Current and Future HPV Vaccines: Promise and Challenges

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PATH

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Contents

Abbreviations and acronyms

AE	Adverse event
AEFI	Adverse event following immunization
AGIN	Anogenital intraepithelial neoplasia
AIS	Adenocarcinoma in situ
APC	Antigen presenting cell
ASCUS	Atypical squamous cells of undetermined significance
BCG	Bacille Calmette-Guérin (vaccine)
CIN	Cervical intraepithelial neoplasia
CTL	Cytotoxic T lymphocyte
DC	Dendritic cell
EMEA	European Agency for the Evaluation of Medical Products
FDA	US Food and Drug Administration
GSK	GlaxoSmithKline
GM-CSF	Granulocyte macrophage–stimulating factor
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
HSV	Herpes simplex virus
HTL	Helper T lymphocyte
IARC	International Agency for Research on Cancer
ICMR	Indian Council of Medical Research
LEEP	Loop electrosurgical excision procedure
LSIL	Low-grade squamous intraepithelial lesion

MVA	Modified vaccinia Ankara virus
NGO	Nongovernmental organization
NIH	US National Institutes of Health
PROVACS	Production of Vaccines from Applied Crop Sciences
RRP	Recurrent respiratory papillomatosis
STI	Sexually transmitted infection
UNICEF	United Nations Children's Fund
VEE	Venezuelan equine encephalitis
VIA	Visual inspection with acetic acid
VILI	Visual inspection with Lugol's iodine
VIN	Vaginal intraepithelial neoplasia
VLP	Virus-like particle
WHO	World Health Organization

Acknowledgments

Executive summary

Two new, highly effective cervical cancer vaccines soon will be on the international market. These vaccines—which target the types of human papillomavirus (HPV) that cause most cases of cervical cancer—may offer the greatest benefits for developing countries, which tend to have limited screening programs for and a higher incidence of cervical disease. The vaccines will not effect an immediate reduction in the incidence of cervical cancer because this cancer develops decades after women are infected with HPV. Ultimately, however, the vaccines have the potential to prevent about 70% of cervical cancers worldwide.

The two new vaccines are Gardasil® (GARDASIL is a registered trademark of Merck & Co., Inc., Whitehouse Station, N.J.) and Cervarix® (CERVARIX is a registered trademark of GlaxoSmithKline [GSK] Biologicals, Rixensart, Belgium). Both vaccines consist of virus-like particles (VLPs) and include the two types of HPV (16 and 18) that cause most cases of cervical cancer and high-grade cervical intraepithelial neoplasia (CIN). Gardasil also includes the two types of HPV (6 and 11) that cause 90% of anogenital warts, which affect men as well as women.

Clinical trials have established that Gardasil and Cervarix:

- Are safe and immunogenic in children as young as nine or ten.

- Offer 100% protection against CIN 2/3 associated with HPV-16 and -18 in young women who have never been infected with HPV and receive all three doses of the vaccine.

- Remain effective for at least four and a half to five years.

Ongoing trials are continuing to collect information about the duration of protection offered by these vaccines and their efficacy in other populations, including men and women previously infected with HPV.

At the same time, academic and industry researchers are actively investigating a second generation of prophylactic HPV vaccines that might be better suited to low-resource settings: ideally, they would be cheaper to produce than VLPs are, have a longer shelf life, would not require a cold chain, would require only a single dose, and could be administered orally or via a nasal spray instead of by injection. Some researchers are trying to refine VLP vaccines, and others are working on entirely different approaches, including protein and peptide vaccines, recombinant live-vector vaccines, plant-based vaccines, and DNA vaccines. Attention also has turned to prime-boost strategies, which sequentially inoculate people with two different kinds of vaccines to elicit a stronger, more complete immune response. Although researchers have generated many potential HPV vaccine candidates, their work remains largely preclinical. It is unclear which, if any, of these vaccine candidates will progress to clinical testing, let alone licensure.

Therapeutic vaccines

Therapeutic vaccines could potentially offer the vast number of women already infected with HPV a less invasive treatment for precancerous lesions and perhaps even more advanced disease. Researchers have developed a wide variety of therapeutic HPV vaccine candidates, including protein and peptide vaccines, chimeric VLP vaccines, recombinant live-vector vaccines, and

DNA vaccines. Many have been tested in small groups of women with CIN 2/3 or other HPV-related disease, but only one has entered Phase 3 clinical trials. So far, these therapeutic vaccines have shown limited efficacy in eradicating established tumors, but researchers are working to enhance their effectiveness with novel adjuvants, prime-boost strategies, and alternate delivery systems.

Introducing prophylactic HPV vaccines

Health authorities in developing countries must weigh the costs and benefits of HPV immunization against screening programs that employ Pap smears, visual inspection, or HPV DNA tests to identify women at risk of cervical cancer. The potential impact of a nationwide immunization program depends on the local burden of disease and the epidemiology of HPV infections, the ability of the health infrastructure to deliver a series of three injections to adolescents, and the cost of the vaccine. Immunization does not entirely eliminate the need for screening, because women already infected with HPV are at risk of developing cervical cancer over the next three decades and also because Gardasil and Cervarix are designed to prevent only the two most common types of oncogenic HPV.

HPV immunization programs face several challenges. First, it is not entirely clear *who* should receive the vaccine and *when.* Females tend to become infected with HPV soon after they become sexually active, so it makes sense to immunize them at a relatively early age, before they become sexually active. It is not yet known, however, whether they will require booster shots later in life or whether a catch-up immunization campaign for older, sexually active women can reduce cancer rates. Another unanswered question is whether boys should be vaccinated; this strategy might create "herd" immunity, but it costs more than vaccinating girls only.

Second, local motivations and concerns can affect the acceptability of an HPV vaccine. For example, some providers and parents in the United States believe that vaccines against sexually transmitted infections (STIs) are appropriate for older adolescents only. Qualitative research can identify and help to address such potential obstacles, for example, by determining whether an HPV vaccine should be positioned as an anticancer or anti-STI measure.

Third, developing world public vaccination programs have little or no experience reaching adolescents, who do not routinely visit health care providers. HPV vaccination programs may require new strategies to reach their target audience, such as administering vaccines in school, conducting mass campaigns, or creating a new standard for adolescent health visits that combines vaccines with other health interventions of benefit to this age group.

Building support for and implementing an HPV immunization program will require energetic advocacy and education. At the international and national levels, advocates must convince government officials, nongovernmental organizations (NGOs), funding organizations, medical professional associations, and medical schools of the value of the vaccines. At the district and local levels, advocates must address the authorities, administrators, and providers who operate the health care delivery system. At the community level, they must win over the support of local opinion leaders, foster broad discussion about the vaccines, and teach parents and their children the importance of being vaccinated against HPV.

Advances in the prevention of cervical cancer are developing at a fast pace. This dynamic and exciting area of research will most certainly continue to grow and effect changes in prevention strategies and therapies for cervical cancer and other conditions in which HPV is implicated. The information in this paper can be considered a "snapshot" of where we are in 2006—but readers are urged to seek updated information as new vaccines are developed and results of clinical trials become available.

I. Introduction

The discovery that a virus—the human papillomavirus (HPV)—causes cervical cancer has opened new avenues to prevention and treatment of this disease. Researchers at academic research centers, pharmaceutical companies, and biotechnology firms around the world have taken advantage of advances in genetic engineering and vaccine development to develop a wide array of prophylactic and therapeutic HPV candidate vaccines. The first prophylactic HPV vaccine (Merck and Co.'s Gardasil) was licensed and on the US market in 2006, and a second vaccine is soon to follow. Research continues on a second generation of prophylactic HPV vaccines that might be better suited to low-resource settings and on immunotherapy for HPV-related cervical disease.

This publication provides a "snapshot" of the current situation in a rapidly evolving field. It offers a technical update on the development of prophylactic vaccines to prevent cervical cancer as well as an overview of therapeutic vaccine development. It also discusses key issues related to vaccine introduction, with emphasis on the two new prophylactic vaccines. It is hoped that the information found herein will help immunization experts, health policymakers, and program planners or managers in developing countries (particularly those involved in cancer prevention and adolescent or reproductive health programs) to understand better the technical issues related to vaccine development. In addition, this publication outlines the anticipated challenges to making new cervical cancer prevention vaccines broadly available in these countries.

A. HPV-related disease

Worldwide, HPV is the most common STI, affecting an estimated 50% to 80% of sexually active women at least once in their lifetime (Crum et al. 2003, Koutsky 1997). However, the prevalence of HPV infections at any given time varies widely among and even within countries. For example, the prevalence of HPV infections of all types ranges from less than 2% of women in Spain to more than 25% in Nigeria according to data collected by the International Agency for Research on Cancer (IARC) (Clifford et al. 2005b). The prevalence of HPV in a country can also change rapidly with shifts in lifestyle and sexual practices (WHO 2005a).

Each year, HPV causes about half a million cases of cervical cancer, about four-fifths of which are in developing countries (Franceschi 2005, Parkin et al. 2006). The incidence of cervical cancer varies more than tenfold between the lowest and highest national rates. Sexual behavior has a large impact on the incidence of cervical cancer, as does the existence of screening programs. The incidence is generally higher in developing countries, in part because they lack the comprehensive precancer screening programs that have dramatically reduced the incidence of cervical cancer in industrialized countries (Parkin et al. 2005a) (see Figure 1.).

Figure 1. Estimates of the number of cases and incidence of cervical cancer, 2002.
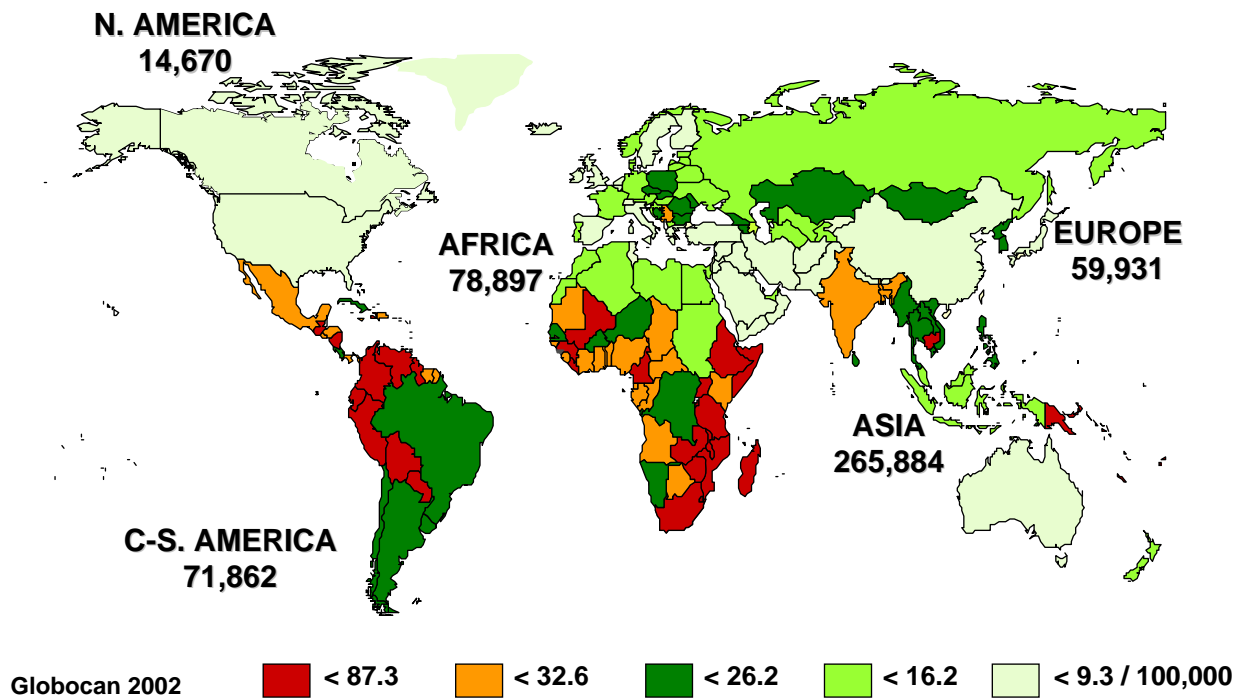
If not detected and treated in a timely way, cervical cancer is almost always fatal. An estimated 274,000 women die of cervical cancer annually (Ferlay et al. 2004). Mortality rates are about four times higher in developing than in developed countries, and cervical cancer is the leading cause of cancer deaths among women in most developing countries (Ferlay et al. 2004).

Women are generally infected with HPV in their teens, 20s, and early 30s, but the vast majority clear the virus naturally (Franco and Harper 2005, Moscicki et al. 2006). HPV infection progresses to cervical cancer rarely and slowly; approximately 5% to 10% of women infected with oncogenic (cancer-causing) HPV ultimately develop persistent infections; these women have an increased risk of developing high-grade precancerous lesions and, if the lesions are not treated, cervical cancer (Bosch et al. 2002, Ho et al. 1998, Hopman et al. 2000, Munoz and Bosch 1996). Generally, it can take 20 years or longer for infections to progress to cancer; as a result, the incidence of cervical cancer begins to rise after age 35 to 40 and does not peak until women reach their 50s and 60s (Miller 1992, Parkin et al. 2005b).

Vaccines against cervical cancer also have the potential to prevent other cancers that are caused by the same types of HPV, including a subset of head and neck cancers, notably oropharyngeal cancer (Herrero et al. 2003, Kreimer et al. 2005), and half or more of anogenital cancers outside the cervix, including cancer of the vulva, vagina, penis, and anus (Carter et al. 2001, Daling et al. 2002 and 2005, Gross and Pfister 2004, van der Avoort et al. 2006). Although much rarer than cervical cancer, anal cancer is of growing concern, especially among men who have sex with men, because HIV infection increases men's and women's susceptibility to HPV-related diseases of all kinds. Antiretroviral therapy does not reduce those risks and may even increase the cancer burden as it increases life expectancy (Chin-Hong and Palefsky 2005).

Although most HPV vaccine research has focused on cervical cancer, some vaccine developers have targeted other diseases related to different strains of HPV. Two types of HPV (6 and 11) can cause genital warts and recurrent respiratory papillomatosis (RRP). Genital warts, which affect both men and women, have provided researchers with 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 also because warts are not life threatening (Lacey et al. 2006). RRP is a rare but life-threatening disease that can require patients to endure multiple surgeries each year to remove warts that block their upper airways. It has attracted the interest of vaccine developers because it may qualify for orphan drug status and fast-track licensing in the United States (Nventa Biopharmaceuticals Corporation 2006).

B. Prophylactic and therapeutic vaccine strategies

A vaccine that prevents HPV-related cervical disease—and hence cervical cancer—potentially offers great benefits to the developing world. Cytology-based screening for precancerous lesions, followed by treatment, has proven effective in reducing the incidence of cervical cancer in industrialized countries. However, it has had limited success in low-resource settings because these areas often lack the skilled providers, supplies, and infrastructure necessary for effective screening and treatment based on traditional approaches (Ashford et al. 2004, Franceschi 2005). Prophylactic immunization offers a potentially inexpensive, logistically simple, and effective strategy to reduce the burden of cervical cancer. A *prophylactic* vaccine works primarily by stimulating antibody-mediated (or humoral) immunity; that is, the vaccine induces neutralizing antibodies capable of recognizing and inactivating HPV before the virus infects host cells (Zinkernagel 2003). Prophylactic vaccines cannot make an immediate impact on the incidence of cervical cancer, however, because this cancer develops several decades after infection with HPV.

Unlike prophylactic vaccines, a *therapeutic* vaccine could help the vast number of women who are already infected with HPV. Used alone or in combination with standard therapies, a therapeutic vaccine could help prevent low-grade disease from progressing and cause existing lesions to regress; some also believe it has the potential to control the spread of metastatic cancer and prevent recurrence of cervical cancer after treatment (Chu 2003, Stanley 2003). To be effective, therapeutic HPV vaccines must prompt cell-mediated immunity because antibodies cannot reach and eliminate the virus once it has been incorporated into host cells (Chu 2003, Ling et al. 2000, Maclean et al. 2005).

