HPV Vaccines: Promise and Challenges

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Biotechnology firms, pharmaceutical companies, and academic researchers are working to develop vaccines against the types of human papillomavirus (HPV) that cause most, if not all, cases of cervical cancer. Some are designing prophylactic vaccines to prevent initial infections with HPV; if successful, these vaccines ultimately could eliminate the public health problem of cervical cancer. Others are focusing on therapeutic vaccines to control the progress of established disease or prevent its recurrence in women who already have cervical dysplasia or cancer. Vaccine developers face many technical challenges, in part because the HPV virus itself has evolved various strategies for evading the immune response.

A safe, effective, and affordable vaccine to prevent cervical cancer must meet several programmatic challenges. First, it must be multivalent; that is, it must be effective against several of the most common types of HPV associated with cervical cancer. Second, the vaccine must offer long-lasting protection against HPV infection, preferably without booster shots. Third, a vaccine suitable for developing countries must minimize financial and logistical demands on health care systems. The ideal vaccine would have low production costs, a long shelf life, and no need for a cold chain; it also would require only a single dose and would be administered orally or via a nasal spray rather than by injection.

A prophylactic HPV vaccine also would need to be administered before children become sexually active. Reaching them in early adolescence may be difficult since adolescents often are not in regular contact with the health care system, nor are they all in school. Reaching individuals during infancy demands a vaccine safe for infants and young children, and one that remains effective, preferably without boosters, for more than 30 years. Because HPV is sexually transmitted, a vaccine to prevent cervical cancer likely will be more effective if it is administered to boys as well as girls. Even after a universal HPV immunization program is in place, screening for precancerous lesions probably could not be eliminated completely, as some women will continue to be at risk for cervical cancer.

One type of prophylactic vaccine, based on virus-like particles or VLPs, is almost ready to enter Phase III clinical trials, which means that a vaccine against a single or a few types of HPV could be available in as soon as five years. However, ten years will likely be needed to formulate a safe and effective multivalent vaccine suitable for universal immunization. Furthermore, vaccines based on injection of purified VLPs
are relatively costly to produce and therefore may not be the ideal vaccine for developing countries, where the majority of cervical cancer deaths occur. Recombinant live vector, DNA, and edible vaccines, none of which have entered clinical trials, may better meet the programmatic challenges outlined above. As for therapeutic vaccines, many peptide vaccines and one recombinant live vector vaccine have been studied in Phase I/II trials, but the cancer patients have been too ill to judge the vaccines’ true impact. Trials targeting women with high-grade precancerous lesions likely will be undertaken in the near future.

In summary, HPV vaccine development holds great promise for reducing the impact of cervical cancer on the world’s women. Given the current state of technology, however, many experts believe that it could be between 10 and 20 years before an effective, affordable, and acceptable vaccine will be available for widespread use in cervical cancer prevention programs.
I. Introduction

The discovery that human papillomavirus (HPV) causes the vast majority of cervical cancers opens exciting new possibilities for controlling this disease, which is the second most common cancer among women worldwide. Vaccines that protect against HPV infection, if administered prior to initiation of sexual activity, theoretically would prevent women from developing cervical cancer later in life. Compared with the current strategy of regularly screening women for precancerous lesions and treating them as necessary, immunization should offer a cheaper, logistically simpler, and more effective intervention that places fewer demands on the health care system as well as on women. HPV immunization offers a long-term solution to cervical cancer, especially in developing countries, where it is especially difficult to effectively implement screening and treatment programs that reduce cervical cancer deaths.

Researchers at pharmaceutical companies, biotechnology firms, and academic research centers around the world are actively developing candidates for both prophylactic and therapeutic HPV vaccines.1,2 They are taking advantage of recent advances in genetic engineering and new approaches to vaccine development to devise a wide array of candidate vaccines, some of which have entered early clinical trials. In the process, researchers are contributing to a growing understanding of HPV itself and its interactions with the human immune system. Given the strong scientific foundations already laid in HPV vaccine research, it appears likely that a safe and effective vaccine against cervical cancer ultimately will become available.

To be effective, a vaccine will need to strengthen the body’s immune response to HPV infections at the genital mucosal surfaces, a response which is less understood than many other aspects of the immune system.3,4 A prophylactic vaccine would work primarily by stimulating antibody-mediated immunity, therefore inducing neutralizing antibodies capable of recognizing and inactivating HPV before the virus infects host cells. This strategy requires sustained, high levels of antibodies at mucosal surfaces over long periods of time. Some scientists believe that sustaining those levels will be difficult, and they recommend that prophylactic vaccines also stimulate a cell-mediated (or cellular) immune response capable of eliminating early stages of infection in host cells.4,5

Prophylactic vaccines cannot make an immediate impact on the prevalence of cervical cancer, which usually takes 20 years or longer to develop after initial infection with HPV.5 In contrast, a therapeutic vaccine theoretically could help women who are
already infected with HPV. Therefore, medical researchers also are investigating therapeutic vaccines for use as an adjunct to standard therapies. Such a vaccine could (1) help prevent low-grade disease from progressing, (2) cause existing lesions to regress, (3) control the spread of metastatic cancer, and/or (4) prevent recurrence of cervical cancer after treatment. Therapeutic HPV vaccines must prompt cell-mediated immunity in order to be effective, since antibodies cannot reach and eliminate the virus once it has been incorporated into host cells. Combined with the fact that no therapeutic vaccines currently exist for other diseases, this makes therapeutic HPV vaccine development a challenging task.

While most candidate HPV vaccines are designed to prevent or alleviate cervical cancer, some focus on genital warts, which generally are caused by different types of sexually transmitted HPV. Genital warts provide a useful model to test the principles of cervical cancer vaccines because the impact of a vaccine on the incidence, regression, and recurrence of warts can be assessed quickly (and because warts are not a life-threatening illness). In addition, genital warts are a public health concern in their own right.
II. Challenges in HPV Vaccine Development

The promise of HPV vaccines does not come without challenges. Some of these reflect characteristics of the virus itself and its interaction with cervical cancer. Others reflect the challenges of stimulating an effective immune response to a mucosal infection.

Because HPV does not cause disease in animals, it is difficult to conduct the animal research needed for vaccine development. Some researchers instead study naturally occurring mammalian papillomaviruses, including cottontail rabbit papillomavirus, canine oral papillomavirus, and bovine papillomavirus. Other researchers study mouse models that graft human materials into immunologically suppressed mice or incorporate specific HPV genes or proteins into model systems. None of these animal models completely mimic the interaction between HPV and human host cells, so it is unclear how well the results of animal studies apply to clinical infections in humans.

Scientists do not know precisely which elements of the human immune system are important in preventing or resolving HPV infections. Although there is evidence that immune response does play a role in controlling HPV infections, it is not known why HPV infections persist in some individuals and regress naturally in others. Vaccine developers are testing a broad range of hypotheses about what makes for an effective immune response to HPV, including testing the relative advantages of stimulating antibody- and/or cell-mediated immunity.

