Prophylactic and Therapeutic Vaccines for Genital Papillomavirus Infection

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The development of potential therapeutic and prophylactic vaccines for human papillomavirus (HPV) infection is a very exciting area of HPV research. There are a number of features of HPV biology that makes the development of a vaccine particularly difficult, although there are several examples of vaccines that have had spectacular success in the prevention of other viral diseases. Our poor understanding of the immune response to HPV infection is the first problem. We do not understand the mechanism by which spontaneous clearing of warts is generated and therefore cannot particularly target this pathway in the development of a vaccine. Furthermore, there is no in vitro culture system nor an animal model for HPV. Another problem is that replication and RNA transcription. The viral capsid proteins (L1 and L2) are produced in the upper layers of differentiating epithelium. Human papillomavirus-containing epithelial cells are shed from the surface of the skin. A relatively large area of skin, probably in the order of 2–3 mm² (Steele and Gallimore, 1990), can be infected by the progeny of a single HPV-infected stem cell. As there is no cell lysis, there is no release of viral proteins and poor antigen presentation to the immune system. Antibody to both capsid proteins of the virus are produced, however, during the course of HPV infection (Christensen et al, 1992). After an average of 6 mo following infection this acquired immunity occurs and may be protective against reinfection, but is not likely to be therapeutic.

The progression of HPV infection to clinical disease appears to be regulated by host immune responses to HPV. Both humoral and cell-mediated immune responses are elicited by HPV. A crucial role in modulating the effects of HPV, such as lesion persistence and spontaneous regression, is played by cellular immunity, particularly the T cell system. The fact that immunosuppressed individuals (transplant, lymphoma, HIV disease) have enhanced HPV proliferation and an increased frequency of HPV infection and associated disease (Kast et al, 1996) illustrates the importance of cellular immunity. In addition, reduced numbers of Langerhans cells are found in CIN (Sherman et al, 1998), and dense infiltrates of T lymphocytes and macrophages are found in regressing warts.

In benign lesions viral DNA remains extrachromosomal. The viral DNA is incorporated into the host chromosome in most cervical cancers. Two of the viral genes, E6 and E7, are consistently retained and expressed in cervical cancers. These same HPV types show transforming activity (Schlegel et al, 1988) in cultured cells. Continued growth requires these...
transforming proteins that may also act as tumor rejection antigens.

**CLINICAL DISEASE**

Most clinically apparent genital HPV infections are benign. Subclinical genital infections with HPV are extremely common. A spectrum of disease results from genital HPV infection ranging from asymptomatic infection to invasive cervical cancer. Based on their association with cervical cancer, HPV types have been divided into low and high risks. External genital warts are the most common clinical expression of infection with low-risk HPV (Beutner et al., 1998a). The giant condyloma of Buschke-Lowenstein caused by HPV type 6 (and sometimes HPV 11) is a rare verrucous carcinoma of the external genital area. Weeks, months, or years may be required from the time of HPV infection to development of warts. Warts, once clinically apparent, may persist, spread, grow, spontaneously regress, and/or recur. A rare but devastating condition caused by perinatally acquired laryngeal low-risk HPV infection is termed juvenile laryngeal papillomatosis (JLP) and is usually due to infection with HPV 6 or 11.

The clinical manifestations of high-risk HPV types include abnormal pap smears, low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), atypical squamous cells of uncertain significance (ASCUS), and atypical glandular cells of uncertain significance (AGUS) or, on biopsy, cervical intraepithelial neoplasia, carcinoma in situ, and invasive cervical cancer. Some anogenital cancers are strongly associated with infection with certain HPV types. Almost all invasive cervical cancers and cervical intraepithelial neoplasia (CIN) contain HPV DNA. Weeks to decades may pass between infection to the appearance of cervical dysplasia and cervical cancer. As the vast majority of women infected with high-risk HPV never develop cervical cancer, these HPV types are considered necessary but not sufficient for the development of cancer. Certain cofactors are necessary but are currently not well understood. Thus, it is believed that if the acquisition of HPV could be prevented, cervical cancer could be greatly reduced, if not eliminated.

The clinical manifestations of the high-risk HPV types on the external genital area are papular lesions (Bowenoid papulosis) or erythematous scaly patches (Bowen’s disease), which histologically have been classified as squamous cell carcinoma in situ or vulgar intraepithelial neoplasia, or penile intraepithelial neoplasia; or, in the vagina, as vaginal intraepithelial neoplasia. Rarely, invasive squamous cell cancer of the penis, vulva, or vagina results.