Perhaps the most effective HPV vaccine strategy calls for a vaccine that possesses both prophylactic and therapeutic properties. A chimeric vaccine of this kind could both prevent new HPV infections and clear existing infections and cervical lesions. Such a vaccine would benefit and could be administered to both sexually inexperienced young people and older women already harboring HPV (Franceschi 2005). It would produce a quicker impact on cervical cancer rates than can a purely prophylactic vaccine.

II. Prophylactic vaccine research

A. Key questions for vaccine development

*1. How many and which types of HPV should a prophylactic vaccine target?*

More than 100 types of HPV have been identified. Although it is not clear exactly how many types are associated with cervical cancer, at least 15 are considered oncogenic; two other types cause most genital warts (Munoz et al. 2004). Whereas most research suggests that antibodies raised against one kind of HPV are unlikely to offer strong protection against other types (Maclean et al. 2005), emerging results from vaccine trials suggest that some cross-protection might be possible. Therefore, preventing most cervical cancer cases is likely to require a multivalent vaccine, that is, a combination vaccine effective against multiple types of HPV.

Both the prevalence of HPV infections and the distribution of HPV types vary geographically (Clifford et al. 2006). However, at every stage of infection and disease, both HPV-16—and, to a lesser extent, HPV-18—are more likely than other types of HPV to persist and progress (Castle et al. 2005, Molano et al. 2003, Schiffman et al. 2005). As a result, these two types account for an estimated 35% of low-grade squamous intraepithial lesions (LSILs) worldwide, but are responsible for double that amount of squamous cell cervical cancers (Clifford et al. 2003a, 2005a). A recent study suggests that HPV-16 and -18 may account for an even greater proportion (about 85%) of adenocarcinomas (Castellsague et al. 2006). Although most cases of invasive cervical cancer in every region of the world are associated with HPV-16 or -18, IARC data have found that about one quarter are associated with 16 other types of HPV (see Figure 2.), and their distribution varies by region (Clifford et al. 2003b).

Based on these epidemiologic data, a bivalent vaccine against HPV-16 and -18 could prevent an estimated 71% of cervical cancers worldwide, although—based on limited HPV data available worldwide—the vaccine's impact could be less in some areas than in others. For example, it could potentially prevent 74% of cancers in Asia but only 64% in sub-Saharan Africa (Munoz et al. 2004).

The most obvious way to increase the protection offered by a vaccine—and simultaneously to reduce regional variations in its effectiveness—is to add more types of HPV to a multivalent vaccine. For example, a vaccine against the seven most common types of oncogenic HPV (16, 18, 45, 31, 33, 52, and 58) could prevent from 85% to 91% of cervical cancers in different regions of the world (Munoz et al. 2004). Although technically feasible, adding more types of HPV to a vaccine increases manufacturing challenges and costs and, as a result, might reduce vaccine access and affordability in countries where it is most needed.

Figure 2. Global distribution of HPV types in cervical cancer.
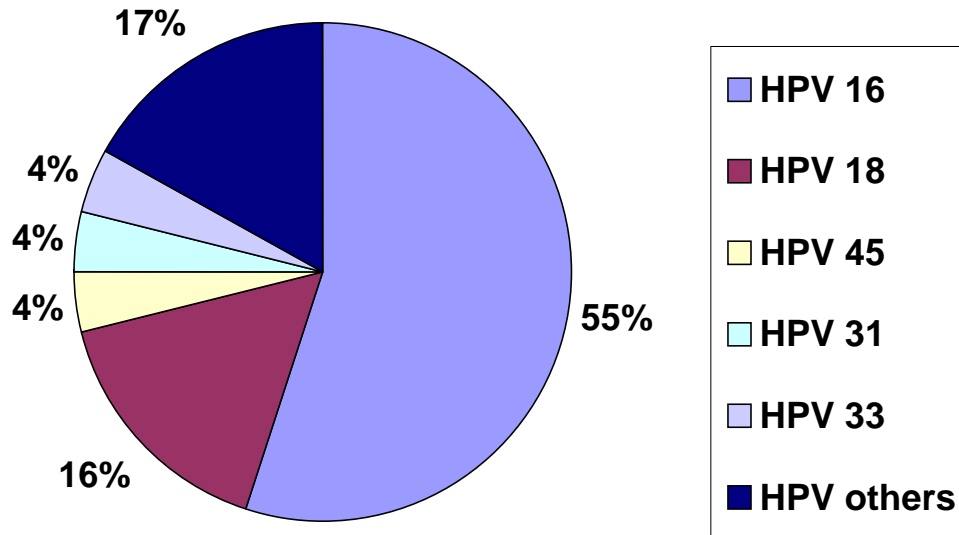
Adapted from Clifford et al. 2006.

Another approach would be to formulate different vaccines that match the distribution of HPV types in specific regions. Regional formulations would reduce the number of HPV types included in any one vaccine but would complicate manufacturing. It is unclear at this time whether regional variations in the distribution of HPV warrant the effort.

Rather than tinkering with the composition of a multivalent vaccine, other researchers are searching for cross-protective antibodies effective against a range of HPV types. Although this research is in the early stages, such a vaccine might be simpler and less expensive to produce than a multivalent vaccine against specific types of HPV (Pastrana et al. 2005). (For more information on vaccine candidates that potentially offer cross-protection, see the section on prophylactic protein and peptide vaccines, page 24).

2. Will vaccinating against HPV-16 and -18 change—for better or worse—the prevalence of other high-risk types of HPV?

Many women are infected with multiple types of HPV. For example, in a series of IARC prevalence surveys, the proportion of HPV-infected women with multiple infections ranged from 11.5% in Turin, Italy, to 42.4% in Ho Chi Minh City, Vietnam (Clifford et al., 2005b). However, interactions between infections with different types of HPV are not well understood. Both antagonistic and synergistic interactions may be possible (Liaw et al. 2001, Mendez et al. 2005, Silins et al. 1999).

Some experts are concerned that other oncogenic HPV types will take the place of HPV-16 and -18 after immunization begins, thus reducing the expected impact of a vaccine on cervical disease (Goldie et al. 2003, Hughes et al. 2002). Even if replacement occurs, however, its effect would be lessened by the fact that other high-risk types of HPV are less oncogenic than HPV-16 and -18.

Other experts raise the possibility that immunization against HPV-16 might reduce the likelihood of infection with HPV types that are not included in vaccine, thereby amplifying the expected impact of a vaccine (Elbasha and Galvani 2005, Mendez et al. 2005). It is not clear, however, whether the current vaccines provide cross-protection against HPV types not included in the formulation. For the bivalent vaccine, protection has been reported in HPV-naïve women against new-incident infections by two other genotypes. For the quadrivalent vaccine, neutralizing antibodies against genotypes 31 and 45 have been demonstrated following vaccination. Ongoing studies are tracking this question.

3. How important is mucosal versus systemic immunity?

Despite considerable research, the role of different elements of the immune system in preventing or resolving HPV infections remains unclear (Franceschi 2005). Clinical trials of first-generation prophylactic HPV vaccines have found that high levels of circulating neutralizing antibodies protect against HPV infection. Because HPV enters the body through the mucosal membranes and does not spread systemically, however, it is possible that HPV vaccines that induce mucosal immunity would be equally or even more effective while offering easier ways to administer a vaccine (Holmgren et al. 2003, Nardelli-Haefliger et al. 1997).

Evidence that exposure to an antigen at one mucosal surface site can elicit an immune response at a distant site has prompted researchers to investigate the effectiveness of nasal, aerosol, and oral delivery of HPV vaccines (Nardelli-Haefliger et al. 2005, Tomson et al. 2004). Although they are simpler and safer to administer than injections, however, mucosal vaccines face challenges in inducing a long-lasting immune response. Notably, HPV VLP oral vaccines require much higher antigen levels to be effective in animal models. They also have the potential to induce a state of immunologic unresponsiveness known as *oral tolerance,* although this is unlikely, given that HPV VLPs directly activate B cells and other antigen-presenting cells (APCs) (Liu et al. 2002; Production of Vaccines from Applied Crop Sciences [PROVACS], 2005, Yang R et al. 2005).

4. At what age should people be vaccinated?

In most areas, HPV prevalence peaks before the age of 25, reflecting the fact that women tend to become infected with HPV soon after they become sexually active (Moscicki 2005). Thus, it makes sense to immunize people against HPV at a relatively early age, before they become sexually active. It is also important to consider the duration of protection afforded by a vaccine. For example, some have speculated that the first generation of prophylactic HPV vaccines will offer about 10 years of protection, after which boosters would be required. If this proves accurate, vaccinating infants would not be cost-effective (Taira et al. 2004), and the cost-effectiveness of 10-year boosters beginning around age 20 to 25 would require additional examination.

Given these constraints and the relatively high immunogenic response among younger girls (10 to 15) compared with older adolescents, vaccine developers are recommending vaccination at age 10 to 12 years in Europe and North America, where many teenagers begin sexual intercourse before marriage and at an early age. Elsewhere, if cultural norms governing sexual behavior delay the age of first intercourse until late adolescence or marriage, it might be preferable to vaccinate girls and boys at a somewhat later age. Regardless of the age at first immunization, vaccination programs must consider the need for boosters. The prevalence of HPV declines with

age in many populations, but in other areas, it remains at a steady level or rises to a second peak in middle age (because of age-related immune suppression or reinfection), implying that women remain at risk for infection and cancer (Franceschi 2005).

Some question remains as to whether an HPV vaccination program should initially include older, sexually active adults as part of a catch-up campaign designed to accelerate the impact of immunization on the prevalence of cervical cancer (Lowndes and Gill 2005). Answering this question requires epidemiologic research on how many older women have been exposed to the HPV types in a vaccine, clinical research to determine whether vaccinating previously infected women might prevent reinfection or limit the persistence of existing infections, and modeling to determine the impact of vaccinating older women on the incidence of cervical cancer (Pagliusi and Aguado 2004).

5. Should males as well as females be vaccinated?

Men play an important role in transmitting HPV, as they do in all STIs, which suggests that immunizing males may be important for creating herd immunity and reducing the incidence of cervical cancer, even though cervical cancer affects only women. This reasoning is the basis for global recommendations to vaccinate both sexes against rubella, even though the disease has no serious outcomes for males. Furthermore, vaccinating both girls and boys may avoid stigmatizing females as the source of STIs and improve the social acceptability of HPV vaccine in some areas of the world. However, vaccinating only females would be less costly than vaccinating both sexes.

Mathematical models of HPV transmission and vaccine impact have reached different conclusions about the cost-effectiveness of immunizing both males and females (Goldie et al. 2006). One study calculated that female-only vaccination is 60% to 75% as effective as universal vaccination (Hughes et al. 2002). Other studies found that vaccinating males made almost no difference in the impact on cervical cancer except when vaccine coverage was low or the duration of protection was short and no boosters were available (Barnabas et al. 2006, Garnett et al. 2006, Taira et al. 2004). A third model has concluded that herd immunity effects made universal vaccination more cost-effective than female-only vaccination (Elbasha and Dasbach 2005). All these models rest on a series of assumptions that are far from certain regarding the vaccine (i.e., its efficacy and duration of protection), its administration (cost and coverage), and local patterns of HPV transmission.

Sharing the health benefits of an HPV vaccine with men offers another reason for vaccinating both sexes. Depending on its formulation, an HPV vaccine may prevent genital warts, anal cancer, and head and neck cancers—all of which affect men as well as women—in addition to preventing cervical cancer. However, vaccines do not always induce the same immune response or confer the same level of protection in both sexes (Bass 2003, Stanberry et al. 2002). Therefore, if HPV vaccines are to be administered to boys or men, males must be included in clinical trials establishing a vaccine's safety, immunogenicity, and efficacy.

6. How expensive or complicated is it to produce, transport, and administer an HPV vaccine?

The first generation of prophylactic HPV vaccines to enter the market, which are based on VLPs, require a cold chain for distribution and are administered in three doses over a six-month period. Although challenging, these conditions do not preclude their use in developing countries, which

routinely employ cold chains and deliver multidose vaccines as part of their national vaccination programs. Researchers are actively working to develop other kinds of HPV vaccines that may more closely approach the ideal characteristics of an HPV vaccine—indeed, of *any* vaccine—for low-resource settings.

B. First-generation VLP vaccines: Gardasil and Cervarix

A major breakthrough in HPV vaccine research came with the discovery that the major capsid protein L1, which comprises the outside coat or shell of HPV particles, self-assembles into VLPs when expressed in eukaryotic cells. L1 interacts with the surface molecules of human epithelial cells during the early stages of infection to gain entry for the viral DNA. Because it is present during the initial infection, it is an ideal target for a prophylactic vaccine.

VLPs closely resemble native HPV particles and include the conformational epitopes that induce neutralizing antibodies. Therefore, the immune system perceives VLPs as an infectious virus and responds accordingly; however, VLPs are not infectious because they do not include viral DNA (Tomson et al. 2004).

Both first-generation prophylactic HPV vaccines are L1 VLP vaccines. Merck & Co. has produced a candidate vaccine called Gardasil, and GlaxoSmithKline Biologicals has produced a vaccine called Cervarix. Many studies of these vaccines have been published in peer-reviewed journals, but ongoing clinical research into Gardasil and Cervarix also generates a constant stream of new data that are initially presented at conferences and in company press releases. To present the most complete and up-to-date picture of these vaccines, this section includes these new data (i.e., as of December 2006) (see Tables 1. and 2. on pages 21 and 22 for a summary).

1. Formulation and manufacture

Both Gardasil and Cervarix consist of L1 VLPs, but the details of their formulation and manufacture differ. Gardasil is a quadrivalent vaccine: It includes the two types of HPV (16 and 18) that cause most cases of cervical cancer and high-grade CIN as well as the two types of HPV (6 and 11) that cause 90% of anogenital warts worldwide. Merck is testing the vaccine on men as well as women because the protection it offers against genital warts offers a tangible benefit to men (Villa et al. 2005) and also because universal vaccination of both sexes might have a greater impact on the incidence of cervical cancer than vaccinating women only (Hughes et al. 2002).

In contrast, Cervarix is a bivalent vaccine that includes the two most common causes of cervical cancer (HPV-16 and -18). GSK views Cervarix purely as a vaccine against cervical cancer and plans to administer it to women only (Monteyne 2005).

Gardasil and Cervarix also differ in their *adjuvants,* which are substances added to a vaccine to enhance its impact by stimulating immune responses. In Gardasil, each type of VLP is purified and adsorbed on aluminum-containing adjuvant (amorphous aluminum hydroxyphosphate sulfate). This adjuvant was the first approved for human use, and it has an extensive safety record in other vaccines.

Cervarix is formulated with a novel adjuvant, AS04, developed by the Corixa Corporation to strengthen and prolong the immune response to vaccines. Along with aluminum hydroxide, AS04 includes MPL[®] (3-deacylated monophosphoryl lipid A), a derivative of the lipid A

molecule found in gram-negative bacteria and a potent immune system stimulant. GSK is using AS04 in several vaccines under development, and a vaccine containing the adjuvant has already been approved in the European Union (Corixa 2005). Research in animals and women has found that, compared with an identical GSK vaccine formulated with aluminum hydroxide only, a GSK HPV-16 L1 VLP vaccine formulated with AS04 induces higher levels of specific antibodies and, in human subjects, the gap persists for 3.5 years (Giannini et al. 2005). What this difference in antibody levels may mean for protection against disease is as yet unclear, however.

Gardasil is manufactured in a yeast system, whereas Cervarix is produced in an insect cell system. Both VLP vaccines are much more expensive to produce than, for example, common viral vaccines propagated in tissue culture, such as rabies, polio, hepatitis A and B, measles, mumps, and rubella (Brinkman et al. 2005).

