A review of cross-protection against oncogenic HPV by an HPV-16/18 AS04-adjuvanted cervical cancer vaccine: Importance of virological and clinical endpoints and implications for mass vaccination in cervical cancer prevention

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Abstract

Human papilloma virus (HPV)-16 and -18 are responsible for approximately 70% of invasive cervical cancers worldwide. Other oncogenic HPV types account for almost all the remainder. Importantly, HPV-45 and -31 account for approximately 10%. HPV-18 and -45, along with HPV-16, are found in over 90% of endocervical adenocarcinomas. HPV-45 is the third most frequent HPV type in cervical carcinoma and adenocarcinoma. The AS04-adjuvanted vaccine Cervarix™ was developed against HPV-16 and -18 focusing on preventing cervical cancer by inducing durable protection against new infection. In clinical trials, it shows evidence of cross-protection against other important oncogenic HPV types using a range of clinicopathological and virological endpoints. The current evidence suggesting the cross-protective effect comes from its overall impact on precancerous lesions and on 12-month or more persistent oncogenic HPV infection, together with specific evidence of protection against incident and new persistent infection lasting 6 months or more with individual HPV types. The use of virological endpoints for such studies is discussed, in particular for cross-protection evaluation, in view of the lower frequency of many important oncogenic HPV types other than HPV-16 or -18 in precancerous lesions and the frequent presence of multiple HPV infections. Both of these factors complicate the interpretation of type-specific, vaccine-induced protection against cervical intraepithelial neoplasia (CIN) lesions, in which other HPV DNA types are found along with HPV-16 and -18. The observed high level of overall protection against clinicopathological endpoints, including CIN2+ in the vaccinated subjects (regardless of their HPV DNA status), predicts a potentially broader impact of the vaccine in the prevention of HPV-related precancers that goes beyond HPV-16 and -18. The prevention of persistent infections by individual types such as HPV-45 provides specific information on the protection against that type, using an alternative endpoint that relates to both precancer and cancer development. Together with sustained protection against HPV-16 and -18, protection against HPV-45 could offer an additional effect on invasive cervical cancer and may have an important impact on endocervical adenocarcinoma, which is not effectively prevented by screening and is becoming increasingly important in young women.

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HPV-16 is the predominant HPV type in all regions across the globe and is associated most with the more frequent squamous cell carcinoma (SCC) of the cervix, followed by HPV-18, -45 and -31 [1]. Infections with HPV-16 and -18 confer a higher risk of developing a cervical cancer [3–5]. Although HPV-16 is the most important type in adenocarcinoma, HPV-18 along with HPV-45 also contribute to adenocarcinoma globally. HPV-18 plays a greater role in endocervical adenocarcinoma than in SCC, and has been found in 30–40% of adenocarcinomas, while HPV-45 is noted in about 6% of cases in most large studies and meta-analyses [6,7], and about 12% in one recent global report [8]. HPV-45 represents the third most prevalent type in adenocarcinoma [1,6,7]. Adenocarcinoma accounts for approximately 10–12% of the global cancer burden, but its importance varies widely. It is not prevented effectively by cytological screening, and is increasing in relative importance and incidence especially in young women in Europe and North America, where, in some countries, it amounts to over 25% of all invasive cervical cancer, and up to 40% in some populations [9], and is also associated with a higher frequency of recurrence and a poor outcome [10,11].

To prevent cervical cancer, HPV vaccination is primarily aimed at HPV-16 and -18. The GSK AS04-adjuvanted cervical cancer vaccine, Cervarix™, is composed of HPV-16 and -18 L1 protein virus-like particles (VLP), in which the VLPs are morphologically and antigenically similar to natural papilloma virions of these types. The original aim of protection through immunization was to generate a strong type-specific immune response protective against cervical disease outcomes associated with HPV-16 and -18. Cervarix™, has been shown to induce a robust and sustained antibody response and protection against cervical HPV-16/18 infections, persistent infections and cervical intraepithelial neoplasia (CIN) related to the vaccine types (HPV-16 and -18) [12–14]. Furthermore, Cervarix™, has shown evidence of protection against infections that extended beyond HPV-16 and -18, i.e. with other oncogenic types that are phylogenetically ‘related’ to HPV-16 and -18, such as HPV-45 and -31 [15]. This observation has public health implications for potentially expanding the range of protection of this vaccine against the HPV types commonly encountered in cervical cancer and those important in adenocarcinoma, in particular HPV-45.

