[Frontiers in Bioscience 8, s333-345 May 1, 2003]

A REVIEW OF HUMAN PAPILLOMAVIRUS VACCINES: FROM BASIC SCIENCE TO CLINICAL TRIALS

J. Michael Berry [1] and Joel M. Palefsky [2]

[1] University of California, San Francisco, Box 0512, 521 Parnassus Ave., San Francisco, CA, [2] General Clinical Research Center, Box 0126, 505 Parnassus Ave, Room M1203, University of California, San Francisco, San Francisco CA 94143

TABLE OF CONTENTS

1. ABSTRACT

Human papillomavirus (HPV) infection leads to a spectrum of disease from genital warts to precancerous lesions to cervical and anal cancer and is a worldwide public health problem of epidemic proportions. Unique to HPV-related neoplasia, the presence of specific viral antigens such as the L1 capsid structural protein and the oncoproteins E6 and E7 provide opportunities for vaccine therapy. Although difficult to precisely define, the natural immune response to HPV is vitally important and defects in cell mediated immunity correlate with increased risk of disease and cancer. In preclinical animal models, both prophylactic and therapeutic vaccines have effectively induced HPV-specific cell mediated immune responses protecting animals from viral challenge or eliminating established tumors. Most prophylactic vaccines are virus-like particles (VLP) composed of the L1 structural protein. Phase I trials have demonstrated safety and immunogenicity, but limited efficacy data are available. Therapeutic vaccine trials are reviewed including E6 and E7 vaccines comprised of peptides, fusion proteins, encapsulated plasmid DNA, and recombinant vaccinia virus. All of the vaccines appear to be safe, well tolerated, and preliminary data indicates that most are clinically effective. Multiple trials are in progress and more mature data are expected within the next few years.

2. INTRODUCTION

Human papillomavirus (HPV) infection is the most common sexually transmitted disease and it is second only to human immunodeficiency virus (HIV) infection in causing morbidity and mortality. Infection with at least one type of HPV is estimated to occur in more than 75% of sexually active adults. HPV is associated with a spectrum of disease ranging from benign anogenital warts to anogenital cancer, including cervical, vulvar, vaginal, penile, and anal cancer. In underdeveloped countries with limited access to health care and in the absence of widely available cervical cancer screening programs, cervical cancer is a leading cause of cancer deaths in women and these deaths are potentially preventable. Given the worldwide distribution of HPV infection, an effective means of preventing new infections and disease is of paramount importance and it is clear that developing an effective vaccine to prevent and/or treat HPV infection would have an enormous impact on public health globally.

Unique to HPV-related neoplasia and unlike many cancers and precancerous diseases, the presence of specific viral antigens provides an opportunity for vaccine therapy. Furthermore, a number of phase I and II clinical trials of HPV vaccines have been completed and large scale phase III trials are just beginning. Thus, it appears that great strides have been made in developing safe, well tolerated, and effective vaccines and the ability to prevent HPV infection or to effectively eradicate established disease seems on the horizon.

This review will summarize the salient biology and natural history of HPV infection with an emphasis on the role of the immune system and natural immunity and how this has led to the development of a number of candidate HPV vaccines (see Table 1). HPV infects only epithelial cells and is not a systemic infection, which implies that immunity at the mucosal level may be more biologically relevant than systemic immunity. Traditionally, vaccines are generally thought of in a preventive context. A vaccine that prevents HPV infection

Table 1. HPV Vaccine Clinical Trials

Pharmaceutical Comp.	Prophylactic or Therapeutic	Antigen	Type of Vaccine	HPV type
Merck	P	L1	VLP	6, 11, 16, 18
NCI	P	L1	VLP	16
Merck	P	L1	VLP	16
Merck	P	L1	VLP	11
Stressgen	T	E7	fusion protein (HspE7)	16
Xenova	T	E6, E7	vaccinia virus (TA-HPV)	16, 18
Zycos	T	E7	encapsulated plasmid DNA	
Xenova	P, T	L2, E6, E7	fusion protein (TA-CIN)	16
GlaxoSmithKline	T	E7	fusion protein (D16E7)	16
NCI	P, T	L1, L2, mutated E2, mutated E7	chimeric VLP	16
Not Available	T	E7	peptide	16

from occurring seems plausible based not only on preclinical studies using animal models, but also preliminary clinical results. Particularly with HPV-associated disease, however, the concept of a therapeutic vaccine is not only rational, but has also been shown to be effective in early clinical trials. The rationale for selecting various possible antigens, different types of vaccines, routes of administration, measurement of surrogate endpoints, and the results of recently completed clinical trials will be discussed.

3. BIOLOGY OF HPV

Papillomaviruses are DNA viruses that are species-specific. Many different mammals harbor papillomaviruses, but cross-infection does not occur. They are also site-specific within the epithelium of a given host, meaning that some types cause only cutaneous warts in humans, other types cause plantar warts, and other types cause disease specifically in the anogenital region. More than a 100 different types of HPV have been identified, including approximately 30 types that infect only the anogenital mucosa. HPV infection is associated with a spectrum of dysplasia, including low-grade disease such as genital warts or condyloma to high-grade dysplasia, which is considered to be pre-cancerous to frankly invasive cancers. HPV types are classified as low-, intermediate-, or high-risk depending on their association with benign or malignant disease. For example, HPV types 6 and 11 are considered low-risk types and associated with condyloma or low-grade dysplasia, also known as low-grade squamous intraepithelial lesions (LSIL). However, types 16 and 18 are associated with high-grade dysplasia, also known as high-grade squamous intraepithelial lesions (HSIL) and more than 50% of the invasive cancers. High-risk types of HPV have been identified in more than 99% of cervical cancers (1) and almost 90% of anal cancers (2).

The HPV genome has been sequenced and consists of an approximately 8,000 base pair circular double-stranded DNA (3). This knowledge coupled with the characterization of the life cycle of HPV provides several specific targets for potential vaccines. There are a series of early genes, E1-E7 that encode proteins involved in viral replication, transcription and cell cycle control. The late genes L1 and L2 encode the major and minor capsid structural proteins, respectively. The early proteins E6 and E7 have been well described and are thought to be responsible for the oncogenic potential of HPV. E6 binds to native cellular p53, which when joined by a third molecule, E6-associated protein, forms a complex that results in ubiquitination of p53. This in turn leads to proteosomal degradation of p53. Loss of p53 function leads to impaired cell cycle control, increased cellular proliferation, decreased apoptosis and decreased p53-induced DNA repair. When the cells are exposed to DNA damaging events, loss of p53 may result in chromosomal instability due to continued cell cycling with lack of DNA repair. The resulting accumulation of host chromosomal changes may be the actual driving force behind progression of low-grade dysplasia to high-grade dysplasia or cancer. Among its many other functions, E7 binds to the retinoblastoma gene product (Rb) with similar loss of cell cycle control. The combined loss of p53 and Rb function appears to be synergistic in the process of malignant transformation.

Both p53 and Rb are thus tumor suppressor genes and their inactivation has been implicated in a number of cancers. E6 and E7 are attractive targets for vaccines because they are expressed throughout the life cycle of HPV and most of the therapeutic vaccines have been directed against E6 or E7. In contrast, the antigens that have been used in a most of the prophylactic vaccines are L1 and L2, which are capsid proteins synthesized late in the life cycle of HPV. L1 is the more abundant of the two capsid proteins and is commonly expressed in terminally differentiated epithelial cells as mature virus particles are assembled prior to release of infectious virion.

4. NATURAL HISTORY OF HPV INFECTION AND IMMUNITY

It is clear based on the natural history of HPV infection that the immune system plays an important role in controlling HPV. Compared with the large number of persons exposed and infected with HPV, only a small percentage develops cancer. HPV is sexually transmitted and the majority of people who develop disease acquire HPV shortly after initiating sexual activity. This infection

may be silent and asymptomatic or be manifest as LSIL or genital warts. For most persons, infection is transient and an immune response is initiated, clearing their infection, at least to the level of detection by currently available tests (4). It is not clear if small foci of latent infection in clinically normal tissues remains in most infected individuals, or if HPV infection is truly cleared.

Most cervical LSIL will resolve spontaneously and therefore women are often observed for a period of time prior to treatment of LSIL. Persistence of infection with high-risk types of HPV due to an inadequate immune response is the hallmark of increased risk of progression to HSIL. Attempts to directly measure or specifically define the natural immune response to HPV have been problematic. Because HPV infection occurs in the anogenital mucosa, important local responses may not be reflected systemically.