2. Distribution and administration

Like many other vaccines, both Gardasil and Cervarix are sensitive to high and low temperatures and require a cold chain to retain their potency; neither can be frozen. Merck and GSK are studying the stability and immunogenicity of the vaccines under different storage conditions.

Both Gardasil and Cervarix are administered as a series of three intramuscular injections administered over a six-month period (at months 0, 2, and 6 and months 0, 1, and 6, respectively). Merck and GSK both advocate vaccination in female adolescents before they become sexually active, around age 10 to 12 years in North America and Europe.

Results from clinical trials of Gardasil and Cervarix showing higher antibody levels in younger participants strengthen the rationale for immunizing younger adolescents. Antibody levels for all four types of HPV included in Gardasil were significantly higher among boys and girls aged 9 to 15 years than among women and adolescent girls aged 16 to 23 years (Barr 2005, Merck press release 5/19/05). Similarly, in studies of Cervarix, antibody levels for HPV-16 and -18 were at least twice as high in girls aged 10 to 14 years as in women and female adolescents aged 15 to 25 years (Dubin 2005).

A study of a monovalent HPV-16 VLP vaccine produced by Merck suggests that the vaccine may also benefit some older, sexually active women who are infected with HPV but in early stages of the disease. A subgroup analysis of a small number of women who were positive for HPV-16 DNA at enrollment, but seronegative for HPV-16, found that these women were less likely to develop moderate or high-grade CIN 2/3 if they received the vaccine (Mao et al. 2006).

3. Safety and immunogenicity

Phase 2 clinical trials have established the safety and immunogenicity of Gardasil and Cervarix in girls and women both in the short run and over a period of several years (Harper et al. 2006, Mao et al. 2006). Both vaccines have generally been well-tolerated and do not appear to be associated with severe adverse events following immunization (AEFIs). The most common mild AEFIs were local discomfort at the injection site, including pain, swelling and redness, headache, and low-grade fever (Barr 2006, Harper et al. 2004, Villa et al. 2005). Although AEFIs have not had any impact on compliance and continuation among female subjects over 15 years of age, Phase 3 trials of Gardasil involving younger subjects found that a small number of 10- to 15-year-olds (0.3%) did discontinue injections because of adverse effects (AEs). These younger

subjects had a significantly higher risk of fever within 15 days after injection, although the episodes were brief and not serious (Merck press release 5/19/05). A Phase 3 trial of Cervarix found that girls aged 10 to 14 years tolerated the vaccine and had similar rates of AEs as young women and female adolescents aged 15 to 25 years (Dubin 2005).

In a Phase 2 trial of different dosages of Merck's quadrivalent vaccine, all the vaccinated female subjects developed neutralizing antibodies to each type of HPV. Serum concentrations of antibodies to HPV-6, -11, -16, and -18 were measured using a competitive immunoassay (Luminex Corporation, Austin, Tex., United States) (Opalka et al. 2003). Antibody titers were determined in a competitive format—known, type-specific phycoerythrin-labelled, neutralizing antibodies compete with serum antibodies from the participant for binding to conformationally sensitive, neutralizing epitopes on VLPs (Christensen et al. 1996). Compared with women in the placebo group who had cleared natural HPV infections, antibody titers among vaccinated women were 7 to 105 times higher one month after receiving the last dose of vaccine. Titers declined after that point, but at 36 months (i.e., 30 months after receiving the last injection), they remained higher among vaccinated women than women who had cleared natural infections (Villa et al. 2006a). Longer follow-up of a monovalent HPV-16 VLP vaccine produced by Merck shows that antibody titers reached a stable plateau by 48 months and remained higher among vaccinated women (Mao et al. 2006).

Similarly, in a Phase 2 trial of Cervarix, 100% of the female subjects who followed study protocols seroconverted to HPV-16 and 99.7% to HPV-18 one month after receiving the last dose of vaccine (antibodies were detected with a type-specific enzyme-linked immunosorbent assay [ELISA], using type-specific recombinant VLPs as coating antigens). One month after receiving the last dose of vaccine, antibody titers in vaccinated women were 80 to 100 times greater than those seen in natural HPV infections (Harper et al. 2004). Antibody titers initially declined over time but plateaued after 18 months (i.e., 12 months after receiving the last injection). At 51 to 53 months, vaccine-induced titers were 17 times greater than those associated with a natural HPV-16 infection and 14 times greater than those associated with a natural HPV-18 infection (Harper et al. 2006).

Bridging studies have established that Gardasil is immunogenic in boys and girls as young as nine years (Barr 2005, Merck press release 5/19/05) and that Cervarix is immunogenic in girls as young as ten years (Dubin 2005).

4. Efficacy

Phase 3 clinical trials for both vaccines are global in scope and involve prospective, randomized, double-blind controlled designs. Control groups in trials of Gardasil are receiving a placebo, and control groups in trials of Cervarix are receiving a hepatitis A vaccine. Trials for both vaccines screen subjects for HPV-16 or -18 at enrollment; women who test positive are excluded from the primary analysis of efficacy.

Merck launched Phase 3 efficacy trials of Gardasil in December 2001. In 2005, investigators reported interim results for the FUTURE II trial, which involves 12,167 female subjects aged 16 to 26 recruited at 90 centers in Brazil, Colombia, Denmark, Finland, Iceland, Mexico, Norway, Peru, Poland, Singapore, Sweden, the United Kingdom, and the United States (Merck press

release 10/6/05). Almost two thirds (65%) of the subjects described in the interim results are from Europe, 26% from Latin America, 20% from Asia/Pacific, and 8% from North America.

GSK began Phase 3 efficacy trials of Cervarix in mid-2004 (GSK press release 5/19/04). The PATRICIA trial involves 18,000 female subjects aged 15 to 25 at 178 sites in Australia, Belgium, Brazil, Canada, Finland, Germany, Italy, Mexico, Philippines, Spain, Taiwan, Thailand, the United Kingdom, and the United States (ClinicalTrials.gov, 12/8/05). The US National Cancer Institute (NCI), in collaboration with several Costa Rican institutions, has recruited an additional 7,467 women aged 18 to 25 years in the Guanacaste Province of Costa Rica for a community-based Phase 3 study of Cervarix (NCI/PDQ 8/5/05). The NCI study will examine the effect of the vaccine on the population over time and the mechanism of immunity as well as the efficacy of the vaccine (Harper 2005a).

Because cervical cancer can take decades to develop after infection with HPV, and because it would be unethical not to treat women who develop precancerous lesions, invasive cervical cancer is not a feasible or appropriate endpoint for clinical trials of HPV vaccines (Pagliusi and Aguado 2004, Pratt et al. 2001). However, earlier stages of disease may not reflect a vaccine's true efficacy against cervical cancer because most infections and early stage lesions clear naturally and do not progress to cancer. Hence, Phase 3 clinical trials for Gardasil and Cervarix employ compromise endpoints: advanced clinical disease that falls short of cancer, including moderate- and high-grade CIN 2/3 and adenocarcinoma in situ (AIS) associated with HPV-16 or -18 (Harper et al. 2004, Merck press release 10/6/05).

Interim results of the FUTURE II trial indicate that the efficacy of Gardasil is extremely high. A per-protocol analysis (i.e, an analysis limited to women who received all three doses of vaccine, remained free of HPV-16 and -18 through the vaccination period, and had no major protocol violations) found the efficacy of Gardasil to be 100% over a mean of 17 months following vaccination. No cases of CIN 2/3 or AIS were detected in 5,301 vaccinated women, compared with 21 cases in the placebo group ($n = 5,258$) ($P < .001$) (Merck press release 10/6/05). Incorporating an additional 7,000 patient records from earlier Phase 2 trials into the analysis also yielded 100% efficacy: no cases of CIN 2/3 or AIS detected in 8,487 vaccinated women, compared with 53 cases in 8,460 women receiving placebo ($P < 0.001$) (American Association for Cancer Research [AACR] 2005). A secondary, intention-to-treat analysis is a better reflection of real world conditions, however, because it includes women who violated the protocol, did not receive all three injections, or became infected with HPV-16 or -18 during the vaccination period. This analysis followed up on subjects for an average of two years, beginning 30 days after the first dose of vaccine. In this larger group, Gardasil reduced the risk of CIN 2/3 and AIS by 97%, with one case in the vaccine group ($n = 5,736$), compared with 36 cases in the placebo group ($n = 5,766$) (Merck press release, 10/6/05). After adding data from the Phase 2 trials to the analysis, investigators calculated Gardasil's efficacy at 99%, with one case in the vaccine group ($n = 9,342$) and 81 cases in the placebo group ($n = 9,400$ placebo) (AACR 2005).

Another analysis of the Phase 3 trials of Gardasil examined its impact on genital warts, vaginal dysplasia, and vulvar dysplasia. The vaccine was 100% effective after a mean of 20 months in a per-protocol analysis, with no cases in 2,261 vaccinated women compared with 40 cases in the placebo group ($n = 2,279$) ($P < .001$). An intention-to-treat analysis calculated the efficacy of the vaccine at 95%, with three cases in the vaccine group ($n = 2,620$) and 59 cases in the placebo

group ($n = 2,628$). No cases of high-grade vaginal or vulvar intraepithelial dyplasia were observed in vaccinated women (Harper 2005b).

Initial results from Phase 3 trials of Cervarix are expected in 2007; however, results from a Phase 2b trial suggest that Cervarix is also highly effective. The study enrolled 1,113 female subjects aged 15 to 25 at study sites in North America and Brazil, randomly assigned them to the vaccine or a placebo, and followed up with them for up to 27 months (Harper et al. 2004). The per-protocol analysis, which included 366 female subjects in the vaccine group and 355 in the placebo group, found 100% efficacy against persistent infection with HPV-16 and -18 and 93.5% efficacy against cytologic abnormalities (including LSIL, high-grade squamous intraepithelial lesion [HSIL], and atypical squamous cells of undetermined significance [ASCUS]) associated with those HPV types. The intention-to-treat analysis found 95.1% efficacy against persistent infection and 92.9% against cytologic abnormalities. Two cases of disease were detected in 560 of those who received the vaccination, compared with 27 cases among the 553 in the placebo group ($P <.0001$) (Harper et al. 2004). A per-protocol analysis of the combined intial and extended follow-up phases of the study conducted at 4.5 years found vaccine efficacy of 94.7% ($P <.0001$) against incident HPV-16/18 infection, 100% against persistent HPV-16/18 infections ($P =.0007$), 92.6% against LSIL associated with HPV-16 or -18 ($P <.0001$), and 100% ($P =.0035$) against CIN 1/2/3 and invasive cell carcinoma associated with HPV-16 or -18 (Harper et al. 2006).

Results of a study of a monovalent VLP vaccine against HPV-16 produced by Merck indicate that it does not protect against other types of HPV. Vaccination did not reduce the incidence of infection with HPV-18 compared with the placebo group (Brown et al. 2004), nor did it reduce overall rates of CIN 1/2/3 beyond what would be expected given the proportion of lesions caused by HPV-16 (Mao et al. 2006).

A combined analysis of initial and extended follow-up data from the Phase 2b trial of Cervarix suggests that the vaccine may offer some protection against infection with HPV types other than HPV-16 and -18 (Harper et al. 2006). Intention-to-treat analyses found 94% efficacy against incident infection with HPV-45 and 55% efficacy against incident infection with HPV-31; these are the third and fourth most common types of HPV associated with cervical cancer. The meaning and magnitude of possible cross-protection are not yet clear (including whether protection will extend to type-specific CIN), and further study will be needed to identify the immune mechanism at work.

None of the clinical trials have followed up subjects long enough to establish the duration of the vaccines' effectiveness. A study of a monovalent HPV-16 L1 VLP vaccine produced by Merck that included 3.5 years of follow-up after vaccination found that the vaccine continued to provide 100% protection against CIN throughout this period: in the per-protocol analysis, no cases of CIN 1/2/3 were found among 755 vaccinated women, compared with 24 cases among 750 women in the placebo group (Mao et al. 2006). An extended follow-up of GSK's Phase 2 clinical trial has established that the protection Cervarix offers is sustained for at least 4.5 years. Analysis of the combined initial and extended follow-up phases of the study found 100% vaccine efficacy against CIN 1/2/3 and invasive cell carcinoma associated with HPV-16 and -18: no cases among 481 vaccinated women were found, compared with 8 cases among 470 women in the placebo group ($P =.0035$) (Harper et al. 2006).

It is possible that the HPV VLP vaccines will protect younger girls longer than older adolescents and adults because younger girls have higher initial antibody levels (Dubin 2005).

Preliminary results from a challenge study in which vaccinated women were given a fourth dose of the Merck vaccine five years after enrollment suggest that immune memory is induced by vaccination (Villa et al. 2006b).

Ultimately, clinical trials must continue follow-up of subjects for many more years to establish how long the vaccines' protective effect lasts, whether and when booster shots are needed, and the ultimate impact of the vaccines on invasive cervical cancer.

5. Research plans

The aforementioned large-scale Phase 3 trials are establishing the efficacy of Gardasil and Cervarix in young women and adolescent girls aged from about 15 to 25 years; however, further clinical research is required to answer key questions for practical vaccination strategies (Franco et al. 2006a, Hildesheim et al. 2006). Both companies are recommending vaccination of young adolescents and are conducting bridging studies to establish the safety, immunogenicity, and tolerability of their vaccines in younger children. A bridging study of Gardasil currently under way involves 4,800 boys and girls aged 9 to 15, and a Cervarix trial is recruiting girls as young as age 10 (Barr 2006, Monteyne 2005). At the other end of the age spectrum, both companies are conducting studies involving older women (i.e., aged 24 to 45 for Gardasil and 26 to 55 for Cervarix) to determine whether the vaccines could offer health benefits to women likely to have already been exposed to HPV (Barr 2006, Monteyne 2005).

Two additional questions about who should be vaccinated are under investigation. To shed light on whether boys should be vaccinated, Merck is collecting data on Gardasil's immunogenicity among 9- to 15-year-old boys and the vaccine's effectiveness against infections and genital warts in boys and men aged 16 to 26 (Barr 2006). Other research will examine the safety and immunogenicity of the VLP vaccines in HIV-positive individuals, which is especially important for countries where the prevalence of HIV is high and an individual's infection status is not always known. The US National Institutes of Health (NIH) is sponsoring a study of Gardasil in preteen HIV-positive boys and girls (Bernard 2005, PATH 2005); GSK is planning to collect safety data on HIV-positive women; and Merck and academic researchers are planning a study comprising HIV-positive women in Senegal.

For the managers who design and operate vaccination programs in developing countries, further research on the number and spacing of doses is also a priority. Experts have speculated about the possibility of reducing the number of doses from three to two and also about making the schedule more flexible, for example, permitting annual injections rather than administering doses according to standard protocols (0, 1, and 6 months or 0, 2, and 6 months) (WHO 2005a and 2006a). Changes of this kind would greatly reduce costs and simplify delivery of an HPV vaccine. To elucidate these issues, clinical trials of the vaccines are including follow-up of participants who received fewer than three doses or had pregnancy-related delays between doses. Also, in Vietnam, PATH is studying alternative dosage schedules with one of the first-generation VLP vaccines.

Merck and GSK have begun testing to determine whether the HPV vaccine can be co-administered with other childhood vaccines, including vaccines against hepatitis B, tetanus, diphtheria, and pertussis (WHO 2005a). Co-administration would help health systems to integrate a new HPV vaccine into the immunization schedule and also to save money by providers administering the HPV vaccine along with other vaccines during a single visit.

Both companies will conduct follow-up of vaccinated women for an extended period to establish the duration of protection offered by Gardasil and Cervarix and to determine the need for booster shots (Monteyne 2005). Merck will use the Nordic Cancer Registries to follow up on 5,800 FUTURE II study subjects enrolled in the region through 2015 and to examine the long-term impact of Gardasil on the prevalence of precancerous lesions and cervical cancer (Barr 2006). A post-licensure population-based evaluation of Gardasil is also planned in Scandinavia (Barr 2006, WHO 2005a).