The oncogenic potential of the high-risk HPV types is frequently found in the anus as well as the cervix, because both structures histologically have transformation zones where columnar epithelium changes to stratified squamous epithelium. Anal HPV infection has best been characterized in men, and its natural history is the subject of ongoing studies.

**EPIEDEMILOGY/EXPECTED IMPACT OF AN EFFECTIVE VACCINE**

It has been estimated that 1% of sexually active persons in the U.S.A. have visible genital warts (Koutsky et al., 1988). Because HPV 6 and 11 cause the majority (>90%) of genital warts, it is feasible to develop an effective bivalent vaccine for these low-risk infections. Genital warts consume healthcare resources and are emotionally troubling for the patients, although they are rarely associated with significant mortality or morbidity. Thus, immunization is an important goal. HPV infects about 50% of young sexually active women (Bauer et al., 1991; Koutsky et al., 1992; Ho, 1998). The leading cause of cancer death in women under the age of 50 worldwide is cervical cancer (Rowen and Lacey, 1998). In countries where screening is unavailable or underused the impact of HPV infection is greatest. Vaccination against HPV is an attractive goal due to this strong association between sexually acquired HPV and cervical cancer. The majority of cervical carcinomas is associated with relatively few HPV types and animal models have consistently shown that both prophylactic and therapeutic vaccination is feasible. The worldwide public health implication of both preventive and therapeutic vaccines not only includes saving lives, but also decreasing the cost of screening and treating premalignant cervical disease. The annual cost of HPV-related disease has recently been estimated by the Institute of Medicine to be $10 billion (Eng and Butler, 1996).

**HISTORY/BACKGROUND OF VACCINE RESEARCH AND OTHER STRATEGIES FOR CONTROL**

The inability to culture HPV and host tropism of the virus has hampered vaccine development. Cottontail rabbit papillomavirus (CRPV) studies established over 60 years ago that antibodies elicited by the injection of intact virions protect against experimental challenge by the homologous viral type (Shope, 1935). Intact virions displaying immunodominant epitopes were found to be needed to induce protective antibodies (Shope, 1935; Kidd, 1938; Pilacinski et al., 1986; Jin et al., 1990; Ghim et al., 1991). It was demonstrated that the L1 major capsid protein of HPV expressed in eukaryotic cells self-assembles into virus-like particles (VLP), which resemble authentic virions without the viral genome and its transforming genes. Neutralizing antibodies generated against confirmational epitopes found on the surface of native virions and VLP are sufficient to prevent infection both in vitro and in animal models.

Immunotherapy is another strategy for HPV control. Cells involved in the host defense can be collected from patients with cervical cancer or a histocompatible donor and then grown and activated ex vivo by cytokines such as recombinant IL-2 and transferred back to the cancer patient as therapy in a process known as adoptive cellular transfer (Hines et al., 1998). This approach (Boursnell et al., 1996; Krul et al., 1996) provided some protection against HPV 16 and 18 E6 and E7-positive tumors in mouse models.

**VACCINE STRATEGIES**

**Prophylactic vaccines** Protective anti-HPV antibodies to prevent infection are induced by HPV subunit vaccines. Antigenic targets in most prophylactic vaccine studies consist of L1 and L2. Fusion proteins, vaccinea virus recombinants, plasmids, and VLP are used in preparation of these vaccines. Molecular techniques (Rose et al., 1993) are used to produce VLP that are spherical 50 nm structures resembling hollow viral capsids. These VLP lack oncogenic DNA, but possess structurally intact viral capsid proteins and may be used in enzyme-linked immunosorbent and hemagglutination assays to detect humoral responses to HPV (Sherman et al., 1998).

**Therapeutic vaccines** Therapeutic vaccination may be used to eliminate residual cancer, cause regression of existing CIN or warts, or prevent progression of infection or low-grade disease to high-risk lesions. Potential targets in the development of therapeutic vaccines include HPV E6 and E7 epitope peptides selectively maintained and expressed during malignant progression. Human leukocyte antigen (HLA)-specific, human cytotoxic T lymphocytes (CLT) induced against E6 and E7 peptides have been shown to cause lysis of HLA-specific HPV-positive cervical carcinoma cell lines (Feltkamp et al., 1993; Alexander et al., 1996) in vivo studies.

