Immune response to human papillomavirus after prophylactic vaccination with AS04-adjuvanted HPV-16/18 vaccine: Improving upon nature

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Abstract

An AS04-adjuvanted human papillomavirus (HPV)-16/18 vaccine has demonstrated excellent efficacy for up to 6.4 years in preventing related persistent infections and cervical precancerous lesions in both Phase II and Phase III clinical trials. The immune responses induced by HPV vaccination are different to those after natural infection. The virus has learned to largely evade our immune system through centuries of co-evolution with the consequence that the immune responses after natural infection, aiming to control or eradicate the virus, are attenuated. However, the AS04-adjuvanted HPV prophylactic vaccine, designed with a new Adjuvant System approach, is able to improve upon the natural immune responses to the virus as it induces strong and sustained neutralizing antibody levels that are several times higher than after natural infection, while creating immune memory through higher frequency of memory B cells. These immunological effects represent the basis for the protection afforded by the AS04-adjuvanted HPV-16/18 vaccine and its observed long-term efficacy against development of precancerous lesions to prevent cervical cancer.

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Introduction

Cervical cancer is a significant cause of morbidity and mortality in women globally. It is estimated that up to 80% of women will acquire a human papillomavirus (HPV) infection in their lifetime [1]. Although most adult women recover completely from their HPV infection, about 5–10% will not clear the virus and develop persistent infection. Persistent infection with an oncogenic type of HPV is an important early precursor event in the progression to cervical precancerous lesions and cervical cancer [2–5].

As many as 15 HPV types have been identified as being of oncogenic risk to the cervix. Of these, HPV-16 and -18 are most commonly found in cancerous cervical tissue [6], accounting for 70% of the cervical cancer cases worldwide [7,8]. HPV-45 and -31 represent the third and fourth most prevalent types responsible for an additional 10% of all cervical cancer cases, while the risk associated with the remaining oncogenic HPV types is lower. Studies have also shown that HPV persistent infection occurs at a higher percentage with HPV-16 than with other oncogenic HPV types [9] and that the risk of progression to cervical cancer is higher for HPV-16, -18 and -45 than for other HPV types [8].

Despite the high natural HPV infection rate in adolescent female populations, most HPV infections of the cervix are asymptomatic, with reported clearance rates of approximately 30% at 6 months and approximately 50% at 12 months [10]. The degree of protection and the duration of immunity induced by natural incident infection are not known, but only 50–60% of women develop serum antibodies to HPV after natural infection [11], and some of these women are still susceptible to re-infection with the same HPV type. As a result of the asymptomatic pattern of HPV infection in women and the
lifelong risk of being infected, the primary goal for an HPV vaccine is to ensure long-term protection.

If the immune system fails to clear or control an incident infection with oncogenic HPV, the infection may become persistent. A persistent infection is generally defined as the continued detection of viral DNA of the same HPV type in the cervix for at least 6–12 months [12,13] and can lead to the development of high-grade cervical intraepithelial neoplasia (CIN2/3) that can progress to invasive cancer. The risk of squamous intraepithelial lesion or CIN development following persistent HPV-16 or -18 infection is high (odds ratio of 169 for HPV-16/18) [14]. Therefore, in order to provide strong and sustained protection against incident and persistent infections with HPV-16 and -18 and associated precancerous lesions, prophylactic HPV vaccination needs to improve on natural immunity. The aim of HPV vaccination is to induce a first line of immunological defense by eliciting large amounts of antibodies that will be able to prevent the virus from entering the cells at the site of infection.

**The human papillomavirus: a virus which evades host defenses**

Classical chronic viral infections, such as those caused by the hepatitis B virus (HBV) or herpes simplex virus (HSV), share persistent viremia during the course of the infection. HBV primarily causes a systemic infection, while HSV causes both mucosal and systemic infection. The rapid presence of viral particles in the bloodstream allows the immune system to recognize and mount a rapid and strong response directed against the pathogen, including both the secretion of high levels of circulating antibodies able to neutralize and control the pathogen and a cellular response. In addition, for most viral infections, time of disease coincides with the time of productive infection.