If HPV-45 and -31 were added to the vaccines, they would offer protection against about 80% of cervical cancers worldwide (Franco and Harper 2005). Any decision to add more types, however, will depend on whether Cervarix and Gardasil provide any cross-protection against HPV types other than HPV-16 and -18; the duration of cross-protection; whether and how immunization will affect the prevalence of other oncogenic types of HPV; and the feasibility of adding more types from a manufacturing, immunogenicity, and the cost-benefit perspective (Harper 2005a).

6. Projected timeline for in-country licensing

As of late 2006, Gardasil has been registered in more than 50 countries, and registration of Cervarix will likely begin in 2007. In some countries, prelicensure "bridging" studies will be required to demonstrate safety and/or immunogenicity in local populations. For example, both Merck and GSK plan to undertake prelicensure studies in India in collaboration with the Indian Council of Medical Research (ICMR) (Sharma 2006). These studies will involve a few hundred women in several locations and are expected to take a year to complete. Assuming they produce positive results (e.g., the immune response observed in Indian women is not inferior to that recorded in Phase 3 clinical studies) the vaccines may be licensed in India by the end of 2007.

Table 1. First-generation HPV vaccines.

	Gardasil	Cervarix
Manufacturer	Merck & Co., Inc.	GlaxoSmithKline Biologicals
Vaccine	L1 VLP vaccine based on recombinant yeast technology	L1 VLP vaccine based on recombinant baculovirus technology
HPV types	6, 11, 16, and 18 Protects against cervical cancer and genital warts	16 and 18 Protects against cervical cancer
Adjuvant	Alum (aluminum salt)	AS04 (alum plus proprietary adjuvant MPL)
Administration	3 injections at months 0, 2, and 6	3 injections at months 0, 1, and 6
Target audience	Adolescent girls and boys	Adolescent girls
Cold chain required?	Yes (cannot be frozen)	Yes (cannot be frozen)
Clinical trials under way	✓ FUTURE II and other efficacy studies of about 25,000 girls and women aged 15–26 in 33 countries ✓ Adolescent immunogenicity and tolerability study of 4,800 boys and girls aged 9–15 ✓ Efficacy and tolerability study of women aged 24–45 ✓ Efficacy study of boys and men aged 16–26	✓ PATRICIA efficacy study of 18,000 girls and women aged 15–25 in 14 countries ✓ NCI efficacy study of 7,467 women aged 18–25 in Costa Rica ✓ Adolescent safety and immunogenicity study of girls as young as age 10 ✓ Efficacy study of women aged 26–55
Regulatory submission	Licensed in > 50 countries (late 2006)	None as of late 2006, but submissions filed in numerous countries

Note: MPL: 3-deacylated monophosphoryl lipid A; NCI: National Cancer Institute; VLP: virus-like particle.

Table 2. Interim results of FUTURE II clinical trial (Gardasil) and Phase 2b clinical trial and other studies (Cervarix)

	Gardasil	Cervarix
Safety	✓ No serious AEs from vaccine ✓ Most common AEs are local discomfort at injection site and headache	✓ No serious AEs from vaccine ✓ Most common AEs are local discomfort at injection site and headache
Immunogenicity	✓ 100% of women develop antibodies to all four HPV types ✓ Antibody levels remain higher than in natural HPV infections at 36 months ✓ Antibody response is stronger in girls and boys aged 10–15 than in women and girls aged 16–23	✓ 100% of women develop antibodies to HPV-16 and -18 ✓ Antibody levels remain higher than in natural HPV infections at 18 months ✓ Antibody response is stronger in girls aged 10–14 than in girls and women aged 15–25 ✓ Antibody levels rise faster, higher, and last longer when vaccine is formulated with AS04 than with alum alone
Efficacy	✓ 100% efficacy against CIN 2/3 and AIS associated with HPV-16/18 in per-protocol analysis ✓ 99% efficacy against CIN 2/3 and AIS associated with HPV-16/18 in intention-to-treat analysis ✓ 100% efficacy against genital warts, vaginal dysplasia, and vulvar dysplasia in per protocol analysis ✓ 95% efficacy against genital warts, vaginal dysplasia, and vulvar dysplasia in intention-to-treat analysis	✓ 96% efficacy against persistent infection with HPV-16/18 in per protocol analysis and 94.4% in intention-to-treat analysis ✓ 100% efficacy against CIN 1/2/3 and invasive cell carcinoma associated with HPV-16/18 in combined initial and extended follow-up analysis ✓ 94.2% efficacy against incident infection with HPV-45 and 54.5% efficacy against incident infection with HPV-31

Note: CIN: cervical intraepithelial neoplasia; AE: adverse effect.

C. Second-generation prophylactic vaccines

Although clinical trials of Gardasil and Cervarix have been extremely promising, these first-generation VLP vaccines may not be the ideal vaccine candidates, especially in low-resource settings. Researchers are actively working to develop other prophylactic HPV vaccines that may be:

- Effective against a broader range of HPV types.
- Have a long shelf life.
- Effective and long-lasting with a single dose and no boosters.
- Able to elicit a mucosal immune response.
- Manufactured, distributed, and administered in developing countries, rather than being expensive imports, which would result in the vaccines being less expensive and easier to use.
- Both prophylactic and therapeutic.
- Stable at a wide range of temperatures so that a cold chain is not required.
- Administered orally or via nasal spray rather than via injection, thereby eliminating the need for sterile needles and highly trained providers (Francheschi 2005, Jones 1999, PATH 2001).

Some researchers are working to refine VLP vaccines, others on entirely different kinds of vaccines, such as protein and peptide vaccines, recombinant live vector vaccines, plant-based vaccines, and DNA vaccines. Attention is also directed toward prime-boost strategies, which sequentially inoculate people with two different kinds of vaccines to elicit a stronger, more complete immune response. Although a great deal of research activity is currently devoted to exploring the potential of these vaccines, research to date has been mostly preclinical, and it is unclear which, if any, of these vaccine candidates will progress to clinical testing, let alone licensure.

1. Refining VLP vaccines

a. More HPV types. Gardasil and Cervarix, which are quadrivalent and bivalent, respectively, have demonstrated the technical feasibility of producing a multivalent VLP vaccine. Whereas adding more HPV types to a VLP vaccine would prevent more cases of cervical cancer, it is not clear whether the small increases in protection would justify the increased cost of production (Schiller 2005). Further research is also needed to determine whether including more types of HPV in a multivalent VLP vaccine would affect the strength and duration of the immune response to each specific type (Franco and Harper 2005). It is encouraging, however, that the response to HPV-16 VLPs did not diminish when Merck moved from a monovalent to a tetravalent vaccine.

b. Adjuvants. One of the primary differences between Gardasil and Cervarix is the adjuvant employed. Researchers are investigating other adjuvants to enhance the immune response of VLP vaccines. For example, researchers at Pochon CHA University (South Korea) have encapsidated plasmid DNA expressing interleukin-2 (IL-2) within HPV-16 L1 VLPs (Oh et al. 2004). In mice, this cytokine genetic adjuvant has significantly enhanced the mucosal and systemic immunogenicity of HPV-16 L1 VLP vaccines.

c. Mucosal delivery systems. Intranasal, aerosol, or oral immunization may be more effective at inducing mucosal immunity; they are also easier to administer, less likely to transmit infection,

more acceptable to patients, and simpler to manufacture (Holmgren et al. 2003, Nardelli-Haefliger et al. 2005). Building on extensive preclinical research on these delivery systems, researchers at the Centre Hospitalier Universitaire Vaudois (Lausanne, Switzerland) conducted a clinical trial comparing the administration of HPV-16 L1 VLPs to female subjects via a nasal spray, an aerosoal (nebulized vaccine was inhaled through a mouthpiece), or a combination of an injected priming dose followed by an aerosol boosting dose (Nardelli-Haefliger et al. 2005). Aerosol vaccination proved far more immunogenic than nasal vaccination. It produced serum antibody titers comparable to those induced by intramuscular vaccination in many volunteers and also induced a mucosal immune response. Other researchers have established proof of principle for the oral delivery of VLPs in plant or yeast extracts in mice, although responses were weak even using high doses (Gerber et al. 2001, Rose et al. 1999, Sasagawa et al. 2005).

d. Heat stabilization and slow-release formulations. The need for a cold chain and for multiple injections increases costs and reduces ease of administration for first-generation VLP vaccines. Heat stabilization of VLPs could eliminate the need for a cold chain (Brandau et al. 2003), and a slow-release formula could reduce the number of injections needed. However, both technologies are as yet unproven for HPV VLPs (Schiller 2005).

e. Chimeric VLPs. By fusing E6, E7, or both to the capsid proteins in VLPs, researchers have created chimeric VLPs that can induce cytotoxic T cells as well as neutralizing antibodies in mice (Greenstone et al. 1998, Muller et al. 1997). The resulting therapeutic effect could serve as a second line of defense in a prophylactic vaccine, enabling the body to eliminate early cellular infections that antibodies miss. Thus, a chimeric VLP could be used both prophylactically and therapeutically (Ling et al. 2000). For further discussion of chimeric VLPs, see the section on therapeutic vaccines (page 31).

2. Protein and peptide vaccines

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 then produces large quantities of the chosen protein or peptide. Researchers have succeeded in isolating the specific HPV epitopes that elicit the desired immune response, making HPV protein and peptide vaccines possible (Davies 2005). This kind of vaccine is safe, easy to make at low cost, and stable. Proteins offer some advantages over peptides: they can elicit the same immune response from different individuals and bypass some potential safety issues associated with peptides (Ling et al. 2000, Tomson et al. 2004).

Once purified, protein and peptide vaccines lack the microbial components that trigger the immune system. Therefore, they prompt weaker immune responses than whole pathogens do, and they may require multiple immunizations to produce long-lasting protective immunity (NIAID Task Force 1998). Researchers are testing various strategies to enhance the potency of protein and peptide vaccines, such as combining them with an adjuvant, fusing them with one another or with a heat-shock protein, or finding new ways to present the epitope to T cells (Davies 2005, Ling et al. 2000, Tomson et al. 2004).

Early attempts to generate L1-based protein and peptide vaccines in bacteria were unsuccessful because neutralizing antibodies to L1 recognize conformation-dependent L1 structures and L1

was purified in a denatured form. However, researchers at the Georgetown University School of Medicine in Washington, DC, have demonstrated that a fusion of L1 capsid protein and glutathione S-transferase (GST) expressed in bacteria can be recovered in its native state and used to prevent infection in a canine oral papillomavirus model (Yuan et al. 2001). Fusing GST to L1 simplifies purification and also helps maintain the protein's immunogenic properties by stabilizing the conformation. This fusion protein forms pentameric capsomeres, which are substructures of the HPV protein coat but do not assemble into VLPs. The bacterial system used to produce the fusion protein is potentially more economical and therefore better suited to developing countries than current VLP production systems. In mice, HPV-16 L1 capsomeres have induced potent immune responses similar to completely assembled VLPs and also have induced regression of established tumors (Ohlschlager et al. 2003). An important question is whether they can match the remarkably consistent induction of high-titer antibody responses in humans by VLPs.

Other researchers are focusing on L2, the minor HPV capsid protein. Animal research suggests that L2 contains cross-neutralizing epitopes that induce neutralizing antibodies against multiple types of HPV (Embers et al. 2004, Kawana et al. 2003, Pastrana et al. 2005, Varsani et al. 2003b). During in vitro assays, L2 from bovine papilloma virus proved especially effective at inducing cross-neutralizing antibodies to epitopes shared by multiple types of HPV, including 16 and 18 (Pastrana et al. 2005). Thus, L2-based vaccines potentially could eliminate the need for a multivalent vaccine; however, L2 polypeptides evoke a much weaker immune response than do L1 VLPs (Tomson et al. 2004). Developing an effective L2 vaccine may require using adjuvants or some other technique to enhance immune response (Roden 2005).

Most work on HPV protein and peptide vaccines is for therapeutic use. However, researchers at the University of Tokyo (Japan) have developed a synthetic L2 peptide vaccine that is administered nasally. An initial clinical test on 13 human volunteers found that the vaccine was safe and well tolerated. It generated antibodies to both HPV-16 and -52 in four of five recipients receiving a higher dose, but it was not immunogenic at lower doses (Kawana et al. 2003).

3. Recombinant live-vector vaccines

HPVs are not efficiently propagated in tissue culture, and they contain oncogenes, which makes it impractical to develop inactivated or attenuated live virus vaccines of the kind used as prophylactic vaccines against other viral diseases. Instead, researchers have added HPV genes to other bacteria and viruses to create recombinant live-vector vaccines. These genetically engineered vectors express an HPV antigen, such as L1, along with antigens from the host vector; the combination stimulates an immune response against both the vector and HPV. The L1 proteins that are produced self-assemble into VLPs.

Recombinant live-vector vaccines combine the advantages of subunit and live, attenuated vaccines. Because they express only selected HPV genes, they are relatively safe. Like other live, attenuated vaccines, however, they can be highly immunogenic, produce long-term protection with a single inoculation, and stimulate strong cell-mediated immunity as well as antibody-mediated immunity (Tomson et al. 2004). Recombinant live-vector vaccines also are potentially less expensive to manufacture than VLP vaccines are and, especially if administered mucosally, cheaper to dispense (Maclean et al. 2005, Schiller 2005).

Recombinant live-vector vaccines have some disadvantages (Tomson et al. 2004). Live vectors, even attenuated ones, may not be safe for immunocompromised individuals. This problem is especially important in developing countries where it may not be feasible to determine an individual's HIV status before vaccination. Also, the body's immune response to the vector may prevent it from being used more than once: in many cases, neutralizing antibodies developed after the first vaccination respond immediately to any subsequent inoculation of the same vector. Because many of the vectors under study are already used in other vaccines, they may face a widespread, preexisting immunity against the vector in the general population. Finally, the immune response to the vector may overshadow the immune response to HPV. Thus, recombinant live-vector vaccines may be best suited to a prime-boost strategy (Jabbar et al. 2000, Tobery et al. 2003).

Many different viruses and bacteria can be used as a vector in a recombinant vaccine. Some researchers are working with attenuated vectors already in use as vaccines, such as vaccinia and bacille Calmette-Guérin (BCG), because these vectors are already accepted by licensing authorities and companies already have experience producing them. Others are selecting vectors based on their immunologic properties to elicit a desired effect (Tomson et al. 2004). For example, some HPV researchers are studying vectors that naturally colonize mucosal tissues or can be administered nasally or orally—such as adenovirus, *Salmonella* spp., and *Shigella* spp.— in hopes that they may prompt a strong response from the mucosal immune system. All work so far on recombinant live-vector vaccines to prevent HPV is preclinical.

a. Bacteria. *Salmonella* bacteria can deliver antigens to both mucosal and systemic immune systems and induce cell-mediated, humoral, and secretory immunoglobulin A (IgA) antibody responses (Maclean et al. 2005). Researchers at the Centre Hospitalier Universitaire Vaudois (Lausanne, Switzerland) are conducting preclinical research on a recombinant attenuated *Salmonella* bacteria that expresses the HPV-16 L1 capsid gene and thus produces HPV-16 VLPs. Earlier experiments demonstrated that nasal immunization induced L1 antibodies in both oral and vaginal secretions (Nardelli-Haefliger et al. 1997). Subsequent improvements have yielded a strain with more stable L1-expressing plasmids that induces high titers of neutralizing antibodies after a single nasal or oral immunization (Baud et al. 2004). One of the strains, Ty21, has been given to millions of individuals worldwide to prevent typhoid fever. These results could pave the way to clinical trials.

Researchers at the University of Queensland (Australia) and the University of Cape Town (South Africa) have investigated genetically engineered BCG bacteria for use as HPV vaccines (Jabbar et al. 2000, Institute of Infectious Disease and Molecular Medicine, 2005). BCG, which is used for tuberculosis vaccines, offers two advantages: it can be produced relatively inexpensively, and the technology to do so is already available in some developing countries. However, a study of recombinant BCG vaccines expressing HPV-6 L1 or HPV-16 E7 found that they elicited relatively weak immune responses in mice; the researchers concluded that the recombinants could be useful only as part of a prime-boost strategy (Jabbar et al. 2000).

