

CervarixTM: a vaccine for the prevention of HPV 16, 18-associated cervical cancer

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Abstract: Cervical cancer continues to be the second largest cause of cancer deaths in women worldwide. Persistent infection with high-risk types of human papillomavirus (HPV) is a necessary cause of cervical cancer. Thus, prophylactic vaccination against HPV is an attractive strategy to prevent cervical cancer. Current strategies for the development of safe and effective preventive vaccines are based on the induction of neutralizing antibodies against the major capsid protein, L1 of HPV. CervarixTM is one of the preventive HPV vaccines that has been approved in the Europe and Australia and is currently under review by the US Food and Drug Administration. Cervarix is composed of HPV16 and HPV18 L1 virus-like particles (VLPs) formulated in ASO4 adjuvant. Vaccination with Cervarix has been shown to protect women against a high proportion of precursor lesions of cervical cancer caused by these two HPV types. This review explores the various features of this new vaccine candidate and discusses the future directions in the field of HPV vaccine development.

Keywords: HPV, L1, VLP, vaccine, Cervarix

Introduction

Cervical cancer continues to be a major health care problem worldwide. Cervical cancer is the second leading cause of cancer death in women in developing countries, although cytological screening programs have substantially reduced its toll in developed countries (Parkin et al 1999). It is known that oncogenic human papillomaviruses (HPVs) are the primary causal agent of cervical cancer (Walboomers et al 1999). More than 99% of cervical cancers and over 90% of their precursor lesions, squamous intra-epithelial lesions (SIL), contain HPV DNA (Walboomers et al 1999). Although more than 200 genotypes of HPV have been identified, a small number of genotypes are highly associated with cancer, especially HPV16 and HPV18 (de Villiers et al 2004). These are termed “high-risk” types and are frequently associated with SIL (also called cervical intraepithelial neoplasia [CIN]), the precursor lesions of cervical cancer (for review see Roden and Wu (2006)).

HPVs are non-enveloped icosahedral viruses, with a circular, double-stranded DNA genome. The genome of this small DNA virus encodes two classes of genes; early and late. The early gene products regulate viral DNA replication (E1, E2), viral RNA transcription (E2), cytoskeleton reorganization (E4) and cell transformation (E5, E6, E7), whereas the late proteins (L1, L2) are structural components of the viral capsid. Expression of the viral proteins is tightly regulated and associated with the differentiation of infected epithelial cells. E2 is the master regulator that regulates the expression of all the other viral genes, and is particularly involved in the repression of E6 and E7. The viral oncogenes E6 and E7 are responsible for transformation. During progression, the HPV genome integrates into the host chromosomal DNA, leading to the disruption of the viral E2 gene and an inability to express the late genes associated with high grade disease. Since E2 is a transcriptional repressor

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of E6 and E7, loss of E2 leads to upregulation of E6 and E7 genes. The elevated expression of E6 and E7 proteins results in the disruption of cell cycle regulation and leads to genomic instability, thereby contributing to the progression of HPV-associated cervical cancer. Notably, their expression is necessary to maintain the transformed phenotype (for a review, see zur Hausen (2002)).

A thorough knowledge of these concepts of HPV virology is essential for the rational development of vaccines against HPV. Vaccination could be implemented in the form of preventive vaccines, which generate neutralizing antibodies to block HPV viral infection or in the form of therapeutic vaccines, which eliminate infection by inducing a virus-specific T cell-mediated response. Current strategies for the development of safe and effective preventive vaccines are based on the induction of neutralizing antibodies against the major and minor capsid proteins, L1 and L2 of human papillomavirus. The newly licensed preventive HPV vaccine, Gardasil® (Merck and Co, Inc.), which is an L1-based vaccine, has both a remarkable safety profile and clinical efficacy against the HPV genotypes from which it was derived. Continued efforts are being made in the field of L1-based vaccines in order to improve their efficacy, by increasing the breadth of protection and reducing the cost of these vaccines for wider access and effective prevention of HPV infections.

