phenotype.

Integration of the viral genome into a host cell chromosome also leads to loss of E5 expression. In papillomaviruses that cause fibropapillomas, such as bovine papillomavirus type 1, the E5 open reading frame encodes the major transforming viral protein. E5 associates with intracellular membranes and transforms cells by activating receptor tyrosine kinases such as platelet-derived growth factor receptor β through a ligand-independent mechanism. HPV E5 proteins may have similar activities, and disruption of E5 expression affects the life cycle of high-risk HPV. The fact that E5 expression is not generally detected in cervical cancers after viral genome integration demonstrates that E5 is not necessary for the maintenance of the transformed phenotype.

**Biological Activities of HPV Oncoproteins:**

The oncogenic activities of high-risk HPV E6 and E7 genes in tissue culture and transgenic mouse model systems have been documented extensively. Expression of high-risk HPV E6 and E7 genes in primary human keratinocytes effectively facilitates their immortalization. When grown under conditions that allow stratification and the formation of skin-like structures, high-risk HPV E6/E7 immortalized cells display histomorphological hallmarks of high-grade squamous intraepithelial lesions, well-established precursors of cervical cancers. At low passage numbers, however, high-risk HPV immortalized cells are nonneoplastic. They can undergo malignant progression after extended growth in tissue culture or when additional oncogenes such as ras or fos are expressed.

The development of cervical cancers in a transgenic mouse model in which HPV-16 E6/E7 is expressed in basal epithelial cells is dependent on long-term exposure to low doses of estrogen. Similarly, progression of high-risk HPV-positive cervical lesions is often a slow process that occurs at a low frequency and requires the acquisition of host cellular mutations. The rate of spontaneous mutagenesis in normal human cells is exceedingly low, but the expression of high-risk HPV E6/E7 proteins dramatically augments genomic instability. Therefore, expression of the high-risk HPV E6/E7 genes not only is necessary for the induction of premalignant alterations but also directly contributes to malignant progression by subverting genomic stability.

**HPV-host cell interactions** (Host cell- Cervix-Cell)

**Cell surface binding: Receptors**

Host cell entry of HPV is initiated by binding of the virus particle to cell surface receptors. It has been suggested that virions bind initially to the basement membrane prior to transfer to the basal keratinocyte cell surface. It is important to note that the entry of HPV in vitro is initiated by binding to a cell surface receptor in contrast to the in vivo situation where the basement membrane has...
recently been identified as the primary site of virus binding. The analyzing virus-cell interactions and uptake mechanisms, much can be learned about the biology of HPV replication and entry pathways, providing a means to discover unique ways for exploiting or interfering with the viral pathogenesis. Like other viruses, HPVs are obligatory intracellular parasites and must deliver their genome and accessory proteins into host cells and subsequently make use of the biosynthetic cellular machinery for viral replication. The journey of a HPV particle from the cell surface to the cytosol and nucleus consists of a series of consecutive steps that move it closer to its site of replication. The viral capsid plays a key role in the establishment of the viral infection.

The entry of HPV in vitro is initiated by binding to a cell surface receptor in contrast to the in vivo situation where the basement membrane has recently been identified as the primary site of virus binding. Binding of HPV triggers conformational changes, which affect both capsid proteins L1 and L2, and such changes are a prerequisite for interaction with the elusive uptake receptor. Most HPV types that have been examined, appear to enter the cell via a clathrin-dependent endocytic mechanism, although many data are inconclusive and inconsistent. Furthermore, the productive entry of HPV is a process that occurs slowly and asynchronously and it is characterised by an unusually extended residence on the cell surface.

**HPV Diagnosis:** (Human Papilloma Virus)/ Cervical Cancer
HPV just by examining your warts. But there are also several tests they can use if you don’t have symptoms you can see.

**Vinegar solution test:** This test uses a vinegar (acetic acid) solution. Your doctor applies it to your genital area. If you have lesions in the area, they’ll turn white.

**Pap test:** Your doctor uses a swab to collect samples from your cervix or vagina. They send the samples to a lab to see if you have abnormal cells. Abnormal cells can lead to cancer.

**DNA test:** If you’re a woman over 30, your doctor may recommend this test along with a Pap test. They look at the DNA of the cells of your cervix to see if you have the type of HPV that can lead to cancer.

