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The impact of anti HPV vaccination on cervical cancer incidence and HPV induced cervical lesions: Consequences for clinical management

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Summary

Cervical cancer is the second most common cause of cancer-related deaths in women worldwide. Screening for cervical cancer is accomplished utilizing a Pap smear and pelvic exam. While this technology is widely available and has reduced cervical cancer incidence in industrialized nations, it is not readily available in third world countries in which cervical cancer incidence and mortality is high. Development of cervical cancer is associated with infection with high risk types of human papillomavirus (HPV) creating a unique opportunity to prevent or treat cervical cancer through anti-viral vaccination strategies. Several strategies have been examined in clinical trials for both the prevention of HPV infection and the treatment of pre-existing HPV-related disease. Clinical trials utilizing prophylactic vaccines containing virus-like particles (VLPs) indicate good vaccine efficacy and it is predicted that a prophylactic vaccine may be available within the next five years. But, preclinical research in this area continues in order to deal with issues such as cost of vaccination in underserved third world populations. A majority of clinical trials using therapeutic agents which aim to prevent the progression of pre-existing HPV associated lesions or cancers have shown limited efficacy in eradicating established tumors in humans possibly due to examining patients with more advanced-stage cancer who tend to have decreased immune function. Future trends in clinical trials with therapeutic agents will examine patients with early stage cancers or pre-invasive lesions in order to prevent invasive cervical cancer. Meanwhile, preclinical studies in this field continue and include the further exploration of peptide or protein vaccination, and the delivery of HPV antigens in DNA-based vaccines or in viral vectors. Given that cervical cancers are caused by the human papillomavirus, the prospect of therapeutic vaccination to treat existing lesions and prophylactic vaccination to prevent persistent infection with the virus are high and may be implemented in the near future. The consequences for clinical management may include a significant reduction in the frequency of Pap smear screening in the case of prophylactic vaccines, and the availability of less invasive and disfiguring treatment options for women with pre-existing HPV associated lesions in the case of therapeutic vaccines. Implementation of both prophylactic and therapeutic vaccine regimens could result in a significant reduction of health care costs and reduction of worldwide cervical cancer incidence.

Key words: Human Papillomavirus; Vaccine; Cervical cancer.

Introduction

Cancer of the uterine cervix is the second most common cause of cancer-related deaths in women worldwide. Approximately 510,000 new cases and 288,000 deaths are reported annually [1]. Nearly 80% of cases and cervical cancer mortality occur in developing countries in which access to routine gynecological examination and Papanicicoloau (Pap) smear screening is difficult [1]. In the United States Papanicicoloau (Pap) smear screening has reduced cervical cancer mortality by 70% in the last 50 years [2]. However, despite the availability of Pap smear screening, approximately 13,000 new cervical cancer cases and 4,500 deaths occur yearly in the United States making cervical cancer the tenth most common cancer in women in the US [3, 4].

The development of cervical cancer is associated with infection with high risk types of human papillomaviruses (HPV). HPV is detected in 99.7% of cervical carcinomas [5]. There are over 30 known HPV types that infect the anogenital region [6, 7]. These types are classified as being low, intermediate or high-risk based on their in-vitro ability to interact with cell cycle proteins and clinical association with causing cervical cancer. Low risk HPV types like 6 and 11 are associated with causing the sexually transmitted disease of genital warts [8]. High-risk HPV types, like 16, 18, 31, and 45 are closely associated with anogenital malignancies and have been implicated in the etiology of most, if not all cervical cancers with high risk types HPV 16 and 18 accounting for nearly 70% of cases [9]. Interestingly, while approximately 30-50% of the general population is positive for HPV DNA, infection with HPV is asymptomatic in most people largely due to host immune system's ability to control the virus and usually does not lead to cancer [9]. In contrast to many other cancers, the fact that cervical cancer is highly associated with a viral infection and that host immune responses control the infection in a majority of infected subjects, vaccination against HPV viruses may represent a unique opportunity to either prevent or treat cervical cancer. The purpose of this article is to discuss both prophylactic and therapeutic anti HPV strategies that are currently in clinical and pre-clinical trials and the impact of successful vaccination on the current standard of care for women with cervical cancer and HPV-associated cervical lesions.

A. HPV biology

The lifecycle of genital HPV encompasses infection of cervical squamous epithelium and is closely linked to keratinocyte differentiation [10]. In one form of HPV infection, the site of initial infection is thought to be the proliferating basal epithelial cells in which the viral genome is maintained as a low copy episome. As the keratinocyte undergoes differentiation, viral gene expression and genome amplification increase until late gene expression of the L1 and L2 proteins occurs in terminally differentiated superficial cells. This form of infection leads to koilocytosis, nuclear enlargement, dyskeratosis, multinucleation, and in some cases low grade SIL. At this point such lesions may regress, persist, or progress [11]. The other form of infection is non-permissive, transformable infection in which viral replication does not occur. This type of infection may be found in both squamous cells and glandular tissue. In this situation, viral DNA persists as either an extra chromosomal element or becomes integrated into the host cell chromosome at a random site [12].

HPV is a small, non-enveloped, DNA virus with icosahedral symmetry and 72 capsomeres surrounding the genome [13]. The genetic information of HPV viruses is encoded in a double stranded, circular genome that is approximately 8 Kb long [13]. The genome can be divided into three major regions: early genes, late genes, and control genes [14].

The E1 gene functions to regulate DNA replication through its interactions with the long control region (LCR) in which the E1 protein forms a complex with the E2 protein to stabilize its binding to the origin of replication [15, 16]. The E2 protein, even though it interacts with E1 to initiate replication, primarily functions to control transcription. However, in the case of E6 and E7, the oncogenic genes of HPV, E2 functions to repress their production by interacting with E1 to favor replication. Although not fully studied, a common theme is that when HPV integrates into the host cell chromosome, it does so at the E1/E2 region [17, 18]. The disruption of these genes, especially E2, results in a deregulation of E6 and E7 gene products, which leads to cellular transformation [19, 20]. E6 and E7 are the major oncogenic proteins involved in HPV carcinogenesis. Both proteins interfere with the normal mechanisms of cell cycle control [21]. HPV types that are low risk for causing cancer have weak interactions with the cell cycle control components, p53 and Rb, whereas high-risk types have stronger binding affinities for these cellular components. The E6 protein of high-risk HPV types forms a complex with the cellular protein E6 associated AP or E6 AP. The E6 AP complex then binds to and degrades p53 through ubiquitin-directed proteolysis [22, 23]. P53 is essential for DNA repair as it mediates either G1 growth arrest or apoptosis in response to DNA damage in the cell. The E7 protein of high-risk HPV types binds the retinoblastoma tumor suppressor protein (pRb) with higher affinity than low-risk types. The binding of the E7 protein to pRb causes Rb to release the transcription factor E2F. This results in activation of certain E2F dependent genes required for DNA synthesis and cell cycle control [24, 26]. The effect is deregulated cell cycle control and uncontrolled cellular proliferation. As mentioned previously, in the best characterized cases, when the virus integrates into the host chromosome, the E1/E2 genes, which regulate E6 and E7 protein production, are interrupted with a resultant increase in both of these oncogenes. This is the proposed method of HPV transformation in cervical cancer.