Unlike *Salmonella* and BCG, a recombinant *Shigella* vaccine could be safe for immunocompromised persons because the infection remains localized. Researchers at Xi'an Jiaotang University (China) have constructed a recombinant attenuated *Shigella* strain that expresses HPV-16 L1. Preclinical testing suggests that it could be a good candidate for an oral HPV vaccine. Mucosal immunizations in guinea pigs, administered in the eye, have elicited

specific neutralizing antibodies systemically and at mucosal sites, including the intestine and vagina (XF Yang et al. 2005).

b. Viruses. Considerable research is being undertaken on recombinant adenoviruses because a single dose delivered orally or nasally can induce both cell-mediated and humoral immune responses, providing for an effective and economical vaccine (Berg et al. 2005). However, a study comparing the immunogenicity of different types of HPV vaccines in monkeys concluded that an adenoviral recombinant might be more effective as a therapeutic than prophylactic vaccine because it produced strong cell-mediated responses but relatively weak neutralizing antibodies (Tobery et al. 2003).

Researchers at the Wistar Institute (Philadelphia, Pa.) have developed a recombinant adenovirus that induces both serum and vaginal antibodies in mice after intranasal immunization. To increase the magnitude and duration of the vaginal antibody response, they are testing prime-boost regimens in which an intramuscular injection of DNA vaccine is followed by intranasal immunization with an adenoviral recombinant (Kowalczyk et al. 2001).

Researchers at the National Defense Medical Center (Taipei, Taiwan) have produced a recombinant adeno-associated virus encoding HPV 16 L1. Tests in mice suggest that a single injection of the recombinant along with an adjuvant is as effective as a series of three injections with a VLP vaccine (Liu et al. 2005a, 2005b). The adjuvant used is an adenovirus encoding murine GM-CSF (granulocyte macrophage–colony-stimulating factor), a medication given to increase white blood cells.

BioVex Limited (Woburn, Mass., United States and Abingdon, United Kingdom) has engineered the herpes simplex virus (HSV) to serve as an antigen delivery platform and plans to use it as the basis for vaccines against a range of diseases, including HPV. Preclinical testing in mice has begun on two HPV-16 vaccines, one of which includes L1, L2, and E2 for prophylactic use (Thomas et al. 2005).

4. Plant-based vaccines

Using genetic engineering, scientists have inserted the genes for human pathogens—such as HPV, hepatitis B, and cholera—into a variety of crop plants, including lettuce, potatoes, and tobacco. These transgenic plants then produce and accumulate disease antigens in their tissues. Alternate approaches involve inserting the gene sequence for a desired antigen either into the bacterium *Agrobacterium tumefaciens* or into a virus that commonly infects plants. When adult plants are infiltrated with the bacterium or infected with the virus, they synthesize and accumulate the desired proteins.

If edible plants are used, their fruits and vegetables could theoretically serve as an edible vaccine. Proof-of-concept studies have shown that HPV VLPs administered orally to mice produce type-specific antibody responses in serum and genital mucosal secretions (Gerber et al. 2001, Rose et al. 1999), and hepatitis B surface antigen in potatoes is capable of boosting serum immunity in preimmunized human volunteers (Thanavala et al. 2005). Given the need for consistent, uniform doses, however, plant-based vaccines will more likely consist of capsules that contain purified and concentrated dry plant tissues or soluble dry powders of highly purified antigens—both of which could still be administered orally (PROVACS 2005).

Using crop plants to produce vaccines is especially appealing to developing countries, which may find it challenging to produce, buy, store, distribute, and administer traditional vaccines (Rigano and Walmsley 2005, Warzecha et al. 2003). Plants offer a simple, inexpensive way to mass produce vaccines that can eliminate the need for costly pharmaceutical manufacturing facilities and high-paid staff—and potentially allow many developing countries to grow their own supplies of vaccines rather than import them. Without the pressure to recoup large capital investments, vaccines could be priced close to the marginal cost of production and made available quickly in developing countries (PROVACS 2005). Other potential advantages of plant-based vaccines include oral administration, heat stability, and the ability to make combination vaccines (PROVACS 2005, Rigano and Walmsley 2005, Warzecha et al. 2003).

However, plant-based vaccines face a host of serious challenges, which may explain why none has yet proceeded to Phase 2 clinical trials (Kirk and Webb 2005, Rigano and Walmsley 2005). Researchers have so far focused on two key technical challenges: developing plant systems that reliably express a desired antigen and identifying adjuvants or other techniques to heighten the immunogenicity of the vaccines produced. Issues of commercial feasibility—that is, how to produce and process, both safely and cost-efficiently, a plant-based vaccine—have been neglected, even though they will determine whether the cost savings and other advantages ascribed to plant-based vaccines will be realized (PROVACS 2005). Ultimately, the development of plant-based vaccines will require a multidisciplinary effort, involving the expertise and active participation of agricultural biotechnology companies as well as pharmaceutical companies (Kirk and Webb 2005).

Work on plant-based HPV vaccines remains at the preclinical stage. However, at least five groups of academic researchers have developed transgenic potato and tobacco plants that express the L1 major capsid protein from human or canine papillomavirus (Biemelt et al. 2003, Caldwell 2004, HL Liu et al. 2005b, Varsani et al. 2003a, Warzecha et al. 2003). The protein produced by the plants correctly self-assembles into VLPs that are essentially identical to those produced by yeast and baculovirus systems. Feeding the potato tubers to mice or injecting rabbits with VLPs derived from tobacco plants has elicited a weak immune response (Biemelt et al. 2003, Varsani et al. 2003a, Warzecha et al. 2003). Researchers at the University of Cape Town have overcome one major challenge to commercial production of plant-based HPV vaccines by increasing the concentration of protein produced in plant tissues. They have obtained yields of up to 1 g of L1 protein per kilogram of tobacco leaves (Rybicki et al. 2005).

Two groups have constructed HPV vaccines using tobacco mosaic virus (TMV). This RNA virus can express genes of interest in the cytoplasm of plant cells without entering the plant's nucleus, plastids, pollen, or seeds—thus avoiding widespread concerns regarding genetically modified plants (LSBC 2005). A TMV vector expressing HPV-16 LI constructed by researchers at the University of Cape Town has produced HPV capsomeres and VLPs in infected tobacco plants and elicited a weak immune response in rabbits immunized with a concentrated plant extract (Varsani et al. 2006). The Large Scale Biology Corporation (Vacaville, CA) is developing a series of TMV-based vaccines. Findings from an initial proof-of-concept study of a TMV vector expressing rabbit papillomavirus L2 suggests it may have both prophylactic and therapeutic benefits (Palmer et al. 2005).

5. DNA vaccines

"Naked" DNA is among the newest approaches to vaccine development. Using recombinant DNA technology, HPV genes are added to small, circular DNA structures called *plasmids* that are found in bacteria. After the plasmids are mass produced in bacteria, they are purified and then injected into vaccine recipients—intramuscularly in saline solutions, encapsulated in biodegradable polymeric microparticles, or by propelling DNA-coated gold beads into skin cells with a "gene gun" (Leitner et al. 2000, Ling et al. 2000, Maclean et al. 2005). Human cells take up the plasmid DNA and then produce the selected HPV antigen.

DNA vaccines have some advantages over other kinds of vaccines. They induce both cell-mediated and antibody-mediated immunity, and they can also induce long-lasting immunity given that the host cells can sustain antibody production for a sustained period (Davies 2005, Ling et al. 2000). However, physical limitations on how much DNA can be injected may limit the immune response to these vaccines.

DNA vaccines also may be cheaper and easier to produce and distribute than other vaccines (Davies 2005, Maclean et al. 2005). Bacteria can generate large numbers of genetically engineered plasmids rapidly, reliably, and relatively inexpensively. DNA vaccines are stable at ordinary room temperatures, eliminating the need for a cold chain. They also have a long shelf life and can be stored dry or in an aqueous solution.

Whereas some experts stress that DNA vaccines are safer than live recombinant vaccines, others have raised concerns that the injected DNA might become integrated into the host genome, potentially inactivating tumor suppressor genes or activating oncogenes (Davies 2005, Ling et al., 2000, Peng et al. 2005a). Vaccine developers have responded with strategies to minimize this possibility, for example, by injecting the plasmid DNA into skin cells with a short lifespan rather than muscle cells or, in the case of therapeutic vaccines, mutating E7 to eliminate the potential for oncogenic transformation while preserving critical epitopes (Ling et al. 2000).

Limited potency has posed a problem in preclinical research on DNA vaccines (Maclean et al. 2005). In response, some researchers are investigating the use of adjuvants, including genetic adjuvants that deliver DNA encoding immunostimulatory sequences along with antigen sequences. For example, South Korean researchers have improved the immunogenicity of an HPV L1 DNA vaccine by fusing it to a chemokine and secretory signal peptide (Kim et al. 2003). Others are investigating the use of DNA vaccines in combination with peptide or recombinant live vector vaccines as part of prime-boost strategies (Kowalczyk et al. 2001). However, a study comparing various HPV-16 L1 vaccines in monkeys concluded that DNA vaccines may be better suited for therapeutic than prophylactic use because they induced potent cell-mediated responses but weak neutralizing antibodies (Tobery et al. 2003).

III. Therapeutic vaccine research

A. The promise of immunotherapy

Therapeutic vaccines offer a potentially less invasive alternative to current options for treating precancerous lesions. Current treatments for HPV-associated disease rely primarily on ablation or excision, which do not eradicate the underlying HPV infection. When the disease is not localized (that is, it exists regionally), these therapies may cause significant morbidity and recurrence rates are high. Ablative therapies are more effective when the disease and infection are local, as is the case in cervical intraepithelial neoplasia, but the possibility of recurrence remains (Chu 2003, Stanley 2003). Conventional medical excisional treatments such as loop electrosurgical excision procedure (LEEP) and cone biopsy procedures also may affect a woman's fertility and carry a risk of serious complications (Brinkman et al. 2005). Immunotherapy offers an attractive alternative treatment strategy because it can address the underlying HPV infection as well as visible lesions, target all HPV-associated lesions regardless of location, and induce long-lasting immunity, thus preventing recurrence (Chu 2003, Stanley 2003).

Low-grade cervical disease is homogeneous, and the lesions are genetically stable. This would make it possible for an effective therapeutic vaccine to consistently clear lesions and prevent the recurrence of disease (Stanley 2003). High-grade cervical disease and invasive cancer pose much greater challenges for therapeutic vaccines: high-grade disease is heterogeneous, lesions are genetically unstable, and HPV gene expression varies between patients and even within an individual patient. This makes it likely that individual responses to a therapeutic vaccine will vary widely, ranging from no response to partial or full clearance of disease (Stanley 2003). Therefore immunotherapies for HSIL and malignant disease will probably be used as adjuncts to conventional treatments.

It is difficult to judge whether therapeutic HPV vaccine candidates have had a real effect on disease because most trials have not been placebo-controlled. To date, clinical trials of therapeutic HPV vaccines have shown little or no correlation between immune responses to the vaccines and clinical outcomes (Brinkman et al. 2005, Chu 2003). The vaccines also have shown, at best, limited efficacy in eradicating established tumors, although the fact that they have mostly been tested in advanced stage cancer patients with compromised immune systems may have limited their impact (Brinkman et al. 2005). See Table 3 (page 38) for a summary of clinical research on therapeutic HPV vaccines.

B. Vaccine strategies

Prophylactic vaccines, including first-generation VLP vaccines, protect people from infection by generating neutralizing antibodies. Therapeutic vaccines must employ different strategies because cellular immunity, particularly antigen-specific T-cell mediated immunity, is thought to be required for clearance of established HPV infection and lesions (Chu 2003, Ling et al. 2000, Maclean et al. 2005).

Therapeutic vaccines also must target different HPV proteins than prophylactic vaccines. Once HPV is integrated into human host cells, the capsid proteins L1 and L2 are no longer present. Instead therapeutic vaccine candidates target proteins that are expressed during later stages of

disease, generally E6 and E7. These two oncoproteins bind the tumor suppressor genes *p53* and *pRB*, are expressed throughout the viral life cycle, are involved in the malignant transformation of HPV-infected cells, and are required for continued tumor growth (Davies 2005, Tomson et al. 2004).

Fewer researchers have investigated the replication proteins E1 and E2, which are necessary for HPV to replicate within the epithelial cells before the virus is integrated into the host DNA. Because they are expressed earlier in the progress of an HPV infection than E6 and E7, however, they may be the best targets for therapeutic vaccines designed to treat low-grade disease associated with HPV, such as genital warts and low-grade dysplasias (Stanley 2003).

C. Therapeutic vaccine candidates

1. Chimeric VLPs

Researchers have constructed chimeric VLPs that fuse all or part of HPV-16 E7 with L1 in an effort to create a vaccine that offers both prophylactic and therapeutic benefits (Davies 2005). Preclinical work on chimeric VLPs conducted in several countries has found that these chimeric VLPs have good immunogenicity, inducing E7-specific cytotoxic T cells as well as neutralizing antibodies (Greenstone et al. 1998, Johns Hopkins 2004b, Wakabayashi et al. 2002). They have protected mice from tumor challenge and led to the regression of existing tumors (Jochmus et al. 1999, Liu et al. 1998, Schafer et al. 1999).

MediGene AG (Martinsreid, Germany, and San Diego) has conducted a double-blind placebo-controlled trial of a chimeric vaccine in patients with CIN 2/3; the vaccine consisted of HPV-16 VLPs combining L1 and E7 (WHO, 2005b). Whereas the chimera induced consistently high levels of L1 antibodies, T-cell responses to E7 were weak and variable. Lesion size decreased by 50%, but there was no correlation between clinical responses and assays of cell-mediated immunity. The relatively weak T-cell responses may suggest a failure to effectively boost primary responses and preexisting immunity to L1 or E7 in patients.

2. Protein and peptide vaccines

More protein and peptide vaccines have entered clinical trials than any other kind of therapeutic HPV vaccine. One vaccine candidate produced by Nventa Biopharmaceuticals (formerly StressGen Biotechnologies, Victoria, Canada)—HspE7 or SGN-00101—has entered Phase 3 clinical trials. It is a fusion of a BCG heat-shock protein (Hsp 65) and HPV-16 E7 (Nventa Biopharmaceuticals 2006). Like other stress proteins, the heat-shock protein heightens the immune response by directing antigens to specialized antigen-presenting cells and by activating cytotoxic T cells. Phase 1, 2, and 3 clinical trials involving nearly 400 subjects have established the safety of HspE7 and tested its efficacy against genital warts, cervical dysplasia, cervical cancer, anal dysplasia, and RRP. Even though the vaccine consists of HPV-16 proteins, it has proven effective against illnesses related to HPV-6 and 11, demonstrating its potential to serve as a broad spectrum therapeutic vaccine against diseases caused by a variety of HPV types. Nventa is currently conducting Phase 3 clinical trials in special populations and against rare diseases, including RRP in children and anal dysplasia, because orphan drug and fast track status offers the shortest path to market in the United States. At the same time, clinical trials testing the efficacy of HspE7 against cervical dysplasia and cervical cancer are proceeding under the auspices of the NCI and various medical institutions in the United States (NCI/PDQ 11/4/04,

3/3/05, 9/30/05, 10/6/05). One such study found that lesions regressed or cleared in 69% of 21 women with CIN3 who received the vaccine and that the vaccine seemed to be effective against other types of HPV than 16 (Einstein et al. 2005).