This paper reviews the problem of demonstrating and measuring efficacy of cross-protection and summarizes the results published so far on Cervarix™. The review explores the limitations of clinicopathological precursor endpoints based on associating HPV infections with CIN when there are HPV-16/18 and other types present and considers the value of virological endpoints, particularly those based on the concept of persistence of HPV infection underlying progression to precursor and cancer. Persistent infection as detected by the continuing presence of HPV DNA in a cervical cytological sample over months or years provides a potentially powerful way of assessing protection against multiple HPV types. The published data generated in the Phase IIb and Phase III clinical trials of Cervarix™, and the potential importance of these for cancer prevention, are reviewed in the light of these issues.

**Importance of virological endpoints to evaluate cross-protection**

The impact of vaccination against HPV-16/18 infections on the precancerous lesions they cause and potential subsequent impact on cancer have been measured in a number of ways. The evaluation of the impact of a vaccine on preventing CIN, especially high-grade precursor (CIN2+) associated with HPV-16/18 DNA in the precancer tissue biopsy, has been the primary objective of all recent HPV vaccine efficacy trials. Efficacy against such clinicopathological endpoints is not, however, the only useful objective when it comes to understanding the impact of cross-protection on prevention of cervical cancer itself. Clinicopathological endpoints such as CIN2+ provide a cross-sectional picture of one important stage in the development of cervical cancer. CIN2+ is an important endpoint as it represents the current treatment threshold for women in cervical screening. However, the development of cervical cancer is a complex multistage process. HPV types differ in their natural histories in terms of risk of progressing from incident infection to invasive cervical cancer [4,5,16]. This is particularly important in assessing fully vaccine impact on cross-protection for a wide range of oncogenic types in relation to cancer prevention, for example, with HPV-18 and -45, both of which are significantly more common in cancer than high-grade precancers (CIN2+). Efficacy data relating to persistent infections with HPV-18 and -45 provide an alternative approach, delivering valuable information on the impact of the vaccine in preventing another important biological stage in the progression to cervical cancer [17]. World Health Organization guidelines on HPV vaccines [18] recognize the difficulties, and note that vaccine efficacy data on cross-protection associated with HPV-16/18 vaccination could be explored by examining the incidences of morphological lesions (e.g. CIN of any grade, CIN2/3 or adenocarcinoma in situ [AIS]) due to the HPV types in question, together with viral persistence, since the numbers of cases of CIN2+ associated with these other HPV types are small [18].

There is also another reason why virological endpoints are potentially very important guides to type-specific cross-protection: it is now recognized that multiple HPV infections are common, and that CIN lesions are frequently associated with the detection of more than one HPV type using HPV DNA testing [19–21]. This makes a CIN endpoint associated with HPV DNA insufficient when assessing the extent and importance of type-specific cross-protection. The problem arises because if HPV-16 or -18 DNA is present with another HPV type in a lesion, it is not possible, on the basis of DNA presence alone, to determine which HPV type is actually the cause of the lesion. If an arbitrary assumption is made of the role of one HPV type or the other (e.g. always due to HPV-16/18 if present, equal distribution of role, or always due to the other HPV type), the true impact of cross-protection cannot be measured, and, importantly, cross-protection may be overestimated. The importance of multiple infections and the assessment of causality of the lesion were discussed during the Advisory Committee on Immunization Practices meeting in October 2007 where experts noted that in multiple infections
including HPV-16/18, efficacy against types 16 and 18 could be inflating the apparent efficacy against the other types [22]. Given that multiple infections have been reported in up to approximately 40% of women with HPV infection [23], the impact of arbitrarily changing the assumptions made about the role of different types in multiple infections can alter the efficacy estimate substantially. This limits the value of such an approach in assessing type-specific cross-protection against HPV types beyond HPV-16 and -18.