HPV enters the body through the mucosal membranes and does not spread systemically. Therefore, a vaccine against HPV will be most effective if it induces a strong immune response at the mucosal surface (mucosal immunity), although some researchers argue that a systemic immune response might be sufficient. The mucosal immune response also must remain effective throughout the menstrual cycle, which may pose a challenge since evidence suggests that hormone levels affect immunologic activity.

The mucosal immune response is less understood than the systemic immune system. However, there is evidence that exposure to an antigen at one mucosal surface site (for example, the nose or gastrointestinal tract) can elicit an immune response at a
distant site, such as the vagina or cervix. Therefore, researchers are investigating whether intranasal or oral immunization would be more effective than injections at causing the vaginal and cervical secretion of HPV antibodies. While simpler and cheaper than injections, current mucosal vaccines do not induce a long-lasting immune response. Also, the amount of antigen absorbed from current mucosal vaccines is low and varies widely among individuals.10

Approximately 90 types of HPV that infect the genital tract have been identified.4 Two types (HPV-6 and HPV-11) account for 90 percent of genital wart cases, while as many as 15 to 20 types may be associated with cervical cancer. Four types of HPV (16, 18, 31, and 45) accounted for 80 percent of all cervical cancer cases in a study of more than a thousand patients with invasive cervical cancer in 22 countries.12 However, the distribution of HPV types varied substantially by region. For example, the prevalence of the most common type, HPV-16, ranged from 32 percent to 78 percent of cervical cancer cases in various countries, while HPV-18, the second most common type, ranged from 0 to 49 percent. Some types of high-risk HPV are significant only in certain regions; for example, clusters of HPV-39 and HPV-59 have been found in Central and South America.

Because HPV types differ significantly at the genetic and protein level, antibodies raised against one kind of HPV generally do not protect against other types.9 Preventing a majority of cervical cancer cases therefore will require a multivalent vaccine, that is, a combination vaccine effective against the common carcinogenic types of HPV (including types 16, 18, 31, and 45).5 Some researchers also have proposed including HPV-6 and HPV-11 in a multivalent cervical cancer vaccine, because the protection this would offer against genital warts would give men an incentive to take the vaccine.11 More epidemiological research is needed to further clarify the types of HPV that are most prevalent in various regions and countries.13

Clinical trials to assess the efficacy of “cervical cancer” vaccines are complicated by the slow and uncertain development of the disease and the multiple types of HPV.4 It can take decades to develop cervical cancer after HPV infection, and the majority of women infected clear the virus naturally and do not develop cervical cancer. Thus, trials measuring the efficacy of a candidate vaccine based on its impact on the
incidence of cervical cancer would take decades and would have to enroll extremely large numbers of women. Such trials also would raise a serious ethical issue: researchers cannot let women who develop abnormal Pap smears over the course of a study go untreated and possibly develop cervical cancer.\textsuperscript{11}

Therefore, researchers have considered using an earlier stage of disease, such as high-grade squamous intraepithelial lesions (HSIL) or cervical intraepithelial neoplasia (CIN) II/III, as the endpoint of clinical trials. Some even suggest using low-grade squamous intraepithelial lesions (LSIL) or CIN I as endpoints. The earlier the stage of disease chosen as an endpoint, the larger the proportion of women infected with HPV who meet the diagnostic criteria will be. At the same time, the earlier the stage of disease chosen as an endpoint, the less it will reflect the vaccine’s true efficacy against cervical cancer. The problems are twofold:

- Precancerous cervical lesions are associated with a much wider range of HPV types than cancer; therefore, a vaccine tailored to prevent high-risk HPV cancer may not have a great effect on the overall incidence of CIN II/III.\textsuperscript{7} This problem could be overcome by typing the HPV infections of study participants.

- Fewer than one percent of women who are infected with HPV-16 (the type most commonly associated with cervical cancer) and who have early stage lesions go on to develop cervical cancer,\textsuperscript{3} and the cofactors linked to this progression are not yet clear. Reductions in early-stage disease, even in lesions associated with high-risk types of HPV, may not eliminate all cases that progress to cancer.

Of course, preventing infection with targeted HPV types also should be an endpoint of a prophylactic vaccine trial. New HPV-DNA test options will facilitate the assessment of this endpoint in large-scale trials.\textsuperscript{14} Ideally, detection methods should be able to distinguish an immunological response to a natural infection from a response to the vaccine.
Since few types of HPV can be propagated in tissue culture, it is not possible to develop inactivated or attenuated live virus vaccines as with some other viral diseases.\textsuperscript{7,11} Therefore, HPV vaccines currently under development are part of a new generation of vaccines that employ genetic engineering. The ability to manipulate and transfer genes from one organism to another is critical for HPV vaccines, given that the virus itself cannot be routinely grown in culture. Recombinant genetic engineering also allows the production of subunit vaccines that include only a portion of a disease-causing organism; since they do not contain the cancer-inducing viral genes, these may be safer and create fewer side effects than vaccines made of whole organisms.\textsuperscript{10}

Researchers are investigating the following five approaches to producing HPV antigens and delivering them to vaccine recipients:

- **Recombinant live vector vaccines**: A harmless host virus or bacteria is genetically engineered to produce an HPV antigen. The immune system responds both to the host organism and the HPV antigen.

- **Protein and peptide vaccines**: An organism, such as yeast, is genetically engineered to produce an HPV protein or peptide. (Small peptides also are synthesized chemically.) After this antigen is purified, it is combined with an adjuvant that helps trigger the immune system.

- **Virus-like particles (VLPs)**: Cultured cells are genetically engineered to produce HPV capsid proteins (see below) which self-assemble into empty shells resembling virus particles.

- **“Naked” DNA vaccines**: HPV genetic material is inserted into bacterial plasmids. When these circular DNA structures are used in a vaccine, the DNA is expressed in human cells that then produce an HPV antigen.

- **Edible vaccines**: Plants are genetically engineered to express HPV antigens in fruits and vegetables. Eating the foods leads to immunization in the gastrointestinal tract.

Each of these approaches is explored in greater detail in Chapters IV through VIII.
Tables 1 and 2 describe a number of ongoing efforts to develop HPV vaccines using these technologies. Specific efforts aimed at each approach are described in the following sections.

Regardless of the approach they take, researchers must first choose which HPV antigens to include in their candidate vaccines. Three categories of HPV proteins are potential targets for vaccines; each is expressed during different stages of infection and disease.

The capsid proteins L1 and L2 make up the outside coat or shell of HPV particles. They interact with the surface molecules of human epithelial cells during early stages of infection to gain entry for the viral DNA. Because they are present during the initial infection, they are ideal targets for a prophylactic vaccine. While neutralizing antibodies to both capsid proteins have been found, there is thirty times as much L1 as L2 on the shell of HPV particles, and the predominant immune response is to epitopes on L1. Therefore, most candidate vaccines have targeted L1 rather than L2. Once HPV is integrated into tumor cells, however, the capsid proteins are not always present. This means L1 and L2 are not reliable targets for a therapeutic vaccine.