HPV infection and the subsequent immunological consequences differ from this classical view of viral infections. HPV does not cause viremia or systemic infection. The virus has a specific tropism either for the squamous epithelium of the skin or mucosal sites. After transmission, mainly by sexual contact, HPV remains located in the epithelial cells of the mucosa. Lacking a bloodstream phase, HPV is barely exposed to the systemic immune system, and antibody levels induced after natural infection are very low and probably not protective during primary infection; they increase only in individuals with persistent infections, i.e. at later stages of the disease [15].

The natural infectious cycle of genital HPV is adapted to the differentiation program of the cells it infects. HPV infects basal keratinocytes that mature vertically through the epithelium to the cervical lumen. In infected cells, viral protein expression and keratinocyte differentiation are controlled in a coordinated fashion, such that the mature virions are produced only in the most superficial layers of the epithelium. As these virus-laden cells desquamate, the viruses are released into the environment [16]. The time from infection to viral release is approximately 3 weeks, which coincides with the time for basal keratinocytes to undergo complete differentiation, desquamation and natural cell death [17].

The adaptation of HPV to the differentiation program of keratinocytes is an important mechanism to evade the host’s immunity. The virus by itself is not cytolytic for the superficial cells. Rather, the fully differentiated epithelial cells undergo a programmed cell death and desquamation. This natural cell death does not present a danger signal to the immune system and therefore is not accompanied by inflammation. Moreover, HPV proteins that would be recognized as foreign by the immune system are expressed only at low levels and are not secreted and hence are not visible to the immune system [18–20]. As a result of these and other host evasion mechanisms, the local innate immune responses aiming to control or eradicate the virus are attenuated.

**HPV and the immune system**

**How the immune system fights against a pathogen**

Upon natural infection, the host immune system generally employs both innate and adaptive immunity to recognize and fight foreign agents (Fig. 1). The innate immune system is able to provide early and non-specific protection against a large variety of pathogens. This response is stereotyped in nature, and does not intensify following repetitive infectious events. In contrast, adaptive immunity, encompassing the humoral and the cell-mediated immune responses, generates pathogen-specific effector cell responses and provides pathogen-specific immunological memory, allowing a more rapid and vigorous response upon a second encounter with the same pathogen.

Local inflammation is the principal marker of the innate immune response, which is activated by local cell death and injury at the site of infection. It involves the recruitment of phagocytic cells (neutrophils, dendritic cells and macrophages) and the release of soluble mediators (such as cytokines and complement). Dendritic cells serve as the bridge between the innate and adaptive immune responses (Fig. 1). These cells take up and process microbial antigens while migrating to secondary lymphoid organs, such as the draining lymph nodes, where they become fully-functional antigen-presenting cells (APC) and interact with T helper lymphocytes that are part of cell-mediated immunity (CMI). T helper lymphocytes are able to regulate the two different arms of the adaptive immune system. They can promote differentiation of cytotoxic T lymphocytes, a class of effector T cells able to migrate to the infected site and kill pathogen-infected cells. A fraction of the T helper lymphocytes and of the cytotoxic T lymphocytes becomes memory T cells and forms the T cell memory pool.

T helper lymphocytes also activate the humoral arm of the immune system, which results in the production of pathogen-specific antibodies (Fig. 1). Although the immune response needed for different pathogens may vary, a strong antibody response is the cornerstone for protection against most viral diseases. Activation by T helper lymphocytes induces the differentiation of B lymphocytes into plasma cells that will secrete pathogen-specific antibodies able to bind the extra-cellular pathogen, flagging it for destruction by the phagocytic cells. The antibodies may also neutralize the virus by binding to the viral capsid proteins, thereby preventing the virus from entering the host cells. Some plasma cells become long-lived
plasma cells and continuously secrete antibodies to maintain a minimum level of protective antibodies long after the first contact with the pathogen. A fraction of B cells differentiates into memory B lymphocytes to form the humoral memory, which ensures that a rapid and amplified antibody response is initiated upon re-infection by the same pathogen.