Several studies have examined the association between serologic response to HPV and cervical dysplasia. In most of these studies, the clinical significance of a serological response to the HPV 16 L1 protein did not necessarily correlate with the presence or absence of infection or disease (5). Antibodies to HPV 16 L1 were detected in 33% of HPV negative women, 55% of HPV infected but healthy women, and 72% of women with cervical dysplasia. Other investigators looking at cell-mediated immunity showed that women who cleared their HPV 16 infection were more likely to have a specific cytotoxic T lymphocyte response to E6 than those with persistence of HPV 16 infection, but this relationship was not shown for E7 (6). Seventy-five percent (30/40) of women who cleared their HPV infection had a response to E6, E7 or both compared with 56% (5/9) of the women who had persistent HPV 16 infection. Specifically looking at E6, however, 0 of 9 women with persistent infection had a response to E6. All of the responses were to E7, which suggests that a cytotoxic T cell response to E6 may be more clinically significant. Other investigators have correlated the presence of HPV 16 specific memory cytotoxic T cell precursors with the absence or level of dysplasia and showed that paradoxically these memory T cells were not seen in women who had cleared their infection, but predominated in women with high-grade lesions or cervical cancer. They concluded that the role of naturally occurring HPV 16 E6/E7 specific memory cytotoxic T cell precursors play is still not clear (7).

An extensive review of the natural immunity to HPV is beyond the scope of this paper, but has been done by Moscicki and colleagues (8). Some of the reasons suggested for this inability to clearly demonstrate the nature of the immune response to HPV include the transient or short-lived nature of an effective immune response, downregulation of human leukocyte antigen (HLA) molecules on dysplastic cells, the small number of memory cells that may exist in the peripheral blood and the possibility of tolerance.

In addition, variation in the experimental methods used in different laboratories to demonstrate immune response leads to difficulty in interpreting the significance of some of the reported findings. For example, some of the peptides used to demonstrate reactivity *in vitro* may not represent epitopes clinically important in clearance of infection *in vivo*. The use of *in vitro* restimulation protocols permits detection of small number of effector cells in the peripheral blood, but the significance of these cells is unclear. The detection of these cells could indicate the presence of an effective immune response. Alternatively, the presence of HPV-specific T lymphocytes in the presence of disease in some instance may reflect persistence of infection and hence exposure to adequate amounts of antigen to generate a response, but not necessarily one that was effective to eliminate the disease. Distinguishing between these two possibilities requires long-term prospective studies of large numbers of women.

Certainly in those patients who have persistent infection, there must be some degree of immunological tolerance induced. These observations are helpful in understanding why immunization may not always be effective and to stimulate research in developing novel ways of presenting antigens to the immune system in order to generate more effective immune responses. It is clear for multiple reasons that the exact nature of the immune response remains elusive despite the clinical fact that regressions of disease do occur and many of the people infected with HPV appear to clear their infections. This demonstration of an immune response coupled with animal studies showing efficacy and now, successful clinical trials, provide realistic hope to the search for an effective HPV vaccine.

It is well established that there is an increased level of HPV-related disease in immunocompromised patients, which is indirect evidence of the importance of immunity in controlling HPV. Prior to HIV infection, increased anogenital neoplasia had been described in patients with disorders of cell-mediated immunity such as organ transplant patients receiving immunosuppressive medications, patients with iatrogenic immunosuppression and those with congenital immunodeficiencies. For example, a 100-fold increase in cancers of the vulva or anus has been reported in renal transplant patients (9). The incidence of anal cancer in HIV-seropositive homosexual men is estimated to be 75 to 80 per 100,000, which is nearly twice that of HIV-seronegative homosexual men. This increased incidence of anal cancer can be compared to a rate of 0.8 per 100,000 in the general population and is greater than the incidence of cervical cancer in women prior to the introduction of widespread screening with cervical Pap smears of 35/100,000 (10).

Although there is a four-fold increase in cervical cancer among HIV-seropositive women compared with the general population and cervical cancer was deemed an AIDS-defining condition in 1993 by the Centers for Disease Control, the nature of the exact relationship of cervical cancer and HIV infection remains controversial. Additional epidemiological studies have not supported an increased incidence of cervical cancer associated with AIDS and it is likely that the increased incidence is related

to similar risk factors for acquisition of HPV as HIV (11). Multiple studies, however, have documented an increase in the prevalence of HPV infection and dysplasia of the cervix and anus among patients infected with HIV and that this increased risk of anogenital dysplasia can be correlated with the degree of immunosuppression as measured by low CD4+ lymphocyte counts and high HIV viral loads (12-19). Highly active antiretroviral therapy (HAART) has greatly reduced the morbidity and mortality of HIV infection, because of some degree of immune reconstitution and improvement in immune function. Therefore, HPV infection and anogenital dysplasia might be expected to improve as well. Paradoxically, HAART has had little impact on anal HPV infection or anal dysplasia at least during the first 6 months of HAART therapy. Moreover, patients treated with HAART are more likely to have high-grade disease compared with patients never treated with HAART, even when matched for CD4 counts (20). One way of explaining this paradox is that recovery of cell-mediated immunity leading to regression of anal dysplasia may require more than 6 months of therapy or that patients who required HAART therapy were more profoundly immunosuppressed compared to patients never treated with HAART. Regardless, the data suggest that if people with HIV infection are living longer as a result of HAART therapy, they continue to be at risk for the development of anal cancer. It is clear from these data that a cell-mediated immune response is important in controlling HPV.

5. GENERAL APPROACHES TO VACCINE DEVELOPMENT

There are two main types of HPV vaccines being developed, prophylactic or preventative vaccines and therapeutic vaccines. Prophylactic vaccines are designed to prevent infection from occurring and most commonly are directed at late structural proteins, such as L1 that makes up the viral capsid. The most common type of prophylactic vaccine is comprised of virus-like particles (VLP), which are easily synthesized proteins that aggregate into structures resembling native viral capsids. This type of vaccine is an ideal sub-unit vaccine that does not contain DNA or oncogenic proteins and in that way is similar to the hepatitis B vaccine (21). VLPs are immunogenic and safe and several L1 VLPs are in clinical trials. In addition to standard intramuscular or subcutaneous routes, other routes of administration are being investigated, such as intranasal inoculation in an attempt to optimize the immune response, particularly mucosal immunity. The ultimate measure of efficacy for a prophylactic vaccine is to prevent the development of cancer. Since there is normally a long latency period between infection and HSIL and cancer, developing and validating surrogate endpoints will be important in designing clinical trials to evaluate prophylactic vaccines.

Therapeutic vaccines are generally directed at E6 and E7 since these proteins are expressed in most HSILs and cancers. The ideal vaccine will engender a cell-mediated immune response generating HPV-specific cytotoxic T lymphocytes. Early or first-generation vaccines have been directed against a single antigenic epitope such as a peptide fragment of the HPV 16 E7 protein and are considered monovalent vaccines. Generally, cross-reactivity amongst types of HPV is low, so that newer approaches are geared towards developing polyvalent vaccines that contain multiple antigenic epitopes from both E6 and E7 as well as from other HPV types, such as HPV 18. Some vaccines in development are hybrids or chimeras and are intended to be both prophylactic and therapeutic. The same concept of type specificity applies to prophylactic vaccines, meaning that an HPV 6 L1 vaccine may exert little protection against HPV types 11, 16, or 18.

Another obstacle to overcome in developing one vaccine "that fits all" is the intimate role of HLA molecules in the immune response. The most potent professional antigen presenting cells (APCs) are dendritic cells, which circulate in the peripheral blood and are responsible for initiating a cellular immune response. Foreign extracellular proteins are taken up by APCs, processed intracellularly within lysosomes and then presented with class II HLA molecules, generating a T helper response. To stimulate a cytotoxic T cell response, peptide fragments must be associated with class I HLA molecules, which usually occurs when intracellular proteins are degraded and transported to the endoplasmic reticulum. Techniques have been developed to enhance class I responses, including the use of viral vectors and modifying antigens chemically (22). Therefore, since some antigens are HLA restricted, then vaccines utilizing these antigens will be optimized only for certain specific HLA haplotypes. Potential ways of bypassing this problem include polyvalent vaccines for multiple HLA types and antigenic epitopes or using larger proteins that span a range of immunogenic epitopes. Other approaches designed to stimulate a specific cell-mediated response include novel fusion proteins, naked DNA, liposome encapsulated plasmid DNA, and recombinant vaccinia virus vaccines.