The oncoproteins E6 and E7 continue to be expressed during later stages of disease. They bind p53 and pRB, which are human tumor suppressor genes. The oncoproteins are involved in the malignant transformation of HPV-infected cells and are thought to be required for continued tumor growth. They are the primary targets of therapeutic vaccines, most of which have been designed to treat later stages of disease.

The replication proteins E1 and E2 are necessary for HPV to replicate within cells before the virus is integrated into the host DNA. Because E1 and E2 are expressed in higher levels than E6 and E7 early in the progress of an HPV infection, several researchers have suggested that they may be the best targets for a therapeutic vaccine designed to treat early stages of disease, such as low-grade dysplasias.
Using live but weakened (or attenuated) disease-causing organisms as vaccines is a traditional approach to vaccine production with significant advantages. Whole organisms induce a strong immune response, including both antibody- and cell-mediated immunity, and generally require fewer injections. Since HPV cannot be raised in culture, researchers have added HPV genes to other bacteria and viruses to create recombinant live vector vaccines. These host vectors express an HPV antigen along with their own antigens, which stimulates an immune response against both the vector and HPV.

Recombinant live vector vaccines combine the advantages of subunit and live, attenuated vaccines. Because they express only selected HPV genes, they would be relatively safe. Like other live, attenuated vaccines, they could produce long-term protection with a single inoculation, and they could stimulate strong cell-mediated immunity as well as antibody-mediated immunity.

Recombinant live vector vaccines have some significant disadvantages, however. Live vectors, even attenuated ones, are not safe for use in immunocompromised individuals (particularly vector vaccines using viruses). This poses a special problem in developing countries, where it may not be feasible to determine an individual’s HIV status before immunization, and where other factors such as malnutrition may depress the immune system. Also, the body’s immune response to the vector prevents it from being used more than once; neutralizing antibodies developed after the first vaccination respond immediately to any subsequent inoculation. Many of the vectors under study are already used for other vaccines, which means there may be a widespread, pre-existing immunity against the vector in the general population. Another problem is that the vector usually expresses low levels of foreign (HPV) antigens, so that the immune response to the vector may overshadow the immune response to HPV.

Either a virus or a bacterium can be used as the vector in a recombinant live vector vaccine so long as it is harmless. The key is selecting a vector that is capable of infecting humans without causing clinical illness. Some researchers are working...
with attenuated vectors already in use as vaccines, such as vaccinia and Bacille Calmette-Guérin (BCG), because these already are accepted by licensing authorities and companies have experience producing them. Other HPV researchers are studying vectors that naturally colonize mucosal tissues and/or can be administered orally, such as adenovirus or Salmonella, in hopes that they may prompt a strong response from the mucosal immune system.¹⁰

Cantab Pharmaceuticals plc (Cambridge, United Kingdom) is the farthest advanced in developing a recombinant live vector vaccine for HPV. They have conducted four clinical trials on a therapeutic vaccine called TA-HPV, which is a recombinant vaccinia virus that expresses E6 and E7 from HPV-16 and HPV-18.² TA-HPV is designed for use as an adjunct therapy for cervical cancer; its objective is to eliminate residual tumor cells that can lead to the recurrence of disease after conventional therapy.¹⁷ Phase I trials on a small number of women with advanced cervical cancer found the vaccine had few side effects, and some women produced cytotoxic T-cells as well as HPV antibodies.¹⁸ A Phase II trial in Europe administered two doses of TA-HPV (before and after surgery) to 29 women with early-stage cervical cancer. This trial exceeded the immunological endpoint; that is, there was an HPV-specific immune response in 10 percent or more patients.¹⁷ An additional Phase II trial to demonstrate a clinical endpoint was scheduled to begin in 1999, after which Cantab plans to move on to Phase III efficacy trials.

Transgene S.A. (France) is developing therapeutic recombinant vaccinia vaccines for a variety of cancers, including cervical cancer. To increase the safety of its vaccines, the company has developed a highly attenuated strain of Modified Vaccinia Ankara (MVA), which cannot grow in mammalian cells, although it stimulates a strong immune response.¹⁹ Transgene’s vaccines express the interleukin-2 (IL2) gene as well as tumor antigens in order to prompt stronger cell-mediated immunity; IL2 controls the growth and function of many kinds of cells, including cytotoxic T-cells. In the fall of 1999, the vaccine entered Phase I clinical trials in the United States and Switzerland with patients with CIN III.²⁰

The European Commission is supporting the development of recombinant vaccinia vaccines for HPV in China.²¹ Dr. L. Ruan at the Institute of Virology, Chinese Academy of Preventive Medicine (Beijing), is developing an HPV-16 L1/E7
recombinant vaccinia virus and is testing its ability to induce strong mucosal immunity. Dr. Y. Zhang at the Chinese Academy of Medical Sciences (Beijing) has developed an HPV-58 E7 recombinant vaccinia virus that has prevented tumor growth in mice. The effort to develop a vaccine for HPV-58 is unique to China, since this type of HPV is less common elsewhere.

Wyeth-Lederle Vaccines & Pediatrics (Madison, NJ, USA) is actively working on recombinant live vector vaccines for several viruses, including HPV, that use a proprietary vector system licensed from AlphaVax, Inc. (Durham, NC, USA). None of these vaccines has entered clinical trials. The AlphaVax system is based on work conducted by researchers at the University of North Carolina/Chapel Hill and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID). These researchers derived a vector from an attenuated form of Venezuelan equine encephalitis (VEE). According to AlphaVax, VEE overcomes many of the limitations found in other vectors. It expresses high levels of antigens; naturally targets them to the antigen processing cells of the immune system; induces strong antibody-mediated, cell-mediated, and mucosal immune responses; continues to be effective when used for multiple inoculations; and has a high margin of safety. VEE could be used to deliver either therapeutic or prophylactic vaccines.

A joint program of the Cancer Association of South Africa (CANSA) and the University of Cape Town (South Africa) is investigating a genetically engineered BCG bacterium that will express HPV proteins, as are researchers at the University of Queensland in Australia. BCG holds several advantages for developing countries. Because BCG is already used for tuberculosis vaccines, the technology to produce such a vaccine is available in South Africa and elsewhere, and is relatively inexpensive. The vaccine also has proven to be stable in animal testing of guinea pigs and mice vaccinated with recombinant BCG expressing HPV-16 L1 and L7 proteins have demonstrated both an antibody- and cell-mediated immune response. Researchers at the University of Cape Town also are investigating edible vaccines for HPV (see below) and plan to move the more promising of these two approaches into clinical trials within three years. Researchers at the University of Queensland have compared recombinant BCG vaccines with protein vaccines (see below) and concluded that the BCG approach is less promising because it induces relatively weak immune responses. Researchers at the Wistar
Institute (Philadelphia, PA, USA) in Dr. Hildegund Ertl’s laboratory are developing vaccines against a variety of diseases that attack the mucous membranes, including HPV. Their goal is to design vaccines that produce strong mucosal immunity, and thus provide better protection than many systemic vaccines. Preclinical studies of prophylactic vaccines have found that intranasal immunization with a recombinant adenovirus expressing HPV-16 L1 induces both serum and vaginal antibodies. Wistar researchers also are investigating the use of recombinant adenoviruses and vaccinia for a therapeutic HPV-16 E6/E7 vaccine.