<table>
<thead>
<tr>
<th>List of HPV:</th>
<th>Protein Functions</th>
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<tbody>
<tr>
<td>E 1</td>
<td>Viral DNA –replication and Transcription</td>
</tr>
<tr>
<td>E 2</td>
<td>Viral DNA –replication and Transcription, repressor of E6/E7</td>
</tr>
<tr>
<td>E 4</td>
<td>Viral DNA –replication</td>
</tr>
<tr>
<td>E 5</td>
<td>Immune recognition (MCH-Major Histo compatibility Complex)</td>
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<tr>
<td>E 6</td>
<td>P53 degradation, alteration of cell cycle regulation, apoptosis resistance.</td>
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<tr>
<td>E 7</td>
<td>Retinoblastoma(PRb) degradation, re-entry into S phase cell cycle, P16 Overexpression.</td>
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<tr>
<td>L 1/L 2</td>
<td>Major/Minor – Viral Capsid Protein</td>
</tr>
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**Immunologic Aspects of HPV Infection:**
The majority of immunocompetent individuals infected with HPV are able to clear the viral infection and remain asymptomatic. It is, however, controversial whether the virus is truly eliminated from the patient or suppressed to such an extent that it is undetectable with current sampling and analytic approaches. In a minority of patients, HPV infection persists and causes clinically detectable lesions that can progress to invasive cancer over a long time period, typically measured in years to decades. 9.13 It is not entirely clear why HPV infection persists in certain individuals, but deficits in cellular immunity, such as treatment of solid organ transplant patients with immunosuppressants and those coinfected with HIV, are associated with increased persistence and HPV related disease. Conversely, the prevention of new infections by vaccination with L1 viral like particles (VLPs) appears to be primarily affected by neutralizing antibodies. In animal models, passive transfer of serum immunoglobulins from L1 VLP-immunized animals is sufficient to confer protection upon naïve animals. Furthermore, the measurement of in vitro neutralization titers in sera is the best available correlate to assess protection in patients for vaccine types and also cross-protection against non-vaccine types, although no minimal titer for protection has been defined as yet.

**HPV Infection Cycle:**
As mentioned previously, HPV has a predilection for epithelial cells of the cervix, which are stratified into a non-differentiated basal monolayer and a suprabasal differentiated non-proliferating epidermis. The basal layer sits above the basement membrane, below which is the cervical stromal layer. Dividing immature basal cells move upward through to the epidermal layer where they are shed as part of the natural process of epithelial maturation. Traumatic micro-abrasions, such as occur during sexual intercourse, expose the naïve basal layer cells to HPV. 14,15 Cell entry is not well understood but is believed to be receptor-mediated and multiple reports have implicated heparin sulfate as a candidate molecule involved in this process. 16,17 HPV replication is dependent upon and utilizes the normal replicative machinery of the cervical cells, which is subverted by two viral proteins, E1 and E2. The virus is maintained at typically ~100 episomal copies per basal cell and the initial infection triggers a burst of viral
replication up to this level. Basal cells that are infected with HPV continue to divide and each form two daughter cells containing viral genomic material. One cell of the pair remains in the basal layer and retains its dividing capacity, therefore acting as a repository for replication of the virus, which requires active cell division to maintain its life cycle. The other daughter cell continues upward through the suprabasal layer, where it differentiates and eventually is shed from the epithelial surface. In order to ensure that cervical cells are maintained in a state of constant growth and division, HPV early proteins are expressed, which stimulate and propagate cell growth via the actions of the E5, E6 and E7 genes. Upon cellular differentiation in the suprabasal layer, the viral genome is replicated to 10,000 or greater copies/cell, and expression of the late viral genes E4, L1 and L2 is triggered. The L1 (major) and L2 (minor) proteins form the capsid structure around the genomic material of the virus. Once this assembly is complete within the cells, the mature viral particles are released from the epithelial cells during terminal shedding from the epithelial surface. It is postulated that the E4 viral protein facilitates the release and spread of HPV from the keratin cage within keratinocytes by collapsing keratin filaments in the dying squames. It should be noted that carcinogenic progression is not part of the normal HPV life cycle, but rather a non-productive ‘dead end’ that is only associated with a small subset of virus types and occurs only after a long period of infection. The vast majority of infections are benign and self-limited.