6. PRECLINICAL ANIMAL MODELS

Because HPV is species-specific, vaccine research has been hindered in part by the lack of a good animal model for HPV infection and disease and the lack of an efficient way to propagate the virus in the laboratory. There are several animal models, however, that approximate human disease and in which vaccination strategies have been successfully demonstrated (23). The cottontail rabbit papillomavirus (CRPV) causes cutaneous warts in both domestic and cottontail rabbits. Some of the warts regress, but in those that persist, half will develop cancers. Two types of bovine papillomavirus (BPV) are known to induce papillomas, which under certain conditions will progress to carcinomas. The third model is known as the canine oral papillomavirus (COPV), a virus that causes oral papillomas in young beagle dogs, which then regress rather quickly. Although there are no known types of papillomavirus that infect rodents, mouse cells can be transformed by high-risk HPV oncoproteins, E6 and E7. These transformed cells develop into tumors when implanted into laboratory mice. Effective L1 VLP prophylactic vaccines that induce neutralizing antibodies have been investigated in rabbits, cows, and dogs. The

advantage of animal models is in the development of new conceptual approaches, which then form the basis for clinical trials.

7. PROPHYLACTIC VACCINE CLINICAL TRIALS

Virus-like particle (VLP) vaccines have been shown to be protective against high-dose virus challenge in several animal models. The NCI has completed a phase I safety and immunogenicity trial using an HPV 16 L1 VLP vaccine (24). Sixty-eight of 72 normal subjects were enrolled**.** These subjects were expected to be at low risk for HPV infection based on sexual history, received all 3 doses of either placebo, 10 mcg. of vaccine without adjuvant, 10 mcg. of vaccine with alum adjuvant, 10 mcg. of vaccine with M59 adjuvant, or 50 mcg. of vaccine without adjuvant or with alum or M59. The vaccine was well tolerated at all doses administered either with or without adjuvant and the most common reactions reported were headache and injection site pain described as mild or moderate. The vaccine was immunogenic and all vaccine recipients seroconverted and developed antibodies to HPV 16 L1 VLPs, but no placebo recipients developed antibodies. The best response was seen in subjects who received the 50 mcg. dose without adjuvant and titers were 40 times higher than antibodies detected in patients who had been previously exposed to HPV 16 as a result of a naturally occurring infection. The antibodies generated were of the IgG1 class and were shown to be neutralizing in a pseudovirion neutralizing assay.

The NCI HPV 16 L1 VLP vaccine is one of the first prophylactic vaccines to be evaluated in a large randomized, double blind, placebo controlled trial in 21,000 women in Costa Rica (25). These women have been well characterized as part of a large natural history study of HPV infection and cervical neoplasia that has been performed in Guanacaste, Costa Rica over the last 10 years. The primary endpoints in this study will be persistence of HPV 16 infection and development of dysplasia. Women will be followed with Pap smears, HPV testing and serology for 4 years to determine vaccine efficacy. A third arm of the trial will be added in the form of a chimeric VLP vaccine that is currently in phase I and II trials. This chimeric vaccine is composed of an HPV 16 L1 VLP and a recombinant fusion protein consisting of HPV 16 L2 joined with the non-structural proteins, HPV 16 E2 and E7. Because of concerns regarding oncogenicity, both E2 and E7 have been mutated to disable their DNA and retinoblastoma gene product binding sites. The chimeric VLP may also have some therapeutic activity and may cause regression of lesions that are pre-existing or that develop if the L1 VLP does not induce sterilizing immunity. In animal models these chimeric VLPs can generate cytotoxic T lymphocytes and cause tumor regressions.

Although immunogenicity has been clearly demonstrated with the HPV 16 L1 VLP vaccine, it remains to be seen how well systemic immunization and neutralizing antibodies will work at the level of the genital mucosa. In the animal models that have demonstrated resistance to viral challenge, virus is applied to damaged, abraded skin or oral mucosa and not genital mucosa. Infection of the basal cell layer of the cervix is thought to be due to microabrasions, which in turn would facilitate the transudation of serum antibodies. The NCI group in collaboration with Swiss researchers, however, have recently demonstrated that significant titers of antibodies can be found in cervical secretions of women after vaccination with HPV 16 L1 VLP vaccine (26). They compared titers in women taking oral contraceptives to women not taking them and found that antibody levels in the cervix varied much more than in serum and were 0.5 to 50% of serum titers. Among women not taking contraceptives there was even more variation, with a 10 to 100 fold decrease in VLP specific IgG at the time of ovulation that was felt to be due to the increased volume of secretions seen at the time of ovulation. This same group also reported results from a small pilot study looking at mucosal delivery of the vaccine using either a nasal spray or an aerosol (27). The aerosol was more effective, and 3 of 6 subjects who received 50 mcg. of the HPV 16 L1 VLP vaccine via this route had serum antibody titers similar to intramuscular administration of the same dose. This trial is ongoing and researchers are evaluating a 250 mcg. dose given by aerosolization. Furthermore, these researchers found HPV 16 L1 VLP-specific IgA secreting cells in the peripheral blood and both IgG VLP-specific antibodies and secretory IgA VLP-specific antibodies in cervical secretions. Based on these promising results, these investigators plan to evaluate the combination of an intramuscular injection and aerosol administration.

Merck has at least 3 candidate VLP vaccines in clinical trials: HPV 16 L1, HPV 11 L1, and a quadrivalent vaccine that includes HPV 6, 11, 16, and 18 L1 VLPs. Preliminary data indicate that subjects who received the quadrivalent vaccine developed neutralizing antibody titers to each of the individual types that were comparable to those produced by a monovalent vaccine. The vaccine was well tolerated without significant systemic side effects (28). Safety, immunogenicity, and extended follow-up data from two randomized, double blind placebo controlled trials of their HPV 16 L1 VLP vaccine are available. One trial enrolled 109 healthy young women with no detectable evidence of HPV 16 infection, either by DNA testing or serology. The other trial included 480 women whose HPV DNA status was not known, however approximately 25% were known to have had HPV 16 antibodies, presumably due to current or prior natural infection with HPV 16. Women received 3 intramuscular injections at month 0, 2, and 6. Serial anti-HPV 16 titers were determined over 24 months of follow-up. A range of doses of 10, 20, 40, or 80 mg. of vaccine were evaluated in each of the groups and responses were similar among dose levels, with the peak response occurring at month 7, one month after the last immunization. Titers slowly decreased over time. At month 18, however, values in immunized women were still much higher than in women who had received placebo and who had positive serology because of previous natural infection with HPV 16. The vaccine was well tolerated with injection site erythema and headaches being the most commonly reported effects, however no serious adverse

effects were reported. There were no differences in the adverse effects reported by subjects who had previously been infected with HPV compared to those with negative serology (29). Similar data were presented for a phase I study of an HPV 11 L1 VLP vaccine in a group of 140 women indicating that it was both immunogenic and well tolerated (30). Three injections were administered at 0, 2, and 6 months and to evaluate the effect of a booster vaccination, approximately half of the subjects received an additional vaccination at month 12. HPV 11 L1 VLP specific antibody titers initially increased after the booster, but within a year there were no significant differences in the titers of subjects who received all 4 doses versus those who only received 3 doses.

Although there are a fair amount of data on safety and immunogenicity of VLP vaccines, limited data regarding clinical efficacy have been published to date. At the 19th Papillomavirus Conference in Brazil, Dr. Laura Koutsky presented preliminary results on the efficacy of the Merck HPV 16 L1 VLP vaccine (Koutsky, L unpublished data). These data were developed retrospectively in a group of women who had no detectable evidence of HPV 16 as described above. Of 129 control subjects who received either the HPV 11 L1 VLP vaccine or placebo, 9 subjects tested positively for HPV 16 DNA compared with none of 66 subjects who received the HPV 16 L1 VLP vaccine. These early results indicate that this HPV 16 L1 vaccine appears to be effective to prevent new infections with HPV 16. Additional data on efficacy are expected from a number of trials in the near future, but it seems likely that the ability to prevent HPV infection from occurring is on the horizon.