L1-based vaccines

The expression of recombinant L1 in mammalian (Hagensee et al 1993; Heino et al 1995), insect (Kirnbauer et al 1992; Rose et al 1994), yeast (Sasagawa et al 1995), and even bacterial cells (Nardelli-Haefliger et al 1997) was shown to generate virus-like particles (VLPs), which were morphologically and immunologically similar to native virions (Kirnbauer et al 1992; Rose et al 1994). Studies in various animal models showed that these L1 VLPs induced high titers of neutralizing serum antibodies, specifically immunoglobulin G (IgG), and protected against cutaneous or mucosal papillomavirus challenge. Furthermore, it was demonstrated that L1 VLPs are immunogenic and protective, and this protection is mediated by L1-specific neutralizing antibodies (Breitburd et al 1995; Suzich et al 1995; Christensen et al 1996; Kirnbauer et al 1996). Interestingly, vaccination with L1 capsomeres, the pentameric subunit of VLPs, is also protective (Yuan et al 2001), but not as immunogenic as VLPs in the absence of adjuvants.

Clinical trials were conducted using HPV L1 VLPs and vaccination with these VLPs produced using baculovirus was

shown to be well tolerated and highly immunogenic (Evans et al 2001; Harro et al 2001). The HPV16 L1 VLP vaccines induced serum antibody titers that were about 40-fold higher than the level observed in natural infection even without an adjuvant and these antibodies were highly neutralizing (Evans et al 2001; Harro et al 2001). Similar results were observed using L1 VLPs derived from other HPV types (Evans et al 2001; Ault et al 2004; Brown et al 2004; Fife et al 2004). In 2002, a landmark clinical trial was conducted by Koutsy et al which showed that vaccination with HPV16 L1 VLPs formulated in the adjuvant alum provided 100% protection from the natural acquisition of persistent HPV16 infection over an average of 17.4 months (Koutsy et al 2002). Importantly, all nine cases of incident HPV16-related CIN were confined to the placebo group, indicating that vaccination protects against HPV-related disease. This high degree of efficacy of L1 VLP vaccines for protection against persistent infection and cervical disease relating to the same HPV type infection has also been shown in other clinical studies (Harper et al 2004; Harper et al 2006; Mao et al 2006; Villa et al 2006a; Villa et al 2006b, Paavonen et al 2007). Thus, the steady progress in the field of L1 VLP vaccines led to the development of two successful vaccine candidates.

Cervarix™

Cervarix™ (GlaxoSmithKline) has already been approved in the Europe and Australia and is currently under review by the US Food and Drug Administration. Cervarix is a L1 VLP vaccine that includes HPV types 16 and 18, the two major serotypes that are involved with cervical cancer. Thus, the vaccine has been developed to protect against infection from the two major cancer-causing types of HPV; HPV16 and 18, which together are responsible for approximately 70% of all cervical cancers (de Villiers 1989). It has been produced using insect cells infected with recombinant baculovirus and formulated in the proprietary adjuvant ASO4, which consists of alum combined with a TLR4 ligand, MPL (3-O-desacyl-4'-monophosphoryl lipid A). The vaccine requires three intramuscular doses. The crucial efficacy endpoints have been protection from HPV-related SIL and persistent HPV infection by the HPV types used to derive the vaccine. Results of efficacy trials have indicated that the vaccines are well tolerated, highly immunogenic, and capable of generating high titers of neutralizing antibody to the HPV types 16 and 18, that are included in the vaccine, thus resulting in a high efficacy against CIN2+ lesions containing HPV 16 and HPV 18 (Harper et al 2004; Paavonen et al 2007).

In a recent report on a phase III double-blind, randomized controlled trial, Cervarix demonstrated an efficacy of 90.4% against CIN2+ lesions containing HPV 16 and 18 (Paavonen et al 2007). There is also some amount of cross-protection with the HPV types 31 and 45, thus leading to protection against approximately 80% of cervical cancers (Harper et al 2006). In addition, these vaccines have been shown to be effective over a 5-year period (Harper et al 2006; Gall et al 2007).

Issues regarding Cervarix

Although it has been established that Cervarix has an excellent safety profile and demonstrates significant protection against persistent infection with both HPV 16 and HPV 18 infections, there are some concerns regarding various aspects of the vaccine. Many of these issues are also applicable to Gardasil.

Type-restricted protection

Cervarix has been developed to protect against infection from HPV types 16 and 18, which together cause approximately 70% of all cervical cancers. Furthermore, HPV16 and HPV31, and HPV18 and HPV45 have very closely related genotypes (for review see Roden and Wu 2006; Roden et al 2007), suggesting that vaccination with Cervarix may provide cross-protection against HPV types 31 and 45. Indeed, vaccination with Cervarix has been shown to generate partial cross-protection against HPV types 31 and 45 (Harper et al 2006). However, the overall protection against cervical cancer provided by Cervarix is probably only up to 80% since this vaccine provides little or no protection against other high-risk HPV types, such as HPV33, HPV52 and HPV58 (Harper et al 2006). Furthermore, cross-protection may not last as long as protection against the type included in the vaccine.

An important approach to resolve the issue of type-restricted protection is the employment of multivalent vaccines, which has been implemented in a number of licensed vaccines against other pathogens. However, this raises the cost and complexity of manufacture with progressively decreasing returns. The implementation of multivalent VLP vaccines will require that there is no interference with the responses compared to vaccination with individual types. It has been suggested that a vaccine comprising the eight most prevalent HPV types detected in cancer might be required for >90% protection against cervical cancer, assuming complete type-specificity of protection (Muñoz et al 2004). However, given partial cross-protection, this number is

probably an over-estimate. The oncogenic HPV types that are present in different parts of the world are relatively consistent, which suggests that such a multivalent vaccine would be useful worldwide (Clifford et al 2005). Merck is currently testing an octavalent HPV VLP vaccine targeting 6 oncogenic HPV types and is likely to at least partially cover several related HPV types.

An alternative approach to highly multivalent vaccine preparations for broad protection is the employment of a conserved and cross-protective antigen, such as L2. Vaccination with the minor capsid protein L2 has been shown to induce broadly cross-neutralizing antibodies in animal models and has shown promise in this regard (Roden et al 2000; Embers et al 2002; Gambhir et al 2007). Therefore, efforts to improve the immunogenicity of L2 and development for clinical testing of L2 vaccines are underway. The ability to produce L2 in *Escherichia coli* and the potential to use a single antigen suggest that L2 vaccines have potential as a low cost alternative or complement to the L1 VLP vaccines. Clinical trials are currently being planned to evaluate the safety of HPV L2 polypeptide vaccination in healthy women.

Length of protection

Although the duration of protection generated by Cervarix is not clear at this point, recently available data has indicated that Cervarix is highly efficacious against HPV-16/18 up to 5.5 years and prevents most CIN2+ lesions, and also shows continued cross-protection against HPV-45 and HPV-31 incident infections (Gall et al 2007). Similar data are available for Gardasil. It will be important to continue to follow the same group of patients over time in order to acquire a comprehensive picture of the length of protection.

Age of vaccination

In a recent phase III trial using Cervarix in healthy volunteers of different age groups, higher antibody levels were observed in the pre-teen/adolescent group compared to those observed in women 15–25 years old (Pedersen et al 2007). This indicates that the elevated levels demonstrated in this younger age range may result in longer duration of protection. Similarly, another study using the quadrivalent HPV L1 VLP vaccine demonstrated robust anti-HPV neutralizing antibody responses that were significantly higher in 10- to 15-year-old girls and boys compared to 16- to 23-year-old females (Block et al 2006). For optimal effect, it is critical to vaccinate adolescents against infection with cancer-causing HPV types 16/18 well before the initiation of sexual activity with a

vaccine of sustained efficacy. The current recommended age group of vaccination for Cervarix is 10–25 years.

Limitations in low resource areas

An important limitation of Cervarix is its high cost compared to other vaccines, which is also true in case of Gardasil. Since cervical cancer has a high prevalence in developing countries, vaccines need to be made available in low-resource areas in order to impact the incidence of cervical cancer worldwide. Cervarix is expected to cost about US\$100 per dose and Gardasil costs US\$120 per dose in the US. Both these vaccines require three doses to complete the vaccination regimen. Thus, these vaccines may not be ideal for low-resource areas and in developing countries. Dramatically tiered pricing would be necessary for their implementation. Furthermore, these vaccines require refrigeration for storage, which might be problematic in remote and low-resource areas. Thus, in low-resource settings, the relative benefits of these vaccines may be restricted by poor coverage. In order to generate impact on the incidence of cervical cancer, it is therefore necessary to develop cost-effective, stable and effective preventive vaccines that are capable of inducing broader protection against most HPV types and which are suitable for low-resource areas.