After merging with Cantab Pharmaceuticals, the Xenova Group (Slough, United Kingdom) has continued to develop Cantab's fusion protein vaccine to treat cervical dysplasia. The vaccine, named TA-CIN, is a genetically engineered fusion of L2, E6, and E7 proteins from HPV-16 that is designed to generate a strong cellular immune response against HPV-infected cells. It is targeted to patients with cervical dysplasia to prevent the onset of invasive cervical cancer. The vaccine's safety and immunogenicity have been established in a Phase 1 trial (de Jong et al. 2002). Phase 2 trials have focused on using TA-CIN as part of a prime-boost strategy with TA-HPV because preclinical studies suggested this was a more effective regimen (van der Burg et al. 2001). One prime-boost clinical trial in the UK administered TA-CIN as a primer in patients with high-grade anogenital intraepithelial neoplasia (AGIN) (Smyth et al. 2004) while another trial administered it as a booster in patients with vaginal intraepithelial neoplasia (VIN) (Davidson et al. 2004). Although both prime-boost regimens are immunogenic, their clinical benefits have not been clear or consistent. In January 2005, Cancer Research Technology Limited (CRT) licensed TA-CIN from Xenova. CRT plans to find a partner to commercialize the vaccine and will facilitate further Phase 2 trials in England examining a combination treatment of TA-CIN and an immune modulator (Xenova 2005).

CSL Limited (Parkville, Australia) and its research partner, the University of Queensland Diamantina Institute for Cancer, Immunology and Metabolic Medicine (Woolloongabba, Australia), have conducted an initial safety and immunogenicity trial on a fusion protein vaccine. The CerVax vaccine consists of a fusion of HPV-16 E6 and E7 proteins along with ISCOMATRIX, a saponin-based adjuvant that induces humoral and cellular immune responses (CSL 2005). In a Phase 1 study of 31 patients with CIN (mostly CIN3), the vaccine was well tolerated, induced HPV-16 E6 and E7 specific immunity, and reduced the viral load in some subjects (Frazer et al. 2004).

Academic institutions around the world, including the National Cancer Institute (Bethesda, MD), the Norris Cancer Center at the University of Southern California, the Université Libre de Bruxelles (Belgium), the Leiden University Medical Center (Netherlands), and the University of Queensland (Woolloongabba, Australia) have developed many other peptide vaccine candidates and tested them in Phase 1 and 2 trials (Hallez et al. 2004, Muderspach et al. 2000, Van Driel Ressing et al. 1999, WHO 2005b). These vaccines target E6 and/or E7, generally require multiple inoculations, and often are administered as an adjunct to conventional therapy such as radiation. Although many patients have demonstrated an immune response to the vaccines, fewer have shown clinical benefits. Similarly, two trials of a therapeutic fusion protein vaccine for genital warts (HPV-6 L2 and E7) developed by GSK found that the vaccine induced an adequate immune response but did not increase the efficacy of conventional therapies (Vandepapeliere et al. 2005). GSK is also conducting preclinical research on a therapeutic protein vaccine for cervical disease that consists of HPV-16 E7 protein and a proprietary adjuvant (WHO 2005b).

Many researchers continue to investigate fusion proteins and novel adjuvants that may enhance the efficacy of therapeutic protein and peptide vaccines (Hallez et al. 2004, Torrens et al. 2005). For example, Innogenetics (Gent, Belgium) and IDM Pharma (Irvine, Calif.), which was formerly known as Epimmune, have created a polyepitope construct (Innogenetics, 2006). The

composition of the vaccine, which includes 91 cytotoxic T-lymphocyte (CTL) epitopes and 31 helper T-lymphocyte (HTL) epitopes from E1, E2, E6, and E7 in four types of HPV (16, 18, 31, and 45), is designed to stimulate a multispecific cellular response (De Winter 2005). BT Pharma (Labège-Innopole, France) has developed a vaccine platform based on a recombinant protein, the adenylate cyclase (CyaA) of *Bordetella pertussis*, that can target and activate dendritic cells (DCs) (BT Pharma, 2004). Preclinical research on mice has found that a CyaA HPV-16 E7 vaccine is immunogenic, triggers the regression of tumors, and is comparable in efficacy to a strongly adjuvanted peptide (Preville et al. 2005).

A group of researchers at Georgetown University (Washington, DC), the German Cancer Center (Heidelberg, Germany), the Ludwig Institute for Cancer Research (São Paulo, Brazil), and the University of Colorado (Denver) has received an award from the Grand Challenges in Global Health initiative to investigate the potential of capsomeres as an inexpensive therapeutic vaccine for HPV. Compared with VLPs, capsomeres are less costly to manufacture because they are expressed by *Escherichia coli bacteria*; they are also more stable because they can be freeze-dried into a powder. Preclinical testing of chimeric L1-E7 capsomeres in mice has demonstrated their immunogenicity and suitability for use as a combined prophylactic-therapeutic vaccine (WHO 2005b). Plans are under way to select, produce, and test a chimeric capsomere vaccine candidate in a Phase 1 clinical trial.

3. Recombinant live-vector vaccines

Researchers around the world are trying to devise strategies to enhance the therapeutic potency of recombinant live-vector HPV vaccines. In the meantime, three candidates are in or about to enter clinical trials.

Xenova Group (Slough, United Kingdom) is the farthest advanced in developing a recombinant live-vector vaccine for HPV. Its therapeutic vaccine candidate, TA-HPV, is a recombinant vaccinia virus that expresses E6 and E7 from HPV-16 and HPV-18. The vaccinia virus can accommodate large recombinant gene insertions, does not persist in the host, and is highly efficient in infection and in expressing recombinant genes—but older people may have preexisting antibodies to vaccinia that limit the immune response (Davies 2005). TA-HPV is designed as an adjunct therapy for cervical cancer. Its objective is to eliminate residual tumor cells that can lead to the recurrence of disease after surgery or radiotherapy. Clinical trials have tested TA-HPV alone and in combination with a peptide vaccine (TA-CIN) as part of a prime-boost strategy. Phase 1 and 2 trials comprising women with cervical cancer have established the safety and immunogenicity of the vaccine (Borysiewicz et al. 1996, Kaufman et al. 2002). Two additional Phase 2 trials have examined the vaccine's efficacy in women with VIN. After two vaccinations, 5 of 12 women in one trial (Baldwin et al. 2003) and 8 of 18 in the other (Davidson et al. 2004) experienced at least a 50% reduction in lesion diameter. The European Organization for Research and Treatment of Cancer is conducting a multicenter Phase 2 trial of TA-HPV in combination with surgery to treat women with early cervical cancer (NCI/PDQ 12/29/05). Two clinical trials of prime-boost strategies involving TA-HPV (one using it as the primer and the other using it as the booster) have found that the regimens induce both humoral and cellular immunity, but their impact on clinical outcomes is less clear (Davidson et al. 2004, Smyth et al. 2004).

Transgene (Strasbourg, France) is developing a series of vaccines based on the modified vaccinia Ankara (MVA) virus, a highly attenuated poxvirus that has been extensively tested as a smallpox vaccine and that stimulates a strong immune response. Transgene's therapeutic HPV vaccine candidate, MVA-HPV-IL2, expresses HPV-16 E6 and E7 and the cytokine interleukin 2 (IL-2) (Liu et al. 2004). IL-2 functions as an adjuvant, helping to stimulate specific T-cell responses and nonspecific cellular responses. A Phase 2 trial involving 27 women with CIN2/3 found that, after three doses of MVA-HPV-IL2 and five weeks of follow-up, partial clinical or histologic responses were seen in 5 of 15 patients treated with a high dose; the low dose showed no impact (Transgene press release 3/18/04). Another Phase 2 trial that administered a low dose of the vaccine to 20 patients with VIN3 also found no response. Based on these results, Transgene has launched a Phase 2 trial in women diagnosed with CIN2/3 that will administer higher doses of the vaccine and will follow up on patients for 6 months to allow them more time to mount an immune response (Transgene press releases 11/30/04 and 2/23/05). Phase 1 and 2 trials are also planned to test aerosol delivery of the vaccine because mucosal vaccination may induce more efficient local cellular immune responses in the genital tract. Studies of mice and macaques have already established the safety and immunogenicity of this mode of delivery (WHO 2005b).

Researchers at the Instituto Mexicano del Seguro Social in Mexico City have tested an MVA E2 recombinant virus vaccine in a Phase 1/2 clinical trial involving 78 women with CIN1/2/3; 36 of the women were assigned to vaccine therapy and 42 to cryosurgery (Corona Gutierrez et al. 2004). The vaccine was administered locally, injected directly into the uterus, once a week for six weeks. All the women who were vaccinated developed E2-specific antibodies and had a specific cytotoxic response against HPV-transformed cells. Precancerous lesions were completely eliminated in 34 women who received the vaccine and greatly reduced in the two others. In addition, the vaccine eliminated all evidence of HPV infection in half the women and reduced the viral load in the remaining women to about 10% of original levels. No apparent side effects were noted.

Advaxis (North Brunswick, N.J.) is developing a series of cancer vaccines based on an attenuated *Listeria monocytogenes* bacteria, a common environmental pathogen (Advaxis 2006b). The advantages of *Listeria* as a vector are its ability to elicit both cytotoxic and helper T-cell responses and to deliver antigens to both MHC-I and MHC-II pathways (Davies 2005). Preclinical development of the vaccine at the University of Pennsylvania found that recombinant *Listeria* vaccine caused regression of HPV-positive tumors in mice (Sewell et al. 2004). Advaxis' vaccine candidate to treat cervical cancer, Lovaxin C, expresses HPV-16 E7. In 2006 the company received approval from regulatory authorities to begin a Phase 1/2 clinical trial of Lovaxin C in Belgrade, Jerusalem, and two sites in Mexico (Advaxis 2006b).

BioVex Limited (Woburn, Mass., United States, and Abingdon, United Kingdom) has produced a therapeutic HPV-16 vaccine, including E2, E6, and E7, based on its herpes simplex virus (HSV) antigen delivery platform. To increase the immunogenicity of the vaccine, which is currently being tested in mice, researchers have created versions that express GM-CSF and fuse the E2, E6, or E7 to sequences that can boost cellular immune responses (Thomas et al. 2005).

Academic centers in China and the United States also are conducting considerable preclinical research on viral recombinants for immunotherapy, primarily involving vaccinia. Vaccinia vaccines developed at the Chinese Academy of Preventive Medicine (Beijing) express wild or mutant HPV-16 E7 (Zhi et al. 2001), and researchers at Guangxi Medical University (Nanning,

China) have integrated L1, L2, E6, and E7 into a vaccinia virus so that it can serve both prophylactic and therapeutic purposes (Huang et al. 2005). Researchers at the Chinese Academy of Medical Sciences (Beijing) and Peking Union Medical College (Beijing) have developed an HPV-58 E7 recombinant vaccinia virus that has inhibited tumor growth in mice (Luo et al. 2003). Their effort to develop a vaccine for HPV-58 is unique to China because this type of HPV is uncommon elsewhere.

In the United States, researchers at the Johns Hopkins School of Medicine (Baltimore, Md.) have tested various targeting strategies to increase the effectiveness of recombinant vaccinia vaccines. Linking calreticulin (CRT) to HPV-16 E7 has proved most effective (Hsieh et al. 2004). Researchers at the Wistar Institute (Philadelphia, Pa.) have concluded that a recombinant adenovirus expressing E7 was more effective than vaccinia recombinants or other adenoviral recombinants (He et al. 2000). Both the Hopkins and Wistar researchers also are testing prime-boost strategies that use recombinant vaccines in combination with DNA vaccines.

4. DNA vaccines

Researchers are working to overcome problems with the potency of therapeutic DNA vaccines by deploying them as part of prime-boost strategies or by combining them with adjuvants that reduce the amount of DNA needed to obtain an immune response (Maclean et al. 2005). New and varied delivery systems are also increasing the effectiveness and feasibility of DNA vaccines (Tomson et al. 2004). So far, only one therapeutic DNA HPV vaccine has entered clinical trials.

MGI Pharma (Bloomington, Minn.), formerly known as Zycos, has created a microparticle system that encapsulates DNA in a synthetic polymer for administration as an intramuscular injection and permits 30 to 60 days of active gene expression. Its therapeutic HPV vaccine candidate, ZYC101a, contains plasmid DNA that encodes fragments from the E6 and E7 proteins of HPV-16 and -18. Two Phase 1 trials in women with cervical or anal dysplasia have established the safety and immunogenicity of the vaccine. ZYC101a is notable because it has been the subject of one of the few double-blind, randomized, placebo-controlled studies of a therapeutic HPV vaccine. Results from this Phase 2 trial comprising 161 women with CIN 2/3 and from an open-label trial of 18 women with CIN 2/3 suggest that the vaccine can benefit women under the age of 25 (MGI Pharma 2004). In this age group, histologically confirmed resolution of CIN 2/3 took place in 70% of women receiving the vaccine in the Phase 2 trial versus 23% of control group (Garcia et al. 2004). A pivotal program is now under way to evaluate ZYC101a in young women with high-grade cervical dysplasia.

Preclinical research at Johns Hopkins University has developed a variety of strategies that can enhance the potency of DNA vaccines. These include (Kim et al. 2004, Peng et al. 2005a and 2005b Wu 2005):

- Four intracellular targeting strategies that route the antigen to desired subcellular compartments within APCs by using the sorting signal of lysosome-associated membrane protein (LAMP-1) or by linking plasmid DNA to CRT, to the *Mycobacterium tuberculosis* heat-shock protein (HSP70), or to the translocation domain of *Pseudomonas aruginosea* exotoxin A.
- An intracellular spreading strategy that increases the number of DCs expressing E7 by using Herpes simplex virus type 1 VP22 proteins.
- Co-administering antiapoptotic factors to enhance survival of DCs.

These strategies work for E6 as well as E7 and have increased the therapeutic effects of candidate vaccines. The Cervical Cancer SPORE program at Johns Hopkins is planning to launch Phase 1/2 clinical trials of DNA vaccines employing some of these strategies (Johns Hopkins 2004a).

Scientists at the Wistar Institute are also engaged in preclinical research investigating ways to heighten or modulate the immune response to DNA vaccines. Recent successful efforts include linking E6 with a viral leader sequence that targets antigens toward desired processing pathways (Wlazlo et al. 2004) and fusing DNA with herpes simplex virus type 1 (HSV-1) gD protein (Lasaro et al. 2005).

PowderMed Ltd (Oxford, United Kingdom) assumed Chiron's powder injection DNA vaccine technology in 2004 and is developing a variety of DNA-based immunotherapies, including a vaccine for genital warts. The company is currently conducting preclinical studies of an optimized therapeutic plasmid that includes E2 from HPV-6 and -11 (PowderMed 2005). Their proprietary delivery system precipitates DNA plasmids onto microscopic gold particles, which are then propelled by pressurized helium gas at near supersonic speeds into the epidermis via a "gene gun." The powdered vaccine is stable at ambient temperature, simplifying storage and transportation, and the system requires 1,000-fold less DNA than injections.

Some researchers are experimenting with encapsulating plasmid DNA in papillomavirus capsids to form HPV pseudovirions. Pseudovirions, which do not replicate, may provide a safer and better way to deliver therapeutic DNA vaccines. The capsid may protect the DNA from nuclease activity or act as an adjuvant. Because pseudovirions also contain VLPs, they may function as a combination prophylactic and therapeutic vaccine (Ling et al. 2000). The recent development of a simple strategy for producing a high titer of pseudovirus stocks in cultured cells makes this approach amenable to large-scale production (Buck et al. 2004).

5. RNA replicon vaccines

RNA replicons are self-replicating genetic vaccines designed to overcome some of the immunogenicity and safety concerns associated with naked DNA vaccines. They are based on RNA viruses, which have the ability to replicate prolifically in the cytoplasm of the host cells they infect (Leitner et al. 2000). Replicon vaccines replace the genes for the virus' own structural proteins with genes for the desired antigen. Although RNA replicons express HPV antigens in large amounts for an extended period, they cannot reproduce themselves, and ultimately they cause the destruction of transfected cells, thereby reducing concerns that HPV DNA will be integrated into the host genome (Ling et al. 2000). They are as stable and as easily prepared as conventional naked DNA vaccines.