**ANIMAL MODELS**

Because HPV is species-specific, it does not cause disease in animals, so extrapolating data to humans must be done with caution. Encouraging results have been obtained, however, using species-specific PV. Experimental infection ex vivo can be prevented with immunization with proteins produced in bacteria or immunization with vaccinia vectors that express L1 and/or L2, although low levels of neutralizing antibodies are induced (Pilacinski et al., 1986; Jarrett et al., 1991; Lin et al., 1992). On the other hand, VLP vaccines are strongly immunoegenic. Experimental
infection with native virus specific to beagle dogs (COPV) (Bell et al., 1994; Suzich et al., 1995), cottontail rabbits (CRPV) (Donnelly et al., 1991; Lin et al., 1993; Breitburd et al., 1995; Christensen et al., 1996), and calves (BPV) (Kirnbauer et al., 1996) can be prevented by prior immunization with homologous VLP. Both species and type specific protection is produced by neutralizing antiserum. Conformational epitopes present on the surface of VLP also help determine protection.

Systemic vaccination in animal models has produced protection of mucosal surfaces against the natural transmission of PV. HPV-11 L1 VLP-specific IgG present in the cervicovaginal secretions of monkeys parenterally immunized with HPV-11 L1 VLP is sufficient to neutralize HPV-11 in the athymic mouse xenograft system (Lowe et al., 1997). Therapeutic vaccines may target early antigens, including E1, E6, and E7 proteins. Regression of tumors derived from HPV 16-transformed oncogenic cell lines (Meneguzzi et al., 1991) have been observed in rats vaccinated with vaccinia virus expressing HPV 16 E7. Prevention and regression of CRPV tumors were both achieved using an E1 vaccine derived from recombinant Listeria monocytogenes (Jensen et al., 1997). A mechanism to eliminate cells undergoing productive viral infection is provided by T cell responses induced by HPV-16 L1 VLP (Dupuy et al., 1997; Luxton et al., 1997).

Studies with animal models have not evaluated authentic routes of PV infection or the longevity of immune responses, or established that tumors can be eliminated with vaccination, despite these encouraging results.

**CLINICAL STUDIES**

**Immunology and efficacy studies** Currently numerous clinical studies are underway throughout the world and a few have obtained data as described below.

In a phase I study with 65 healthy volunteers a vaccine specific for HPV type 11 was found to be both safe and immunogenic (Reichman et al., 1998). The major capsid protein, L1, was placed in a recombinant virus, expressed in insect cells, and VLP were produced. Volunteers seronegative for HPV-11 were given vaccine composed of 3, 9, 30, or 100 µg of VLP or placebo at 0, 4, and 16 wk. Neutralizing antibody titers against HPV 11 of 1:1000 or greater were achieved in seven of 10 subjects who received the 3 µg dose, nine of 10 who received the 9 µg dose, all 12 who received the 30 µg dose, and all 10 who received the 100 µg dose. The vaccine was well tolerated at all doses.

No significant toxicities, an antivaccine antibody response in all eight participants, and HPV-specific antibody response in three of eight participants resulted from a recombinant vaccine virus expressing E6 and E7 epitope peptides of HPV 16 and 18 in a phase I/II study in advanced cervical carcinoma (Borysiewicz et al., 1996).

Good humoral responses were produced in open-label studies with an L2 E7 fusion protein vaccine, incorporating an alun adjuvant combined with conventional therapy for the treatment of anogenital warts in men, although lymphoproliferative responses were variable (Lacey et al., 1997; Rowen et al., 1997).

**Vaccine development** A number of vaccines are in various stages of development (Table I). There are a number of observations that would seem to indicate that effective HPV vaccination is feasible although modern pivotal efficacy trials with HPV have not been completed. These observations are as follows: 1 more persistent and more frequent clinical expression of HPV infection (which is usually more difficult to treat) results from immunosuppression; 2 vaccine studies with animal papillomavirus systems have produced favorable results; 3 many infected humans never express their clinical lesions; 4 external genital warts, nongenital warts, and HPV-related cervical lesions often regress spontaneously; 5 infiltrates of regressing warts often contain CD4 lymphocytes and macrophages; 6 autologous vaccines have been reported to be successful in humans.

The possibility that a genital HPV vaccine might not be effective, on the other hand, is suggested by the following factors that make HPV vaccines particularly challenging:

1 There is no clear definition of protective immunity, nor is the necessary response to particular antigens well understood. Antibodies appear to protect against infection in animal systems, and cell-mediated immune responses are required for wart regression or protection against the development of warts.

2 With HPV the route and frequency with which the immunized subject is exposed naturally may be more chronic and repetitive than with other viral infections for which we have effective vaccines. The level of immunity required to protect against frequent genital exposure is unknown. Viral infections for which we currently have vaccines are not characterized by frequent (potentially daily) exposure for years.