**HPV Virology:** *(Human Papilloma Virus)*

E1 and E2. The initial events after HPV achieves cell entry and delivery of the genome to the nucleus trigger the expression of the HPV early genes E1 and E2. The E1 and E2 genes activate viral replication through interaction with specific sequences in the HPV genome origin of replication. By binding to sites that are proximal to its promoter, E2 leads to the recruitment of E1 to the HPV origin of replication. E1 binding to E2 increases stable binding to the AT-rich sequences within the origin of replication through the formation of a binding complex. E2 contains about 360 amino acids and possesses a DNA binding domain as well as a transactivation domain, and it can be spliced into a truncated variant lacking the transactivation domain. Thus, through alternative splicing, E2 is capable of both activation and repression of its target proteins in HPV. Interestingly, E2 also downregulates E6 and E7. Since E2 expression is typically lost during viral integration and carcinogenic progression, E6 and E7 are relieved of the suppressive influence of E2, leading to elevated expression of these viral oncogenes. By repression of an early promoter, E2 self-regulates levels of E1 and E2 transcription to maintain a stable viral copy number (~102/cell) in the basal epithelium. As keratinocyte differentiation progresses in the upper layers of squamous epithelia, the transcription of E1 and E2 switches to a different (late) promoter that is not modulated by E2, thus allowing for vegetative replication and increased (~104/cell) viral genomic copies. Following this, expression of the capsid antigens leads to the formation of viral particles by encapsidation of the nucleosome-bound viral genomes. E5, as part of the early replication process, is the gene expressed. E5 is a small, hydrophobic, single membrane-spanning protein that complexes with platelet-derived growth factor receptor and epidermal growth factor receptor to stimulate cell growth. E5 also inhibits apoptosis, and maintains the epithelial cell in continuous replication. Much of the specific activities and function of E5 are poorly understood, however, it appears that this gene is involved mainly in early events and has an unknown role in the later phases of viral replication. Although it has onco-gevic activity, E5 expression is usually absent in malignant cervical cells. E5 is expressed in early events and has an unknown role in the later phases of viral replication. Although it has onco-gevic activity, E5 expression is usually absent in malignant cervical cells.

**Treatment and prevention of HPV infection:**

Current therapies for HPV-associated lesions are mostly ablative and cytotoxic in nature (cryotherapy, surgical excision, topical application of cytotoxic agents, etc), although genital warts can be treated by topical application of the immunomodulator the recently approved Polyphenon®E ointment made of catechins extracted from green tea. As a prophylactic measure, a quadrivalent vaccine based on recombinant virus-like particles from HPV types 6, 11, 16 and 18. To this vaccine has been shown to be safe and highly effective in preventing the development of pre-cancerous cervical lesions associated with HPV16 and HPV18, and genital warts caused by HPV6 and HPV11. An analogous bivalent vaccine directed against HPV16 and HPV18 was expected to be submitted for US Food and Drug Administration approval in 2007. However, because immunological cross-reactivity between HPV types is limited, these vaccines will not protect against all anogenital HPV types nor eliminate the need for Pap screening. Nevertheless, because they are effective against the most prevalent low- and high-risk types, they are expected to significantly reduce the disease burden associated with anogenital HPV infections.

**HPV and Vaccination:**

The use of vaccines that activate cytotoxic cells and stimulate cell immunity responses effectively prevents viral infection. There are different vaccines against viral infections, such as prophylactic and therapeutic vaccines. Studies have shown that most viral vaccines have been used successfully for vaccination-induced humoral immune responses. Similar results were obtained from prophylactic HPV vaccines, showing protection against persistent infections and premalignant neoplasia by inducing neutralizing antibodies (mostly IgG). Therapeutic vaccines are different from prophylactic vaccines. They stimulate cell-mediated immunity (especially CD8+ T cells) rather than neutralize antibodies. To date, no therapeutic vaccines are approved for use in viral infection. Although, various researcher teams are trying to develop a safe and effective therapeutic vaccine. Different methods have been investigated for the synthesis and development of therapeutic vaccinations, including nucleic acid-based, peptide-based, protein-based, cell-based, and live-vector vaccines, all of which are currently in clinical trials. Clinical trials of HPV therapeutic vaccines show that they are safe and efficient in treating cervical cancer while also having limitations. A bacterial vector vaccine, ADXS11-001, and a DNA vaccine, VGX3100, are both in phase III clinical trials, indicating that they have promising potential. pNGVL4aCRT/E7 (NCT01493154), VGX-3100 (NCT01304524), and GX-188E...
(NCT02139267) (48) are based on E6/E7 gene vaccination against HPV and used electroporation as a delivery technique. Many clinical trials are now looking into the role of checkpoint inhibitor therapy. The combination of a therapeutic HPV vaccine and anti-PD1 therapy has been proven to be effective so far (49). It is critical to design various platforms for simultaneous use in order to prevent the restrictions of each platform. By reducing T-cell inhibition and enhancing proinflammatory cytokines, combination therapy may solve some of the potential drawbacks of therapeutic vaccinations. Peptide-based vaccines, for example, can be used as a booster for viral-based vaccinations to prevent antivector immunity.