**Natural immune response against HPV infection**

Despite the ability of HPV to evade the host’s immune system and to down-regulate innate immunity, a primary infection is naturally cleared in approximately 90% of cases. The precise sequence of immune events following HPV infection in the cervical tract is not fully understood, but it is clear that first innate and cell-mediated immunity and then humoral immunity play a role in the immune response at the site of HPV infection in the genital tract [21–23]. A cell-mediated immune response (effector T lymphocytes) directed against the early E2 and E6 HPV proteins occurs first, as reflected by the infiltration of specific helper and cytotoxic T lymphocytes, macrophages, and the local production of proinflammatory cytokines [21,24,25]. Approximately 8 months after infection, low levels of neutralizing antibodies to the major capsid protein L1 may appear in the serum of infected individuals, and specific IgG and secretory IgA are found locally in the cervical mucosa, but at very low levels [23,26,27]. Antibodies generated in response to a persistent primary infection may prevent successive rounds of auto-inoculation within the female genital tract. Induction of CMI would be expected to prevent further outgrowth and eventually induce regression of individual lesions. Although HPV infection can lead to seroconversion (induction of measurable specific antibodies in the serum), in some subjects antibody levels after natural infection appear to be insufficient for long-term protection [28].

**Vaccine-induced immune response against HPV infection**

The first challenge for an HPV vaccine is to demonstrate protective efficacy through a systemic immune response for a virus that enters only via the mucosal route and remains localized there. The mechanism by which vaccine protects against HPV infection has not been fully elucidated, but experimental data have shown that high levels of HPV-specific neutralizing serum antibodies are key for reliable protection against HPV infection [29–31]. Neutralizing antibodies are also considered in a WHO publication “to be the major basis for protection by VLP-based vaccines in humans” [32]. Not only
are anti-HPV antibodies important, but some experimental data also indicate that they are sufficient to afford protection. Indeed, passive transfer of serum or IgG from vaccinated animals to naïve animals has been found to confer protection against animal papillomavirus infection [31,33]. HPV transmission and infection occur at the cervical epithelium, where HPV can be counteracted by local IgG, the main immunoglobulin present in the female genital tract. In vaccinated subjects, antibodies represent the first line of defense. They are already present at the time of exposure. These neutralizing antibodies are not produced locally, but transudate or exudate from serum to the cervical mucus (Fig. 2) [34–37]. The antibodies must reach the cervical mucosa, but they also need to be present at sufficient levels and in a timely and spatially orchestrated manner to neutralize the virus before it enters the cells.

However, the protective level of anti-HPV antibody is not known. After natural infection, some seroconverted individuals have become infected with the same type of HPV [28], indicating that the antibody levels induced after primary infection may not always be sufficient for long-term protection. Although the minimum level for protection can only be established when a sufficient number of breakthrough cases are observed through clinical trials, a direct correlation between antibody levels and protection is intuitively obvious. In that respect, antibody titers close to or inferior to those induced by natural HPV infection may not be sufficient. A high level of specific antibodies in the cervical mucosa at the time of HPV exposure is the best guarantee for protection.

After immunization, exposure of individuals to the same HPV type is not likely to significantly boost the antibody level, since the epithelial localization and invasion process of the virus will make it invisible to the pool of memory cells at an early stage. Notably, a boost of the systemic response has been demonstrated after parenteral antigen challenge only and not after re-infection [38]. Therefore, to offer continued protection against oncogenic HPV types, vaccination should induce not only a strong but also a sustained antibody response at the systemic level and at the site of primary infection [39–41]. This does not exclude that vaccine-induced CMI also plays a role in viral defense of immunized individuals. Enhanced vaccine-induced CMI would be expected to function in support of antibody production and maintenance of memory B cells, and not as an independent effector mechanism.

Traditionally, the induction of memory B cells is considered a crucial factor for the long-term efficacy of vaccine-induced protection [41]. Indeed, a sustained specific antibody production does not only reflect the generation of long-lived plasma cells but also the induction of memory B cells able to regenerate the pool of antibody-secreting cells. In this respect, recent studies have demonstrated a positive correlation between the frequency of antigen-specific memory B cells and antigen-specific serum antibody levels in studies of vaccines against tetanus toxoid [42], smallpox [43] and hepatitis B [44]. Prophylactic vaccination against HPV-16/18 with an AS04-adjuvanted vaccine has been shown to induce high frequencies of memory B cells [39]. This may be the basis for the generation of long-lived plasma cells and hence the high and sustained antibody responses observed up to 6.4 years for both HPV-16 and -18 after VLP vaccination [45–48].