8. THERAPEUTIC VACCINE CLINICAL TRIALS

Clinical trials of several different types of therapeutic vaccines have been completed and will be reviewed. One of the earliest reported results of a therapeutic vaccine is from a trial in patients with advanced cervical cancer, who were immunized with a peptide vaccine manufactured at the Netherlands National Institute of Public Health and Environmental Protection in Bilthoven (31). Nineteen patients with advanced cervical cancer recurrent or refractory to conventional treatment participated in a dose escalation phase I study. The vaccine consisted of two HPV 16 E7 peptides (amino acids 11-20 and 86-93), representing two cytotoxic T cell epitopes with high binding affinity for HLA-A*0201 combined with a pan-DR binding T helper peptide and suspended in an adjuvant similar to incomplete Freund's adjuvant. Eligibility criteria included positivity for HLA-A*0201 and persistent tumor containing HPV 16. The vaccine was well tolerated with no significant adverse effects reported. Many patients experienced an absolute lymphopenia, which was present in most prior to vaccination and likely reflected the advanced stage of their cancers or the effects of previous chemotherapy or radiation therapy. There was no consistent correlation between vaccine dose and clinical outcome. Two patients, however, had stable disease for up to 1 year after vaccination, 2 patients experienced tumor regressions after receiving chemotherapy after vaccination and the others died of progressive disease. Immunologically, no HPV-specific cytotoxic T lymphocytes were detected in any of the patients, however, 4 of 12 patients evaluated developed T helper peptide proliferative responses (32). The investigators concluded that peptide vaccination is feasible and should be investigated further in patients who were not as profoundly immunocompromised.

A phase I dose escalation trial of a similar peptide vaccine developed under an Investigational New Drug application held by the Cancer Therapy Evaluation Program of the Nation Cancer Institute was reported in 18 women with either high-grade cervical or vulvar dysplasia, who were HLA-A2 positive and who had detectable HPV 16 (33). The first 10 patients were treated with an HPV 16 E7 peptide (amino acids 12-20) suspended in incomplete Freund's adjuvant and the remaining 8 patients had an additional peptide added that consisted of an HPV 16 E7 peptide (amino acids 86-93) linked to a helper T-cell peptide epitope and a lipid tail. The vaccine caused a moderate local reaction categorized as grade I or II in 17 of 18 patients, but persistent granulomas were only seen in patients who received the highest dose level. Grade II systemic systems consisting of fever, lethargy, nausea, arthralgias, or myalgias occurred, but there was no correlation of symptoms with dose, and no grade III or IV toxicity was reported. Twelve of 18 patients had clearance of HPV 16 DNA by polymerase chain reaction (PCR), 3 had a decrease in signal intensity, and 3 had no change. The vaccine was immunogenic and 10 of 16 patients tested had specific E7 reactivity demonstrated by cytokine release and cytolytic assays. In 6 biopsy samples that were examined after vaccination, there was a significant increase in dendritic cells infiltrating the specimens compared to pre-vaccination samples. Moreover, 3 complete responses were seen and an additional 6 patients had a more than 50% decrease in the size of their lesions measured colposcopically. This vaccine appears to be safe, immunogenic, and to have therapeutic activity, which will require validation in randomized phase II trials.

Borysiewicz and colleagues in conjunction with Cantab Pharmaceuticals have developed a recombinant vaccinia virus vaccine known as TA-HPV, which encodes E6 and E7 from HPV types 16 and 18 with a mutation introduced to inactivate the E7 Rb binding site (34). Eight patients with advanced cervical cancer received a single dose of TA-HPV by scarification and were then kept in isolation. No clinically significant side effects were observed and there was no environmental contamination. All patients developed antibodies to vaccinia and 3 of 8 patients developed antibodies to HPV 18 E7. These HPV-specific antibodies were felt to be due to the immunization since tumors from these patients were known to contain HPV 16 but not HPV 18. 1 of 3 patients developed HPV-specific cytotoxic T lymphocytes. The study was too small to draw conclusions about efficacy, although 2 of the 8 patients were alive and tumor-free for 15 and 22 months, respectively, after vaccination. Two additional phase I trials of TA-HPV have been completed in 12 women with CIN II and 29 women with early stage cervical cancer

prior to radical surgery (35). No significant toxicity was reported in any of the 49 patients, although most patients had local reactions at the vaccination site. Immunologically, HPV-specific cytotoxic T lymphocytes were demonstrated in 3 of 12 patients with CIN III and 4 of 29 with early stage cervical cancer. Since the vaccine was administered primarily in an adjuvant setting, clinical efficacy could not be determined.

More recently, Davidson and colleagues in conjunction with Xenova, who merged with Cantab Pharmaceuticals in 2001, have vaccinated a group of 18 women with TA-HPV, who had vulvar intraepithelial neoplasia III (VIN III) and detectable HPV 16 (36). Patients were evaluated over a 6-month period with clinical exams, documentation of any symptoms, photographs, measurement of disease, peripheral blood mononuclear cells (PBMC) for immunological assays, and biopsies for histology, immunohistochemistry, and HPV DNA status. Before and after vaccination, PBMC were incubated with HPV peptides consistent with known or predicted T cell epitopes. After this *in vitro* stimulation Elispot assays were used to detect gamma-interferon producing HPV-specific CD8+ lymphocytes. TA-HPV was well tolerated and no vaccine-related adverse effects were reported. Preliminary results indicate a range of responses including symptomatic improvement, shrinkage of lesions, regression of lesions histologically, and absence of detectable HPV in some patients. A group from Addenbrooke's Hospital in Cambridge, UK presented similar data on 12 women with high-grade dysplasia vaccinated with TA-HPV at the British Society of Investigative Dermatology meeting held in Norwich, UK in March 2002. The vaccine was safe, well tolerated, and 1 woman had a complete regression of her lesion and 4 demonstrated at least a 50% decrease in size of their lesions for a response rate of 42% (37).

TA-CIN is a second vaccine developed by Xenova that is a recombinant fusion protein composed of HPV 16 L2, E6, and E7 (38,39). Preclinical studies using a mouse model demonstrated the ability of TA-CIN to induce HPV 16 specific antibodies, CD4+ T helper cells, and cytotoxic T lymphocytes, which protected the animals from challenge by HPV 16-positive tumor cells and also eliminated minimal residual disease. A phase I, blinded, dose escalation study has been completed in a group of healthy volunteers comprised of 30 men and 10 women. Three dose levels were evaluated consisting of 26 mg., 128 mg., or 533 mg. and 2 of 10 subjects at each dose level received placebo. At the 533 mg. dose level, 10 women were evaluated in addition to the 10 men. The vaccine was given as an intramuscular injection at month 0, 1, and 2 and samples were taken to assess immunogenicity concurrently and at month 3. No serious or severe adverse effects were reported and there was no dose-response relationship detected between the dose of TA-CIN administered and the extent of adverse events reported. All of the vaccinated subjects developed HPV 16-specific antibodies and the highest titers were seen after all 3 vaccinations, although subjects in the high-dose cohorts developed good IgG responses after 2 doses. Elispot assays demonstrated HPV 16-specific T cells that were reactive to E6 and E7 and lymphoproliferative responses were seen in all vaccinated subjects. The investigators concluded that TA-CIN administered at doses up to 533 mg. was both well tolerated and immunogenic. Furthermore, additional preclinical studies using TA-CIN in conjunction with the recombinant vaccinia virus vaccine TA-HPV indicate that this combined approach may be the optimal method to induce an HPV-specific T cell mediated immune response and will be investigated in future trials.

Preclinical studies of a recombinant fusion protein vaccine developed by GlaxoSmithKline (GSK) show that it effectively eradicates established tumors in mice and protects them from a second challenge given two months later (40). The fusion protein is composed of one third of the Hemophilus influenzae capsular lipoprotein D joined to the HPV 16 E7 protein and termed PD-E7. The advantage of using a whole protein is that it encompasses both CD4 and CD8 epitopes and obviates the need for HLA typing. Because whole proteins are weakly immunogenic, methods of increasing immunogenicity and increasing tumor-specific immunity have been developed including recombinant fusion proteins and strong adjuvants. PD-E7 was evaluated in three different adjuvants, which contained the immunostimulants MPL and QS21 in liposomes, an oil and water emulsion, or aluminum salts. SBAS 2, the oil and water emulsion was the most effective of the adjuvants and has been shown in other studies to induce cytotoxic T lymphocytes and a Th1 type of response. Mice were injected subcutaneously with tumor cells and then subsequently vaccinated. Unvaccinated mice had rapid growth of tumor similar to mice that received adjuvant alone. Although PD-E7 without adjuvant caused some slowing of tumor growth, PD-E7 with adjuvant rendered 60 to 100% of animals tumor-free. The vaccine induced both a humoral and cellular immune response with IgG antibodies, specific cytotoxic T lymphocytes, and lymphoproliferative responses.

Based on that preclinical work GSK has developed a recombinant fusion protein vaccine that consists of a mutated HPV 16 E7 protein joined to part of the Hemophilus influenzae protein D (D16E7) mixed with the GSK adjuvant AS02B. Women whose biopsies revealed cervical intraepithelial neoplasia III (CIN III) and who were positive for HPV 16 DNA received 3 vaccinations given 2 weeks apart. Of 6 women enrolled, preliminary results were reported for 4 women who were evaluable because they had received all 3 vaccinations (41). Two patients had partial regressions, defined as a decrease in lesion size measured colposcopically. In one of these regressions, a marked infiltration of lymphocytes was seen in the post-vaccination biopsy specimen compared to barely detectable lymphocytes seen in pre-vaccination biopsies and HPV 16 DNA became non-detectable. These Belgian researchers developed a protocol to assess HPV 16 E7 cellular immunity by incubating PBMC overnight with or without antigens, then freezing supernatants to look at cytokine production, and fixing and staining cells for cell surface markers and intracellular cytokines. Flow cytometry was then used to determine gamma-interferon producing CD4+/CD8-, CD4-/CD8+ and CD3-/CD16-56+

cells and interleukin-5 producing CD3+/CD4+ cells. Using this assay, 3 women had a pre-existing CD8+ T cell response to D16E7 and 2 women had gamma-interferon producing CD4+ T cells demonstrated. Post-vaccination samples incubated overnight with D16E7 showed that the number of gamma-interferon producing CD8- (4/4) and CD4- (3/4) cells were either induced or increased. Interleukin-5 producing cells were not induced by the vaccination, signifying that this was mainly a Th1 type response, but all patients developed low titers of anti-E7 IgG antibodies that were not present prior to vaccination. Studies are ongoing with more patients to further correlate histological, immunological, and virological responses.

9. THERAPEUTIC VACCINE CLINICAL TRIALS FOR ANAL DYSPLASIA

At UCSF, there is a cohort of approximately 800 HIV-seronegative and HIV-seropositive homosexual and bisexual men with anal dysplasia in a longitudinal natural history study and several hundred additional men and women with anal dysplasia that are followed in the UCSF Dysplasia Clinic. Over the last 10 years, we have pioneered the technique of high-resolution anoscopy, using a colposcope to examine the anus with 3% acetic acid and Lugol's solution in a manner similar to colposcopy of the cervix, vagina, and vulva. Colposcopic findings have been correlated with HPV DNA status, anal cytology, and anal histology (42,43). The same types of HPV that are involved in cervical dysplasia and cancer are also involved in anal dysplasia and cancer and although detection and management of anal disease is similar, protocols and standards of care are less well established. Epidemiologically, there is a significant problem of anal cancer in homosexual men that is estimated to occur at a rate of 35/100,000 HIV-seronegative men, which is similar to the rate of cervical cancer prior to the introduction of widespread screening of women with cervical Pap smears (44). Surgical treatment of anal dysplasia using high resolution anoscopy to guide fulguration of all microscopically visible disease is an effective treatment modality for HIV-seronegative subjects, but is associated with significant short-term morbidity and pain in most patients (45). Even though patients are treated, they still require continued follow-up because of a risk of recurrence of dysplasia, which in HIV-seropositive patients may be as high as 80%. Therefore, a better means of non-surgical management is needed. HIV-seronegative patients with high-grade anal dysplasia from either the natural history study or dysplasia clinic comprise an ideal group of subjects to evaluate therapeutic HPV vaccines because their disease is well characterized and can be followed longitudinally using well established methods and the results of two trials are discussed below.

Stressgen Biotechnologies Inc. has developed a therapeutic vaccine that has completed phase II trials and based on preliminary results presented in a poster at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy appears to be an effective non-surgical treatment for anal HSIL (46). The vaccine is a recombinant fusion protein termed HspE7 and consists of heat shock protein 65 (Hsp65) from Mycobacterium bovis variant BCG fused to HPV 16 E7. In preclinical studies immunization with HspE7 induces cytotoxic T lymphocytes and a cell-mediated immune response (47). TC-1 cells are a mouse tumor cell line that contains HPV 16 E6 and E7. When mice are inoculated with TC-1 cells they develop tumors, creating an animal model that mimics cervical carcinoma. When mice are immunized with HspE7 prior to challenge with TC-1 cells, the mice transiently develop tumors, which then completely resolve. Furthermore, these immunized mice are able to resist a second challenge administered 49 days later. HspE7 immunization of mice with established TC-1 tumors results in complete regression of tumors and increased survival compared with non-immunized mice. Interestingly, the immune response to HspE7 appears to be due to CD8+ cells independent of CD4+ cells based on studies in knockout mice depleted of either CD4+ or CD8+ cells. It is speculated that antigen presenting cells, such as dendritic cells have receptors for heat shock protein, which account for this CD4+ -independent response.

In the first part of this phase II clinical trial of HspE7 trial, subjects were randomized to receive either low-dose, 100 mcg. of HspE7 or placebo monthly for 3 injections. Eighty-six HIV-seronegative subjects with biopsy-proven anal HSIL were enrolled at two sites, UCSF or New York. Patients were assessed every 3 months with high-resolution anoscopy and biopsy of any lesions suggestive of HSIL. Three low-dose, 100 mcg. subcutaneous injections of HspE7 were no more effective than placebo. No complete responses were seen six months after the initial injection at this dose level and there was no difference between the two groups in the number of subjects with a partial response, defined as a regression from HSIL to LSIL on biopsy.

The second part of the study was an open-label study of 3 high dose, 500 mcg. subcutaneous injections of HspE7 given monthly. Patients were eligible to participate in the open label study if they still had HSIL at 3 months after their original injection of either 100 mcg. HspE7 or placebo or if they had either LSIL or HSIL at 6 months after their original injection. Eighty of the original 86 patients received the 3 high-dose injections of HspE7. The vaccine was well tolerated and there were no serious drug-related adverse effects, although the majority of subjects experienced mild to moderate reactions at the injection site. Within the first 6 months after entry into the open label high dose study, partial responses or regressions to LSIL were seen in 61 of 80 (76%) subjects, but no complete responses were seen. A complete response was defined as no dysplasia demonstrated on either cytology or biopsy. With continued follow-up, complete responses were first seen beginning at 9 months after initiation of the high dose study, or in other words, 7 months after the last high-dose injection. At 15 months of follow-up 12 of 22 (55%) patients had developed complete responses. These complete responses were durable with 12 of 14 (86%) remaining in remission at 15 months and 2 patients relapsed with LSIL. An example of

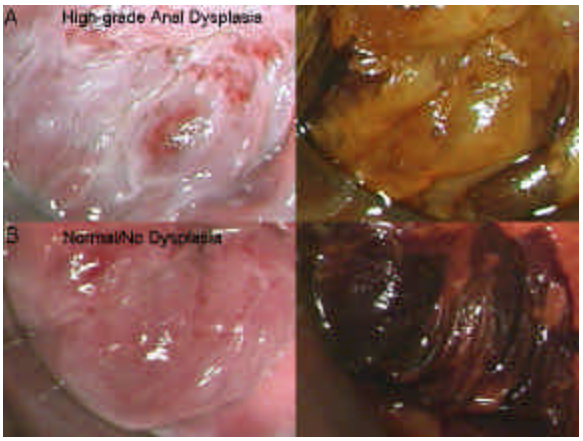

Figure 1. Resolution of anal high-grade dysplasia after treatment with the Stressgen therapeutic vaccine, HspE7. This subject had been followed in the UCSF Anal Neoplasia Study since 10/91 and initially had normal exams and Pap smears until 12/98, when a Pap smear revealed condyloma. High-grade anal dysplasia was first diagnosed in 7/99, when a biopsy at 2:00 revealed moderate dysplasia and confirmed in 11/99 with biopsies at 9:00 (the region depicted in the pictures) and 3:00 indicating severe dysplasia. In 3/00 the patient enrolled in the Stressgen HspE7 vaccine study and received either placebo or 3 injections of 100 mcg. of Hspe7 spaced a month apart. The pictures in row A are from his screening visit, the Pap smear was LSIL, a biopsy at 9:00 shown in the picture revealed atypia suggestive of HSIL and severe dysplasia at 3:00 (not shown). This picture is the best representation of HSIL and the result of atypia most likely represents sampling error since this same area was biopsied at his 6 month visit and revealed moderate dysplasia, which is consistent with the diagnosis of severe dysplasia from the same area in 11/99. The pictures on the right are after application of 3% acetic acid, which makes the abnormal areas turn white or opaque and help to reveal abnormal vascular changes, which are hallmarks of HSIL. The pictures on the right are after application of Lugol's iodine solution, which stains normal anal epithelium dark, mahogany brown as seen in row B and glycogen-poor areas of HSIL are stained yellow as in row A. At 6 months in 9/00, since he still had HSIL at 9:00, he enrolled in the second phase of the study and received 3 injection of 500 mcg. of HspE7. In 4/01, 6 months after beginning the open label high-dose HspE7, Pap smear and biopsies were LSIL and since 6/01 all biopsies and Pap smears have been normal. The picture in row B are from 6/02 and the biopsy of this same area at 9:00 is normal.

a complete response is shown in Figure 1. Although HspE7 is thought to produce HPV 16-specific immunity, the majority of responses were seen in subjects who did not have HPV 16 DNA detected (47 of 54) suggesting cross-reactivity to other types. A randomized, double-blind, placebo-controlled phase III trial of high-dose HspE7 in a similar group of patients has completed accrual and is in progress.

The UCSF group recently completed a phase I study of an encapsulated plasmid DNA (pDNA) vaccine made by ZYCOS, Inc. designated as ZYC101 in a similar cohort of patients with anal high-grade dysplasia (48). Although the primary goal was to evaluate safety in this dose escalation study, patients were evaluated for histological, immunological, and virological responses. Therapeutic activity was demonstrated in 3 of the 12 patients evaluated. The pDNA consists of the early promoter region of the human cytomegalovirus added to facilitate expression of RNA, which consists of a class II HLA secretory leader signal sequence joined to a 13 amino acid sequence proximal to the carboxy-terminus of the HPV 16 E7 protein that covers several overlapping HLA-A2 associated T cell epitopes. The resulting pDNA is encapsulated in 2 micron polylactide co-glycolide (PLG) microspheres, which is an optimal size for phagocytosis by APCs. PLG is a biodegradable polymer used in various pharmaceutical products such as suture material. The advantage of encapsulating the pDNA compared with naked DNA vaccines is a passive targeting of APCs including dendritic cells. Uptake of the pDNA then leads to E7 epitope transcription and translation into E7 peptides in the endoplasmic reticulum. In association with class I molecules, these epitopes are transported to the cell surface where they can stimulate cytotoxic T lymphocyte responses.

Twelve eligible subjects were enrolled, comprised of immunocompetent adults with anal HSIL who were HLA-A2 positive and had infection with HPV 16 documented by PCR. Beginning with the lowest dose, 3 subjects were enrolled in one of 4 dose levels: 50, 100, 200, or 400 mcg. of DNA, but there was no escalation of doses for individual patients. Subjects received 4 intramuscular injections of a single dose every 3 weeks at weeks 0, 3, 6, and 9. At baseline and at weeks 12 and 24, all subjects had an anal Pap smear for cytology and high-resolution anoscopy with biopsy of the most colposcopically abnormal areas within the anal canal or peri-anal region. PCR testing for HPV 16 was done at baseline and week 24.

ZYC101 was well tolerated and no significant adverse events occurred, although injection site pain was commonly reported. Systemic side effects consisting of mild fatigue, malaise, and fever were reported by a few patients. No histological responses were seen at the 50 and 100 mcg. doses. Although no complete responses were seen in any of the patients, 3 patients had partial responses demonstrating therapeutic activity of ZYC101: 1 of 3 patients at the 200 mcg. level had atypia at week 24, 1 of 3 patients at the 400 mcg. level had atypia at week 12 and 24, and another patient at the 400 mcg. level had a benign biopsy at week 12 and LSIL at week 24. Two of 12 patients cleared their HPV 16 infection meaning that there was no detectable HPV 16 at week 24. Since one of these patients continued to have persistent HSIL, clearance of HPV did not correlate with histological response.

Immunological response to vaccination with ZYC101 was assessed using the Elispot assay, which detects gamma-interferon production by antigen-specific T

lymphocytes. PBMC were isolated from patients and allowed to react with generic APCs that had been pulsed with the particular immunogenic peptide being evaluated. Ten of 12 subjects demonstrated a 2-fold increase in HPV 16 E7 specific lymphocyte response levels using this Elispot assay, which evaluated 3 of the 6 possible epitopes in ZYC101. Immunological response defined in this manner, however, did not correlate with histological response. Both of the immunological non-responders had high baseline levels and therefore it may have been difficult technically to demonstrate a 2-fold increase. However, when the Elispot assay was performed on cells that had been expanded from the immunological non-responder, who had a partial histological regression and also had clearance of his HPV 16 infection, an immunological response was demonstrated. Another possible explanation for the apparent lack of response is that the assays only evaluated 3 of the 6 possible epitopes. These results are encouraging and demonstrate evidence of therapeutic activity of ZYC101 in patients with anal HSIL who are HLA-A2 positive and have HPV 16 infection, but additional phase II and III studies will be required to document its true efficacy.

A phase I/II dose escalation study of ZYC101 has also been completed in a group of women with cervical HSIL at Brigham and Women's Hospital in Boston with similar results. ZYC101 was biologically and clinically active in these patients and well tolerated with no significant adverse effects reported. Zycos has developed a new vaccine called ZYC101a that has an expanded repertoire including multiple high risk HPV epitopes and is optimized for other haplotypes in addition to HLA-A2. It is currently being evaluated in a phase II, double-blind, placebo-controlled study in women with cervical HSIL.

10. CONCLUSIONS AND PERSPECTIVE

There is no doubt that HPV infection, HPV-associated dysplasia, anal cancer, and cervical cancer are major public health problems worldwide. Increasing knowledge about the natural history of anogenital dysplasia and natural immunity to HPV infection have led to the development of prophylactic and therapeutic HPV vaccines, which are clearly efficacious in preclinical animal models. Although there are much more data available from human clinical trials on safety and immunogenicity than efficacy, data on effectiveness continues to emerge and results from trials currently in progress are expected shortly. Data have been presented from a number of clinical trials evaluating a variety of different vaccines with no evidence of any significant vaccine-related adverse effects and good evidence of immunogenicity. The prophylactic HPV 16 L1 VLP vaccine developed by Merck may be effective to prevent new infections of HPV 16 from occurring. Based on preliminary results, all of the therapeutic vaccines appear to have some clinical activity and have produced complete responses in a subset of patients and meaningful regressions of disease in others. Validation of these results will require larger, randomized, placebo-controlled trials, many of which are in progress and have completed accrual. Based on this, it is clear that a number of safe, well tolerated, and effective HPV vaccines are likely to be added to our clinical armamentarium in the near future.

Many questions remain unanswered. Will ongoing clinical trials of VLP vaccines, particularly polyvalent and chimeric vaccines continue to show efficacy? Will immunity be sterilizing? What is the optimal route of administration that will engender significant mucosal protective immunity? Are there some antigens that are better than others? What is the optimal vector that will stimulate cell-mediated immunity and present antigens in such a way that all arms of the immune system are activated? What will be the durability of response? Will it last a lifetime? As more clinical trials mature, evaluating why some people do not respond to a particular vaccine will provide invaluable information. How common is escape, is it related to host factors, viral factors, or a combination of the two? Assuming that HPV vaccines work extremely well against the most common types of high-risk types of HPV, 16 and 18, will there be a significant decline in the incidence of cervical and anal cancer? Assuming that immunity is type-specific, will other less common types of HPV emerge and reach a similar level of clinical significance? Will it be possible to develop effective vaccines for the large population of people who suffer from HPV disease because of compromised immune systems? Certainly the question of whether it is possible to develop a clinically effective prophylactic or therapeutic human papillomavirus vaccine seems to have been answered and there is great hope for the future, however, much more research remains to be done.