Although current L1 VLP vaccines, Cervarix and Gardasil are produced in insect cells and yeast respectively, higher levels of production of the vaccine in *E. coli* may be a cheaper alternative. The expression of L1 in *E. coli* produces high levels of capsomers (Li et al 1997; Rose et al 1998; Chen et al 2000) and vaccination with such capsomers induces neutralizing antibodies (Rose et al 1998; Fligge et al 2001) and protects dogs from experimental canine oral papillomavirus challenge (Yuan et al 2001). In addition, the L1 capsomere vaccine is likely stable at ambient temperatures. However, its immunogenicity relative to VLPs is unclear. Clinical trials are currently being planned to evaluate the safety and immunogenicity of L1 capsomere vaccines formulated in alum. Alternatively, the inclusion of L1 in other vaccines, such as typhoid, tuberculosis or measles vaccines might represent a cost-effective and practical alternative that could also provide immunity to HPV (Reuter et al 2002; Baud et al 2004a,b; Govan et al 2006). In addition, the use of live vectors for the delivery of L1 VLPs is attractive in remote and low-resource areas as immunity could be spread, but safety remains an issue with such vectors. Thus, further steps need to be taken in order to improve the cost effectiveness of HPV vaccines

for successful implementation in low resource areas where they are most needed.

The requirement for needles and current three-dose regimen for the current VLP vaccines also presents a formidable obstacle to the delivery of these vaccines in low-resource areas. Many possible needle-free alternatives, including nasal inhalation and transdermal vaccination are being considered as feasible options (Nardelli-Haefliger et al 2005; Rechtsteiner et al 2005). The regimen also presents a problem in low-resource areas where regular follow-up is highly unlikely. Thus, it is important to develop a vaccine formulation that does not require three doses to generate protective humoral immunity.

Lack of therapeutic benefit

An important obstacle to the rapid elimination of cervical cancer is the current prevalence of established HPV infections and HPV-associated disease. The existing HPV L1 VLP vaccines, Gardasil and Cervarix do not generate therapeutic effect against pre-existing HPV infection. Since infected basal epithelial cells and cervical cancers cells do not express detectable levels of capsid antigen (L1 and/or L2), preventive HPV vaccines targeting L1 and/or L2 are unlikely to be effective in the elimination of pre-existing infection and HPV-related disease. This is a serious concern since there is currently a considerable burden of HPV infections worldwide. It is estimated that it would take approximately 20 years from the implementation of mass vaccination for highly effective preventive vaccines to impact the cervical cancer rates due to the prevalence of a significant population with existing HPV infections and slow process of carcinogenesis. Thus, in order to accelerate the control of cervical cancer and treat currently infected patients, it remains important to develop therapeutic vaccines against HPV in addition to improving the efficacy of preventive vaccines such as Cervarix.

Comparisons between Cervarix and Gardasil

Cervarix serves as an important competitive product relative to the recently licensed L1-based preventive HPV vaccine, Gardasil (FDA 2006a, b). Table 1 discusses the various aspects of comparison between the two vaccines. Gardasil is a quadrivalent vaccine that includes HPV types 6, 11, 16 and 18. The types 16 and 18 are major high-risk HPV types, while the types 6 and 11 are the low-risk HPV types that are associated with a majority of benign genital warts and laryngeal papillomas. In comparison, Cervarix includes only the two most important high-risk types, HPV 16 and 18.