Wyeth Pharmaceuticals (Collegeville, Penn.) has created therapeutic replicons from an attenuated Venezuelan equine encephalitis (VEE) alphavirus vector. VEE offers many advantages: It is safe; expresses high levels of antigens; naturally targets the antigens to the antigen-processing cells of the immune system; induces strong antibody-mediated, cell-mediated, and mucosal immune responses; and continues to be effective when used for multiple inoculations. Preclinical research on VEE virus replicon particles that deliver HPV-16 E6 or E7 found that the vaccine was immunogenic, offered protection from a tumor challenge, and

eliminated established tumors in a mouse model (Cassetti et al. 2004, Velders et al. 2001). More recently, Wyeth has generated and begun testing virus replicon particles that express a fusion E6-E7 protein from HPV-18, -45, -31, -33, -52, and -58 (Pullen et al. 2005).

Researchers at Johns Hopkins University (Baltimore, Maryland) and Chang Gung Memorial Hospital (Taipei, Taiwan) have conducted preclinical research on therapeutic HPV vaccines that employ Semliki Forest virus replicons (Hsu et al. 2001) and Sindbis virus replicons (Lin et al. 2003). To enhance the potency of their replicon vaccines, both groups fused E7 with a heat-shock protein, and one group also tested the replicons as part of a prime-boost strategy with a recombinant vaccinia vector.

6. Dendritic-cell vaccines

DC vaccines offer another way to enhance T-cell-mediated immunity against tumors; unlike the other approaches discussed herein, however, they are patient specific (Tomson et al. 2004). To make a DC vaccine, physicians harvest DCs from the patient, load them with antigens such as E6 or E7, and then inject these pulsed DCs back into the patient, where they can make and display relevant tumor antigens (Davies 2005, Figdor et al. 2004). In addition to pulsing DCs with peptides derived from E6 and E7, researchers have successfully transduced genes coding for E6 and E7 into DCs (Ling et al. 2000). Although researchers have begun testing DC vaccines on patients with cervical cancer (Chu 2003, Ferrara et al. 2003), this report does not detail these efforts because these vaccines must be individually produced for each patient and thus are highly labor-intensive and too expensive to make a substantial public health impact in most settings.

Table 3. Clinical research on therapeutic HPV vaccines*

Organization (vaccine)	Target	Type	Current Status
Medigene	HPV-16 L1, E7	Chimeric VLP	One clinical trial of patients with CIN 2/3 conducted
Nventa Biopharmaceuticals (HspE7, also known as SGN-00101)	HPV-16 E7	Fusion protein	Phase 3 trials under way against recurrent respiratory papillomatosis, anal dysplasia, and cervical disease
Xenova (TA-CIN)	HPV-16/18 L2, E6, E7	Fusion protein	Phase 2 trials of prime-boost strategy conducted
CSL Limited (CerVax)	HPV-16 E6, E7	Fusion protein	Phase 1 trial has established safety, immunogenicity, and reduction in viral load
Université Libre de Bruxelles	HPV-16 E7	Fusion protein	Phase 1/2 trial of women with CIN 1 and 3 conducted
GlaxoSmithKline	HPV-6 L2, E7	Fusion protein	Two clinical trials of patients with anogenital warts conducted
National Cancer Institute	HPV-16 E6, E7	Peptide	Phase 1 trials of various peptide vaccines among patients with advanced cervical cancer
Norris Cancer Center	HPV-16 E7	Peptide	Phase 1 trial of women with high-grade CIN and VIN conducted
University of Leiden	HPV-16 E7	Peptide	Phase 1/2 dose-escalation study of women with cervical carcinoma conducted
Innogenetics and IDM Pharma	HPV-16/18/31/45 E1, E2, E6, E7	Polyepitope construct	Phase 1 trial
Xenova (TA-HPV)	HPV-16/18 E6, E7	Recombinant vaccinia virus	Phase 2 trial of women with early cervical cancer is recruiting patients Previous Phase 2 trials conducted in patients with VIN and with prime-boost strategy
Transgene (MVA-HPV-IL2)	HPV-16 E6, E7	Recombinant MVA virus	Phase 2 trial of higher doses under way; prior trials found highly dose-dependent clinical response
Instituto Mexicano del Seguro Social	HPV-16 E2	Recombinant MVA virus	Phase 1/2 trial has established safety, immunogenicity, reduction of viral load, and regression of lesions
Advaxis (Lovaxin C)	HPV-16 E7	Recombinant *Listeria* bacteria	Phase 1/2 trial to begin in 2006
MGI Pharma (ZYC101a)	HPV-16/18 E6, E7	DNA vaccine	Phase 2 trials suggest clinical benefits for young women.
Johns Hopkins University	HPV-16 E7	DNA vaccine	Phase 1/2 trial currently recruiting women with CIN 2 or 3.

Note: CIN: cervical intraepithelial neoplasia; MVA: modified vaccinia Ankara; VIN: vaginal intraepithelial neoplasia; VLP: virus-like particle.

*Whereas efforts were made to be comprehensive, this list is not complete and may not be up to date.

IV. Programmatic issues for the developing world

A. Suitability of HPV vaccines for developing countries

In developing HPV vaccines, researchers' primary objectives are safety and efficacy, that is, creating a vaccine that prevents disease, without serious adverse effects, under the conditions provided by carefully controlled clinical trials. However, safety and efficacy alone do not make a vaccine appropriate for nationwide use by the public sector in developing countries. The vaccine's affordability, ease of administration, acceptability, and effectiveness under real-world conditions also are important (Franceschi 2005).

The effectiveness of any HPV vaccine depends in part on the local burden of disease and epidemiology of HPV infection, including the distribution of HPV types in cervical cancer cases (Clifford et al. 2003b). Based on this information, national health authorities can calculate the potential impact of a vaccine candidate on the incidence of cervical cancer in their country (Lowndes and Gill 2005, Pagliusi and Aguado 2004). Authorities also should consider whether local conditions are likely to affect a vaccine's immunogenicity or safety and, if so, conduct regional bridging studies to supplement the clinical trials conducted by vaccine manufacturers. For example, in areas with endemic malaria, hepatitis B, or HIV, bridging studies may be needed to assess whether and how these infections affect the action of an HPV vaccine (Pagliusi and Aguado 2004).

Vaccine effectiveness also depends on the ability of the health infrastructure to deliver a proposed vaccine (Franceschi 2005, Trimble 2005). In the case of Gardasil and Cervarix, for example, this could mean developing a system to reach adolescents with a series of three injections, expanding cold chain capacity (Kane et al. 2006), or both. Even more critical may be the cost of an HPV vaccine: many have expressed concern that health systems in developing countries will not be able to afford to buy imported VLP vaccines (Rashid 2005, Trimble 2005). Experience with introducing other new vaccines in developing countries, such as the vaccines for hepatitis B and *Haemophilus influenzae* type b, shows that outside financial and technical assistance is frequently needed to integrate them into routine vaccination programs (WHO and UNICEF 2005).

In countries already paying for comprehensive screening with Pap smears and treatment for cervical dysplasia, introducing an HPV vaccine has the potential to lower health care costs by reducing the number of abnormal findings that require follow-up and treatment (Lowndes and Gill 2005, Trimble 2005), which would also reduce the emotional and physical burden borne by women. However, because cervical cancer rates are already low in these settings, the additional impact of a vaccine on disease will be limited—and will depend on ensuring that poor and marginalized populations, who are most likely to miss screening and suffer from invasive cervical cancer, are vaccinated (Cohen 2005). Cost-effectiveness modeling exercises in US settings have generally concluded that adding vaccination at 12 years of age to routine screening could reduce cervical cancer rates by about two thirds but would increase overall costs (Goldie et al. 2004, Sanders and Taira 2003, Taira et al. 2004). Deferring screening to a later age or reducing its frequency would considerably increase the cost-effectiveness of a combined vaccination and mass screening strategy (Goldie et al. 2004, Kulasingam and Myers 2003).

The situation is far different in developing countries, where comprehensive screening programs do not currently exist, cervical cancer rates are high, and resources are usually limited. Nationwide vaccination would very likely reduce cervical cancer rates in the long run, but the question is at what cost. In these settings, decision-makers may well consider HPV vaccination in place of, rather than in addition to, conventional screening with Pap smears, or they may consider vaccination and new, simpler approaches to screening based on visual inspection with acetic acid or Lugol's iodine (VIA and VILI, respectively), or HPV DNA tests (Schiffman and Castle 2005).

Researchers have begun to apply cost-effectiveness models developed in the United States to low-resource settings, although the lack of adequate epidemiologic and cost data has hampered these exercises (Goldhaber-Fiebert et al. 2005, WHO 2005a). Models of the Indian setting have found that differences in the incidence of cervical cancer within the country affect which strategy (such as vaccination alone versus vaccination combined with one-time visual or HPV DNA screening) is best. However, preliminary results suggest that a combination of vaccination and limited screening may be cost-effective (WHO 2005a). In contrast, another modeling exercise comparing high- and low-resource settings has concluded that VIA screening is the most effective and least costly way to extend life in low-resource settings; vaccination becomes cost-effective only if the vaccine costs US$2 or less (Kulansingam and Myers 2005). This may be a realistic goal, considering that UNICEF buys the VLP-based vaccine against hepatitis B for approximately US$0.40 per dose.

B. Challenges for introducing a prophylactic HPV vaccine

1. Acceptability of STI vaccines

Public acceptance is essential to the successful introduction of any vaccine. Parental support is particularly important to the success of a HPV vaccine for two reasons. First, it is targeted toward preadolescent or adolescent girls (and possibly boys), which constitute extremely sensitive population segments in many ethnic and cultural communities. Second, parents generally control their children's access to health care and guide their health care decisions (Rosenthal 2005, Zimet 2005). Participation rates in HPV vaccine trials in both developed and developing countries have been high (WHO 2005a). Given the stigma associated with STIs, however, parents may question whether a vaccination against a STI is appropriate for pre-adolescents and young adolescents (Pagliusi and Aguado 2004). Indeed, conservative groups in the United States have expressed concern that offering young girls HPV vaccines may promote sexual promiscuity, and similar worries have been voiced in some developing countries (MacKenzie 2005, Stein 2005). Conversely, the public may welcome a HPV vaccine in countries where awareness of STIs is high and stigma has been reduced as, for example, in South Africa (WHO 2005a). In some parts of the world, vaccinating boys as well as girls may reduce the stigma associated with the vaccine and improve its social acceptability.

To understand local concerns and motivations that will affect the acceptability of HPV vaccination in any given country, qualitative research is essential (Agurto et al. 2005, Sherris et al. 2006). Only with this information can local planners decide the best way to introduce an HPV vaccine. A series of studies in the United States has begun to explore these issues and have found that most parents and adolescents favor vaccines against STIs, including HPV (Boehner et al. 2003, Davis et al. 2004, Hoover et al. 2000, Mays et al. 2004, Olshen et al. 2005, Zimet et al.

2000a, 2000b, and 2005). Positive attitudes toward vaccines in general, physicians' recommendations, high vaccine efficacy, and low cost all have helped to make the vaccines more acceptable. Obstacles to acceptance have included limited knowledge about HPV, resistance to vaccinating preadolescents, and concerns that a vaccine would promote unsafe sex. A survey assessing whether mothers in Mexico were willing to let their daughters participate in HPV vaccine trials found similar results (Lazcano-Ponce et al. 2001). Most mothers (84%) were willing to allow their daughters to participate, and their belief in the usefulness of vaccines in general was the major factor associated with their acceptance of an HPV vaccine. In the United States, Mexico, and other countries, knowledge about HPV and cervical cancer was extremely limited (Sherris et al. 2006).

In June 2006, PATH began its cervical cancer vaccine project, designed to assess various HPV vaccination strategies in the developing world. Operational research is being conducted in four countries: India, Peru, Uganda, and Vietnam. PATH is working closely with local organizations and ministries in each country to explore how the vaccine can best be introduced, especially how it can be delivered to older children and young adolescents—groups not usually reached by health services (except in crisis situations). Cost and sociocultural considerations also are being evaluated. The results will be analyzed and disseminated to help guide HPV vaccine policies in other countries. The project is being conducted in close collaboration with other global partners, such as WHO and the GAVI Alliance, and will provide data for future international decision-making as well (PATH 2006, RHO 2006).

2. Positioning and marketing an HPV vaccine

Given the sensitive social issues that may arise in marketing an STI vaccine to young adolescents, combined with the complex natural history explanation necessary to describe how HPV leads to cancer, it may be more productive to position a HPV vaccine as an anticancer or women's health intervention. Such a marketing strategy might minimize social criticism while garnering important support from cancer organizations and women's health groups (PATH 2001). This is the strategy likely to be adopted by GSK, which is positioning Cervarix as a cervical cancer prevention agent and is proposing to vaccinate females only. In contrast, Gardasil prevents genital warts as well as cancer and may be marketed to both boys and girls in some countries, thus making it difficult to avoid labeling Merck's candidate as an STI vaccine (Zimet 2005). Depending on local concerns, one vaccine may be easier to market than another vaccine in a given country. It is also possible that differences in the vaccines' formulations and marketing strategies will create confusion and misunderstanding (WHO 2005a).

3. Providers' attitudes

In many developed countries, it is not clear which providers will be responsible for delivering a HPV vaccine. Whereas obstetrician/gynecologists have training and experience in treating HPV disease, they are not likely to see pre-adolescents and young adolescents (Sherris et al. 2006). Primary care providers, however, have little knowledge of HPV and cervical disease. Studies in the United States have explored the attitudes of nurse practitioners (Mays and Zimet 2004), family physicians (Riedesel et al. 2005), pediatricians (Kahn et al. 2005, Liddon 2005), and obstetrician/gynecologists (Raley et al. 2004) toward HPV and other STI vaccines. Most providers were willing to recommend such vaccines if they were endorsed by their professional association.

4. Delivering vaccines to adolescents

Immunizing young adolescents against HPV presents a logistical challenge because they do not routinely visit health care providers and public vaccination programs have little or no experience reaching this age group (Pagliusi and Aguado 2004, PATH 2001, Shaw 2005). An HPV vaccination program may require devising new strategies, such as in-school delivery, mass campaigns, or even a separate delivery infrastructure for adolescent health care (WHO 2005a). Given that a number of other vaccines for adolescents have been approved or are in the pipeline, some physicians in the US have suggested creating a new standard adolescent check-up centered on these vaccines (Shaw 2005, Zimet 2005). International health experts also point to the possibility of linking an HPV vaccine with other health interventions that benefit adolescents, such as booster doses of tetanus vaccine, antihelminthics, bednets, or anti-tobacco education (WHO 2005a).

Effectively promoting HPV and other new vaccines also will entail changing the popular perception that vaccines are for infants and teaching people that vaccines are a lifelong intervention (Baltimore and Jenson 2005). The Global Immunization Vision and Strategy (GIVS) for 2006 through 2015, developed jointly by WHO and UNICEF, takes an important step in that direction by recommending that national immunization schedules be expanded beyond infancy to other age groups, including school-age children, adolescents, and adults (WHO and UNICEF 2005). The introduction of an HPV vaccine could provide a model for the new vaccination services and strategies suggested by GIVS (WHO 2006).

Some experts have suggested exploring whether the VLP vaccines would be effective with fewer doses, with longer intervals between doses or in other age groups—all of which could simplify the delivery of an HPV vaccine. For example, both theoretical reasons and clinical trial data suggest that young children may have a stronger response to the vaccine than adults. This raises the possibility of vaccinating children when they are easier to reach, for example, when they enter school or even as infants, with perhaps a single booster dose during adolescence (WHO 2005a).

5. Catch-up campaigns

Although experts agree that vaccinating young people before they become sexually active will maximize the impact of a HPV vaccine, it is less clear whether an HPV vaccination program should include a catch-up campaign for older, sexually active women who are likely to have been exposed to HPV already (Lowndes and Gill 2005). Vaccinating a broader age group, such as all women under age 35, could accelerate a vaccine's impact on cervical cancer rates; however, it could also be quite expensive, depending on how many additional women are vaccinated. An expert consultation convened by WHO has recommended studying whether a HPV vaccine could benefit previously infected women by preventing re-infection or reducing persistent infections (WHO 2006c).