Thus, the combination of results against both type-specific virological endpoints and clinicopathological endpoints (type-specific and non-type-specific) provides a more comprehensive picture of the likely important impact of cross-protection in preventing cervical cancer.

As a virological endpoint, persistent infection is considered the most robust because it requires detection of a particular type of HPV DNA in a cervical cytological sample on more than one occasion, and also indicates the presence of HPV DNA for a sustained period of time. It is now clearly established that persistence of HPV DNA in a cervical cytological sample is associated with the development of cytohistological lesions, including CIN2+ and invasive cancer [24–26]. The continued expression of oncogenic HPV is causally associated with development of cervical cancer [27]. The persistence of infection at the cervix with oncogenic HPV types has been strongly linked to precancerous cervical lesions and invasive cancer (see systematic review by Koshiol et al. [25]). This systematic review outlines the evidence of the association between HPV persistence and precancer and illustrates that persistence is strongly associated with CIN2–3/high-grade squamous intraepithelial lesions (HSIL) and that the strength of the association increases with grade of cervical abnormality, and with increased duration of persistence. This was consistently demonstrated across over 40 studies, despite a wide variation in study methodology and the definition of persistence in the studies considered [25]. Such persistent virological infection endpoints are not influenced or complicated by the presence of multiple HPV types.

The value of employing virological endpoints in cervical cancer vaccine clinical trials therefore lies in exploring the impact of vaccination beyond the virologic data in high-grade CIN. The use of persistent endpoints allows: 1) more precise measurement of protection against individual HPV types by vaccination, 2) the ability to examine type-specific virological endpoints uninfluenced by the presence of multiple HPV types, 3) the ability, through separately addressing individual types, to investigate the impact of phylogenetic relations between different HPV types and HPV-16 and -18 on cross-protection, and 4) the ability to address protection against HPV types that are infrequent compared to HPV-16 or -18 in high-grade precancer (e.g. HPV-45), but which are important in cervical cancer [1,6]. Used in this way virologic endpoints can represent a valuable indicator of protection from infection by less frequent oncogenic HPV types in relation to the potential of vaccination for preventing invasive cervical cancer [1,6].

The use of virological endpoints also provides an opportunity to overcome some of the other limitations of morphological endpoints, such as the low reproducibility between pathologists of HPV-related morphological abnormalities and some grades of cervical precancer, and the uncertainty about their biological outcome. In contrast, the virological endpoints permit the use of validated, more reproducible, and very sensitive sampling and testing procedures.

In the Phase III clinical trial of Cervarix™, an approach has been used which addresses the issue of multiple infections using the combination of persistent HPV infection and HPV DNA in the biopsy, supported by expression of HPV genes in the CIN lesions to assess type causality [14]. When this was done for CIN cases in which multiple types were detected with high frequency in the lesions, the association with persistent infection proved a consistent predictor of type causality compared with HPV gene expression.

**Technical issues in employing virological endpoints**

The methodology used in the clinical trials reviewed below is the SPF10 polymerase chain reaction (PCR) system which enables detection of very low copy numbers of 14 high-risk types and 11 low-risk types, and multiple types simultaneously. The system has been shown to be reproducible and to have a high level of molecular sensitivity and specificity for most oncogenic HPV types [28,29]. There is some variation in detection between different types, but all the types discussed here are detected with high sensitivity and specificity, avoiding problems with false negative results. In liquid-based cytological specimens it has a performance similar to that of some other sensitive PCR-based HPV typing systems for most oncogenic HPV types [30].

The sensitivity is such that the test will also detect HPV DNA which is not active. To differentiate an active HPV infection from the presence of passenger or latent virus, other supplementary approaches are required, such as PCR analysis of laser-microdissected cells associated with lesions to localize HPV DNA, and studies of expression of type-specific HPV E4 or E6/E7 genes, and the use of persistence to assign causality [14].