11. ACKNOWLEDGEMENTS

This paper is dedicated to the memory of Joe Thatcher, whose care, compassion, humor, and dedication to excellence in clinical research and patient care will always be remembered.

12. REFERENCES

1. zur Hausen, H. Papillomaviruses in human cancers. *Proc Assoc Am Physicians*. 111, 581-587 (1999)

2. Frisch, M., B. Glimelius, A. J. van den Brule, J. Wohlfahrt, C. J. Meijer, J. M. Walboomers, S. Goldman, C. Svensson, H. O. Adami, & M. Melbye. Sexually transmitted infection as a cause of anal cancer. *N Engl J Med*. 337, 1350-1358 (1997)

3. Palefsky, J. M. & E. A. Holly. Molecular virology and epidemiology of human papillomavirus and cervical cancer. *Cancer Epidemiol Biomarkers Prev*. 4, 415-428. (1995)

4. Moscicki, A. B., S. Shiboski, J. Broering, K. Powell, L. Clayton, N. Jay, T. M. Darragh, R. Brescia, S. Kanowitz, S. B. Miller, J. Stone, E. Hanson, & J. Palefsky. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. *J Pediatr*. 132, 277-284 (1998)

5. Stern, P. L., M. Brown, S. N. Stacey, H. C. Kitchener, I. Hampson, E. S. Abdel-Hady, & J. V. Moore. Natural HPV immunity and vaccination strategies. *J Clin Virol*. 19, 57-66 (2000)

6. Nakagawa, M., D. P. Stites, S. Patel, S. Farhat, M. Scott, N. K. Hills, J. M. Palefsky, & A. B. Moscicki. Persistence of human papillomavirus type 16 infection is associated with lack of cytotoxic T lymphocyte response to the E6 antigens. *J Infect Dis*. 182, 595-598 (2000)

7. Bontkes, H. J., T. D. de Gruijl, A. J. van den Muysenberg, R. H. Verheijen, M. J. Stukart, C. J. Meijer, R. J. Scheper, S. N. Stacey, M. F. Duggan-Keen, P. L. Stern, S. Man, L. K. Borysiewicz, & J. M. Walboomers. Human papillomavirus type 16 E6/E7-specific cytotoxic T lymphocytes in women with cervical neoplasia. *Int J Cancer*. 88, 92-98 (2000)

8. Scott, M., M. Nakagawa, & A. B. Moscicki. Cell-mediated immune response to human papillomavirus infection. *Clin Diagn Lab Immunol*. 8, 209-220 (2001)

9. Penn, I. Cancers of the anogenital region in renal transplant recipients. *Cancer*. 58, 611-616 (1986)

10. Palefsky, J. M. Anal squamous intraepithelial lesions in human immunodeficiency virus-positive men and women. *Semin Oncol*. 27, 471-479 (2000)

11. Biggar, R. J. & C. S. Rabkin. The epidemiology of AIDS--related neoplasms. *Hematol Oncol Clin North Am*. 10, 997-1010 (1996)

12. Williams, A. B., T. M. Darragh, K. Vranizan, C. Ochia, A. R. Moss, & J. M. Palefsky. Anal and cervical human papillomavirus infection and risk of anal and cervical epithelial abnormalities in human immunodeficiency virus-infected women. *Obstet Gynecol*. 83, 205-211. (1994)

13. Wright, T. C., Jr., T. V. Ellerbrock, M. A. Chiasson, N. Van Devanter, & X. W. Sun. Cervical intraepithelial neoplasia in women infected with human immunodeficiency virus: prevalence, risk factors, and validity of Papanicolaou smears. New York Cervical Disease Study. *Obstet Gynecol*. 84, 591-597. (1994)

14. Sun, X. W., L. Kuhn, T. V. Ellerbrock, M. A. Chiasson, T. J. Bush, & T. C. Wright, Jr. Human papillomavirus infection in women infected with the human immunodeficiency virus. *N Engl J Med*. 337, 1343-1349. (1997)

15. Holly, E. A., M. L. Ralston, T. M. Darragh, R. M. Greenblatt, N. Jay, & J. M. Palefsky. Prevalence and risk factors for anal squamous intraepithelial lesions in women. *J Natl Cancer Inst.* 93, 843-849 (2001)

16. Conley, L. J., T. V. Ellerbrock, T. J. Bush, M. A. Chiasson, D. Sawo, & T. C. Wright. HIV-1 infection and risk of vulvovaginal and perianal condylomata acuminata and intraepithelial neoplasia: a prospective cohort study. *Lancet*. 359, 108-113. (2002)

17. Palefsky, J. M., E. A. Holly, M. L. Ralston, N. Jay, J. M. Berry, & T. M. Darragh. High incidence of anal high-grade squamous intra-epithelial lesions among HIV-positive and HIV-negative homosexual and bisexual men. *Aids*. 12, 495-503. (1998)

18. Palefsky, J. M., E. A. Holly, M. L. Ralston, S. P. Arthur, N. Jay, J. M. Berry, M. M. DaCosta, R. Botts, & T. M. Darragh. Anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual and bisexual men: prevalence and risk factors. *J Acquir Immune Defic Syndr Hum Retrovirol*. 17, 320-326. (1998)

19. Palefsky, J. M., E. A. Holly, C. J. Hogeboom, M. L. Ralston, M. M. DaCosta, R. Botts, J. M. Berry, N. Jay, & T. M. Darragh. Virologic, immunologic, and clinical parameters in the incidence and progression of anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual men. *J Acquir Immune Defic Syndr Hum Retrovirol*. 17, 314-319. (1998)

20. Palefsky, J. M., E. A. Holly, M. L. Ralston, M. Da Costa, H. Bonner, N. Jay, J. M. Berry, & T. M. Darragh. Effect of highly active antiretroviral therapy on the natural history of anal squamous intraepithelial lesions and anal human papillomavirus infection. *J Acquir Immune Defic Syndr*. 28, 422-428. (2001)

21. Schiller, J. T. & A. Hidesheim. Developing HPV virus-like particle vaccines to prevent cervical cancer: a progress report. *J Clin Virol*. 19, 67-74 (2000)

22. Valone, F. H., E. Small, M. MacKenzie, P. Burch, M. Lacy, M. V. Peshwa, & R. Laus. Dendritic cell-based treatment of cancer: closing in on a cellular therapy. *Cancer J*. 7 Suppl 2, S53-61 (2001)

23. Breitburd, F. & P. Coursaget. Human papillomavirus vaccines. *Semin Cancer Biol*. 9, 431-444 (1999)

24. Harro, C. D., Y. Y. Pang, R. B. Roden, A. Hildesheim, Z. Wang, M. J. Reynolds, T. C. Mast, R. Robinson, B. R. Murphy, R. A. Karron, J. Dillner, J. T. Schiller, & D. R. Lowy. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. *J Natl Cancer Inst.* 93, 284-292 (2001)

25. Herrero, R., A. Hildesheim, C. Bratti, M. Schiffman, A. C. Rodriguez, S. Wacholder, E. Freer, J. Schiller, & D. Lowy. Rationale and design of the NCI-Costa Rica HPV 16 vaccine trial. *Proceedings of the 19th International Papillomavirus Confernence, Sept 1-7, 2001*, P-94 (2001)

26. Nardelli-Haefliger, D., D. Wirthner, J. Schiller, D. Lowy, F. Hartmann, & P. DeGrand. Antibody responses in cervical secretions of female volunteers after intramuscular vaccination with purified HPV 16 VLPS. *Proceedings of the 19th International Papillomavirus Confernence, Sept 1-7, 2001*, O-52 (2001)

27. Nardelli-Haefliger, D., F. Lurati, D. Wirthner, F. Spertini, J. Schiller, D. Lowy, F. Hartmann, & P. DeGrand.

Mucosal vaccination of female volunteers with purified HPV 16 VLPS. *Proceedings of the 19th International Papillomavirus Confernence, Sept 1-7, 2001*, P-103 (2001)

28. Brown, D. R., K. A. Ault, C. Wheeler, K. U. Jansen, J. F. Smith, E. Barr, A. Dicello, M. R. Nelson, & R. Railkar. A dose-ranging study of the safety and immunogenicity profiles of a quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP candidate vaccine in young healthy women. *Proceedings of the 19th International Papillomavirus Confernence, Sept 1-7, 2001*, O-51 (2001)