The late genes (L1 and L2) function to form the viral capsid. The L1 protein is the major capsid component comprising 80% of total viral protein and forms the outer pentamer, and the L2 protein, which is the minor capsid component located in the center of the pentamer, may be involved in packaging the viral DNA into the capsid [25]. DNA detection and typing of HPV viruses, unlike other viral families that are typed based on differences in surface antigens, is accomplished based on DNA sequence differences found in the L1 open reading frame (ORF). Less than 90% sequence homology of the L1 ORF to the closest related known HPV type defines a new type [26].

B. HPV immunology

Epidemiological studies involving screening cohorts for the presence of HPV antibodies indicate that neutralizing conformational IgG antibodies directed toward the major capsid antigen (L1) occur in a majority in people having past or current HPV infection. However, these antibody titers are generally low and type specific. Thus antibodies to one HPV type is not protective for other HPV types [27]. However, these data do demonstrate that through vaccination, infection with certain high-risk types can be prevented. In patients with early stage cervical carcinoma and HPV-induced CIN lesions, cytotoxic T-cell responses (CTL) have been demonstrated against HPV-16 and HPV-18 [28-30]. In addition, T-helper cell responses directed against HPV-16 E6 and E7 have been detected in patients with abnormal cervical cytology [31-35]. These studies may indicate that while effective antiviral immunity is generated in a majority of HPV infected individuals, this immunity may develop too late or be of too low of magnitude to prevent the development of cancer in some individuals.

Effective eradication of virally infected cells is generally mediated by a Th1-type immune response characterized by the presence of activated cytotoxic T lymphocytes (CTL) and CD4+ T-helper cells. CD4+ T-helper cells aid in viral eradication and tumor ablation by helping to activate and maintain CTL-mediated responses to tumor antigens through the induction of Th1 cytokines like IFN-gamma. CTLs are considered the major eradicator of both tumor and virally infected cells in the adaptive immune response. Target cells are identified by CTLs as a result of the presentation of aberrant or "non-self" antigens, typically 8-10 amino acids in length and complexed with major histocompatibility complex (MHC) class I molecules on the cell surface of a majority of cells in the body. "Self" proteins are also pre-

sented in the context of MHC I molecules and this allows the cells of the immune system to distinguish between "self" and "non-self" antigens. Aberrant, "non-self" and "self" antigens are endogenous proteins that are degraded by the proteasome complex in the cytosol before they are transported by the TAP transporter into the endoplasmic reticulum for complexing with MHC class I molecules and then transported to the cell surface through the Golgi Complex [36]. CD4+ T-helper cells interact with professional antigen-presenting cells (APC), which present viral and tumor antigens through the MHC II pathway [36]. Viral antigens or components of tumor cells that are present in the extra cellular spaces can become ingested by APCs through endocytosis and subsequently degraded into 12-15 amino acid peptides in endosomal vesicles [38]. Vesicles containing peptides fuse with vesicles containing newly synthesized MHC class II molecules in the cytosol. The MHC class II molecules and the peptide antigen associate and then the bound peptide is transported to the cell surface of the APC [38]. This process is known as exogenous antigen presentation and unlike MHC class I presentation is restricted to professional APCs such as B lymphocytes, macrophages, Langerhans' cells (LCs), and dendritic cells (DCs).

Immature LCs and DCs are professional antigen presenting cells that specialize in antigen capture in the periphery. Immature DCs reside in the dermis, whereas immature LCs are found in the epidermis of the skin and in the epithelial layers of all mucosa [39, 40]. In the genital tract, HPV infection is initiated in basal epithelial cells and LCs are believed to pick up HPV particles and cross-present these antigens to naïve T cells [41]. In the process, LCs should undergo maturation to initiate an adaptive immune response. However, the local cytokine environment in which LCs encounter HPV antigens contributed to by keratinocytes which secrete TGF-beta, and IL-10, [42] may result in a APC that is polarized to Th-2 type immunity which may result in a relatively ineffective response against solid tumors and HPV infected cells [41]. Conversely, under conditions of inflammation, keratinocytes can produce pro-inflammatory cytokines which can promote a Th1-type immune response leading to presentation of viral antigens by mature LCs and the generation of an effective immune response against HPV infection [41]. The effects of the local cytokine environment on primary introduction of HPV antigens may explain why some women resolve HPV infection while others progress. An interesting study by Fausch *et al.* demonstrated that while HPV virus-like particles (VLP) can activate DC they do not do not activate Langerhans' cells [40, 43] indicating an intriguing immune escape mechanism employed by HPV. DCs have emerged as one of the best stimulators of naïve T cells [40]. DCs have a high density of MHC and co-stimulatory molecules and they efficiently process exogenous antigens through the MHC class I pathway in order to stimulate naïve CD8+ T cells through the process of cross-presentation [36]. Targeting HPV antigens to DCs by vaccination, resulting in activation and a subsequent Th1 response, as opposed to antigen capture by LCs in a natural infection may overcome the paradigm in host immunity to HPV infection and lead to lesion regression in HPV infected subjects.