Prophylactic Vaccines

The prophylactic vaccines activate the humoral immunity and production of virus-neutralizing antibodies, inhibit viruses from entering into host cells, and induce effective protection against HPV infection. To date (2021), three prophylactic licensed vaccines for the prevention of high-risk HPV infection are available in most countries: the vaccinations are Gardasil, Cervarix, and Gardasil-9. These vaccines were produced by recombinant DNA technology using the HPV L1 capsid proteins, which self-assembled into the noninfectious form of virus-like particles (VLPs). The VLPs contain no viral DNA genome and no live HPV, which is noninfectious and nononcogenic. The first generation of prophylactic vaccines was approved in 2006 and named Gardasil™ (Merck, West Point, PA, USA) or quadrivalent human papillomavirus recombinant vaccine (53). It has VLPs containing low-risk HPV6 (20 μg) and HPV11 (40 μg) and high-risk HPV16 (40 μg) and HPV18 (20 μg), which are responsible for 90% of genital warts. Cervarix™ (GlaxoSmithKline, Rixensart, Belgium) or human papillomavirus bivalent vaccine recombinant contains VLPs of high-risk HPV16 (20 μg) and HPV18 (20 μg), which cause approximately 70% of invasive cervical cancers worldwide (54). This vaccine was approved in 2009. The bivalent vaccines were produced using baculovirus-infected insect cell Trichoplusia, and the quadrivalent vaccines were made by Saccharomyces cerevisiae that expressed the L1 gene. To improve the efficacy of immune responses for a longer time, the bivalent contain the proprietary adjuvant ASO4, which is constructed from 500 mg aluminum hydroxide and 50 mg toll-like receptor 4 agonists, 3-O-desacyl-4’ monophosphoryl lipid A as an additional immunostimulator. In addition, quadrivalent vaccines used 225 mg amorphous aluminum hydroxyphosphate sulfate (AAHS) as an adjuvant. It has been reported that both vaccines revealed effective safety and immunogenicity profiles.

immunocompromised women or started vaccination between 15 and 45 years, a three-dose program (at 0, 1–2 months, 6 months) is recommended (59–61). In addition, the third dose of vaccine should be undertaken for individuals who do not receive the vaccine before the aged of 15 years. Also, vaccination of 15- to 26-year-old women, 15- to 21-year-old men, and high-risk men up to 26 years of age is recommended in a three-dose series (61, 62). The HPV vaccines are safe, and their local adverse reactions such as pain, swelling, and redness are usually short and reversible. The systemic reactions of the available HPV vaccines, including fever, nausea, dizziness, fatigue, headache, and myalgia are rarely observed after vaccination (63). Several studies revealed that HPV VLPs induced an effective humoral immune response, and HPV vaccination generates 10- to 100-fold higher titers of specific neutralizing antibodies against HPV antigens than natural infection (64–66). However, the levels of produced antibodies are dependent on the sex and age of vaccinated individuals and the type of administrated vaccine. The comparison of seroconversion in males and females aged under 30 years demonstrated higher titers of specific antibodies among females ages 9 and 15 years vs. females who had the vaccine at 16 and 26 years old (67–71). Long-time evaluation of the immunogenicity of HPV16/HPV18 vaccine in the serum of females aged between 15 and 55 years showed high seropositive antibody levels for anti-HPV16 vaccine in all age groups, 10 years after the first dose of vaccination. In contrast, seropositivity rates for anti-HPV18 decreased in the age group of 15–55 years with aging (67). However, anti-HPV16 and anti-HPV18 antibodies were higher than natural infection with HPV in all studied groups and could be detected more than 30 years after vaccination.