Researchers at the Johns Hopkins School of Medicine (Baltimore, MD, USA), including Dr. T.C. Wu and Dr. Drew Pardoll, have developed a molecular targeting system to increase the effectiveness of recombinant live vector vaccines for HPV. A molecular tag (lysosomal associated membrane protein [LAMP]) is added to HPV-16 E7, which transports the HPV antigen to the lysosomes where antigen-presenting cells present it to CD4 T-cells. By activating helper T-cells as well as cytotoxic T-cells, researchers hope to increase the therapeutic potency of this class of vaccines. Tests in a mouse model have found that vaccinia viruses expressing HPV-16 E6/E7 with the LAMP signal are able to abolish existing tumors and prevent new tumors from taking hold, while vaccines lacking LAMP do not. The vaccine is scheduled for Phase I trials in early 2000 in women positive for HPV-16 who have CIN III. In the meantime, the Hopkins researchers are developing a second targeting system for HPV vaccines.

Researchers at both Wistar and Johns Hopkins also are investigating the use of recombinant live vector viruses in combination with naked DNA vaccines (see page 18). This would overcome the problem of ineffective booster vaccination with live
Subunit protein and peptide vaccines rely on genetic engineering techniques to produce antigenic fragments that can evoke an immune response more safely and with fewer side effects than a whole organism. Selected HPV genes are inserted into yeast or another organism, which produces large quantities of the chosen protein or peptide (short peptides also can be made synthetically). Once the peptides are purified, however, they lack the microbial components that trigger the human immune system and therefore prompt weaker immune responses than whole pathogens. To overcome this problem, the peptides are combined with an adjuvant, that is, a substance that stimulates the immune system by producing local inflammation. Because the properties of the adjuvant are critical to the effectiveness of peptide vaccines, researchers are searching for new adjuvants that are more potent than alum (the only one approved for human use to date), that elicit cell-mediated and antibody-mediated immunity, promote mucosal responses, and induce sustained immune memory with a single immunization.

Peptide vaccines are safe, easy to make at low cost, and involve minimal regulatory issues. However, it can be difficult to isolate the specific epitopes that elicit the desired immune response, and the peptides themselves may be misshapen and unable to elicit a potent immune response. Other disadvantages are that peptide vaccines do not generate a strong cytotoxic T-cell response, they may induce tolerance rather than protection, and multiple immunizations may be needed to produce long-lasting protective immunity. Also, small peptides may be unstable in vivo and may not elicit the same immune response from different individuals.

In addition to its work on a recombinant vaccinia HPV vaccine, Cantab Pharmaceuticals is developing two fusion protein vaccines for HPV. Trials of a therapeutic vaccine for genital warts (formerly known as TA-GW and now called TH-GW or pharmaccine) have proceeded through Phase II testing. TH-GW is a fusion of two HPV-6 proteins (L2 and E7) and is given by intramuscular injection in multiple doses. Phase I and II trials first began in 1995 with alum as the adjuvant. The manufacturer reports that these trials found the vaccine was safe and immunogenic, cleared existing genital warts, and reduced recurrence rates. In 1996 the production
process was transferred to SmithKline Beecham Biologicals (London, England), which reformulated the vaccine with a new, proprietary adjuvant (SBAS2) that heightens cell-mediated immune responses. Phase I and II trials in 1997 and 1998 assessed the safety, immunogenicity, optimal dose, and dosing regimen of this new formulation.33 Randomized, double-blind, placebo-controlled Phase IIb trials are currently taking place in multiple sites in Europe, Canada, Scandinavia, Australia, and New Zealand.34 These will assess wart recurrence rates and rates of disease regression following vaccination.

Building on the success of its peptide vaccine against genital warts, Cantab has begun developing a similar fusion protein vaccine to treat cervical dysplasia. By treating early-stage cervical disease, scientists hope to reduce treatment costs and patient trauma. This vaccine called TA-CIN uses a novel adjuvant (NAX-57) developed by NovaVax Inc. (Columbia, MD, USA). Preclinical testing has demonstrated a T-cell response in model systems and examined dosing. Phase I trials were scheduled to begin in 1999.34

Multiple academic institutions around the world, including the National Cancer Institute (Bethesda, MD, USA), the Norris Cancer Center at the University of Southern California, the University of Leiden (Netherlands), and the University of Queensland (Woolloongabba, Australia) are sponsoring Phase I and II trials of various therapeutic peptide vaccines for HPV-16 and HPV-18.35,36 These vaccines target E6 and/or E7, are administered as an adjunct to conventional therapy such as radiation, and generally require multiple inoculations. Although some patients have demonstrated an immune response to the vaccines, initial results have been limited, possibly because the vaccines have initially been tested on patients with advanced cancer, many of whom are immunocompromised. As trials proceed, they will enroll patients with less advanced disease, including high-grade dysplasia, and healthy immune systems that are more able to respond to vaccination.35

StressGen Biotechnologies (British Columbia) is using stress proteins to develop a variety of immunotherapy products, including a therapeutic vaccine to treat cervical dysplasia. This vaccine, HspE7, is a fusion of a BCG heat-shock protein and HPV E7.37 Like other stress proteins, the heat-shock protein heightens the immune response by directing antigens to professional antigen-presenting cells and by activating cytotoxic T-cells. In animal models, HspE7 protects against challenge with
cervical cancer cells, regresses existing tumors, and provides long-term survival benefit. A Phase II study in women with high-grade cervical dysplasia began recently, with patients receiving three doses of the vaccine at two-week intervals.
A major breakthrough in HPV vaccine research came with the discovery that the capsid proteins L1 and L2 (or L1 alone) self-assemble into virus-like particles (VLPs) when expressed in cells. VLPs closely resemble native HPV particles and include the conformational epitopes that induce virus-neutralizing antibodies. Therefore, the immune system perceives VLPs as infectious viruses and responds accordingly.\textsuperscript{7,9} Because VLPs are empty and do not include viral DNA, they are not infectious. VLPs have been produced for at least ten HPV types so far (6, 11, 16, 18, 31, 33, 35, 39, 45, and 58), proving the applicability of this approach for a multivalent vaccine.\textsuperscript{39}