Less sensitive HPV testing systems, such as GP5+/6+ and HC-2, detect HPV DNA only above a certain threshold. It has been known for many years that there is a relationship between viral DNA load and the presence of an active and transforming infection, certainly for HPV-16 [4,5,31–33]. While calibrating tests on this basis may be of use in cervical screening practice in improving specificity, this relationship is not precise or absolute so that use of HPV DNA tests employing this principle leads to loss of sensitivity and to an increased rate of false negative results for HPV association which is inadequate for accurate determination of vaccine performance [34,35].

The approach being applied in GSK trials is to combine sensitive detection of persistent infection and of HPV DNA with precise localization in lesions to assign causality. The strength of association between persistent HPV DNA detection increases with grade of cervical abnormality, and with increased duration of persistence [25]. In the studies reviewed here the type-specific results relate to incident and persistent infection of
Grouping oncogenic HPV types: phylogeny and contribution to carcinogenicity

To evaluate cross-protection requires an understanding of the genetic and immune relationships between oncogenic HPV types. The phylogenetic grouping of HPV types is based on broad genetic similarity. This does not mean that all closely related HPV types will share the same epitopes, but they may, and some do [15,36]. This is important as cross-protection probably results from similar antigenic sites (epitopes) seen by the immune system on closely related papillomaviruses. Such an immune response may be further promoted by the use of a novel adjuvant (e.g. AS04) to stimulate antigen presentation [37].

An alternative way to group the oncogenic HPV types is in relation to their ranking in importance for cervical cancer causation. There is an evident correlation of characteristics for carcinogenicity in experimental studies and epidemiologically measured cancer risk for oncogenic HPV types [15,27,38]. The four oncogenic HPV types 16, 18, 45 and 31 are the most important oncogenic HPV types globally in terms of the proportion of cervical cancer they cause [1,2] with HPV-16,-18 and -45 being most “aggressive” in terms of risk of progression to cancer.

Cross-protection and vaccine efficacy against HPV infection: summary of Cervarix™ data

This review summarizes the efficacy results of Cervarix™ against incident and persistent infection with oncogenic HPV types beyond HPV-16 and -18, individually and grouped. The current analysis for individual type-specific virological endpoints relates to both incident infections for up to 5.5 years (mean 5 years) of follow-up after vaccination, and to protection against persistence of infection (six months or more). Results on protection against clinical endpoints, regardless of HPV DNA type found in the lesion, are also presented because such efficacy data provide direct information on the impact of vaccination on screen-detected precancerous lesions of clinical importance and are not confounded by the presence of multiple HPV types in the lesions. These data complement the virological results with regard to the likely impact of vaccination on current cytological screening strategies and histological outcomes [13,14,39,40]. The presentation of results against both virological and clinicopathological endpoints provides a more comprehensive picture of the likely important impact of cross-protection on prevention of cervical cancer.

Clinical trials

Two clinical trials have evaluated the efficacy of Cervarix™ on other oncogenic types. The first study was an initial efficacy Phase IIb study (580299/001) and an extended follow-up phase (580299/007) of the same study [12,13,39,40]. In the initial Phase IIb efficacy study, 1113 women aged 15–25 years old in North America and Brazil received three doses of Cervarix™ or Al(OH)₃ at months 0, 1, and 6. The extended follow-up of the same study enrolled 776 participants who were HPV-16 and -18 seronegative and DNA negative for 14 oncogenic HPV types (HPV naive) at entry into the initial study. In the second study, a Phase III efficacy study (580299/008), 18,644 naive and non-naive women aged 15–25 years old in 14 countries received Cervarix™ or hepatitis A vaccine at months 0, 1, and 6 [14].

Incident HPV infection

In the extended follow-up efficacy study there was evidence of sustained cross-protection against incident infection with HPV-45 and -31 for the entire follow-up period up to 5.5 years (mean of approximately 5 years after the first vaccination in the primary efficacy study) [13]. High efficacy was seen against type HPV-45 (88%; 95% CI: 60.0–96.4) and substantial efficacy was observed against HPV-31 (54%; 95% CI: 15.9–73.6) [13]. The continued efficacy and increasing divergence between vaccinated and unvaccinated women with time over the 5.5 years is clearly seen in the Kaplan–Meier curves for incident infection (Fig. 1).