29. Koutsky, L. A., L. R. Miller, G. A. Poland, R. Jacobson, G. Suhr, L. Chiacchierini, E. Barr, & K. Jansen. Persistence of HPV 16 antibodies after immunization with HPV 16 L1 virus-like particle (VLP) vaccines. *Proceedings of the 19th International Papillomavirus Confernence, Sept 1-7, 2001*, O-50 (2001)

30. Wheeler, C., D. Brown, K. Fife, J. F. Smith, A. Dicello, R. Wang, E. Barr, G. Suhr, L. Chiacchierini, & K. Jansen. A phase I study of the safety/tolerability and immunogenicity profiles of research lot HPV 11 L1 virus-like particle (VLP) vaccine: persistence of anti-HPV 11 responses. *Proceedings of the 19th International Papillomavirus Confernence, Sept 1-7, 2001*, P-90 (2001)

31. van Driel, W. J., M. E. Ressing, G. G. Kenter, R. M. Brandt, E. J. Krul, A. B. van Rossum, E. Schuuring, R. Offringa, T. Bauknecht, A. Tamm-Hermelink, P. A. van Dam, G. J. Fleuren, W. M. Kast, C. J. Melief, & J. B. Trimbos. Vaccination with HPV16 peptides of patients with advanced cervical carcinoma: clinical evaluation of a phase I-II trial. *Eur J Cancer*. 35, 946-952. (1999)

32. Ressing, M. E., W. J. van Driel, R. M. Brandt, G. G. Kenter, J. H. de Jong, T. Bauknecht, G. J. Fleuren, P. Hoogerhout, R. Offringa, A. Sette, E. Celis, H. Grey, B. J. Trimbos, W. M. Kast, & C. J. Melief. Detection of T helper responses, but not of human papillomavirus- specific cytotoxic T lymphocyte responses, after peptide vaccination of patients with cervical carcinoma. *J Immunother*. 23, 255-266. (2000)

33. Muderspach, L., S. Wilczynski, L. Roman, L. Bade, J. Felix, L. A. Small, W. M. Kast, G. Fascio, V. Marty, & J. Weber. A phase I trial of a human papillomavirus (HPV) peptide vaccine for women with high-grade cervical and vulvar intraepithelial neoplasia who are HPV 16 positive. *Clin Cancer Res*. 6, 3406-3416. (2000)

34. Borysiewicz, L. K., A. Fiander, M. Nimako, S. Man, G. W. Wilkinson, D. Westmoreland, A. S. Evans, M. Adams, S. N. Stacey, M. E. Boursnell, E. Rutherford, J. K. Hickling, & S. C. Inglis. A recombinant vaccinia virus encoding human papillomavirus types 16 and 18, E6 and E7 proteins as immunotherapy for cervical cancer. *Lancet*. 347, 1523-1527. (1996)

35. Adams, M., L. Borysiewicz, A. Fiander, S. Man, B. Jasani, H. Navabi, C. Lipetz, A. S. Evans, & M. Mason. Clinical studies of human papilloma vaccines in pre-invasive and invasive cancer. *Vaccine*. 19, 2549-2556. (2001)

36. Davidson, E., A. Tomlinson, P. Stern, J. Dobson, L. Lack, J. S. Roberts, C. Boswell, J. Hickling, & H. Kitchener. A phase II trial to assess the safety, immunogenicity and efficacy of TA-HPV in patients with high grade vulvar intraepithelial neoplasia (VIN) *Proceedings of the 19th International Papillomavirus Confernence, Sept 1-7, 2001*, P-88 (2001)

37. Xenova. Xenova Group plc press release: successful results of phase IIa trial for therapeutic vaccine TA-HPV. *http://www.xenova.co.uk/PressReleases/pr_20020327_01.html*, (2002)

38. O'Neil, T., Y. Khan, S. E. Chisholm, N. W. Whittle, L. C. Jack, J. S. Roberts, & J. K. Hickling. Safety and immunogenicity of an HPV 16L2E7E6 fusion protein vaccine (TA-CIN) in a phase I clinical trial. *Proceedings of the 19th International Papillomavirus Confernence, Sept 1-7, 2001*, P-100 (2001)

39. Burg, S. V. d., A. deJong, K. Kwappenberg, A. Y. Khan, C. Melief, T. O'Neil, J. Hickling, & R. Offringa. Induction of HPV16-specific T-cell immunity in healthy volunteers: a phase I/II study with the recombinant HPV 16 L2E6E7 fusion protein vaccine TA-CIN. *Proceedings of the 19th International Papillomavirus Confernence, Sept 1-7, 2001*, P-91 (2001)

40. Gerard, C. M., N. Baudson, K. Kraemer, C. Bruck, N. Garcon, Y. Paterson, Z. K. Pan, & D. Pardoll. Therapeutic potential of protein and adjuvant vaccinations on tumour growth. *Vaccine*. 19, 2583-2589. (2001)

41. Hallez, S., F. Maudoux, F. Buxant, I. Fayt, A. Burny, J.-C. Noel, & P. Simon. Use of cytokine flow cytometry to monitor cellular immune reponse to an HPV 16 E7-derived vaccine: phase I/II trial in women with HPV 16 positive grade 3 cervical intraepithelial neoplasia. *Proceedings of the 19th International Papillomavirus Confernence, Sept 1-7, 2001*, P-98 (2001)

42. Palefsky, J. M., E. A. Holly, C. J. Hogeboom, J. M. Berry, N. Jay, & T. M. Darragh. Anal cytology as a screening tool for anal squamous intraepithelial lesions. *J Acquir Immune Defic Syndr Hum Retrovirol*. 14, 415-422 (1997)

43. Jay, N., J. M. Berry, C. J. Hogeboom, E. A. Holly, T. M. Darragh, & J. M. Palefsky. Colposcopic appearance of anal squamous intraepithelial lesions: relationship to histopathology. *Dis Colon Rectum*. 40, 919-928 (1997)

44. Berry, J. M. & J. M. Palefsky. Pathogenesis and clinical manifestations of HIV-associated anogenital neoplasia. In: *The AIDS knowledge base: a textbook on HIV disease from the University of California, San Francisco School of Medicine, and the San Francisco General Hospital*. P. T. Cohen, M. A. Sande, & P. A. Volberding (Eds.) Philadelphia, PA: Lippincott Williams & Wilkins, (1999)

45. Chang, G. J., J. M. Berry, N. Jay, J. M. Palefsky, & M. L. Welton. Surgical treatment of high-grade anal squamous intraepithelial lesions: a prospective study. *Dis Colon Rectum*. 45, 453-458 (2002)

46. Palefsky, J. M., S. E. Goldstone, M. T. Winnett, M. I. Siegel, & J. R. Neefe. HspE7 treatment of anal dysplasia: results of an open label trial of HspE7 and comparison with a prior controlled trial of low dose HspE7. *Proceedings of the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy in Chicago, IL, Dec. 16-19, 2001*, (2001)

47. Chu, N. R., H. B. Wu, T. Wu, L. J. Boux, M. I. Siegel, & L. A. Mizzen. Immunotherapy of a human papillomavirus (HPV) type 16 E7-expressing tumour by administration of fusion protein comprising Mycobacterium bovis bacille Calmette-Guerin (BCG) hsp65 and HPV16 E7. *Clin Exp Immunol*. 121, 216-225 (2000)

48. Klencke, B., M. Matijevic, R. G. Urban, J. L. Lathey, M. L. Hedley, M. Berry, J. Thatcher, V. Weinberg, J. Wilson, T. Darragh, N. Jay, M. Da Costa, & J. M. Palefsky. Encapsulated plasmid DNA treatment for human papillomavirus 16-associated anal dysplasia: a Phase I study of ZYC101. *Clin Cancer Res*. 8, 1028-1037 (2002)

Key Words: Human Papillomavirus Vaccines, Anogenital Neoplasia, Squamous Intraepithelial Lesion, Anal Cancer, Cervical Cancer, Virus-Like Particle, Plasmid DNA Vaccine, Heat Shock Protein, Fusion Protein, Review

Send correspondence to: J. Michael Berry, MD, Assistant Clinical Professor of Stomatology, University of California, San Francisco, Box 0512, 521 Parnassus Ave., San Francisco, CA 94143-0512, Tel: 415-353-7443, Fax: 415-353-7464, E-mail: jmberry@itsa.ucsf.edu