Clinical results after AS04-adjuvanted HPV-16/18 vaccination

HPV prophylactic vaccine

The most successful approach for a prophylactic HPV vaccine has been the use of non-infectious virus-like particles (VLPs), composed of the major virus capsid protein L1. Genetic engineering techniques have been utilized to express these structural proteins, which undergo self-assembly to form empty...
viral capsids. The L1 VLPs have been shown to be morphologically and antigenically similar to authentic virions. Three distinct animal model systems have all demonstrated that L1 VLPs are potent immunogens that can induce protection against virus challenge and associated lesions. Current HPV vaccines for the prevention of cervical cancer have demonstrated very promising efficacy results in recently completed and ongoing clinical trials [38,46,47,49–54].

The L1 VLPs are usually formulated with vaccine adjuvants to enhance the antigen-induced humoral and cellular immune responses. There are two vaccines for the prevention of cervical cancer, which have recently been licensed. One of these two vaccines, Cervarix™ from GlaxoSmithKline, contains L1 VLPs for HPV types 16 and 18 designed for the prevention of related infection and precancerous lesions. The second vaccine, Gardasil™ from Merck, contains L1 VLPs for HPV types 6, 11, 16 and 18 designed for the prevention of precancerous lesions caused by HPV-16/18 and genital warts caused by HPV-6/11. Gardasil™ is formulated with an aluminum hydroxy-phosphate-based adjuvant, whereas Cervarix™ is formulated with an Adjuvant System referred to as AS04, which contains a combination of aluminum hydroxide and the immunostimulant MPL (3-O-desacyl-4′-monophosphoryl lipid A). The AS04 Adjuvant System has been utilized with two other vaccines against viral diseases. The first is a licensed HBV vaccine for hemodialysis patients. The second is a candidate vaccine against herpes simplex virus (HSV) infection. The AS04-adjuvanted HBV vaccine was superior to the corresponding vaccine adjuvanted with aluminum salt alone in terms of its capacity to induce a strong and sustained immune response, e.g. a higher number of subjects being protected and/or a more sustained protection in the long term [55,56]. Evaluation of the efficacy of the AS04-adjuvanted HSV vaccine showed significant protection (73%) against disease in women who were seronegative for HSV-1 and -2 before vaccination [57]. AS04-containing vaccines have been administrated to more than 30,000 subjects, being generally well tolerated with an acceptable safety profile [57–60].

While aluminum salts have been used extensively as adjuvants in vaccines, their mechanism of action is not fully understood and they have not been the optimal choice for several vaccines in development, such as malaria and HSV candidate vaccines [60]. The MPL component of the AS04 Adjuvant System represents an efficient answer to this problem, as this compound is not only able to promote an antibody response but also efficiently augments the cell-mediated arm of an immune response. MPL is the non-toxic form of the cell wall lipopolysaccharide of the gram-negative Salmonella minnesota R595 strain. MPL potentiates specific immune responses to vaccination and has been shown to act through innate immune mechanisms, particularly by activating dendritic cells [61]. When compared with vaccine formulated with aluminum hydroxide alone, although both types of vaccines induced a long-lasting humoral (antibody) response persisting for at least 3.5 years in vaccinated women, vaccination with the AS04 Adjuvant System induced consistently higher antibody levels throughout the observation period [39] (Fig. 3). In addition, AS04 elicited an increased frequency (2.2–5.2-fold) of HPV-16 and HPV-18 L1 VLP-specific memory B cells when compared with aluminum salt formulations [39]. In this respect, inclusion of AS04 in the vaccine formulation will enhance the immune response leading to high antibody levels and high frequency of memory B cells.

Serological response to HPV vaccine

In an ongoing extended follow-up study (580299/007) of an initial Phase Ib primary efficacy trial of the AS04-adjuvanted cervical cancer vaccine in an HPV-16/18 naïve population (580299/001) [46], a strong and sustained anti-HPV-16/18 neutralizing antibody response was induced for up to approximately 5.5 years post-vaccination in young women aged 15–25 years (Fig. 4) [45,48,62,63]. Seropositivity for both HPV-16 and -18 neutralizing antibodies was ≥98% and was sustained throughout this period. At 63–64 months following first
vaccination, geometric mean titers (GMTs) for total anti-HPV-16 and anti-HPV-18 IgG, when compared with baseline GMTs observed in subjects enrolled in a Phase III study (580299/008) who had cleared HPV-16 or -18 infection (seropositive and DNA negative) [51], were at least 11-fold higher for both types at any time point. According to a mathematical modeling study, the vaccine is predicted to generate sustained longevity of anti-HPV-16 and -18 antibody titers, both remaining substantially above those associated with natural infection and lasting for several decades in young women aged 15–25 years [64].

