# Human papillomavirus, it's genes… and cancer vaccines

**Genital human papillomavirus (HPV) infection is causally linked to the development of cervical cancer, a leading cause of cancer-related mortality in women worldwide. Recent studies demonstrate the effectiveness of virus-like particle-based vaccines to induce neutralizing antibodies against HPV and prevent cervical intraepithelial neoplasia.**

Despite the implementation of cervical cytology screening programs over 50 years ago, cervical cancer remains the second most common cause of cancer death in women worldwide. The etiological role of human papillomavirus (HPV) infection in the development of anogenital cancers has been firmly established, and epidemiologic research indicates that genital HPV infections are widespread among adults who have been sexually active and have the highest incidence of any sexually transmitted disease (STD) in the United States (Cates, 1999). More than 100 different HPV genotypes have been identified and approximately 30 have been shown to have a propensity to infect anogenital tract tissues. Mucosotropic HPVs are grouped into low-risk or high-risk categories on the basis of each genotype's association with a benign or malignant disease process, and high-risk HPV DNA is detected in more than 99% of all cancers of the uterine cervix (Walboomers et al., 1999). Although most anogenital HPV infections are transient, persistent infection with a high-risk HPV is associated with anogenital cancers (Wallin et al., 1999). HPV type 16 (HPV-16) is the type most commonly linked with cancer and is detected in over 50% of invasive cervical cancers and high-grade cervical intraepithelial neoplasias (CIN), and in 25% of low-grade CIN.

Koutsky et al.'s recent report of an effective prophylactic HPV-16 vaccine that prevents the subsequent development of CIN culminates more than two decades of extensive preclinical and clinical research (Koutsky et al., 2002). The vaccinated women in this study were not only protected from developing preinvasive disease associated with HPV-16, but also from persistent and transient genital HPV infection. In essence, by neutralizing HPV-16, the vaccine prevents the causative agent from residing in the anogenital tract, thereby reducing the risk of subsequent sexual transmission.

Efforts to develop a vaccine to prevent persistent HPV infection have focused on eliciting humoral immune responses to the HPV capsid proteins using synthetic empty capsids, termed "virus-like particles" (VLPs). The L1 (major capsid protein) organizes itself into naked icosahedrons when it is expressed at high levels in microbial or cellular expression systems using recombinant techniques (Schiller and Lowy, 2001) (Figure 1). These VLPs are morphologically indistinguishable from the authentic virion, are non-infectious, and lack any oncogenic DNA. Preclinical studies in animals demonstrated that vaccination with species-specific VLP induced neutralizing antibodies that were also effective in preventing the development of HPV-induced lesions. In early clinical studies, HPV-16 L1 VLPs were well tolerated and induced neutralizing anti-HPV-16 antibodies (Harro et al., 2001).

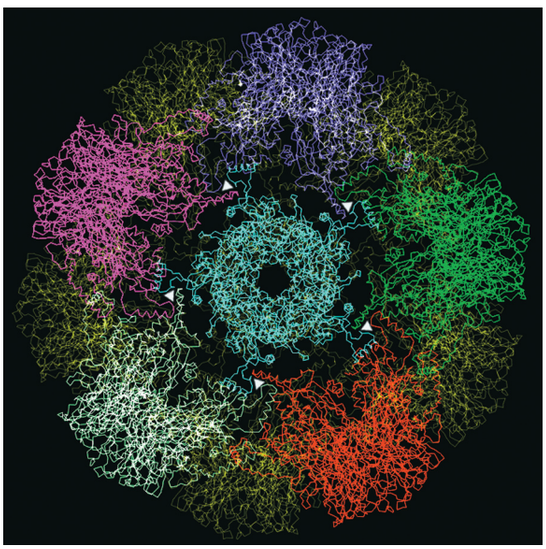

**Figure 1.** The icosahedral assembly of the HPV-16 L1 VLP

The naked icosahedrons are composed of an ordered array of 72 L1 pentamers. Twelve identical pentamers are shown in this assembly, each in a different color. This figure was reprinted from Chen et al., 2000.

Humoral immunity to VLP-based vaccines is not only species specific but also type specific, although divergent variants of HPV types are serologically crossreactive. Since several HPV types are consistently detected in anogenital cancers, a multivalent vaccine is a rational next step for future clinical trials. Because about 70% of HPV-associated cervical cancers contain either HPV-16 or -18, inclusion of at least these two types would be highly desirable. If a multivalent vaccine containing VLPs for HPV-16, -18, -31, -33, and -45 were developed, approximately 85% of cervical cancers could theoretically be prevented (Walboomers et al., 1999). The inclusion of HPV-6 and -11, which cause most genital warts, in a multivalent vaccine might also increase the utilization of a genital HPV vaccine by members of both genders, thereby promoting "herd" immunity.