Cervical cancer screening

The medical standard for diagnosing HPV disease is the Pap smear. A Pap smear is usually taken during the course of a pelvic examination at which time a sample of exfoliated cells is taken from the cervical os. The cervical os represents a sample area of the transition zone where through the process of squamous metaplasia the columnar epithelium of the endocervix is transformed into squamous epithelium. High-risk HPV infection is most likely to begin in the transition zone [4]. Once collected, these cells are stained and examined for the presence of koilocytosis and premalignant lesions. Koilocytic cells have a high nuclear to cytoplasm ratio and the presence of these cells is virtually diagnostic for HPV-related genital tract infection [44]. The cervical lesions that are detected in HPV-derived cervical disease can be graded by two classification systems based on either the cells collected from the Pap smear or biopsy tissue. The first system describes lesions as seen from biopsy specimens as cervical intraepithelial neoplasia (CIN) and gives the lesion a grade of 1-3. CIN1 includes very mild to mild dysplasia. Mild dysplasia is characterized by 20-25% replacement of the epithelium with immature cells and high nucleus to cytoplasm ratios. CIN2 or moderate dysplasia is characterized by 50% replacement with immature cells. CIN3, which includes severe dysplasia and carcinoma *in situ* is characterized by complete or nearly complete replacement of normal epithelial cells [45]. In contrast, the Bethesda classification system, which was recently updated in 2001, describes abnormal results from Pap smears. An atypical squamous cell (ASC) is now divided into two categories. A small percentage of atypical squamous cells of undermined significance (ASC-US) may result in an underlying CIN2 or 3 biopsy. In contrast, the classification, atypical squamous cells can not exclude high-grade squamous intraepithelial lesions (ASC-H) has a predictive value for CIN2 or 3 that is intermediate between ASC-US and HSIL [46]. A low-grade squamous intraepithelial lesion (LSIL) encompasses mild dysplasia and CIN1, while high-grade squamous intraepithelial lesions (HSIL) encompass moderate to severe dysplasia CIN2/3 and carcinoma *in situ* [46]. A meta-analysis conducted by Melnikow *et al.* prior to the reclassification of ASC-US lesions in the 2001 Bethesda system estimates that approximately 68% of ASC-US lesions would regress to normal, 7% would progress to HSIL and 0.25% would progress to invasive cervical cancer, with all other lesions presumably being either ASC-US or LSIL. In the case of LSIL lesions, approximately 47% will regress to normal, 20% will progress to HSIL and 0.15% will progress to invasive cervical cancer. Of HSIL lesions, approximately 35% will regress to normal, 23% will persist as HSIL and 1.44% will progress to invasive cervical cancer within a two-year

period [47]. The 2001 Bethesda classification system also describes abnormal endocervical or endometrial glandular cells using the following classifications: Atypical glandular cells (AGC) favor neoplastic and endocervical adenocarcinoma *in situ* (AIS) [46].

A small portion of ASC-US Pap smears will have CIN biopsy readings; however, a vast majority of these lesions do not represent the presence of dysplasia. When the ASC-US diagnosis is seen on Pap smear specimens, three management options are available: one is to wait and repeat the Pap smear, another option is colposcopy, and the last option is HPV DNA testing. In patients with this diagnosis, the inclusion of HPV testing can eliminate the need for more invasive procedures such as colposcopy [46]. The diagnosis of LSIL in Pap smear specimens can represent any of the three histologic abnormalities seen on subsequent biopsy. Approximately 90% of biopsy specimens following a LGSIL Pap reading will yield CIN1, which is a lesion that usually undergoes spontaneous regression and thus requires no treatment. Approximately 10% of Pap smears that are read as LSIL will demonstrate a grade of CIN2 or 3 on biopsy. Thus it is important to follow up all LGSIL graded Pap smears with colposcopic examination. If CIN lesions or cancer are not detectable by colposcopic examination, repeat cytology is recommended at six and 12 months or HPV DNA testing at 12 months [4]. ASC-H Pap smears are managed similar to LSIL as between 24-94% of these smears will result in CIN2 or 3 lesions [48-50]. These types of lesions have the potential to become invasive cervical cancer and may require treatment. A Pap smear reading of HSIL suggests the finding of CIN2 or 3 or invasive cancer on biopsy tissue. This grade of cytology is generally managed by colposcopy and biopsy [4] and always requires immediate intervention in order to prevent cervical cancer progression [51].

Treatments for cervical dysplasia can be excisional or ablative depending on the extent of the lesion. Ablative treatments such as cryotherapy or laser evaporation therapy are an option for patients in which there is no suggestion of invasive disease or adenocarcinoma, and when colposcopic evaluation is satisfactory with no evidence of the lesion extending into the canal, but for all other cases excisional therapies such as the Loop Electrosurgical Excision Procedure (LEEP) or cervical conization are indicated [4]. A study by Mitchell *et al.* demonstrated that the recurrence rate of women having CIN2-3 who were treated with either cryotherapy, laser vaporization or LEEP was between 13-19% after 37 months [52]. Additionally, the post-treatment five-year survival rate declines from 85% in FIGO Stage I to only 11% in FIGO Stage IV [53]. These statistics underscore the need for development of anti-HPV vaccination strategies in an effort to prevent the occurrence of new cervical cancer cases and to treat existing cervical cancer disease.

HPV vaccines

A. Prophylactic vaccines

Prophylactic vaccination strategies aim to prevent initial infection with the HPV virus by inducing a neutralizing antibody response toward the L1 and the L2 capsid antigens and thus preventing viral entry into susceptible cells. Most vaccines that are currently available to treat viral related illnesses are based on injection of killed or attenuated virus. However, HPV cannot easily be propagated in tissue culture systems because it requires dividing cells in order to grow. Several investigators have reported being able to propagate some HPV types in a culture system using human foreskin epithelial cells grown on a collagen matrix. Currently, this system is extremely technically difficult and overall not practical for use in a clinical laboratory. However, many investigators now use purified recombinant L1 proteins expressed in vaccinia virus, insect cell lines, or yeast expression systems [54-56] that can assemble into virus-like particles (VLP) which mimic the structure of the virion but do not contain viral DNA.

A recent phase I clinical trial was conducted at the John Hopkins University in order to assess the safety and immunogencity of an HPV 16 L1 VLP-based vaccine produced in recombinant baculovirus by the Novavax corporation [57]. In this dose-escalation study, 72 healthy volunteers were randomized to receive either 10 μg or 50 μg doses with or without adjuvant in an intramuscular injection. The results of this study indicated that the vaccine was well tolerated at both dose levels. The majority of vaccinated patients produced antibody titers that were approximately 40-fold higher than those occurring during natural infection. However, the patient group that was vaccinated with the 50 μg dose without adjuvant produced the highest neutralizing antibody titers. A follow-up phase II clinical trial was also conducted using this same vaccine agent and dose without adjuvant in order to access the cellular immune response to the vaccine. After vaccination at months 0, 1, and 6, lymphoproliferation and cytokine responses were evaluated from peripheral blood mononuclear cells (PBMC) in 43 healthy volunteers. At months 2 and 7 after the start of the study, significant increases in both T-cell proliferation and IL-5, IL-10, and IFN-γ cytokine production were detected in vaccinated subjects, suggesting that VLP vaccination may not only result in production of neutralizing antibodies but also may stimulate the Cellular Mediated Immune Response (CMI) response to prevent infection [58].