VLPs, which by definition target the capsid proteins, are ideal for prophylactic vaccines because they induce high levels of neutralizing antibodies.\textsuperscript{11} Immunization with L1 VLPs in rabbits, dogs, and cows has offered 90 to 100 percent protection against experimental high-dose challenge with species-specific mammalian papillomaviruses.\textsuperscript{9,11} It is not yet clear, however, whether systemic immunization with VLPs can protect against natural mucosal HPV infections. Some researchers are examining alternative delivery systems for VLPs, such as intranasal or oral immunization, that might be more effective than injections at inducing mucosal immunity.\textsuperscript{40,42} Other studies indicate that VLPs induce a cervical immune response when they are expressed in live vectors such as vaccinia or Salmonella.\textsuperscript{8,39}

In one model, researchers have created chimeric VLPs that can induce cytotoxic T-cells in mice as well as neutralizing antibodies by fusing E6 and E7 to the capsid proteins in VLPs.\textsuperscript{11,15} Some scientists believe that chimeric VLPs hold the potential of a single vaccine that could be used both prophylactically and therapeutically.\textsuperscript{4} Others argue that a therapeutic effect might be more important as a second line of prevention in a prophylactic vaccine, enabling the body to eliminate early infections that antibodies miss.\textsuperscript{11}

Recent studies have demonstrated that VLPs can be prepared and purified in the large quantities needed for a vaccine,\textsuperscript{7} although there is some question of whether they will be too expensive and logistically demanding for use in developing
countries.\textsuperscript{15,39} For example, VLP vaccines require constant refrigeration and multiple injections to be effective. While VLPs do not require an adjuvant, researchers are examining whether an adjuvant could boost their effectiveness.

MedImmune, Inc. (Gaithersburg, MD, USA) has developed a series of prophylactic VLP vaccines for HPV based on preclinical research with beagles at Georgetown University.\textsuperscript{2,43} The furthest advanced of their vaccines is MEDI-501, an HPV-11 VLP for genital warts that is produced in recombinant baculovirus-infected insect cells. Phase I clinical trials of a three-dose regimen of MEDI-501 with an alum adjuvant found the vaccine was safe and elevated levels of HPV antibodies.\textsuperscript{44} Phase II trials are now underway. MedImmune also has launched Phase I trials of two VLP vaccines: MEDI-503 for HPV-16 and MEDI-504 for HPV-18.\textsuperscript{45} The company plans to combine these two vaccines into a multivalent formulation for Phase II and III clinical trials. MedImmune has an agreement with SmithKline Beecham to commercialize and market all of these HPV vaccines.

Since 1994, Merck Research Laboratories has been working with CSL Limited (Parkville, Victoria, Australia) to develop prophylactic VLP vaccines, beginning with preclinical research on rabbits.\textsuperscript{46} Their ultimate goal is to produce a quadrivalent vaccine for genital warts and cervical cancer that includes HPV-6, 11, 16, and 18.\textsuperscript{1,2} Phase II trials of a three-dose regimen of HPV-16 VLPs in young, healthy women who do not have HPV are underway at multiple sites in the United States, Australia, and the United Kingdom.\textsuperscript{47,48} A large multi-center, U.S.-based Phase III study is likely to begin soon.\textsuperscript{49}

The National Cancer Institute (NCI) and the National Institute of Allergy and Infectious Diseases (NIAID) (Bethesda, MD) have sponsored Phase I clinical trials of HPV-16 VLPs, which were developed based on work with rabbit and cattle models.\textsuperscript{50} The VLPs are produced in recombinant baculovirus-infected insect cells. These initial clinical trials found that the VLPs produced high titers of neutralizing antibodies with only minor side effects. Phase II trials of the vaccine are now underway at Johns Hopkins, and early results are promising.\textsuperscript{51} NCI hopes to begin a bridging study in Costa Rica in the final months of 2000 that will lead to a large-scale,
seven-year efficacy trial of the HPV-16 vaccine against a placebo. This will be a proof-of-principle trial rather than a Phase III trial, since NCI has no intention to commercially license the vaccine and is not developing the multivalent vaccine needed for widespread distribution.\textsuperscript{51}

NCI collaborators at Universitaire Vaudoise, Lausanne, Switzerland also are investigating whether intranasal instillation of VLPs would increase mucosal antibody responses compared with injections. Intranasal immunization with VLPs in mice have elicited IgA and IgG antibodies in the genital tract of female mice\textsuperscript{41} throughout the estrus cycle.\textsuperscript{42}

Preclinical work on chimeric VLPs (CVLPs) that incorporate an HPV-16 E7 peptide as an L1 fusion protein has been conducted at MediGene AG (Germany), NCI, and the University of Queensland (Australia).\textsuperscript{40,50,52} These vaccines include L1 or L2 and E7, and should have both prophylactic and therapeutic effects. Initial results from mouse models have found that these chimeric VLPs have good immunogenicity, whether administered intramuscularly or intranasally.\textsuperscript{40} An NCI vaccine incorporating HPV-16 E7 into VLPs as an L2 fusion protein also induced cytotoxic T-cells in mice, protected them from tumor challenge, and led to the regression of existing tumors.\textsuperscript{50} MediGene has completed animal experiments providing proof of concept for a vaccine against HPV-16.\textsuperscript{53} A Phase I/II clinical trial (jointly induced by MediGene AG and Schering AG) involving healthy volunteers as well as patients with dysplasia is scheduled for 2000. The trial will test how well the vaccine is tolerated and whether it produces an immune response to HPV-16.\textsuperscript{52}
“Naked” DNA is among the newest approaches to vaccine development. Using recombinant DNA technology, HPV genes are added to small, circular DNA structures found in bacteria called plasmids. After the plasmids are mass produced in bacteria, they are purified and then injected into vaccine recipients—either in saline solutions or by propelling DNA-coated gold beads into cells with a “gene gun.” In humans, cells would take up the plasmid DNA and then produce the selected HPV antigen.

Animal tests of DNA vaccines against various pathogens, including HPV, have found that they are potent vaccines with multiple advantages over other kinds of vaccines. They induce cell-mediated as well as antibody-mediated immunity, they raise antibodies against native forms of proteins, and they can induce long-lasting immunity since host cells may continue producing antigens for years. They also simplify the production of multivalent vaccines since purification and characterization of only a single chemical entity, DNA, is needed. DNA vaccines also make it possible to define the immune response, producing exactly the types of T-cells desired, for example.

DNA vaccines also have the potential to be less expensive than conventional vaccines, and easier to produce, distribute, and administer. The development and production of DNA vaccines use generic production and validation techniques, no matter what the pathogen. Adjuvants are not needed. DNA vaccines are stable at both high and low temperatures, eliminating the need for a cold chain, which can account for 80 percent of the cost of vaccination programs in developing countries. They also have a long shelf life and can be stored dry or in an aqueous solution.