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**Table I. Candidate HPV vaccines**

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Candidate</th>
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<tbody>
<tr>
<td>Peptide vaccines</td>
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<tr>
<td>HLA-A0201 HPV-16 lipopeptides</td>
<td>Steller and Schiller, 1996</td>
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<tr>
<td>HPV 16 E7 PADRE</td>
<td>Feltkamp et al, 1993</td>
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<tr>
<td>HPV 16 E7 “ISCAR” conjugate</td>
<td>Tindle et al, 1995</td>
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<tr>
<td>Protein-based vaccines</td>
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<tr>
<td>HPV 16 E6-E7</td>
<td>Mallarios et al, 1999</td>
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<tr>
<td>HPV 16 E7 + BCG H5/65</td>
<td>Zha et al, 1995</td>
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<tr>
<td>HPV 16 E7</td>
<td>Harihara et al, 1998</td>
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<tr>
<td>HPV 16 E2</td>
<td>Heinemann et al, 1999; Hibma et al, 1999</td>
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<tr>
<td>HPV 16 E4 with HbeAg</td>
<td>El Mehdouini et al, 1999</td>
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<tr>
<td>Virus-like particles</td>
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<tr>
<td>HPV 11 L1</td>
<td>Reichman et al, 1999</td>
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<tr>
<td>HPV 6 L1</td>
<td>Zhang et al, 1999</td>
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<tr>
<td>HPV 16 L1</td>
<td>Da Silva et al, 1999</td>
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<tr>
<td>Vaccinia HPV 16-E7</td>
<td>Cooney et al, 1991; Gao et al, 1994</td>
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<tr>
<td>Vaccinia HPV 16 + 18 E6 + E7</td>
<td>Borysiewicz et al, 1996</td>
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<tr>
<td>Vaccinia HPV 16 L1</td>
<td></td>
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<tr>
<td>Salmonella typhimurium HPV 16 E6 and E7</td>
<td>Krul et al, 1996; London et al, 1996</td>
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<tr>
<td>Salmonella typhimurium HPV 16 L1</td>
<td>Nardelli-Halinger et al, 1997</td>
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<tr>
<td>Streptococcus gordovi HPV16 E7</td>
<td>Jensen et al, 1997</td>
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**Table II. Potential clinical trial end-points**

<table>
<thead>
<tr>
<th>End-point</th>
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<tr>
<td>High-risk genital HPV</td>
</tr>
<tr>
<td>Therapeutic</td>
</tr>
<tr>
<td>Prophylactic</td>
</tr>
<tr>
<td>Acquisition of infection</td>
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<tr>
<td>Shortening duration of infection</td>
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<tr>
<td>Decrease in frequency of cytologic abnormalities</td>
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<tr>
<td>Decrease in duration of cytologic abnormalities</td>
</tr>
<tr>
<td>Therapeutic</td>
</tr>
<tr>
<td>Resolution of cytologic abnormalities</td>
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<tr>
<td>Regression of dysplasia (monotherapy or combination therapies)</td>
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<tr>
<td>Regression of cancer (monotherapy or combination therapies)</td>
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<tr>
<td>Low-risk genital HPV</td>
</tr>
<tr>
<td>Therapeutic</td>
</tr>
<tr>
<td>Prophylactic</td>
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<tr>
<td>Acquisition of infection</td>
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<tr>
<td>Development of warts</td>
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<tr>
<td>Therapeutic</td>
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<tr>
<td>Monotherapy or combination therapies</td>
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<tr>
<td>Prevention of recurrence after treatment</td>
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Although both prophylactic and therapeutic vaccines are feasible (Table II), prophylactic vaccines are the most appealing, based on experience with animal PV and in humans, with a number of viral vaccines in clinical trials.

A vaccine to control genital HPV probably would need to involve immunization of both men and women. Our knowledge of detection of high-risk HPV in women, the attack rate of HPV in women, and the natural history of HPV in women, however, is much greater than our information about infectivity, attack rate, and natural history of HPV in men.

Development of vaccination strategy and rational design of clinical trials is difficult without this information. The lack of a readily available standardized and validated serologic test to determine who is “immune” and who is susceptible is another obstacle to HPV vaccine development and implementation. VLP ELISA and pseudoviron infectivity assays serve as surrogate markers. In addition, the detection of viral DNA and cervical lesions will characterize patients at entry and serve as markers to determine vaccine efficacy.