A double-blind randomized clinical trial conducted by Koutsky and colleagues utilized an HPV-16 L1 VLP vaccine formulated by Merck Research laboratories and delivered in aluminum hydroxyphosphate sulfate adjuvant in 2,392 young women aged 16-23 years old who were negative for both HPV-16 DNA and antibodies. Three vaccinations were given at day 0, two months and six months. The median follow-up period was 17.4 months and the primary endpoint was persistent HPV-16 infection defined as the presence of HPV-16 DNA detected at two or more visits. Within this time period, the vaccine demonstrated 100% efficacy, with all nine cases of HPV-16 related CIN lesions occurring in

the placebo group [59]. Currently, a phase III international trial is underway of a VLP vaccine containing HPV types 6, 11, 16, and 18 L1 capsid antigens. It is projected that this vaccine may by available in four to six years and that it can eliminate approximately 70% of invasive cancers, 60% of high-grade CIN lesions, and 90% of genital wart infections [60].

At the 21st international HPV conference and clinical workshop held in Mexico City in February 2004 GlaxoSmithKline and Med Immune announced the results of a pilot efficacy trial of an HPV 16/18 VLP vaccine formulated with 3-deacylated monophosporyl lipid A (AS04) and aluminum salts [61]. A previous clinical trial indicated that the vaccine was well tolerated and induced high titers of neutralizing antibodies and strong cell mediated immune responses to HPV 16 and 18, warranting conducting a pilot study [62]. The pilot study involved 1,113 women with no prior HPV infection between the ages of 15-25 years old who were monitored from 18-27 months by HPV DNA testing, cytology, and antibody levels every six months. Additionally, the women self-sampled for HPV DNA every three months. Considering all samples combined, vaccine efficacy for incident HPV 16 and/or 18 infections was 73.6% and 100% for persistent infections. Six women in the placebo group developed persistent HPV-16 CIN1 or 2 lesions. However, no women in the vaccinated group developed persistent HPV 16/18-related lesions [61]. Due to the encouraging results of this study, GlaxoSmithKline and MedImmune are preparing a phase III clinical trial utilizing this agent over a four-year time period in order to study long-term efficacy of the vaccine agent. In addition, GlaxoSmithKline and MedImmune are currently conducting research on production of HPV-16 VLPs in potatoes and other edible plants and delivering these orally in association with E. coli delivered antigens as adjuvants. The idea behind producing an oral HPV vaccine is that administration does not require an injection or a physician and could be readily delivered in third world countries where an HPV vaccine is needed most [63]. Also oral vaccine administration with bacterial derived adjuvants may stimulate a protective mucosal immune response that may be not induced by either natural HPV infection or vaccination with VLP alone. Utilizing heat labile enterotoxin derived from E. coli or CpG DNA co-administered orally with HPV-16 and 18 VLPs, Gerber *et al.* demonstrated significant increases in anti-VLP antibody titers in genital mucosal secretions and peripheral blood in mice [64]. Another recent preclinical study in mice demonstrated that mucosal administration of a Salmonella recombinant strain expressing HPV-16 L1 VLPs induced anti-tumor immunity in both a therapeutic and prophylactic setting. The investigators speculate that this route of administration may be more effective at inducing long-term immunity and stimulating a CMI response to HPV antigens [65]. Ongoing clinical trials reflect the prospect of available prophylactic HPV vaccination in the near future. However, preclinical studies are still necessary to determine a route of delivery that provides the best immunity against HPV infection and wide spread administration to third world countries where cervical cancer is one of the leading causes of cancer-related deaths in women [65].

One last approach to anti-HPV prophylactic vaccination involves the use of chimeric VLPs containing L1 or L2 recombinant proteins fused to modified E7 or E2 antigens. It is hoped that by containing early HPV antigens this vaccine candidate will have therapeutic properties as well. This technology is being developed by Medigene and Schering in association with the National Cancer Institute and preliminary studies have demonstrated the safety of the vaccine agent in humans [1]. However, Da Silva *et al.* have demonstrated that the existence of neutralizing antibodies to chimeric VLPs may prevent boosting of the CMI response in subsequent vaccinations in a mouse model [66]. Therefore, if multiple vaccinations of the agent are required to achieve either a prophylactic or therapeutic effect, the immunogenicity and efficacy of this strategy remains to be established.

Therapeutic vaccines

1. Peptide and protein-based vaccines

Therapeutic vaccination against HPV attempts to induce strong CTL and T-Helper cell responses to HPV antigens, such as the E6 and E7 oncoproteins, which unlike the L1 and L2 capsid antigens are expressed in precancerous and cancerous tissue. In particular the E7 oncoprotein contains epitopes that are processed and presented in association with the HLA-A2 MHC class I molecule [67]. MHC class I restricted peptide epitopes that have been generated for the E7 protein of HPV 16 [68] have demonstrated immunogenicity in both human and animal models [69, 70]. For these reasons peptides generated from the E7 protein have been used extensively in clinical trials attempting to treat HPV-associated cancers.

A phase I clinical trial sought to examine the efficacy of multiple administrations of an HLA-A*0201-restricted HPV-16 E7 lipopeptide vaccine linked to the non-specific helper peptide PADRE in 12 women with refractory cervical or vaginal cancer [67]. Four subcutaneous injections (s.c.) of the E7 86-93 lipopeptide were given at three-week intervals. Five of seven patients that were evaluable after two vaccinations and two-thirds of patients that were evaluable after all four vaccinations demonstrated a E7 peptide specific CTL response as determined by IFN-γ release assay. However, no clinical responses were associated with vaccination in these studies.

Another phase I-II clinical trial utilized two HPV-16 E7 peptides and the PADRE helper peptide delivered in incomplete Freund's adjuvant (IFA) to perform a dose escalation study in 19 HLA-A*0201+ patients with HPV-16+ cervi-

cal carcinoma [71]. Successive patient groups received 100 μg, 300 μg, or 1000 μg of each peptide s.c. every three weeks for four vaccinations. Utilizing radiological evaluation, two patients demonstrated stable disease for one year after vaccination and two patients demonstrated tumor regression after vaccination and subsequent chemotherapy treatments. All other patients experienced progressive disease. Immunohistochemical evaluation of skin biopsies taken from the site of vaccination demonstrated an influx of CD8+, CD4+, and CD3+ T cells in three patients analyzed. A large portion of the patients in the study demonstrated lymphopenia both before and after vaccination, indicating that this study population was fairly immunocompromised due to the nature of their disease. Immunological analysis from peripheral blood mononuclear cells measured after vaccination indicated that there was no E7 peptide-specific CTL induction in these patients at any dose [72] perhaps indicating that these patients were too advanced to benefit from peptide immunization.