The types of prophylactic HPV vaccines elicited specific immune responses, but the structural similarity between L1 genes of vaccine and nonvaccine HPV types led to long-term cross-reactive immunogenicity against HPV types not included in the vaccine. Previous studies have been reporting a cross-protection against HPV31 and HPV45 types after administration of bivalent (HPV16/HPV18) vaccines. In addition, cross-reactive immunogenicity has been detected against HPV45 for the quadrivalent HPV vaccine (74). These findings suggest that HPV vaccination could induce an effective immune response against nonvaccine HPV types.

Clinical Research For HPV-Vaccine: U S FDA

Synthesis of viral capsid Human Papillomavirus Vaccines:

In June 2006, the United States Food and Drug Administration (FDA) approved the prophylactic quadrivalent HPV vaccine (GARDASIL, Merck and Co., Inc.), for use in women aged 9–26 years old.45 Subsequently, in October 2009, the prophylactic bivalent HPV vaccine (CERVARIX, GlaxoSmithKline) was licensed for use in women aged 10–25 years old.46 The quadrivalent vaccine is composed of recombinant L1 protein-based viral like particles from HPV 6, 11, 16 and 18. Phase II and phase III studies have shown efficacy of 100% in preventing cervical dysplastic lesions in women aged 16–26 years, who were not infected by any of the vaccine HPV types. 47 Efficacy in women with simultaneous vaccine type HPV DNA positivity and seropositivity was about 25%. The most common adverse effects were related to injection site pain (84 vs. 48.6% in treatment and placebo groups respectively). It is expected that the vaccines will achieve a lifetime risk reduction of 20–70% for cervical cancer, in patients aged 12 years. The vaccine is typically given in three doses of 0.5 ml at 0, 2 and 6 months. The bivalent vaccine contains recombinant L1 protein from HPV 16 and 18, and phase III study of 18, 644 females followed for 35 months showed efficacy of up to 93% in prevention of CIN 2 lesions due to HPV 16 and 18.46 Similar to the quadrivalent vaccine, there was a higher rate of complications at the injection site. The dosage schedule is 0.5 ml at 0, 1–2, and 6 months for a total of three doses. New approaches to vaccine development may provide more options for controlling HPV acquisition and transmission in the future. The use of recombinant L2-based prophylactic vaccines (Roden R, unpublished data), as well as the development of drug targets against early protein (E6 and E7) activity are a few examples. The study of HPV has provided valuable information on the mechanisms by which tumor viruses initiate carcinogenesis and the regulation of cell cycle in human cells. Cervical cancer as a disease model presents unique
opportunities in finding a cure, because it is caused by a pathogen with well characterized biological mechanisms and life cycle. The use of cytologic and HPV screening and ablation of premalignant disease has proven very successful at reducing cancer rates in developed nations. The recent introduction of prophylactic HPV vaccines has been another great step forward. Given the huge disease burden in the developing parts of the world, it is evident that population based measures offer the best hope of limiting mortality worldwide. The development of therapeutic cervical cancer vaccines and/or virus-targeted drug therapies is eagerly awaited.

HPV Vaccine Efficacy:
Cervarix induces high anti-HPV-16 and 18 antibody titers and can prevent the incidence of infection for at least 10 years. In addition, Cervarix invokes a significantly high and long-term cross-reactive immunogenicity against HPV-31 and 45. During a 10-year follow-up study, over 85% of participants remained seropositive for anti-HPV-31 and 45 antibodies following three doses of Cervarix. In addition, Cervarix efficiently (>90%, injection prior to HPV exposure) protects against vaccine-targeted HPV related abnormalities and precancerous lesions, including cervical intraepithelial neoplasia 2 (CIN2), CIN3 and adenocarcinoma in situ (AIS) [38,39]. Cervarix also shows efficacy (>60%) in preventing all cervical precancerous lesions regardless of HPV infection or precancerous lesions caused by any HPV types [40,41]. Notably, Cervarix also showed strong protection against oral HPV16 and 18 infections. After a four-year vaccination period, a 93% reduction in the prevalence of oral HPV-16 and 18 infections was reported.