To emphasize the potential importance of Koutsky et al.'s recent report, consider the impact that the hepatitis B vaccine program has had on the incidence of hepatocellular carcinoma, a cancer causally linked with hepatitis B and previously one of the most common cancers in man. Following the universal vaccine program targeting neonates in Taiwan since 1984, the prevalence of childhood hepatitis in that country has decreased by almost 90%, the morbidity has decreased by nearly 80%, and the incidence of hepatocellular carcinoma has declined by a factor of four (Huang and Lin, 2000).

Since most sexually active women have already been exposed to HPV, it is unlikely that humoral immunity induced from a VLP-based HPV vaccine will be effective in treating women who have already been infected. For example, VLPs were ineffective in the treatment of established lesions in several animal studies. This is not surprising, since VLPs induce neutralizing antibodies that circulate in the

serum and recognize extracellular antigens or antigens born on the surface of a cell. The life cycle of an established HPV infection is characteristically intracellular, noncytopathic, and nonlytic. Therefore, therapeutic vaccines are being developed to eradicate existing disease or infection by eliciting cell-mediated immune responses targeting cells expressing tumor-associated or tumor-specific antigens on their surface. For cervical cancer, the viral peptides derived from high-risk HPV E6 and E7 oncoproteins are the tumor-specific antigens because the viral genes encoding E6 and E7 are selectively retained and expressed during malignant progression of virally induced neoplastic lesions. A wide variety of immunologic approaches are candidates for therapeutic, antitumor vaccines. In general, there are four broad categories of therapeutic vaccine strategies: peptide-based, protein-based, nucleic acid based, and cell-based. Therapeutic vaccination has enormous clinical implications and is being investigated intensively (see Steller, 2002 for a review).

As impressive as VLPs seem to be for preventing papillomavirus infections and subsequent diseases, this technology's potential for also *treating* established lesions is perhaps of surpassing importance (Schiller and Lowy, 2001). To increase their therapeutic potential, polypeptides of the nonstructural viral genes have been incorporated within the VLPs as a genetic fusion with either the major (L1) or minor (L2) capsid proteins. These chimeric VLPs, which are morphologically indistinguishable from their parental VLPs, induce cell-mediated immune responses to the fused polypeptides contained within it. Chimeric VLPs containing HPV-16 E7 polypeptides have been shown to induce potent cytotoxic T-lymphocyte responses and to induce the regression of established tumors (Schafer et al., 1999). Recent investigations also indicate that VLPs specifically bind to dendritic cells (antigen-presenting cells) and induce their activation (Rudolf et al., 2001). Since chimeric VLPs retain the conformational L1 surface epitopes required for inducing neutralizing antibodies, they may ultimately be developed as a combined prophylactic and therapeutic vaccine. Furthermore, non-HPV antigens can be incorporated within the VLPs, raising the possibility that chimeric VLPs may be useful as vehicles for the delivery of antigens to treat non-HPV associated diseases.

Several important issues require careful consideration before anti-HPV vaccines become available for mass immunization programs. Female genital HPV infection occurs commonly and cervical cancer remains a leading cause of cancer-related death in women worldwide, but cervical cancer does not develop in the vast majority of women infected with HPV. Therefore, very few women are likely to benefit from the protective effects of a prophylactic HPV vaccine when the aim is to prevent the development of cervical cancer. However, an effective vaccine might dramatically reduce the cost of, and possibly eliminate the need for, screening and surveillance programs, and there would be a clear benefit in the unscreened population. The timing of vaccine delivery is another important issue since a successful prophylactic vaccine will only be effective when it is administered to women before they acquire HPV infection through sexual activity. Like hepatitis B (which is also sexually transmitted), should a prophylactic HPV vaccine be administered universally during childhood? To enhance overall effectiveness and diminish transmission rates, the vaccine should ideally also be administered to males, even though they rarely manifest HPV-associated malignancies.

Because of the large lag-time from incident HPV infection to the development of invasive cervical cancer, it will take several years before the impact of an effective vaccine to prevent cervical cancer can be clinically appreciated. For instance, in North American women, incident genital HPV infection usually occurs during the late-teen age years, yet the mean age of women diagnosed with cervical cancer is 53. Following well-done, long-term clinical studies, the true effectiveness of anti-HPV vaccines and their real impact on HPV-associated diseases will hopefully be demonstrated. Such a vaccine should gradually have a profound effect on health care resource allocation and potentially represents yet another medical advance that will shift human demographics.

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