A recent phase I trial was conducted in women with high-grade cervical or vulvar intraepithelial neoplasia who were HLA-A2 and HPV-16 positive [73]. Eighteen patients were treated with four vaccinations given three weeks apart with escalating doses of the E7 peptide 12-20 in incomplete Freund's adjuvant (IFA). Starting with the eleventh patient, the E7 peptide 86-93 linked to the PADRE helper peptide with a covalently linked lipid tail was added to the vaccine regimen. Three patients demonstrated complete and nine of 17 demonstrated partial regression of dysplastic lesions. Although T cell frequencies remained the same, six of six patients tested by immunohistochemical staining demonstrated an increase in DC infiltrate in dysplastic tissue. Although positive delayed-type hypersensitivity (DTH) responses were not detected in any patients after vaccination, E7 peptide specific cytotoxic T lymphocytes (CTL) responses were detected from PBMCs in ten of 16 patients. Lastly, *in situ* RNA hybridization revealed that all biopsy specimens were positive for HPV viral RNA after vaccination, although at a much reduced level. Thus, while the agent in this study was able to effect complete regression in three patients and partial regression in nine others, demonstration of viral RNA in biopsy specimens post vaccination may be an indication of insufficient follow-up time in order to demonstrate complete viral clearance.

Although in a majority of HPV-peptide-based vaccine trials an HPV-specific CTL response has been detected in some patients, tumor regression has been minimal and these responses do not correlate with clinical outcome. An explanation for the lack of correlation between peptide specific T-cell responses and clinical outcome may involve the general state of the patients' immune system such that more advanced stage cancer patients tend to have decreased immune function that correlates with stage of disease. Many of the studies discussed above included patients with advanced stage cancers. Obviously, it is necessary to examine peptide or protein-based vaccination in patients with early-stage cancers or even pre-invasive lesions in order to access whether in vitro immune reactivity can predict tumor regression or prevent disease progression. One such clinical trial was recently conducted in women having CIN1-3 lesions [74]. The vaccine agent was a fusion protein (PD-E7) containing mutated HPV-16 E7 protein conjugated to protein D of *Haemophilus influenzae* and delivered intramuscularly with AS02B adjuvant. Five out of seven patients in the study demonstrated significant increases in IFN-γ responses and all vaccinated patients had significant titers of E7 antibodies. However, clinical responses were not correlated with immune responses in this study and the investigators suggest that the efficacy of this agent needs to be further examined in a larger cohort of CIN patients having a high and persistent HPV viral load. Similarly, StressGen has developed a recombinant fusion protein consisting of heat shock protein (Hsp 65) from *Mycobacterium Bovis* BCG and the E7 protein of HPV 16 to treat HPV-related diseases. A recent Phase II clinical trial utilizing this agent demonstrated high statistical significance for lengthening the interval between debulking surgeries for patients with recurrent respiratory papillomatosis (RRP). The result from a separate phase II clinical trial demonstrated that HspE7 is likely active in anal intraepithelial neoplasia (AIN) in converting most patients from high-grade to low-grade squamous intraepithelial lesions within three to six months of starting therapy. However, a recent adjunct of this trial involving 133 patients, designed to confirm previous results, indicated that the drug exceeded the treatment effect, but the anticipated placebo effect doubled as estimated by experts through studies of natural history, and thus there was no difference between drug and placebo. In this study there was a highly discordant level of interpreted degree of dysplasia between separate pathologists and thus additional measurements of HPV, such as viral load, need to be evaluated before Phase III dysplasia trials are initiated. In the future, StressGen may also evaluate the use of this agent to treat women with HPV-related CIN lesions [1, 75].

Two recent clinical trials have examined therapeutic HPV-protein-based vaccine strategies in subjects that do not have HPV-related disease. A phase I clinical trial was recently conducted in 40 healthy volunteers [76]. The vaccine agent utilized for these studies, TA-CIN, is a single fusion protein of HPV-16 L2, E6 and E7 proteins and it was administered without adjuvant intramuscularly in three doses. The results indicated that E6 and E7 specific T-cell immunity was induced in eight of 11 evaluable subjects at the highest dose as well as antibody and T-cell proliferative responses. A similar recombinant bacterial fusion protein of HPV-16 E6 and E7 has been made by CSL and was delivered in ISCOMATRIX® adjuvant intramuscularly to 42 healthy volunteers in a Phase I clinical trial. After three injections of the vaccine agent, measurable T-cell responses were detected in 80% of vaccinated subjects and all vaccine recipients demonstrated an HPV-16 E6/E7 antibody response. The preliminary findings in these trials are indeed encouraging but require further evaluation in patients having CIN lesions in order to access clinical efficacy [1, 77].

Preclinical research utilizing peptide-based vaccines has shifted to the examination of peptides generated from other

early genes of HPV that are expressed in cervical cancers delivered with bacterial adjuvants such as CpG oligodeoxynucleotides [78] and to the administration of long peptides or whole protein vaccines as mentioned above. The trend of utilizing long peptides and whole protein is attributable to the desire to allow presentation of more CTL epitopes and T-helper epitopes. As mentioned previously, T-helper cells are necessary to enhance CTL-mediated tumor killing and for the generation of memory responses. A preclinical study examined prime-boost vaccination with a 35 amino acid HPV-16 E7 peptide that included both CTL and T helper epitopes administered with oligodeoxynucleotide-CpG as adjuvant to activate DCs in C57BL/6 mice and compared their results to mice that were immunized with a minimal E7 CTL epitope [79]. The results demonstrated that a more vigorous CTL response and enhanced tumor eradication were seen when vaccinating with the long peptide as compared to vaccination with the minimal CTL epitope. The results of these studies are encouraging and indicate the need for further investigation into vaccination with long peptides and whole proteins and additional exploration into the use of prime boost strategies to vaccinate with peptide-based agents.