There are major psychosocial challenges in addition to the scientific challenges. We do not know if the public is ready to vaccinate their children for sexually transmitted diseases. In addition, small but vocal groups question the value of current childhood vaccinations. There is very little awareness of HPV among the general public, and the risk of acquiring HPV and the reality that cervical cancer is a sexually transmitted disease are unfamiliar concepts outside the medical community.

POTENTIAL VACCINES

Peptide vaccines Peptide vaccines have obvious therapeutic appeal, particularly if capable of binding to HLA and generating cytotoxic T lymphocytes. Peptide vaccines are in early trials as candidate therapeutic vaccines for cervical cancer.

Proteins Production of fusion proteins of all HPV gene products is now possible, but which of these or which combinations should be studied in clinical trials is not known. An HPV6 L2-E7 fusion protein has been shown to be immunogenic, and perhaps therapeutic, in the treatment of genital warts in humans.

Virus-like particles Chimeric VLP can be formed by combining L1 or L1 plus L2 with early proteins. Clinical trials are being conducted with an HPV 11 VLP (Reichman et al., 1998, 1999), an HPV 16 L1 VLP (Zhang et al., 1999), and an HPV 16 L1 VLP (Du Silva et al., 1999).

Viral and bacterial vectors Viral and bacterial vector vaccines are potentially equivalent to live attenuated HPV vaccines. Humoral and CTL responses could potentially be generated with such vaccines, which could be polyvalent. Early clinical trials in human cervical cancer patients are evaluating viral vector vaccines such as a vaccinia vaccine expressing modified forms of E6 and E7 from HPV 16 and 18 (Borysiewicz et al., 1996), which appear to be immunogenic.

Other approaches It is theoretically possible to elicit virus-specific immune responses utilizing direct introduction into the host of viral DNA coding for viral antigens. This approach has only been investigated in the rabbit system thus far.

Low-risk types The goal of vaccination with low-risk types is primary prevention of HPV acquisition. If this goal cannot be achieved, it is hoped that vaccination can at least prevent the development of external genital warts. There is a lack of precise knowledge about infectivity, susceptibility, attack rate, and time from infection to the development of external genital warts. Although the presence or absence of such lesions would represent a clear clinical end-point, designing clinical trials in terms of sample size and duration of follow-up is challenging without this information.

Therapeutic end-points could be achieved with a low-risk HPV vaccine, either by accelerating the resolution of genital warts, enhancing the efficacy of current therapies, or preventing wart recurrence after current therapy has produced a wart-free state. The precise frequency and factors that favor or prevent “spontaneous” resolution of genital warts are poorly understood. Complete resolution of genital warts over a few months appears to be in the range of 0%-20% based on the placebo arm of controlled clinical trials (Beutner et al., 1998b).

Most current therapies have about a 50% complete response rate. Increasing complete responses from 50% to 80% by supplementing current treatment with a vaccine would require a trial using hundreds of patients.

If one hopes to decrease recurrences after conventional therapy, similar challenges exist. There have been no studies prospectively powered and designed to establish the frequency of or time to recurrence following treatment of genital warts with any modality, although published recurrence rates with most modalities appear to be high. In order to design vaccine trials adequately, this information will be required.

High-risk HPV types Prevention of cervical cancer is the obvious aim of vaccination against high-risk HPV types, but it is not a practical end-point for human vaccine trials. Because the biopsy may alter the natural history, histologic end-points are not feasible. Several potential surrogate end-points are of potential use: the acquisition of infection, the virologic persistence, and a decrease in the proportion of subjects who experience cytologic abnormalities.

In theory the best way to prevent cervical cancer is to prevent the acquisition of high-risk HPV infection, but this may also be the greatest challenge. The lack of a serologic test to accurately identify those who are either susceptible or immune is one of the many problems with this end-point. While virgins would be susceptible, limiting enrolment to virgins has some logistic limitations.

The persistence of HPV, not just the acquisition of a short-lived infection, is a critical step in the pathway to cancer, which may help explain why only a minority of those infected develop cervical cancer. Cervical cancer may thereby potentially be prevented using a vaccine to decrease the proportion of the population with persistent HPV. Inherent limitations in terms of interpretation and reproducibility exists with the use of a screening cytologic test as a clinical end-point.

CONCLUSION

We have come a long way on the path to a vaccine considering that HPV cannot be grown in vitro and that until very recently there have been no serologic tests for HPV. Although large-scale clinical trials are now underway, it is difficult to predict how soon we will have effective prophylactic and/or therapeutic HPV vaccines.

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