Quadriavalent Gardasil shows excellent efficacy against cervical HPV infection, cervical cancer precursor lesions, and genital warts caused by the HPV types covered by Gardasil [9,11]. In addition, studies demonstrated that Gardasil significantly decreases HPV infections in the anus, vulva, and penis, as well as in the oral cavity related to HPV vaccine types [12,13,14]. Gardasil has a strong prevention rate (>90%, injection prior to HPV exposure) against CIN 2 or worse (CIN 2+), CIN 3+ and vulvar/vaginal intraepithelial neoplasia grade 2 or worse (VIN/ValIn 2+), caused by HPV 16 and 18 [6,41]. However, the inhibition on CIN 2 + and CIN 3 + caused by any HPV types was lower (20-50%) [6,41]. Comparatively, Gardasil demonstrated less cross-protection effect than Cervarix and the protection efficacy for HPV31, 33, 45, 52, and 58 were 46%, 29%, 7%, 18%, and 6%, respectively. Currently there are six licensed HPV vaccines: three bivalent, two quadriavalent, and one nonavalent vaccine. Those that have been prequalified are being marketed in countries throughout the world. All vaccines are highly efficacious in preventing infection with virus types 16 and 18, which are together responsible for approximately 70% of cervical cancer cases globally. The vaccines are also highly efficacious in preventing precancerous cervical lesions caused by these virus types. The quadrivalent vaccine is also highly efficacious in preventing anogenital warts, a common genital disease which is virtually always caused by infection with HPV types 6 and 11. The nonavalent provides additional protection against HPV types -31, 33, 45, 52 and 58. Data from clinical trials and initial post-marketing surveillance conducted in several continents HPV vaccines to be safe.

Cervavac HPV Vaccine:
Serum Institute of India(SII) Cervavac is the first Quadrivalent(HPV)-Vaccine made in India, Protecting people against HPV types 6,11,16 and 18. Efficacy was studied in HPV Vaccine clinical trials that started in September 2018.

Who should get HPV vaccination?
The Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) develops recommendations regarding all vaccination in the United States, including HPV vaccination. The current ACIP recommendations for HPV vaccination are Children and adults ages 9 through 26 years. HPV vaccination is routinely recommended at age 11 or 12 years; vaccination can be started at age 9 years. HPV vaccination is recommended for all persons through age 26 years who were not adequately vaccinated earlier.

Adults ages 27 through 45 years. Although the HPV vaccine is Food and Drug Administration (FDA) approved to be given through age 45 years, HPV vaccination is not recommended for all adults ages 27 through 45 years. Instead, ACIP recommends that clinicians consider discussing with their patients in this age group who were not adequately vaccinated earlier whether HPV vaccination is right for them. HPV vaccination in this age range provides less benefit because more people have already been exposed to the virus.

Persons who are pregnant. HPV vaccination should be delayed until after pregnancy, but pregnancy testing is not required before vaccination. There is no evidence that vaccination will affect a pregnancy or harm a fetus.

How is the Vaccine Administered?
As per the December 2022 WHO Position on HPV vaccines, WHO recommends the following schedule:
- A one or two-dose schedule for girls aged 9-14
- A one or two-dose schedule for girls and women aged 15-20
- Two doses with a 6-month interval for women older than 21

A minimum of 2 doses and when feasible 3-doses remain necessary for those known to be immunocompromised and/or HIV-infected.

Older will need three doses. The vaccine is recommended for everyone up to 26 years of age.

The HPV vaccine has also been recently approved for those 27 to 45 years old. However, HPV vaccination of people in this age range provides less benefit, as more have been already exposed to HPV. Since the vaccine was introduced, HPV infection rates
have fallen dramatically. Among teen girls, infections with the types of HPV that cause genital warts and cancer have dropped by 86%.

According to Cancer Centre’s, the HPV vaccination is administered as: A two-dose series (0, 6-12 months) for most persons who initiate vaccination at ages 9 through 14 years. A three-dose series (0, 1-2, 6 months) for persons who initiate vaccination at ages 15 through 45 years, and for immunocompromised persons.

How effective is Cervarix?

Cervarix is the first Indian HPV vaccine which acts against four different strains 6,11,16 and 18. Hence it is called a quadrivalent vaccine.