Although there is no risk of infection associated with DNA vaccines, they raise other potential safety issues that need further investigation. Injecting plasmid DNA into the genome of host cells might induce mutations, disrupt cellular genes, or cause other harm. It also is possible that DNA vaccines might induce anti-DNA antibodies and produce autoimmune phenomena. Without an intranasal, oral, or other mucosal
delivery system, DNA vaccines might not elicit mucosal immune responses as well as other types of vaccine. In addition, introducing HPV genes that code for viral oncoproteins poses a risk and it is important that these be fully inactivated.

To increase the potency of DNA vaccines, researchers are investigating the use of adjuvants, including genetic adjuvants that deliver immunostimulatory sequences along with the antigen sequences. Using DNA vaccines in combination with peptide or recombinant live vector vaccines also is under investigation.

In 1995, Wyeth-Lederle Vaccines began collaborating with Apollon, Inc. (Malvern, PA, USA) on prophylactic and therapeutic DNA vaccines for a variety of diseases. Apollon’s GENEVAX technology uses plasmid DNA injected with bupivacaine to facilitate DNA uptake into target muscle cells. Since Wyeth acquired Apollon in 1998, preclinical work has continued on DNA vaccines for herpes, hepatitis, and HIV. A DNA vaccine for HPV is in an earlier stage of development.

Merck is collaborating with Vical, Inc. (Emeryville, CA, USA) on research and development for DNA vaccines for multiple infectious diseases, including herpes, HIV, and hepatitis as well as HPV. Vical’s technology uses a lipid delivery system to facilitate cellular uptake of the plasmid DNA. Preclinical research on cottontail rabbit papillomavirus found that DNA vaccines coding for L1-induced neutralizing antibodies protected rabbits from challenge with the virus.

Scientists at the Wistar Institute are engaged in preclinical research on various ways to heighten or modulate the immune response to DNA vaccines. They are testing the impact of different delivery routes (intramuscular, intradermal, intratracheal) on mucosal immunity and are investigating the use of traditional adjuvants and genetic adjuvants to facilitate the uptake of DNA into cells. In order to target specific cellular pathways and facilitate the presentation of antigens to B- and T-cells, they have developed therapeutic DNA vaccines that express ubiquitin or the lysosome-associated membrane protein along with E6 or E7. Researchers at Johns Hopkins also have applied the targeting strategy they initially developed for recombinant live vector vaccines to DNA vaccines. In addition, researchers at Wistar and Johns Hopkins are testing the use of DNA vaccines to prime the immune system and boost the response to subsequent immunizations with recombinant live vector vaccines.
Plant biotechnology techniques have permitted scientists to insert the genes for human pathogens, such as HPV, hepatitis B, and cholera, into yeast or edible plants such as potatoes, carrots, and lettuce. These genetically engineered plants then produce and accumulate disease antigens in their tissues. Their fruits and vegetables may serve as an edible vaccine, since eating them can induce an immune response.\(^\text{10}\)

A related approach engineers nonedible plants to produce large quantities of an antigen in their leaves. In this case, the antigen is purified and combined with an adjuvant for use as a vaccine, just like the peptide vaccines already described.

Edible vaccines offer several practical advantages of special importance for developing countries, which may have difficulty paying for, storing, distributing, and administering traditional vaccines.\(^\text{58}\) Yeast extract or plants could provide a simple, inexpensive way to mass-produce vaccines. Many developing countries would be able to grow their own supplies of edible vaccines rather than import them. Edible vaccines also do not require costly and complicated cold chains for distribution. Finally, it is far simpler and cheaper to give foodstuffs to vaccine recipients than injections, which require skilled providers and strict attention to infection-prevention measures. Oral administration also has the potential to stimulate the mucosal immune system, which may be essential to the effectiveness of HPV vaccines.

Animal studies of edible vaccines against a variety of infectious diseases have produced promising results.\(^\text{10}\) In the first human trial of an edible vaccine, volunteers ate bite-sized pieces of raw potatoes that were genetically engineered to produce part of an \textit{E. coli} toxin; levels of serum and intestinal antibodies increased as a result.\(^\text{59}\) A mouse study of oral immunization with VLPs against HPV provides conceptual support for using edible vaccines against HPV.\(^\text{60}\)

The joint CANSA and University of Cape Town program in South Africa is investigating various plant expression systems for HPV, including the tomato.\(^\text{25}\) An edible vaccine would be an attractive alternative for South Africa since the technology to process the plants for an edible vaccine is available locally and vaccine costs would be very low.\(^\text{27}\) Results from preclinical studies of edible vaccines will be
compared with the results of work on a recombinant BCG vaccine for HPV (described in Chapter IV). The more promising approach will enter Phase I trials, hopefully within three years.
Although research has progressed farthest on prophylactic VLP and therapeutic peptide vaccines, it is impossible to predict whether these or another candidate will become the first commercially available vaccine against HPV. Continuing research will determine which kind of vaccine is the most effective, practical, and affordable for developing as well as developed countries. The deciding factors will relate to a range of programmatic issues, some of which are described below.

At a minimum, any vaccine must be safe and effective. To be effective, a prophylactic vaccine for cervical cancer must prevent HPV-16 and HPV-18, the two most common forms of high-risk HPV, which together account for nearly two-thirds of cervical cancer cases worldwide. Adding protection against other types of high-risk HPV to a multivalent vaccine would increase its impact. Some researchers have promoted the development of regional vaccine formulations that are tailored to prevent locally prevalent types of HPV. More epidemiological research on the prevalence of various HPV types is required before the need for regionally-tailored vaccines is confirmed, however.

Effectiveness also demands that a prophylactic HPV vaccine produce a long-lasting immune response that protects women during the decades when they are most at risk for HPV infection, that is, from the onset of sexual activity through their twenties and into their thirties. If booster shots are required to maintain protection, costs will rise and coverage decrease.

Because large numbers of women already are infected with HPV, early detection of precancerous changes in the cervix and effective treatment—perhaps in the form of a therapeutic vaccine—will be important for years to come. Therapeutic capabilities may also reinforce the protection offered by prophylactic vaccines, stimulating T-cells to eliminate early lesions when neutralizing antibodies fail to block all of the virus. In fact, the ideal vaccine for cervical cancer might be both prophylactic and therapeutic (e.g., a chimeric VLP) that could be distributed en masse to young women whether or not they are infected with HPV.

While safety and efficacy are essential for a vaccine, containing costs also is important. No vaccine will reach an adequate number of people in developing countries unless it can be produced and distributed cheaply. Some ways to reduce
costs and increase vaccine coverage are:60,61

- developing a vaccine that can be produced in developing countries rather than relying on imports (e.g., a recombinant BCG or edible vaccine);
- simplifying distribution by creating a stable vaccine with a long shelf life that does not require an expensive and logistically complex cold chain (e.g., DNA vaccines);
- developing a vaccine that creates long-lasting immunity with a single dose (e.g., recombinant live vector vaccines);
- formulating an oral vaccine, since it is easier to administer, more acceptable to recipients, and can be less pure than a vaccine formulated for injection.