Persistent HPV infection

In a large Phase III efficacy trial (PATRICIA) (580299/008), cross-protection against six-month persistent infection (defined as the detection of DNA from the same HPV type in two consecutive cervical cytology samples collected over any six-month period) was observed for HPV-45, -31 and -52 (Table 1) (mean follow-up of approximately 15 months) [14]. Furthermore, broad protection was observed against 12-month persistent infections (defined as detection of the same HPV type in all available cytology samples collected over any 12-month period) with 12 combined oncogenic HPV types, not including HPV-16 and -18 (vaccine efficacy 27.1%; 97.9% CI: 0.5–46.8) [14]. These findings extend the evidence of cross-protection against incident HPV infection observed in the extended follow-up Phase IIb study (described above) to the more strongly cancer and precancer-related endpoint of persistent infection [13].

When compared with the point estimates for protection against incident infection in the extended follow-up Phase IIb study, the lower point estimates for HPV-45 and -31 in the Phase III population are likely to reflect the high proportion of persistent infections acquired before completion of the full course of vaccination at month 6 in the Phase III analysis, due to the fact that the efficacy analysis was done on a total vaccinated cohort [14]. In fact, 68% of the six-month persistent infections and 93% of 12-month persistent infections included in the endpoint calculation were detected at month 6, before administration of the third vaccine dose. This observation shows early onset of vaccine protection, before completion of the entire vaccination schedule. However, higher protection may be expected in the longer term and in clinical practice when young girls will have received a full course of three doses of
vaccine with the expectation that most infections will occur after completion of vaccination.

Impact on clinicopathological endpoints

The 5.5-year analysis of the extended efficacy study showed a point estimate of vaccine efficacy against CIN2+, independent of the HPV result on the biopsy, of 68% (95% CI: 6.8–90.8 using the conditional exact method) (Table 2). To further explore the results obtained by conventional statistics where quite large confidence intervals were observed, a Bayesian statistical method was applied (based on the true distribution of vaccine efficacy) which showed 88% probability that the true vaccine efficacy was higher than an expected 50% approximation of the causal contribution of HPV-16 and -18 to these lesions. Although the numbers of CIN2+ endpoints were insufficient to yield tight confidence intervals, the results are consistent with clinically important overall cross-protection against cytological abnormalities and CIN of all grades, including CIN2+, extending beyond that related to HPV-16 and -18 (Table 2).

Discussion

These data illustrate the use of virological and clinicopathological endpoints and provide an overview of the potential role of cross-protection from two important clinical trials. One of these contributes a longer term follow-up of an HPV naive population over a mean of 5 years, and the other evaluates a large number of women both naive and non-naive from 14 different countries with shorter follow-up reported to date (15 months). The pattern of results of cross-protection reviewed here is consistent with the findings that Cervarix™ is highly efficacious against HPV-16/18 CIN2+ and related virological endpoints. Taken together the results indicate that the vaccine can substantially reduce cervical precancer requiring treatment and may have an important impact on cervical cancer [13,14] through both direct protection against HPV-16/18 and cross-protection against other oncogenic HPV types.

The results obtained using the HPV type-specific virological endpoints of incident and persistent infection show very clearly medium to long-term (up to 5.5 years) protection against new infection with HPV-45 and -31, in addition to the very high level of sustained protection against HPV-16 and -18 infections. These studies indicate that there is a cross-protective effect shared among closely related HPV types: between HPV-16 and several closely related types (such as HPV-31, HPV-52 [at least for a few years] and, potentially, HPV-33), and markedly between HPV-18 and -45.