Substantial long-term vaccine efficacy was observed against HPV-16 and/or HPV-18 endpoints, including incident infection (98% [95% CI: 89–100]), 6-month persistent infection (100% [95% CI: 81–100]), 12-month persistent infection (100% [95% CI: 54–100]), and cytological abnormalities greater than or equal to atypical squamous cells of undetermined significance (100% [95% CI: 85–100]) [63]. Vaccine efficacy has also been evaluated in a large, international, Phase III trial (580299/008) conducted in a broad population of young women aged 15–25 years [51]. Vaccinees were uninfected with HPV-16/18 but possibly infected with other oncogenic HPV types or had current or previous low-grade cytological abnormalities prior to vaccination. In an interim analysis, vaccination exhibited high prophylactic efficacy (up to 100%) against CIN grade two lesions or worse that were associated with HPV-16/18.

**Vaccine-induced neutralizing antibodies**

Preclinical models have demonstrated the critical role of neutralizing antibodies in protecting against papillomavirus infection [31,33,65,66]. By preventing persistent HPV-16/18 infection, these antibodies are thought to efficiently prevent cervical cancer development in vaccinated women. It is noteworthy that while total antibody responses can be easily determined by classical antigen-binding enzyme-linked immunosorbent assay (ELISA), neutralizing antibodies can only be

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**Fig. 4.** Geometric mean titers (and 95% confidence intervals) of (A) anti-HPV-16 and (B) anti-HPV-18 total IgG antibodies in women aged 15–25 years included in a Phase II study (580299/007) of AS04-adjuvanted cervical cancer vaccine during a 5.5 year follow-up [69]. Levels of naturally-acquired anti-HPV-16 antibodies [29.8 EU/mL (95% CI: 28.5–31.0)] and HPV-18 antibodies [22.6 EU/mL (95% CI: 21.6–23.6)] are shown by a horizontal line. Geometric mean titers for natural infection were obtained from baseline serum samples of subjects included in a Phase III study (580299/008) who were seropositive and HPV DNA negative for the respective HPV type [51].
identified using pseudovirion-based neutralization assays (PBNA). Anti-HPV-16 and -18 neutralizing antibody titers were specifically assessed in a subset of women throughout the extended follow-up study (S80299/007) [62]. At least 98% of all vaccinees had detectable HPV-16 and -18 neutralizing antibody responses that were maintained up to 5.5 years following first vaccination. The kinetic profile observed for GMTs of neutralizing antibodies against HPV-16 and -18 was similar to that seen with GMTs of total IgG. Furthermore, a high correlation was observed between antigen-binding ELISA and PBNA at all time points up to 5.5 years, indicating that GSK’s antigen-binding ELISA is a valuable tool for measuring vaccine-induced immune response. Taken together with the total antibody ELISA data described above, these findings suggest that the AS04-adjuvanted cervical cancer vaccine elicits high-level, high-quality and long-lasting neutralizing antibody responses in young women aged 15–25 years.

**Vaccine-induced antibodies transudate to the cervical mucosa**

Local and systemic antibody responses play an important role in HPV vaccine-induced responses, as demonstrated by AS04-adjuvanted cervical cancer vaccine, which has been shown to induce high levels of HPV-16 and -18 antibodies in serum and cervicovaginal secretions (CVS) for at least 24 months following the first vaccine dose in females aged 15–55 years [67]. In all age groups, a strong correlation was observed between serum and CVS antibody titers for both HPV-16 and HPV-18 [67]. Thus, systemic vaccination with AS04-adjuvanted vaccine is able to induce serum neutralizing IgG antibodies that transudate to the site of HPV infection in sufficiently high concentrations to bind HPV particles, and may contribute to vaccine efficacy [67]. These results highlight the important relationship between antibody levels at both serological and mucosal sites for prevention of infection in the transformation zone or at the vaginal level. It has previously been shown that vaccine-induced IgG transudates from the serum across the endo-cervical and squamo-columnar surfaces and could therefore play a prominent role in local immunity in the cervicovaginal mucus [35]. In addition to this local protective effect, the systemic presence of vaccine-induced neutralizing antibodies is thought to prevent a new infectious event (at the same or a distant site) through an exudation process due to microtrauma by neutralizing viral particles released by actively infected cells (Figs. 2 and 5). In women infected with HPV-16 or -18, these neutralizing antibodies may bind to virus particles and prevent them from progressing to the transformation zone [36].