Who should receive a prophylactic cervical cancer vaccine, and at what age? Since most women acquire HPV infections soon after becoming sexually active, they need to be vaccinated before they first engage in intercourse, perhaps at ages 10 to 12.4 Achieving universal coverage at this age may be difficult, however, since adolescents do not routinely require or receive medical care. Immunization programs might reach many, but not all, children by working through the schools. It would be logistically simpler, especially in developing countries, to add an HPV vaccine to the existing Expanded Program of Immunization (EPI) for infants; this also could reduce any possible transmission of HPV from mother to child.39 Immunizing infants, however, would require a vaccine that remains effective (without boosters) for at least 20 to 30 years and is demonstrated to be safe in infants and young children. Regardless of the age at which a vaccine is administered, coverage will be greater if only one dose is required.

While women are at risk of cervical cancer, men play a key role in spreading HPV. Reducing the prevalence of HPV in men will help eliminate disease in women. It may be difficult to persuade boys to be vaccinated, however, without some sort of incentive. One solution is to create a vaccine that prevents genital warts as well as cervical cancer, since HPV vaccines must be multivalent in any case.11

Models that explore the impact of vaccines of varying effectiveness administered to target populations with different characteristics will help researchers determine the most promising approaches.14
Once a prophylactic vaccine is in use, should screening programs remain in place to check vaccinated women for cervical lesions? Unless a multivalent vaccine prevents every type of high-risk HPV, small numbers of vaccinated women will continue to develop cervical cancer. Also, certain cases of cervical cancer may not be associated with HPV, and some women may not respond immunologically to the vaccine. Consequently, it may be important for programs that ultimately offer an HPV vaccine to continue offering screening at some level, and to guard against complacency among women who may assume a vaccine offers 100 percent protection (an unlikely scenario with most candidate vaccines).

Another challenge for programs that ultimately offer an HPV vaccine is how to “position” and promote the vaccine. Should it be described as an “anti-cancer” vaccine (which would appeal primarily to women), an anti-STD vaccine (which raises difficult social issues in most cultures), or even an anti-wart vaccine (which may broaden the vaccine’s appeal, particularly in young, sexually-active populations)? These questions are important ones; planning how to promote the vaccine would have a major impact on its ultimate success or failure.
X. Conclusion

With some HPV vaccine candidates about to enter Phase III clinical trials, a viable prophylactic vaccine against one or two types of HPV could be available in as little as five years. Before an HPV vaccine can be commercialized, however, a multivalent formulation must be developed and tested. Given the current state of research efforts, routine use of an HPV vaccine to prevent cervical cancer likely is 10 to 20 years away. The prophylactic vaccines most likely to emerge first are purified VLPs, which may not be the vaccines best suited for the needs of the developing world because of cost and other concerns. Innovative approaches like naked DNA and edible vaccines are more likely to overcome cost and logistical barriers to universal vaccination in developing countries, but will require substantial new investment to develop.

Most of the therapeutic vaccines under investigation are designed to complement conventional therapy for advanced disease, and it is not yet clear how much benefit they will offer (and at what cost) for these women. Therapeutic vaccines designed to clear HPV infections in their earliest stages are less well developed, but they hold greater promise for reducing the suffering and treatment costs associated with cervical disease.

Even with the most optimistic assumptions about when a prophylactic or therapeutic vaccine will be mass-marketed, many hundreds of thousands of women will develop cervical cancer in the coming decades. Therefore, it is important to continue developing appropriate screening and treatment programs for precancerous lesions at the same time as vaccine development efforts move forward.
Glossary

**Adjuvant** – A substance included in some vaccine formulations that enhances its ability to stimulate the immune system.

**Antibody** – Protein molecule produced by B-cells that bind to foreign antigens and mark them for destruction by other immune cells.

**Antibody-mediated immunity (humoral immunity)** – Immune protection provided by soluble factors such as antibodies that circulate in the body’s fluids, primarily blood and lymph.

**Antigen** – Substance that provokes an immune response.

**Antigen-presenting cells** – Various cells, including macrophages and dendritic cells, that present antigen in a form that T-cells can recognize.

**B-cells (B lymphocytes)** – Small white blood cells that mature in the bone marrow and produce antibodies crucial to immune defenses.

**Capsid** – Protein shell covering a viral particle.

**Cell-mediated immunity (cellular immunity)** – Immune protection provided by the direct action of immune cells, including cytotoxic T-cells.

**Clinical trials** – Three phases of study of candidate vaccines in people. Phase I trials include small numbers of volunteers and determine the safety of the vaccine; Phase II trials are open to hundreds of volunteers to test the vaccine for safety, the ability to evoke an immune response, and the ability to prevent disease; Phase III trials are large-scale studies in thousands of people to confirm that a vaccine safely prevents disease with minimal side effects.

**Cytotoxic T-cells (CD8+ T-cells, killer T-cells, cytotoxic lymphocytes, CTLs)** – A type of T-cell that can attack and destroy body cells infected by viruses or transformed by cancer.

**Dendritic cells** – White blood cells found in the spleen and other lymphoid organs that enmesh antigen and present it to T-cells.
**DNA vaccine (naked DNA vaccine)** – Vaccine made of DNA that is not encased or encapsulated, so that genetic material is injected directly into the vaccine recipient.

**Epitope** – A unique shape or marker carried on the surface of an antigen that triggers a corresponding antibody response.

**Helper T-cells (CD4+ T-cells)** – A type of white blood cell that is essential for turning on antibody protection, activating cytotoxic T-cells, and initiating other immune responses.

**Humoral immunity** – See “antibody-mediated immunity.”

**Immunogenic** – Capable of stimulating an immune response.

**Live, attenuated vaccine** – A vaccine consisting of a disease-causing organism whose ability to cause disease has been weakened.

**Mucosal immunity** – Protection against infection of the moist tissues lining body cavities, including the lungs, gastrointestinal tract, and reproductive tract; requires the presence of immune cells and antibodies in the mucosal membranes.

**Neutralizing antibody** – An antibody that reacts with an infectious agent and destroys or inhibits its infectivity and virulence.

**Plasmids** – Small circular DNA structures separate from the chromosomes that replicate stably in bacteria.

**Preclinical** – An early phase of study of a vaccine or drug that is completed before clinical studies are carried out in people; may be conducted in cells or in animals.

**Recombinant genetic engineering (recombinant DNA technology)** – Technique by which genetic material from one organism is inserted into a foreign cell or another organism in order to mass-produce the protein encoded by the inserted genes.
Recombinant vector vaccine – A vaccine consisting of a live, but harmless, bacterium or virus that has been genetically engineered to produce an antigen from another pathogen.

Subunit vaccine – A vaccine that uses a component of a disease-causing organism, rather than the whole organism, to stimulate an immune response.

Systemic immunity – Another term for antibody-mediated or humoral immunity.