Comparison of HPV vaccines:
The HPV vaccines currently being produced are based on L1-VLPs, which only provide type-restricted immunity, neglecting many other oncogenic HPV genotypes. Consequently, the second-generation VLPs, such as L2-VLP and Chimeric L1-L2 VLP, are drawing a lot of attention for their broader genotype coverage [34,35]. In comparison to L1-VLP, the minor capsid protein, L2, contains type-common epitopes that can provide broad cross-neutralizing antibody responses. Notably, Cervarix can confer a degree of cross-protection against some phylogenetically related types of HPV16 and 18 from the same phylogenetic cluster alpha-9 (HPV16-like: HPV31, 33, 35, 52, 58) and alpha-7 (HPV18-like: HPV39, 45, 59, 68) species groups, owing to its unique adjuvant systems.

Mechanism of Vaccinations:
Currently, the licensed HPV vaccines are developed based on a virus-like particle (VLP) of the major papillomavirus capsid protein L1. Since VLPs are merely proteins and do not contain viral genome, these are considered non-infectious and non-oncogenic, and thus are safer than HPV-attenuated vaccines. VLPs can be produced in bacteria, yeast, or insect cells. Cervarix comprises HPV16 and 18 VLPs, monophosphoryl lipid A (MPL), and aluminum hydroxide (together called adjuvant system 04, AS04) as an adjuvant. MPL is a toll-like receptor 4 (TLR4) agonist that can induce high levels of antibodies as compared to Gardasil and Gardasil 9, both of which contain only aluminum hydroxide as an adjuvant and are produced in Saccharomyces cerevisiae yeast. Gardasil contains VLPs against HPV6, 11, 16, and 18, while Gardasil 9 contains VLPs against HPV6, 11, 16, 18, 31, 33, 45, 52, and 58.

In countries in the past decade, have shown promising results in decreasing HPV infection and related diseases, such as warts and precancerous lesions. In this review, we present the updated information about current HPV vaccines, focusing on vaccine coverage and efficacy. In addition, pan-gender vaccination and current clinical trials are also discussed. Currently, more efforts should be put into increasing the vaccine’s coverage, especially in low- and middle-income countries. Provision of education on HPV and vaccination is one of the most important methods to achieve this. Vaccines that target HPV types not included in current vaccines are the next stage in vaccine development. In the future, all HPV-related cancers, such as head and neck cancer, and anal cancer, should be tracked and evaluated, especially in countries that have introduced pan-gender vaccination programs. Therapeutic vaccines, in combination with other cancer treatments, should continue to be investigated.

HPV Vaccine Coverage:
Quadrivalent HPV vaccine, Gardasil (Merck & Co., Kenilworth, NJ, USA), is the first commercially available HPV vaccine licensed by the United States Food and Drug Administration (FDA), in 2006. The bivalent HPV vaccine, Cervarix (GSK, Brentford, UK) was approved by the European Medicines Agency (EMA) in 2007 and by the FDA in 2009. Cervarix protects against the most common oncogenic genotypes of HPV (types 16 and 18), which cause around 70% of cervical cancers. Gardasil, in addition to HPV16 and 18, also targets HPV6 and 11, which cause around 90% of genital warts. In 2014, a nine-valent vaccine, Gardasil 9 (Merck & Co., Kenilworth, NJ, USA), was licensed by the FDA, which offers protection against HPV6, 11, 16, 18, 31, 33, 45, 53, and 58. The five additional types covered by Gardasil 9 could cover HPV types related to another 20% of cervical cancer cases; thus, Gardasil 9 has the potential to protect against approximately 90% of cervical cancers. A comparative modeling analysis predicted that if the global strategy of combined intensive scaled-up HPV vaccination and twice-life time screening is achieved, the incidence of 97% cervical cancers would be reduced by 2100.

In general, HPV national programs cover about 30% of the global target population, with low full-dose coverage in many regions. The HPV vaccine coverage is significantly higher in high-income countries, where about 32% of females aged 10–20 years received the full-dose vaccination by 2014. HPV coverage is more than 60% in countries such as Australia, Denmark, and Sweden. Most low- and middle-income countries remain unprotected, only about 1% of adolescent females in low-income countries received a full course of HPV vaccines [24]. Fortunately, HPV vaccine was successfully introduced in some low- and middle-income countries’ national routine immunization schedules and achieved high coverage, such as in Bhutan and Rwanda. The HPV-vaccination gap also exists between urban and rural residents within low- and middle-income countries. For example, although 65.3% of children are fully immunized in India, the HPV coverage is only 2% in rural villages of Uttar Pradesh, with 72% vaccine coverage concentrated in urban areas. As more than 80% of cervical cancer deaths occur in low- and middle-income countries, the implementation of the HPV vaccine is urgently needed for public health intervention in these countries.