2. DNA vaccines

Recent efforts in the field of HPV tumor immunotherapy have focused on the delivery of peptides in the form of mini-gene vaccines or naked DNA vaccines. DNA-based vaccines are attractive candidates for therapeutic vaccines because of simplicity of delivery, stability, and overall safety. DNA-based vaccines can either stably integrate into the genome or be maintained in an episomal form allowing for extended expression of HPV antigens over a longer period of time than peptide vaccination. Furthermore, administration of DNA-based vaccines has been shown to induce both CTL and antibody responses to HPV antigens [80]. To date, two phase I clinical trials assessing the safety of mini-gene administration in the delivery vehicle have taken place. One study examined this vaccine regimen in 12 patients with HPV-16 associated anal dysplasia [81] and a more recent study examined this vaccination strategy in 15 women with CIN 2 or 3 [82]. The ZYC101 vaccination agent utilized for both of these studies consists of bacterial expression plasmid that expresses a segment of the HPV-16 E7 gene which includes several overlapping CTL epitopes and is fused to a secretory leader sequence derived from the HLA-DRA*0101 locus [81, 82]. The construct was delivered to patients on a dose-escalating scale either by intramuscular (IM) injection [81] or IM and s.c. injection [82] encapsulated in polylactide-co-glycolide particles, which enhances delivery to antigen-presenting cells [81]. All of the patients utilized in both studies were HLA-A2 and HPV-16 positive. After four IM injections at three week intervals, three of 12 patients demonstrated partial regression of anal dysplasia lesions and ten of 12 patients demonstrated increased peptide-specific immune responses as determined by direct ELISPOT assay. All ten of these patients maintained heightened immune responses for at least six months and no serious adverse effects were seen [81]. In a study examining women with CIN, three treatments were given at three-week intervals and colposcopy was performed at each treatment and at a four-month follow-up period. Eleven of 15 women in the study demonstrated peptide-specific T-cell responses as judged by IFN-γ release in ELISA assay and five women demonstrated complete regression of cervical lesions. Four of five of the women who regressed developed cervical IgA responses to the E2 protein of HPV-16, suggesting that HPV infected cells are undergoing lysis as a result of immune stimulation due to vaccination and that this is in effect causing epitope spreading of other HPV antigens that are not included in the vaccine formulation [82]. A follow-up report, utilizing a similar agent designated as ZYC101a was recently presented at the 21st International Papillomavirus Conference. This multi-center study involved 127 women with biopsy confirmed CIN 2/3 lesions. Six months after vaccine administration, a higher proportion of vaccinated subjects resolved their lesions than those in the placebo group. However, this effect was most pronounced in subjects who were less than 25 years of age [83]. The preliminary results of this study are slightly encouraging; however the study is still ongoing in order to measure the durability of the agent's effect over a longer follow-up period.

Preclinical studies using DNA-based vaccine approaches tend to focus on enhancing presentation of HPV antigens via the MHC class I pathway. Several different approaches have been examined including the attachment of ubiquitin to DNA constructs [84, 85], targeting of tumor antigens to centrosomal compartments to enhance MHC class I presentation [86] or the attachment to constructs of calreticulin to target HPV antigens to the MHC I pathway [87], optimization of codon usage for enhanced expression and presentation of HPV oncogenes [88-90], utilization of modified or duplicated genes to increase the number of available CTL epitopes [91-93] or enhancement of intercellular spreading in order to increase the number of cells expressing antigen [94]. All of these approaches have demonstrated increased antigen presentation and superior tumor eradication in mouse models. However, further examination is necessary in order to determine if these effects can be achieved in humans.

3. Delivery of HPV antigens through viral vectors

Delivery of HPV antigens, particularly those derived from the E6 and E7 oncogenes, in recombinant viruses poses an attractive approach for therapeutic vaccination. It offers an advantage over peptide immunization in that CTL epitopes can be processed and presented naturally and are delivered more efficiently to target cells, and it has an advantage over DNA-based vaccines because it increases the efficiency of introducing heterologous genes into target cells

[95]. Viruses like vaccinia, the agent utilized to vaccinate against the disease small pox, are lytic and extremely immunogenic. The lysis of vaccinia virally infected cells insures uptake of the HPV antigens and the vaccinia virus acts as an adjuvant to enhance the immune response to the HPV antigens. One vaccine based on this scheme is the TA-HPV vaccine which was recently examined for immunogenicity and safety in 29 patients with Stage Ib or IIa cervical cancer [96]. The TA-HPV vaccine is a recombinant vaccinia virus that expresses modified forms of the HPV-16 and 18 E6 and E7 oncoproteins. The vaccination was given in two applications ranging from four to 12 weeks apart by dermal scarification and was thought to deliver approximately 2.5 x 10^5 pfu. The results indicated that after a single vaccination four patients developed HPV-specific CTL responses and eight patients developed HPV-specific serological responses. Currently, the investigators of this study are continuing examination of this agent in patients with vulvar and vaginal intraepithelial neoplasia (VIN) [98, 99]. Similarly, Transgene has developed an MVA-HPV-IL-2 vaccine that is currently being tested in three phase II clinical trials for patients with VIN, CIN, or cervical cancer. The MVA-HPV-IL2 agent is based on the modified virus ankara (MVA), a non-propagative highly attenuated vaccinia virus strain expressing modified E6 and E7 proteins as well as IL-2 to enhance both specific and non-specific cellular immune responses. In the study involving patients with CIN lesions partial clinical and/or histological responses were observed in five out of the 15 patients treated with a high dose of the agent while CIN regression was not observed in the 12 patients treated with the low dose. In the study involving patients with cervical cancer two out of 27 patients demonstrated stable disease at six months when treated with a low dose of the agent. In the clinical trial involving patients with VIN, clinical results showed no difference in the patients treated with the vaccine compared to the controlled placebo group. However, given the low dose of the agent that was utilized for this and the cervical cancer study, and given the dose-related effect seen in the CIN2/3 trial, these results were probably due to the low dose used and the advanced stage of the disease of these patients [99]. Despite the encouraging results of these studies, there may be problems involved in immunizing with vaccinia virus. One problem may lie in the fact that a majority of women in the US over the age of 35 have been previously vaccinated with vaccinia virus and women in this age group are at the peak age for cervical cancer acquisition. In the present study, nine patients had prior vaccination with vaccinia virus but none of the four women who developed a CTL response to the HPV antigens had been previously vaccinated. Therefore it is possible that childhood vaccination with vaccinia virus, in an effort to eradicate small pox, may induce a strong and early memory response that may supersede the response to HPV antigens. One way to overcome this challenge may be heterologous boosting. In animal models, Da Silva et al. have demonstrated that the existence of neutralizing antibodies to chimeric VLPs may prevent boosting of the CMI response in subsequent vaccinations [66]. This effect however can be overcome by utilizing different chimeric or heterologous VLPs in prime-boost vaccination strategies [100]. Van der Burg *et al.* demonstrated enhanced CTL immunity in mice by combining TA-HPV, a vaccinia based vaccine containing HPV 16/18 E6/E7, and TA-CIN containing HPV 16 L2, E6 and E7 as a single fusion protein, in a heterologous prime-boost strategy [101]. Currently, this prime-boost regime is being utilized in a phase II clinical trial and preliminary results were reported at the 21st International Papillomavirus conference [102]. Twenty-nine women with high-grade vaginal intraepithelial neoplasia (VIN) received three intramuscular injections of TA-CIN followed by a single dermal scarification with TA-HPV. After twelve weeks 11/27 patients demonstrated increased T-cell proliferation responses to HPV antigens and HPV 16 E6 or E7 specific T cells were identified in 11/25 via IFN-gamma production in ELISPOT assay. Correlation of immunological responses to clinical responses will be reported at the conclusion of that study.

Similarly, preclinical studies have been conducted in mice using adenovirus vectors which have demonstrated good protection against HPV-16+ tumor challenges [103]. However, given that vaccinia and adenoviruses are DNA viruses, another potential draw back of using these vectors to deliver HPV E6 and E7 oncogenes in therapeutic vaccines is the potential integration of these oncogenes into the host genome. Some investigators are attempting to overcome the issues of pre-existing immunity and oncogene integration by using alphavirus vectors in therapeutic vaccine regimens. Alphaviruses such as Sindbis virus, Venezuelan equine encephalitis virus (VEE) or Semliki forest virus (SFV) are cytopathic RNA viruses that express the RNA of the E6 and E7 oncogenes in the cell cytosol and thus eliminate the potential integration of these oncogenes into the host cell chromosome. Also there is no pre-existing immunity to these viruses in a majority of individuals [80, 104]. Additionally, Alpha virus vectors have a very wide host range and can infect a variety of different cell types [105] which makes them attractive candidates for delivering antigenic components of immunizing vaccines.

Administration of Sindbis virus replicon particles containing herpes simplex virus type 1 (HSV-1) tegument protein linked to HPV-16 E7 resulted in the spread of E7 antigen to neighboring cells and caused a significant increase in the number of E7-specific CD8+T cell precursors and a strong anti-tumor effect against E7-expressing tumors in C57BL/6 mice [95]. Similarly, immunization with recombinant SFV encoding a fusion protein of HPV-16 E6 an E7 resulted in complete elimination of established tumors and induced a long-term high level of antigen specific CTL activity in tumor bearing mice [104]. Further advances in using the SFV alpha virus vector may be seen by linking *Mycobacterium tuberculosis* heat shock protein 70 (Hsp70) to HPV-16 E7. In a recent study, SFV vector containing E7/Hsp70 fusion genes generated significantly higher E7-specific T cell-mediated immune responses than SFV vector containing the wild-type E7 gene in vaccinated mice. Furthermore the E7/Hsp70 fusion vaccine demonstrated significant potency against established E7-expressing metastatic tumors in mice [106].

Venezuelan equine encephalitis virus (VEE) replicon particles are unlike other alpha viruses because they have the ability to infect dendritic cells which perhaps leads to better antigen delivery to naïve T-cells present in the lymph nodes [107]. Recent studies utilizing this agent contained mutated, fused E6 and E7 genes of HPV-16 and demonstrated 100% protection from tumor challenge in vaccinated mice. Additionally, eradication of established tumors was observed in 90% of C57/BL6 and HLA-A*0201 transgenic mice during therapeutic vaccination [107]. Although these are preclinical studies, the eradication of tumors in the HLA-A*0201 transgenic mice is significant because these mice bear the most common human leukocyte antigen in the human population. These encouraging results provide incentive for further examination of this agent in clinical trials.

Taken together, results from preclinical studies of tumor eradication with therapeutic alpha virus vaccination strategies are very promising and are likely to be taken into clinical trials in the near future. Ongoing research in delivery of HPV antigens in the form of viral vectors may include other viral vectors like flaviviruses or Kunjin virus replicons which are non-cytopathic and may allow prolonged expression of inserted genes for presentation to the host's immune response for the propose of enhancing T-cell memory [108].

Impact of prophylactic/therapeutic vaccine administration on clinical management of cervical cancer

Therapeutic vaccination against HPV oncogenes in established HPV lesions are attractive treatment alternatives because recurrent lesions are sometimes seen after conventional medical treatments such as LEEP and cone biopsy procedures. Additionally, therapeutic vaccination with high efficacy would provide a less invasive and likely less costly treatment option to patients with HPV-associated lesions and cancers, and is less likely to affect future fertility in premenopausal women. Lastly, once invasive cervical cancer develops, treatment is likely to impact fertility and prognosis worsens with increasing stage. Even though a majority of therapeutic clinical trials discussed above have not been successful at tumor eradication in advanced stage cancer patients, there is the prospect of utilizing therapeutic vaccination to treat women with precancerous lesions in which the immune response has not been significantly compromised. Future and some current clinical trials are utilizing therapeutic vaccine agents to treat pre-cancerous lesions in order to prevent progression of invasive cervical cancer.

The implications of treating HPV-associated lesions with therapeutic vaccination as opposed to current procedures including LEEP and cone biopsy can have far reaching consequences on clinical management. LEEP and cone biopsy procedures can cause damage to the cervix such as stenosis and cervical incompetence leading to miscarriage. Additionally, these procedures can result in more painful menstrual cycles, excessive bleeding after surgery, secondary infection as the result of surgery, and increased difficultly in taking future Pap smears [4]. Moreover, because these are surgical procedures, when anesthesia is indicated, patient fatality is a remote outcome and these procedures are far more costly than a vaccination which could be administered in a doctor's office. Lastly, given that there is a high recurrence rate with these procedures, especially when analyzed over a lifelong period, elimination of the viral infection via vaccination could possibly reduce HPV recurrence leading to a reduction in follow-up visits and eliminate the need for other treatments and procedures or perhaps lower the recurrence rate if vaccination were to be utilized in conjunction with these procedures.

Prophylactic vaccination against oncogenic HPV viruses may be widely available for administration in the next several years with the expectation that vaccination can reduce the overall incidence of cervical cancer and the cost of cervical cancer screening. A study presented at the 21st International Papillomavirus meeting sought to examine the healthcare costs of HPV screening and related disease in a population of females in the United States. At the Kaiser Permanente Health plan the total cost of cervical cancer screening and treatment for HPV-related disease was $26,415 for every thousand female enrollees with routine cervical cancer screening comprising approximately 66%, managing cervical precancers comprising 17%, treating invasive cervical cancers comprising 10%, and management of false positive Pap smears comprising 9% of annual costs [109]. Recently, a large Markov model of the natural history of HPV infection was conducted from a population of women in the United Kingdom in order to determine if vaccination and screening compared to screening with advanced measures such as HPV DNA detection could decrease cervical cancer risk. The results of the study indicated that increasing the sensitivity of screening by adding adjunct testing could decrease cervical cancer incidence by 27%. In comparison, the addition of prophylactic vaccination to existing screening programs could reduce the incidence of cervical cancer between 11-68% depending on vaccine efficacy [110]. Furthermore a study conducted by Goldie *et al.* utilized a computer based model to predict the most effective strategy in terms of cost and reduction in cervical cancer incidence of combined screening and prophylactic vaccination. Their model predicted that the most cost effective strategy was one combining vaccination at age 12 and triennial cytology screening beginning at age 25. This strategy is predicted to reduce the absolute lifetime risk of cervical cancer by 94% compared to no intervention. Because this strategy utilizes a later age at induction of screening and less frequent screening intervals it is likely to reduce the cost of cervical cancer screening [111]. Thus, even though pelvic examination is important for detection of other conditions and sexually transmitted diseases in women, a reduction in years between Pap smear screenings made possible by prophylactic HPV vaccination, could greatly decrease the cost of cervical cancer prevention to industrialized healthcare infrastructures and reduce the incidence of cervical cancers in third world

nations in which these infrastructures are not in place. However, there may be several issues related to implementation of prophylactic HPV vaccination. One issue may lay in the adolescent population that is targeted for vaccination. Parents may be reluctant to vaccinate their child against a sexually transmitted disease fearing premature discussions of sexual activity or being stigmatized and assumed to be sexually active at a young age. Another issue may be whether or not to vaccinate the male population which is involved in transmission of the virus but for whom direct consequences of high-risk HPV infection are rarely seen. It may prove to be extremely difficult to convince a population to be vaccinated in order to prevent a disease that does not directly effect that population. In addition, an important study conducted by Nardelli-Haefliger *et al.* demonstrated that cervical titers of HPV specific IgG and IgA antibodies varied greatly in vaccinated subjects due to the influence of the menstrual cycle [112]. The hormonal influence on the presence of neutralizing HPV antibodies may provide an argument for vaccination of male subjects. However, further research needs to be conducted in order to determine if vaccination in males is necessary to reduce cervical cancer incidence. Parents may also fear that vaccination against a sexually transmitted disease (STD) may produce a sense of invulnerability towards all STDs and encourage risky sexual behaviors in adolescents [113]. Indeed two recent studies conducted on HPV education indicated that a majority of women are unaware of the link between HPV and cervical cancer [114] and that knowledge of HPV was greater in older persons and married adults [115]. This perhaps stresses the need for educational programs about HPV infection and cervical cancer before widespread prophylactic vaccination can be implemented.

Other important issues in prophylactic vaccine development is the fact that VLP vaccination will have no effect on millions of women already infected with HPV and that millions of women will become infected before the vaccine is made available. Even after vaccination is implemented it is estimated that a reduction in the incidence of cervical cancer will not be apparent for at least ten years [116]. This perhaps stresses the importance of continued therapeutic vaccine development. Another important issue lies in the fact that although HPV-16 and HPV-18 are associated with a majority of cervical cancers, other high-risk types like 31, 33, 45, 52, and 58 have also been associated with cervical cancer. It may be possible that other high-risk types will emerge to fill the niche once the current high-risk types have been eliminated. If this were indeed the case, then prophylactic vaccine development will need to continually evolve to meet the challenges of new high-risk, cancer causing viruses.

One last important issue in prophylactic HPV development and implementation may be cost of vaccination. Many common viral vaccines are produced by propagation in tissue culture. However, HPV can not be easily propagated in tissue culture systems and thus VLP vaccines are produced in yeast or insect cell lines, which tend to be much more expensive. Thus, the high cost of VLP vaccination may simply be too high for developing countries where preventative vaccines are needed most. Therefore, a trend in the continuing development of prophylactic HPV vaccines will likely be the application of such vaccines in a more cost effective way or the development of alternative vaccines to cover such underserved populations.

Conclusions

Since infection with high-risk types of human papillomavirus is so closely associated with the development of cervical cancer, several vaccination strategies have been examined in clinical trials for both the prevention of HPV infection and the treatment of pre-existing HPV-related disease. Prophylactic vaccines contain virus-like particles (VLPs) that express HPV viral capsid antigens in a conformational manner and aim to induce neutralizing antibodies to prevent infection with high-risk HPV types. Preliminary findings from these clinical trials indicate good vaccine efficacy and it is predicted that a prophylactic vaccine may be available within the next decade. However, issues such as cost of vaccination in underserved third world populations have propelled preclinical research in this field to look for alternative routes of administration or vaccine candidates. An additional issue that may need to be addressed for prophylactic vaccination in the future includes other high-risk HPV types emerging as major causes of cervical cancer once the most common types, HPV-16 and 18, are eliminated through vaccination.

Therapeutic vaccination aims to prevent the progression of pre-existing HPV associated lesions or cancers by targeting the E6 or E7 oncogenes because of their role in cellular transformation and continuous expression in cervical cancers. A majority of clinical trials using therapeutic agents have shown limited efficacy in eradicating established tumors in humans. The lack of clinical outcome seen in these studies may involve the general state of the patients' immune system such that more advanced stage cancer patients tend to have decreased immune function that correlates with stage of disease. Many of the studies examining therapeutic vaccination included patients with advanced stage cancers. Future trends in clinical trials with therapeutic agents will examine patients with early-stage cancers and pre-invasive lesions in order to prevent invasive cervical cancer. Meanwhile preclinical studies in this field continue and include the further exploration of peptide or protein vaccination, and the delivery of HPV antigens in DNA-based vaccines or in viral vectors. Given that a majority of cervical cancers are caused by the human papillomavirus, the prospects of therapeutic vaccination to treat existing lesions and especially prophylactic vaccination to prevent initial infection with the virus seem highly likely and will be implemented in the near future. The consequences for clinical management may include a significant reduction in the frequency of Pap smear screening in the case of prophylactic vaccines, and will

include the availability of less invasive and disfiguring treatment options for women with pre-existing HPV-associated lesions in the case of therapeutic vaccines. Implementation of both prophylactic and therapeutic vaccine regimens could result in a significant reduction in both healthcare costs and worldwide cervical cancer incidence.

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