C. Advocacy and education

Lack of understanding and political will poses the major barrier to effective cervical cancer prevention activities of all kinds, including HPV vaccination (Sherris et al. 2005). Policymakers, health care providers, and the general public in most developing countries simply do not know enough about HPV and cervical cancer to understand the potential value of a vaccine. They do not appreciate the burden of disease associated with cervical cancer; they do not know HPV

causes cervical cancer; and they are not aware that immunization is emerging as an alternative approach to prevention. As a result, they are unlikely to support an HPV vaccine without systematic advocacy and education (Sherris et al. 2006).

At the international and national levels, advocates must raise awareness and build support for HPV vaccination among government officials, NGOs, international organizations that provide funding or guidance for health care, medical professional associations, and medical schools (Batson et al. 2006). The best way to convince these stakeholders of the value of HPV vaccination is to show them hard evidence, that is, to collect and disseminate readily understood and country-specific information on the impact and cost-effectiveness of HPV vaccines and other cervical cancer prevention activities (Sherris et al. 2005, Tsu and Pollack 2005).

Advocacy is equally important at the local level where services are delivered. Advocates must address the local authorities, NGOs, administrators, and providers who shape local health care services (Wittet 2001). Educational activities are especially important for health care providers, who may know little about HPV infection but have a strong influence on individual decisions to accept or reject a vaccine (Jain et al. 2005). Information about HPV, cervical cancer, and the new vaccines should be integrated into both preservice and continuing education curricula for providers at all levels.

Advocates also should engage respected members of the community, including women's groups, church leaders, teachers, traditional healers, village elders, and informal community leaders, in the development and implementation of an HPV vaccination program (Agurto et al. 2005, Sherris et al. 2006). These opinion leaders not only can sway their neighbors' attitudes and decisions regarding HPV vaccines, they also can ensure that educational activities and immunization services are persuasive, culturally appropriate, and responsive to local needs (Agurto et al. 2005, Tsu and Pollack 2005).

Community outreach and educational activities will be essential to teach parents and their children the importance of being vaccinated against HPV and to foster discussion about the vaccines in the broader community (Rosenthal 2005). Program managers should consider employing the full range of behavior change communications, including print materials, the mass media, community events, peer educators, and others (Agurto et al. 2005, Davis et al. 2004, Sherris et al. 2006). Although the specific content of the messages will depend on the knowledge and attitudes of the local community, it should offer comprehensive information about HPV, including the risks of infection, the cause of cervical cancer, the value of screening (in countries where it is offered), and the benefits of HPV vaccine (Rosenthal 2005, Lazcano-Ponce et al. 2001). All messages should guard against potentially dangerous misperceptions, for example, the belief that an HPV vaccine will protect against other STIs, reduce the risk of AIDS, or eliminate the need for cervical cancer screening (WHO 2005a).

D. Need for continued screening

Even after the introduction of a prophylactic HPV vaccine, it will be important to keep screening programs in place for many years to come (Pagliusi and Aguado 2004, Tsu and Pollack 2005, Franco et al. 2006b). First, large numbers of women already are infected with HPV and at risk of developing cervical cancers over the next three decades. Early detection of pre-cancerous changes in the cervix and effective treatment are critical for them. Second, the new VLP

vaccines are designed to prevent only the two most common types of oncogenic HPV. Some vaccinated women will continue to develop cervical cancer caused by infections with HPV types not included in the vaccines. Third, in developing countries, it may take many years before broad population-based access to a vaccine is possible.

Therefore, in countries that already have screening programs in place, advocates must convince governments and other funders to continue paying for routine screening for cervical cancer along with the new vaccines. In countries that lack screening programs, decision makers must weigh the feasibility and cost-effectiveness of initiating one-time screening of older women along with routine immunization of adolescents. Where screening programs exist, HPV vaccination programs also must teach individual women that being vaccinated does not mean they can forgo screening (Kahn 2005).

In the longer term, however, the introduction of an effective vaccine will undoubtedly change the balance of costs and benefits for routine screening. Policymakers should seriously consider changing the nature of screening regimens for vaccinated women (Trimble 2005, Lowndes and Gill 2005). For example, they might reduce the frequency of screening—even to as little as once per lifetime—or shift from Pap smears to HPV DNA testing (Harper 2005a, Lehtinen and Paavonen 2003, Shaw 2005). Post-licensure studies will be important to determine how best to modify screening recommendations.

V. Conclusion

With the availability of two highly effective HPV vaccines, cervical cancer prevention is entering a new era. Admittedly, much remains to be learned, for example, about the duration of protection offered by the vaccines and their effectiveness in males. Although these current vaccines are highly effective in women, large numbers of women infected with oncogenic HPV other than types 16 and 18 remain at risk of disease. In addition, the vaccines are designed to be delivered in a series of precisely spaced injections to adolescents (although it is possible that changing those intervals might not diminish the vaccine's effectiveness), and the public sector cost of the vaccine is unknown. Yet Gardasil and Cervarix offer an unparalleled opportunity to prevent cervical cancer where it remains most common: in developing countries that lack comprehensive screening programs. After all, vaccine programs have been proven to be one of the most successful public health interventions for overcoming economic disparities in health care.

At the same time, research continues on other fronts. Vaccine developers and academic researchers are actively working on a second generation of prophylactic HPV vaccines that may be cheaper to manufacture, easier to deliver, and protective against a broader array of HPV types. They also are trying to develop therapeutic vaccines that can help treat cervical cancer and other HPV-related diseases.

The advent of prophylactic HPV vaccines presents a range of questions and challenges for health policymakers in the developing world, who must decide what cervical cancer reduction strategy makes most sense for their countries in the face of competing priorities and limited resources. This may mean introducing one of the new vaccines, strengthening or scaling up screening programs (which have a proven track record and have benefited from new techniques like VIA, VILI, and HPV DNA testing), or some combination of the two. The "right" choice depends on the local situation, including the burden of disease, the capacity of the health system and vaccination program, and sociocultural issues.

Fortunately, policymakers can expect help in making informed decisions about whether, when, and how to introduce HPV vaccines. In 2005, WHO created an HPV Expert Advisory Group to develop global guidelines for the introduction of HPV vaccines. These experts have identified information gaps and key questions for vaccine introduction (WHO 2006c) and have also identified health organizations to seek the answers (WHO 2005a). Planned studies on a series of potential "early introducer" countries will generate valuable evidence and practical tips for vaccine introduction (PATH 2006), and HPV vaccine funding mechanisms also are under discussion (WHO 2005a). The combination of effective vaccines and thoughtful guidance based on relevant scientific and programmatic evidence will enable policymakers to take the next step in the fight against cervical cancer.

Appendix 1. Vaccine developers

Advaxis, Inc.
The Technology Centre of New Jersey
Suite 117
675 U.S. Route 1
North Brunswick, NJ 08902
United States
Tel: +1 732.545.1590
Fax: +1 801.459.3596
www.advaxis.com

BioVex Limited
34 Commerce Way
Woburn, MA 01801
United States
Tel: +1 781.933.1409
Fax: +1 781.933.6025
www.biovex.com

BT Pharma S.A.
Rue Pierre et Marie Curie BP 28262
31682 Labège-Innopole Cedex
France
Tel: +33 5 61 28 70 60
Fax: +33 5 61 28 70 69
www.btpharma.com

Centre Hospitalier Universitaire Vaudois (CHUV)
Department of Gynecology
Rue du Bugnon 46
1011 Lausanne
Switzerland
Tel: + 41 21 314 11 11
www.chuv.ch

Chang Gung Memorial Hospital
Department of Pathology
Taipei, Taiwan
www.cgmh.org.tw/

Chinese Academy of Medical Sciences
PO Box 2258
Beijing 100021
China
www.cams.ac.cn

Chinese Academy of Preventive Medicine
Institute of Virology
Beijing 100052
China

CSL Limited
45 Poplar Road
Parkville, Victoria 3052
Australia
Tel: +61 3 9389 1911
Fax: +61 3 9387 8454
www.csl.com.au

Georgetown University Medical Center
4000 Reservoir Road NW
Suite 120
Washington, DC 20007
Tel: +1 202.687.0100
gumc.georgetown.edu/research.html

German Cancer Research Center (DKFZ)
Im Neuenheimer Feld 280
D-69120 Heidelberg
Germany
Tel: +49 6221 42 0
Fax: +49 6221 42 2995
www.dkfz-heidelberg.de/index.html

GlaxoSmithKline (GSK) Biologicals
Rue de l'Institut 89
1331 Rixensart
Belgium
Tel: +32 2 656 9831
Fax: +32 2 656 8000
www.gsk-bio.com

Guangxi Medical University
Department of Gynecology Oncology
Nanning, Guangxi 530021
China

Innogenetics Group
Technologiepark 6
9052 Gent
Belgium
Tel: +32 9 329 13 29
Fax: +32 9 329 19 11
www.innogenetics.com

IDM Pharma, Inc.
9 Parker, Suite 100
Irvine, CA 92618-1605
United States
Tel: +1 949.470.4751
Fax: +1 949.470.6470
www.idm-biotech.com/

Instituto Mexicano del Seguro Social
Av. Paseo de la Reforma 476
Col. Juárez
C.P. 06600 Mexico City
Mexico
Tel: +52 55 5238 2700
www.imss.gob.mx

Johns Hopkins University
Sidney Kimmel Comprehensive Cancer
Center
The Harry and Jeanette Weinberg Building
Suite 1100
401 North Broadway
Baltimore, MD 21231
United States
Tel: +1 410.955.5222
www.hopkinskimmelcancercenter.org

Large Scale Biology Corporation
3333 Vaca Valley Parkway
Vacaville, CA 95688
United States
Tel: +1 707.446.5501
Fax: +1 707.446.3917
www.lsbc.com

Leiden University Medical Center
Department of Gynecology
P.O. Box 9600
2300 RC Leiden
The Netherlands
Tel: +31 71 5269111
www.lumc.nl/

Ludwig Institute for Cancer Research
R. Prof. Antonio Prudente, 109 - 4th Floor
Liberdade
São Paulo, SP 01509-010
Brazil
Tel: +55 11 3388 3200
Fax: +55 11 3207 7001
www.ludwig.org.br/

MediGene AG
Lochamer Str. 11
82152 Martinsried / Planegg
Germany
Tel: +49 89 85 65 29 00
Fax: +49 89 85 65 29 20
10660 Scripps Ranch Blvd., Suite 200
San Diego, CA 92131
United States
Tel: +1 858.586.2240
www.medigene.com

Merck & Co., Inc.
One Merck Drive
P.O. Box 100
Whitehouse Station, NJ 08889-0100
United States
Tel: +1 908.423.1000
www.merck.com

MGI Pharma
5775 West Old Shakopee Road
Suite 100
Bloomington, MN 55437
United States
Tel: +1 952.346.4700
Fax: +1 952.346.4800
www.mgipharma.com

National Cancer Institute (NCI)
Bethesda, MD 20892
United States
Tel: +1 800.422.6237
www.cancer.gov/researchandfunding
researchportfolio.cancer.gov/index.jsp

National Defense Medical Center
Department of Microbiology and Immunology
Taipei, Taiwan

Norris Comprehensive Cancer Center
University of Southern California
1441 Eastlake Avenue
Los Angeles, CA 90033
United States
Tel: +1 323.865.3000
ccnt.hsc.usc.edu/

Peking Union Medical College (PUMC)
Beijing, China
www.pumch.ac.cn

Pochon CHA University
College of Medicine and Research Institute of
Basic Medicine
Kyounggi-do 487-800
South Korea
www.cha.ac.kr

PowderMed Ltd
4 Robert Robinson Avenue
The Oxford Science Park
Oxford OX4 4GA
England
Tel: +44 1865 501500
Fax: +44 1865 501501
www.powdermed.com

Shantha Biotechnics India
3rd Floor, Serene Chambers,
Road No.7, Banjara Hills,
Hyderabad - 500 034
India
Tel: +91 40 23543010, 23548507, 23608843
Fax: +91 40 23548476
www.shanthabiotech.com

Nventa Biopharmaceuticals Corporation
(formerly StressGen Biotechnologies
Corporation)
#120 - 4243 Glanford Avenue
Victoria, British Columbia V8Z 4B9
Canada
Tel: +1 250.744.2811
Fax: +1 250.744.3331
www.nventacorp.com

Transgene S.A.
11 rue de Molsheim
67082 Strasbourg Cedex
France
Tel: +33 3 88 27 91 00
Fax: +33 3 88 22 58 07
www.transgene.fr

Université Libre de Bruxelles
Institut de Biologie et de Medecine
Moleculaires
12 rue des Professeurs Jeener et Brachet
6041 Gosselies
Belgium
www.ulb.ac.be/ibmm/

University of Cape Town
HIV Vaccine Development Group and
Human Papillomavirus Research Group
Groote Schuur Hospital
Observatory 7925
South Africa
Tel: +27 21 4066124
www.iidmm.uct.ac.za/alwilliamson/index.htm

**University of Colorado at Denver and
Health Sciences Center**
13001 E. 17th Place
Aurora, CO 80011
United States
Tel: +1 303.372.0000
www.cudenver.edu/home.htm

University of Queensland
The Diamantina Institute for Cancer,
Immunology and Metabolic Medicine
Princess Alexandra Hospital
199 Ipswich Road
Wooloongabba, Queensland 4102
Australia
Tel: +61 7 3240 5938
Fax: +61 7 3240 5946
www.cicr.uq.edu.au

University of Tokyo
Department of Obstetrics and Gynecology,
Faculty of Medicine
7-3-1 Hongo
Tokyo 113-0033
Japan
www.u-tokyo.ac.jp/index_e.html

The Wistar Institute
3601 Spruce Street
Philadelphia, PA 19104
United States
Tel: +1 215.898.3700
www.wistar.upenn.edu

Wyeth Pharmaceuticals
500 Arcola Road
Collegeville, PA 19426
United States
Tel: +1 610.902.1200
www.wyeth.com

Xenova Group plc
957 Buckingham Avenue
Slough, Berkshire SL1 4NL
United Kingdom
Tel: +44 1753 706600
Fax: +44 1753 706607
www.xenova.co.uk

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 binds to foreign antigens and marks 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 (APCs): Various cells, including macrophages and dendritic cells, that present antigen in a form that T cells can recognize.

Atypical squamous cells of undetermined significance (ASCUS): Pap smear finding that indicates precancerous changes in a minority of cases.

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.

Capsomere: Protein-based cluster making up a discrete subunit of a viral capsid.

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

Cervical intraepithelial neoplasia (CIN): Precancerous changes in the surface layers of the cervix that are graded into three levels of severity: CIN 1, CIN 2, and CIN 3 (which includes carcinoma *in situ*).

Clinical trials: Three phases of study of candidate vaccines in people. Phase 1 trials include small numbers of volunteers and determine the safety of the vaccine. Phase 2 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 3 trials are large-scale studies in thousands of people to confirm that a vaccine safely prevents disease with minimal side effects.

Cytokine: Proteins secreted by cells of the immune system that regulate the intensity and duration of the immune response.

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 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.

Immunotherapy: Treatments that stimulate the body's own immune system to respond to a disease (e.g., cancer).

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

Major histocompatibility complex (MHC): Molecules on cell surfaces that hold and display antigen to cytotoxic T cells (Class I) or helper T cells (Class II).

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.

Pseudovirion: A particle resembling a virus but lacking its genetic information and therefore unable to replicate.

Preclinical: An early phase of study of a vaccine or drug that is completed before clinical studies are carried out in human subjects and 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 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.

RNA replicon: A self-replicating RNA molecule.

Seroconversion: Development of antibodies in the blood against a particular antigen.

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 a disease, it protects against subsequent infection by that organism.

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