**Serological response in early adolescence**

Other studies have shown that immune response to the vaccine is strongest in early adolescent girls. This is important since optimal cervical cancer prevention will require prophylactic vaccination against HPV-16 and -18 in early adolescence, before the onset of sexual activity [52]. In a Phase III clinical trial (107477/012) involving 773 participants (girls, 10–14 years of age) the AS04-adjuvanted cervical cancer vaccine was found to be highly immunogenic with GMTs at Month 7 approximately twice as high as those observed in young women aged 15–25 years in the same study [52]. In a separate Phase III study (104896/013), 626 girls aged 10–14 years received three doses of AS04-adjuvanted cervical cancer vaccine. Eighteen months after the first vaccine dose, GMTs for antibodies to HPV-16 and -18 were at least 5-fold higher than those observed in female vaccinees aged 15–25 years [46,47,68]. Thus, AS04-adjuvanted cervical cancer vaccination in pre-teen/adolescent girls achieves substantially greater antibody responses than those elicited in young women aged 15–25 years, for whom sustained and long-term protection against incident and persistent HPV-16/18 infection and associated cervical precancerous lesions has been established [63,69]. The higher antibody titers observed in 10–14-year-old vaccinees are likely to result in longer antibody persistence [52] and long-term protection [68].

**Serological response in mature women**

Epidemiological studies have shown that incident infection with oncogenic HPV is estimated to occur in 5–10% of women aged 25–55 years [70–72], and although the frequency of new HPV infections decreases with age, women in this age range may remain at risk. In addition, when considering prevalent oncogenic HPV infections the risk of persistence increases in older women [73]. The AS04-adjuvanted cervical cancer vaccine has demonstrated long-term immunogenicity for up to 18 months after the first dose in women over 25 years of age [74]. This Phase III trial (105879/014) was stratified according to age. One hundred percent of women seroconverted after vaccination, with an age-dependent decrease in antibody response that can be attributed to age-related decline in immune function [74]. However, even in the oldest age group, GMTs remained at least 8-fold higher than those observed following natural infection in another study (S80299/008) [51] and were in the same range as reported in young women aged 15–25 years, in whom vaccine efficacy has been demonstrated [46,47]. These data demonstrate that the
AS04-adjuvanted cervical cancer vaccine is highly immunogenic in women up to 55 years of age and may offer protection against new HPV-16/18 infections in a broad age range of women.

**Conclusions**

HPV has learned to evade and down-regulate the host’s innate immune response. Adaptive cell-mediated immunity is then the primary mechanism of defense, as antibody levels observed after natural infection seem unreliable for long-term protection. In order to provide long-term protection against HPV-16 and -18 infection and associated lesions, prophylactic cervical cancer vaccination needs to go beyond and improve on natural immunity.

A cervical cancer vaccine should induce a strong immune response with high and sustained systemic antibody levels that highly correlate with high and sustained antibody levels in the cervix. In contrast to the immune response after natural infection, the antibody responses in the serum and at the cervix after vaccination are the key elements of HPV vaccine-induced responses, together with long-lived plasma cells. The presence of vaccine-induced neutralizing antibodies at the site of HPV infection will be key to preventing virus particles from infecting the transformation zone where cervical cancers usually develop. The AS04-adjuvanted prophylactic cervical cancer vaccine fits this immunological profile, eliciting high and sustained antibody responses against both HPV-16 and -18 together with high and sustained efficacy for up to 6.4 years against HPV-16 and HPV-18 related infections and precancerous lesions.

**Conflict of interest statement**

TFS has received honoraria from GSK as an investigator on HPV vaccine trials, for lecturing on HPV vaccinology, and as member of HPV advisory boards. OL TFS has received honoraria from GSK as an investigator on HPV vaccine trials, and sustained efficacy for up to 6.4 years against HPV-16 and -18 together with high and sustained systemic antibody levels that highly correlate with high and sustained antibody levels in the cervix.

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