Table I Comparisons between Cervarix and Gardasil

Category	Cervarix	Gardasil
HPV types Included	HPV 16 and 18	HPV 16, 18, 6, 11
Production system	Insect cells infected with recombinant baculovirus	Yeast
Adjuvant	ASO4 (aluminium salt + MPL (3-O-desacyl-4'-monophosphoryl lipid A))	Alum
Diseases covered	Anogenital cancers, including cervical, vulval, vaginal, and anal cancers and their associated precursor lesions (and a subset of head and neck cancers)	Anogenital cancers, including cervical, vulval, vaginal, and anal cancers and their associated precursor lesions (and a subset of head and neck cancers) Genital warts and laryngeal papillomas
Available data regarding Length of protection	5.5 years	At least 5 years
Dose	0.5 mL dose containing 20 µg HPV 16 L1 and 20 µg HPV18 L1	0.5 mL dose containing 20 µg HPV6 L1, 40 µg HPV 11 L1, 40 µg HPV16 L1 and 20 µg HPV18 L1
Recommended administration Route and regimen	Three intramuscular injections at 0, 1, and 6 months	Three intramuscular injections at 0, 2, and 6 months
Recommended age group for vaccination	10–25	9–26
Price (US\$)	Approx. \$100 per dose	Approx. \$120 per dose

Thus, Gardasil can be used to prevent not only a majority of cervical cancers, but also genital warts, one of the most common sexually transmitted diseases.

The inclusion of HPV6 and 11 VLPs has been used to help justify vaccination of men, who do not get cervical cancer. Men suffer from HPV-related cancer (including anal, penile, head, and neck cancers) at a lower frequency than women because of the predominance of cervical cancer. This suggests that that cost/benefit ratio is questionable for men since efficacy in men has not yet been demonstrated. The argument that men should be vaccinated to provide herd immunity is also not so strong because the vaccine is so effective in women, suggesting that ensuring broad protection in women would be more cost-effective. However, if only women are vaccinated, then there would be little impact on HPV prevalence in men who have sex with men. Anal cancer is a significant problem, particularly for this population.

Furthermore, Gardasil is produced in recombinant yeast, whereas Cervarix is produced using insect cells, but this does

not seem to affect the immunogenicity of the VLPs. One can imagine that individuals that are allergic to yeast can turn to Cervarix as an alternative option and vice versa.

Another difference between Cervarix and Gardasil is the adjuvant used in the formulation of the final product. Gardasil uses Merck's alum-based adjuvant. In contrast, Cervarix uses the proprietary adjuvant developed by GSK, ASO4. The formulation of Cervarix with the ASO4 adjuvant has been shown in clinical trials to induce a stronger antibody response against HPV types 16 and 18 compared to the same vaccine formulated with aluminium salt alone (Giannini et al 2006). However, head-to-head trials with Gardasil would be needed to confirm this observation. In addition, a higher frequency of HPV L1-specific B cells was observed in individuals immunized with this bivalent L1 vaccine formulated with ASO4 adjuvant compared to the same vaccine formulated with alum. While the antibody responses generated by Cervarix vaccine may be higher than that generated by Gardasil, it is not clear if the observed enhanced humoral immune response translates into a stronger efficacy or longer duration of protection compared to Gardasil. It would be important to determine if the employment of different adjuvants will influence the duration of protection of the two vaccines.

Conclusions

Cervarix, represents yet another success in the development of preventive HPV vaccines. Similar to Gardasil, Cervarix also has an excellent safety profile and high clinical efficacy, possibly protecting against up to 75%–80% of all cervical cancers if the vaccine is fully implemented. Although Cervarix has many similarities to Gardasil, there are also several unique factors that are highlighted, such as the adjuvant formulation, the production system and the HPV types included. The inclusion of the proprietary ASO4 adjuvant in Cervarix has led to a stronger immune response in vaccinated individuals, although it is not clear if this will lead to greater efficacy or longer duration of protection. However, Cervarix, like Gardasil is mainly available in developed countries. Thus, these vaccines are unlikely to reach the people in the low-resource areas, who need them the most. Since more than 80% of all cervical cancer deaths occur in developing countries that lack the resources and infrastructure for cytologic screening and intervention, it is essential to make significant efforts to develop cost-effective vaccines that are stable and can be administered in a simple regimen (heat-stable, needle-free, single vaccination) and thus can be effectively employed

in low-resource areas in order to maximize the impact of vaccination on the global cervical cancer burden. Finally, since HPV also contributes to a large proportion of other cancers, including head and neck, vaginal, vulvar, anal, and penile cancers, it will be of interest to determine if these HPV vaccines are effective in protecting against these cancers as well.

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