T-cells (T lymphocytes) – Small white blood cells that mature in the thymus and orchestrate or directly participate in immune defenses (see “cytotoxic T-cells” and “helper T-cells”).

Vaccine – A substance that contains antigenic components from an infectious organism; by stimulating an immune response, but not disease, it protects against subsequent infection by that organism.
## Table 1. Prophylactic HPV Vaccines Under Development*

<table>
<thead>
<tr>
<th>Organization (Vaccine)</th>
<th>HPV Type &amp; Antigen</th>
<th>Kind of Vaccine</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medimmune, SmithKline Beecham (MEDI-501)</td>
<td>HPV-11 L1</td>
<td>VLP</td>
<td>Phase II trials underway; safety and immune response proven in Phase I trials</td>
</tr>
<tr>
<td>Medimmune, SmithKline Beecham (MEDI-503 &amp; 504)</td>
<td>HPV-16, 18 L1</td>
<td>VLP</td>
<td>Phase I trials underway</td>
</tr>
<tr>
<td>Merck, CSL Limited</td>
<td>HPV-16 L1</td>
<td>VLP</td>
<td>Phase II trials underway in US, UK, and Australia; Phase III trial to begin soon</td>
</tr>
<tr>
<td>National Cancer Institute, NIAID</td>
<td>HPV-16, L1</td>
<td>VLP</td>
<td>Phase II trials underway; large-scale efficacy trial planned to begin in Costa Rica in winter 2000/2001</td>
</tr>
<tr>
<td>MediGene</td>
<td>HPV-16 L1, E7</td>
<td>Chimeric VLP</td>
<td>Phase I/II trial scheduled for 2000</td>
</tr>
<tr>
<td>University of Queensland</td>
<td>HPV-16 L1, E7</td>
<td>Chimeric VLP</td>
<td>Preclinical; systemic and mucosal immune response in mice</td>
</tr>
<tr>
<td>CANSA, University of Cape Town</td>
<td>HPV-16 L1, L7</td>
<td>Recombinant BCG</td>
<td>Preclinical; humoral and cellular immune response in guinea pigs</td>
</tr>
<tr>
<td>European Commission, Chinese Academy of Preventive Medicine</td>
<td>HPV-16 L1, E7</td>
<td>Recombinant vaccinia virus</td>
<td>Preclinical</td>
</tr>
<tr>
<td>University of Queensland</td>
<td>HPV-16 L1, E7</td>
<td>Recombinant BCG</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Wistar Institute</td>
<td>HPV-16 L1</td>
<td>Recombinant adenovirus</td>
<td>Preclinical; intranasal immunization induced serum and vaginal antibodies</td>
</tr>
<tr>
<td>Wyeth-Lederle, AlphaVax</td>
<td></td>
<td>Recombinant Venezuelan equine encephalitis</td>
<td>Preclinical (may also be used to deliver a therapeutic vaccine)</td>
</tr>
<tr>
<td>Merck, Vical Inc.</td>
<td>L1</td>
<td>DNA</td>
<td>Preclinical; neutralizing antibodies induced in rabbits</td>
</tr>
<tr>
<td>Wistar Institute</td>
<td></td>
<td>DNA</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Wyeth-Lederle</td>
<td></td>
<td>DNA</td>
<td>Preclinical</td>
</tr>
<tr>
<td>CANSA, University of Cape Town</td>
<td></td>
<td>Edible (tomatoes)</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>

Note: While efforts were made to be comprehensive, this is not a complete list.
<table>
<thead>
<tr>
<th>Organization (Vaccine)</th>
<th>HPV Type &amp; Antigen</th>
<th>Kind of Vaccine</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cantab, SmithKline Beecham (TH-GW)</td>
<td>HPV-6 L2, E7</td>
<td>Peptide (fusion protein)</td>
<td>Phase Ib trials with novel adjuvant underway to assess wart regression and recurrence rates</td>
</tr>
<tr>
<td>Cantab (TA-CIN)</td>
<td>HPV-16/18 L2, E6, E7</td>
<td>Peptide (fusion protein)</td>
<td>Phase I trials begun in 1999</td>
</tr>
<tr>
<td>National Cancer Institute</td>
<td>HPV-16 E6, E7</td>
<td>Various peptide vaccines</td>
<td>Phase I/II trials underway</td>
</tr>
<tr>
<td>Norris Cancer Institute, University of South Carolina</td>
<td>HPV-16 E7</td>
<td>Peptide</td>
<td>Phase I/II trials underway</td>
</tr>
<tr>
<td>StressGen Biotechnologies (HspE7)</td>
<td>HPV-16 E7</td>
<td>Protein/peptide</td>
<td>Phase II trials underway</td>
</tr>
<tr>
<td>University of Leiden</td>
<td>HPV-16 E7</td>
<td>Peptide</td>
<td>Phase I/II dose-escalation study completed; no adverse side effects</td>
</tr>
<tr>
<td>University of Queensland</td>
<td>HPV-16 E7</td>
<td>Peptide</td>
<td>Phase I/II trials underway; antibody and T-cell responses in some patients</td>
</tr>
<tr>
<td>Cantab (TA-HPV)</td>
<td>HPV-16/18 E6, E7</td>
<td>Recombinant vaccinia virus</td>
<td>Phase II trials of clinical impact underway; immune response demonstrated in prior Phase II trials</td>
</tr>
<tr>
<td>European Commission, Chinese Academy of Medical Sciences</td>
<td>HPV-58 E7</td>
<td>Recombinant vaccinia virus</td>
<td>Preclinical; prevented tumor growth in mice</td>
</tr>
<tr>
<td>Johns Hopkins University</td>
<td>HPV-16 E6, E7</td>
<td>Recombinant vaccinia virus with LAMP</td>
<td>Preclinical; eliminated and prevented tumors in mice; Phase I trials scheduled for 2000</td>
</tr>
<tr>
<td>Transgene (MVA-HPV-IL2)</td>
<td>HPV-16</td>
<td>Recombinant MVA vaccinia virus</td>
<td>Phase I trials underway</td>
</tr>
<tr>
<td>Wistar Institute</td>
<td>HPV-16 E6, E7</td>
<td>Recombinant adenovirus and vaccinia virus</td>
<td>Preclinical; cytotoxic T-cells protect against challenge with tumor cells</td>
</tr>
<tr>
<td>MediGene, Schering AG</td>
<td>HPV-16 L1, E7</td>
<td>Chimeric VLP</td>
<td>Phase I/II trial scheduled for 2000</td>
</tr>
</tbody>
</table>

*Note: While efforts were made to be comprehensive, this is not a complete list.*
Appendix: Vaccine Developers

AlphaVax, Inc.
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www.alphavax.com
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Merck & Co., Inc.  
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National Cancer Institute (NCI)  
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http://rex.nci.nih.gov/RESEARCH/basic/lco/schiller.htm  
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Department of Medical Microbiology
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References


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