![Fig. 1. Cross-protection against incident infection with HPV-31 and -45 up to 5.5 years. Based on cervical samples only from the combined primary efficacy study and the extended follow-up study phase (intention-to-treat [ITT] analyses). ITT analyses included women who had received at least one dose of study vaccine or placebo and were HPV DNA negative for the specific HPV type at month 0 in the primary efficacy study, and who had any data available for outcome measurement in the extended follow-up study phase. Mean follow-up time was approximately 5 years after the first vaccination in the primary efficacy study. Cox regression model, \( p \leq 0.001 \) (log-rank test).](image-url)
A limitation of the interim analysis of the Phase III study is the short follow-up time. Events starting immediately after the first vaccination are included in the analysis and negatively bias an estimate of efficacy in very young, unexposed, adolescent women who would be mostly in pre-puberty, and most likely not to start sexual activity until they had completed the full six-month, three-dose vaccination schedule, in a context of universal mass vaccination. The study, however, provides an indication of early onset of protection that may be beneficial in a population already sexually active and potentially exposed.

Implications of cross-protection for cervical cancer prevention

Based on both clinicopathological and virological endpoints, these results indicate the potential of the HPV-16/18 vaccine adjuvanted with a novel Adjuvant System (AS04) for important and sustained extra benefits for cervical cancer prevention, beyond prevention of HPV-16/18-related cancer, and are likely to add to the sustained, long-term benefit of HPV-16/18 vaccination with Cervarix™ for cervical cancer prevention. Ongoing and further long-term studies starting now after licensure, with continued follow-up of existing cohorts in the studies reported, will provide more detailed information on the extent and duration of cross-protection. Nonetheless, the current results provide consistent evidence of high and sustained efficacy against incident and persistent infection with the important oncogenic type HPV-45, which is particularly important because of the prevalence of this type in invasive cervical carcinoma [1], the high rate of progression from infection to malignancy [10,11], and its role, along with its close relative HPV-18, in cervical adenocarcinoma [1,2,6,7].

There is also evidence of sustained, substantial efficacy against HPV-31 and some broader protection against other HPV-16-related oncogenic HPV types such as HPV-52 (for early infection after vaccination) and potentially, HPV-33. HPV-33 and -52 also rank within the first seven HPV types in frequency in cervical cancer globally in both studies.

Cervarix™ has been registered in Europe via the European Medicines Agency, and in more than 60 countries worldwide. In several countries the regulatory authorities have accepted the evidence provided to date in clinical studies on the cross-protective effect of the vaccine against other oncogenic types, in particular HPV-45 and -31.

Conclusions and future prospects

The possibility that a prophylactic vaccine against HPV16/18 might induce protective immunity against other important oncogenic HPV types potentially offers additional benefits from HPV vaccination. Assessment of this effect is a scientific challenge, particularly as many CIN lesions, including CIN2+ lesions which form the primary endpoint of current vaccine trials, can be associated with DNA of more than one oncogenic HPV type in the biopsy: this may include HPV-16 or -18 DNA and another oncogenic type. Persistence of infection as HPV DNA in cervical samples has been linked to development of cancer and precancer. The use of persistent type-specific HPV infection as an endpoint can help to elucidate the range of types and extent of protection, and help to assign CIN2+ lesions to persistent HPV types. Using the virological approach there was high, sustained efficacy of protection over a mean of 5 years against incident HPV-45 infections in the initial study of Cervarix™ and evidence from a larger study of early protection against persistent infections of 6 months or more. This, together with evidence of broad protection against oncogenic non-16/18 HPV types persisting over 12 months or more, and against CIN2+ irrespective of HPV types, indicates that cross-protection is an important phenomenon. Longer term assessment of the Phase III population and Phase IV studies, such as the one currently in progress in Finland, should provide additional data on vaccine efficacy or effectiveness against persistence of individual HPV types such as HPV-45 for 12 months or longer. Through the effect on HPV-45, along with the protection against HPV-18, Cervarix™ HPV16/18 vaccine with AS04 adjuvantation may ultimately turn out to be particularly valuable in improving prevention of adenocarcinoma which is now increasing in frequency in young women in screened populations, and is not effectively reduced by cytological screening.

Conflict of interest statement

DJ is a former Director of Clinical Research for HPV vaccines and a consultant to GSK Biologicals, Rixensart, Belgium.

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