Immune Evasion:
Like many viruses, HPV has developed mechanisms to escape immune surveillance. This is of particular significance when considering that cervical carcinogenesis is linked to HPV persistence. One means by which HPV escapes immune detection is built into its natural life cycle. Primary infection occurs in the basal cells of the stratified epithelium where viral genomes are maintained.
only at very low levels. Viral proteins are also very weakly expressed and confined mainly to the nucleus of basal cells. Increased protein expression only occurs as keratinocytes migrate through the upper layers of the epithelium where the adaptive immune system has limited access. Finally, newly assembled viral particles are released by natural shedding, a process that does not involve cell lysis, thereby preventing dendritic cell activation, pro-inflammatory cytokine liberation, and antigen presentation by Langerhans cells in the proximal layers of the epithelium. HPV has also evolved mechanisms to counteract intracellular antiviral defense pathways. An interferon (IFN) response is usually triggered upon viral infection, which proceeds through IFN-α and IFN-β secretion, both of which harbour anti-proliferative and pro-apoptotic abilities. Interferon immunoregulatory effects were shown to be directly inhibited by HPV, both by reducing interferon expression and by interfering with its signalling pathways. Gene expression profiles studies have shown that HPV-31 downregulates IFN-responsive genes. Furthermore, E6 is known to physically interact with IRF-3 and inhibit its transactivation ability, and by doing so blocks IFN-β expression. HPV-18 E6 has also been proposed to interact with and impair activation of Tyk2, an important mediator of IFN-receptor signalling. Simultaneously, E7 can interfere with the IFN response by inhibiting IRF-1 and ISGF-3. Another innate intracellular antiviral response is mediated by the double-stranded RNA protein kinase (PKR), an IFN-inducible protein, which is also targeted by HPV. This protein kinase has been shown to be mis-localized in HPV-infected cells and its phosphorylation level reduced in cells expressing E6 and E7, suggesting a modulation of this pathway by HPV. Moreover, E5 has been proposed to interfere with MHC1 mediated antigen presentation by deregulating endosomal acidification and trafficking, and thus, prevents immune recognition. Altogether, these immune evasion mechanisms probably function in concert to facilitate viral persistence, a known risk factor for cancer progression.

Conclusions:
HPV is a common virus that can be easily transferred from person to person. Some types of HPV cause different cancers. It is essential to know how to prevent HPV infection or HPV-related cancers. Vaccination is an effective method for the early prevention of HPV infections. There are safe and highly effective prophylactic vaccines to prevent many HPV-related disorders. The bivalent and quadrivalent HPV vaccines appear to be significantly effective in preventing HPV infection after introducing vaccines into vaccination schedules. Bivalent vaccines produced higher immunogenicity against HPV infection than quadrivalent vaccines. A booster vaccine dose and adjuvants can improve the immune responses through neutralizing antibodies. Young women aged 15 to 26 showed higher levels of neutralizing antibodies so, they are the primary target for receiving HPV vaccines. Recent studies confirmed that administration of nonavalent HPV vaccine before starting sexual activity provides effective protection against multiple HPV subtypes. Recent studies of HPV vaccines tend to focus on the production of vaccines based on L1 and L2 capsid proteins in live viral or bacterial vectors, with cost-effective production systems. In addition, combined prophylactic and therapeutic vaccines could be prevented and treat HPV-related diseases. The next generation of HPV vaccines will reduce many limitations related to available vaccines and is a major step toward the fight against cervical cancer. Soon, developed vaccines will likely generate good protection against various types of HPV, being based on recombinant vectors, and maybe administrated by inhalation or through oral route. However, further clinical investigation is required to develop and validate cost-effective new vaccines with proper immunogenicity against various types of HPV. Understanding the specific pathological mechanisms of HPV is helpful in the development of more effective vaccines that are frequently used in the clinic.

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REFERENCE: