Sustained efficacy and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine up to 7.3 years in young adult women

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A B S T R A C T
We report efficacy and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine up to 7.3 years post-vaccination. The study was conducted in a population (N=433) of women enrolled in Brazilian centres from an initial placebo-controlled study. Women were aged 15–25 years at first vaccination. During the most recent year of follow-up, approximately 7 years after initial vaccination, no cases of infection or cytohistological lesions associated with HPV-16/18 were observed in the vaccinees. Vaccine efficacy (95% confidence interval) up to 7.3 years was 94.5% (82.9, 98.9) for incident infection, 100% (55.7, 100) for 12-month persistent infection and 100% (−129.8, 100) for cervical intraepithelial neoplasia grade 2+.
Antibody titres for total IgG and neutralising antibodies remained several folds above natural infection levels and ≥96% of women were seropositive. Vaccine safety was similar to placebo. This is the longest follow-up study for a licensed cervical cancer vaccine.

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1. Introduction
Cervical cancer is the second most common malignancy in women worldwide. The latest estimates from the International Agency for Research on Cancer indicate that it is responsible for almost 300,000 deaths per year [1]. The Latin America and Caribbean region shows a higher prevalence of human papillomavirus (HPV) infection than in North America, Europe and overall worldwide [2,3]. Prevention and early diagnosis programmes in the region have had limited success, hampered by many factors including under-recognition of cervical cancer as a preventable condition, inadequate resources, sub-optimal programme management and weak or absent national policies [4]. The incidence of cervical cancer in Latin America and the Caribbean is predicted to increase by more than 75% in the next 20 years [5]. In Brazil, 18,430 new cases are foreseen during 2010 [6].

It is now well recognised that HPV infection plays a causative role in cervical cancer [7]. Approximately 15 oncogenic HPV types have been identified, of which HPV-16 and HPV-18 are the most commonly detected in cervical cancer. Together, these two types are associated with approximately 70% of cervical cancer worldwide [2]. Other common types include HPV-31, -33, -45, -52, and -58 [2]. Of these, HPV-18 and HPV-45 make a relatively larger contribution to adenocarcinoma compared with squamous cell carcinoma [2]. Prophylactic HPV vaccines are now available and vaccination programmes are being widely implemented, with young adolescent girls being the primary target group for most programmes. Catch-up programmes in older girls and young women are also in place in many countries. It is essential that HPV vaccines offer long-term protection because women remain vulnerable to HPV infection for as long as they are sexually active.

The HPV-16/18 vaccine from GlaxoSmithKline Biologicals is adjuvanted with the Adjuvant System AS04 (comprising aluminium hydroxide and 3-O-desacyl-4′ monophosphoryl lipid A [MPL]) [8]. Studies comparing the HPV-16/18 AS04-adjuvanted vaccine with HPV vaccines adjuvanted with aluminium have shown that the AS04-adjuvanted vaccine produces a greater immune response, with higher and more sustained antibody titres and a higher
frequency of memory B-cells and T-cells [9–11]. An extensive clinical trial programme has shown that the HPV-16/18 AS04-adjuvanted vaccine is highly immunogenic, efficacious against HPV-16/18 infection and associated cytohistological lesions, and generally well tolerated [10–18]. Cross-protection against non-vaccine oncogenic HPV types has also been demonstrated [14,16]. The clinical development programme of the HPV-16/18 AS04-adjuvanted vaccine has included long-term evaluation. We report here an interim analysis of a long-term study of women who had participated in an initial vaccination study, with efficacy and immunogenicity data up to 7.3 years post-vaccination.

2. Materials and methods

2.1. Study objectives

The primary objective of the study was to evaluate long-term vaccine efficacy in prevention of incident cervical infection with HPV-16 and/or HPV-18 in young women. Secondary objectives were to evaluate vaccine efficacy against persistent infection and cytological and histopathological abnormalities associated with HPV-16/18, vaccine efficacy against incident and persistent infection and cytological and histopathological abnormalities associated with non-vaccine oncogenic HPV types, and long-term vaccine safety and immunogenicity.

2.2. Study design and participants

Women were originally enrolled into a double-blind, randomised, multicentre initial study (NCT00689741; HPV-001). From this initial study, women who received all three doses of vaccine or placebo and whose treatment allocation remained blinded were invited to take part in a follow-up study (NCT00120848; HPV-007). Of those, women participating at Brazilian study centres were invited to continue into the current follow-up study (NCT00518336; HPV-023) (Fig. 1). Detailed methodology of the preceding studies has been reported previously [12,13]. Five centres are participating in the current follow-up study. The study started in November 2007 and will last for 3 years (up to approxi-

mately 9.5 years post first vaccination). Here, we report data up to 7.3 years post first vaccination from an interim analysis.

In the initial study, women were randomised 1:1 to receive either the HPV-16/18 AS04-adjuvanted vaccine (Cervarix®, GlaxoSmithKline Biologicals, Rixensart, Belgium) or placebo in a 0-, 1- and 6-month schedule. The vaccine and placebo have been described previously [12]. Treatment allocation has remained blinded throughout the initial and both follow-up studies. Women aged 15–25 years, healthy, HPV-16 and -18 seronegative by enzyme-linked immunosorbent assay (ELISA), HPV DNA-negative by polymerase chain reaction (PCR) for 14 oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) in exocervical or ectocervical cells, and with normal cervical cytology at screening were enrolled in the initial vaccination study. Inclusion and exclusion criteria related to this study have been fully described previously [12].

Follow-up evaluations performed in the current study were conducted in accordance with the current version of the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice guidelines and regulatory law in Brazil. The study protocol and informed consent documentation were approved by the Independent Ethics Committee or Institutional Review Board of each study centre and the National Ethical Committee for Research in Brazil. Written informed consent was obtained from each participant before any study procedure was performed.

2.3. Procedures and endpoints

2.3.1. Virology and cytohistopathology

Cervical samples were collected every 6 months for HPV DNA typing. Gynaecological examinations were performed and cytology samples collected every 12 months. Collection of cytology and histopathology specimens and the clinical management algorithm for abnormal cytology results and colposcopy referral have been previously described [12,13]. A broad spectrum PCR SPF10-LiPA25 system was used to test cervical samples and biopsy material for DNA from 14 oncogenic HPV types [19]; positive specimens were tested by line probe assay and type-specific HPV-16 and HPV-18
Vaccine efficacy was calculated against the following clinical endpoints associated with (1) HPV-16/18 and (2) any oncogenic HPV type: incident infection, 6-month and 12-month persistent infection, cytological abnormalities (atypical squamous cells of undetermined significance or worse [≥ASC-US] and low-grade squamous intraepithelial lesions or worse [≥LSIL]), histopathologically-confirmed cervical intraepithelial neoplasia (CIN) grade 1 and above (CIN1+) and CIN grade 2 and above (CIN2+). Incident infection was defined as the first detection of an HPV type in a woman previously negative for that type. Persistent infection was defined as detection of the same HPV type in two consecutive samples over a minimum of 5 months (6-month definition) or 10 months (12-month definition). CIN1+ was defined as CIN1, 2, 3, adenocarcinoma in situ, and invasive carcinoma; CIN2+ excluded CIN1.

2.3.2. Immunogenicity

Blood samples were collected at months 0, 7, 12 and 18 during the initial study and on a yearly basis during the follow-up studies. As women were enrolled into the follow-up studies independently of the date of first vaccination, results from the follow-up studies are allocated to 6-month intervals relative to the time of the first vaccination of each woman. Total IgG antibody titres to HPV-16 and HPV-18 were measured by ELISA as previously described [12,13,20]. Neutralising antibody titres to HPV-16 and HPV-18 were also assessed using the Pseudovirus-based Neutralisation Assay (PBNA) [21,10] in a subset of subjects.

2.3.3. Safety

Serious adverse events (SAEs), medically significant adverse events (i.e. AEs or SAEs prompting emergency room or physician visits that are not related to common diseases), new onset chronic diseases (NOCDs) and new onset autoimmune diseases (NOADs) were recorded. For assessment of NOCDs, all AEs reported during the trial were compared with a pre-defined list of potential chronic diseases derived from the Medical Dictionary for Regulatory Activities (MedDRA). Determination of whether a chronic disease was of new onset was based on blinded review of the reported symptoms and the subject’s prevaccination medical history by a GSK physician. A separate list, restricted to potential autoimmune events which excluded allergy-related events or isolated signs and symptoms and events not considered to be autoimmune in origin, was used to identify NOADs among events identified as NOCDs. Pregnancies and their outcomes were also recorded.

2.4. Statistics

Two interim analyses after 1 and 2 years of follow-up were specified by the study protocol. The efficacy analysis presented here includes data from the first year of the current follow-up study. In addition, a combined efficacy analysis was performed on the enrolled population, including data from the preceding studies, with a total follow-up period of up to 7.3 years after first vaccination. To illustrate the kinetics of the immune response, immunogenicity data are presented since first vaccination for the population enrolled in the current follow-up study. Safety data are presented for the first year of the current follow-up study.

The overall alpha value for all analyses was 0.05 (two-sided test). Alpha values were adjusted for the two interim analyses: for the first and second interim analyses, \( \alpha = 0.001 \) (two-sided), and for the final analysis, \( \alpha = 0.049 \) (two-sided). Based on an estimated 6% cervical infection rate, minimum 80% vaccine efficacy, 360 women enrolled in the trial and 10% discontinuation rate per year, the power at the end of the trial was estimated at 83%. Combined analyses (HPV-001, HPV-007 and HPV-023) were descriptive. The conditional exact method was used to estimate vaccine efficacy and exact 95% confidence intervals (CI) around the rate ratio (ratio of the event rates in the vaccinated versus placebo group). The calculation took into account the follow-up time of the subjects within each group. Vaccine efficacy was defined as 1 minus the rate ratio.

The primary analysis of efficacy was performed on the according-to-protocol (ATP) cohort for efficacy for virological endpoints. For cytological endpoints, however, the primary analysis was performed on the total vaccinated cohort (TVC) because of the limited number of cases obtained in this study. The primary immunogenicity analysis and the primary safety analysis were performed on the ATP cohort for immunogenicity and the TVC, respectively. The ATP immunogenicity and efficacy cohorts included women who met all eligibility criteria, complied with study procedures in the current and preceding studies, and had data available for at least one vaccine antibody blood sample (ATP immunogenicity cohort) or data available for the efficacy measure considered (ATP efficacy cohort). The TVC included women who were enrolled in the current study, had received at least one dose of study vaccine or placebo in the initial study and for whom endpoint measures were available. All women enrolled in the initial study were randomised and received at least one dose of vaccine or placebo in accordance with the randomisation schedule [12].

Women were not included in immunogenicity assessments if HPV infection was detected for the type under consideration during the study periods in order to exclude any influence of a natural infection on the immune response. Women were censored from efficacy assessments once a specific endpoint was met in the current or preceding studies.

3. Results

A total of 433 women enrolled in the current follow-up study (506 women from the population enrolled at Brazilian centres took part in the initial study and 448 had continued to the first follow-up study) (Fig. 1). Most women (n = 428) completed the first year of the current follow-up study to the time of the present analysis (Fig. 2).

Demographic characteristics in the placebo group and the vaccine group were similar. Age at first vaccination was similar in the whole population of women who entered the initial study, the population who continued to the first follow-up study and the population who continued to the current follow-up study (Table 1). The mean age at entry to the current follow-up study was 26.5 years and the population was racially diverse (Table 1). There was some variability in the racial distribution compared with the preceding studies due to the fact that the current study was conducted exclusively at Brazilian centres (Table 1). The mean follow-up time since first vaccination in the initial study was 7.0 years (2561.6 days; standard deviation: 70.3 days). A total of 395 women were included in the ATP efficacy cohort and 304 in the ATP immunogenicity cohort (Fig. 2). Demographic characteristics were similar to the TVC (data not shown).

3.1. Incident and persistent infection

No cases of incident HPV-16/18 infection were observed in the HPV-16/18 vaccine group during the first year of the current follow-up study. Two cases of incident HPV-16/18 infection were seen during this period in the placebo group. In the combined analysis of the preceding studies and the current follow-up study, vaccine efficacy remained very high against incident infection with HPV-16/18 up to 7.3 years (94.5% [95% CI: 92.9, 98.9%]) (Table 2). Considering incident infection with individual non-vaccine oncogenic types,
Table 1
Demographic characteristics of women in the initial study, the first follow-up study and the current follow-up study (TVC).

<table>
<thead>
<tr>
<th>Study</th>
<th>Initial study (HPV-001)</th>
<th>First follow-up study (HPV-007)</th>
<th>Current follow-up study (HPV-023)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-16/18 vaccine</td>
<td>(n = 560)</td>
<td>HPV-16/18 vaccine</td>
<td>HPV-16/18 vaccine</td>
</tr>
<tr>
<td>Placebo</td>
<td>(n = 553)</td>
<td>Placebo</td>
<td>Placebo</td>
</tr>
<tr>
<td>Age at entry to initial study, years</td>
<td>20.4 (2.8)</td>
<td>20.5 (2.7)</td>
<td>20.3 (2.9)</td>
</tr>
<tr>
<td>Age at entry to follow-up study, years</td>
<td>NA</td>
<td>NA</td>
<td>23.2 (2.9)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>23.2 (2.8)</td>
</tr>
<tr>
<td>Black</td>
<td>43 (7.7)</td>
<td>41 (7.4)</td>
<td>33 (8.4)</td>
</tr>
<tr>
<td>White/caucasian</td>
<td>389 (69.5)</td>
<td>384 (69.4)</td>
<td>251 (63.9)</td>
</tr>
<tr>
<td>Asian/Oriental</td>
<td>9 (1.6)</td>
<td>4 (0.7)</td>
<td>8 (2.0)</td>
</tr>
<tr>
<td>Other</td>
<td>119 (21.3)</td>
<td>124 (22.4)</td>
<td>101 (25.7)</td>
</tr>
</tbody>
</table>

Data shown are mean (standard deviation) for age and number (%) for race.

Vaccine efficacy up to 7.3 years was 41.1% (−40.1, 76.3) and 72.2% (20.5, 92.0) against HPV-31 and HPV-45, respectively. For any oncogenic type including HPV-16 and HPV-18, vaccine efficacy against incident infection was 26.6% (−0.7, 46.5) (Table 2).

No cases of persistent infection with HPV-16/18 were seen in either the HPV-16/18 vaccine group or the placebo group during the first year of the current follow-up study. In the combined analysis, vaccine efficacy against 6-month and 12-month persistent infection with HPV-16/18 was 100% (79.5, 100) and 100% (55.7, 100), respectively. Vaccine efficacy against 6-month and 12-month persistent infection with any oncogenic HPV type was 18.8% (−23.7, 46.8) and 19.4% (−41.0, 54.1), respectively (Table 2). Few cases of persistent infection with HPV-31 or HPV-45 were seen in either the vaccine or placebo group.

3.2. Cytohistological abnormalities

No cases of ≥ASC-US or ≥LSIL associated with HPV-16/18, and no cases of CIN1+ or CIN2+ associated with either HPV-16/18 or with any oncogenic HPV type, occurred during the first year of the current follow-up study in either the vaccine or placebo group.

In the combined analysis, high vaccine efficacy (>94%) was seen against any cytohistological abnormality ≥ASC-US or ≥LSIL associated with HPV-16 and/or HPV-18 up to 7.3 years (Table 3). Substantial...
Table 2
Vaccine efficacy against incident and persistent infection with HPV-16/18 or any oncogenic HPV type in cervical samples up to 7.3 years after first vaccination (ATP efficacy cohort).

<table>
<thead>
<tr>
<th></th>
<th>HPV-16/18 vaccine</th>
<th>Placebo</th>
<th>Vaccine efficacy, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number of women</td>
<td>Women reporting ≥ 1 event</td>
<td>Total number of women</td>
</tr>
<tr>
<td>Incident infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-16/18</td>
<td>193</td>
<td>3</td>
<td>175</td>
</tr>
<tr>
<td>Any oncogenic type</td>
<td>179</td>
<td>80</td>
<td>158</td>
</tr>
<tr>
<td>Persistent infection (6-month)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-16/18</td>
<td>193</td>
<td>0</td>
<td>175</td>
</tr>
<tr>
<td>Any oncogenic type</td>
<td>179</td>
<td>47</td>
<td>158</td>
</tr>
<tr>
<td>Persistent infection (12-month)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-16/18</td>
<td>193</td>
<td>0</td>
<td>175</td>
</tr>
<tr>
<td>Any oncogenic type</td>
<td>179</td>
<td>27</td>
<td>158</td>
</tr>
</tbody>
</table>

Combined analysis of the initial study, first follow-up study and current follow-up study in the population of women who were enrolled at Brazilian centres in the current follow-up study. ATP efficacy cohort (women who met all eligibility criteria, complied with study procedures in preceding and current studies and had data available for efficacy measures).

Table 3
Vaccine efficacy against cytohistological lesions associated with HPV-16/18 or any oncogenic HPV type up to 7.3 years after first vaccination (TVC).

<table>
<thead>
<tr>
<th></th>
<th>HPV-16/18 vaccine</th>
<th>Placebo</th>
<th>Vaccine efficacy, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number of women</td>
<td>Women reporting ≥ 1 event</td>
<td>Total number of women</td>
</tr>
<tr>
<td>≥ASC-US</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-16/18</td>
<td>224</td>
<td>1</td>
<td>219</td>
</tr>
<tr>
<td>Any oncogenic type</td>
<td>224</td>
<td>48</td>
<td>219</td>
</tr>
<tr>
<td>≥LSIL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-16/18</td>
<td>224</td>
<td>1</td>
<td>219</td>
</tr>
<tr>
<td>Any oncogenic type</td>
<td>224</td>
<td>27</td>
<td>219</td>
</tr>
<tr>
<td>CIN1+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-16/18</td>
<td>219</td>
<td>0</td>
<td>212</td>
</tr>
<tr>
<td>Any oncogenic type</td>
<td>219</td>
<td>6</td>
<td>212</td>
</tr>
<tr>
<td>CIN2+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-16/18</td>
<td>219</td>
<td>0</td>
<td>212</td>
</tr>
<tr>
<td>Any oncogenic type</td>
<td>219</td>
<td>5</td>
<td>212</td>
</tr>
</tbody>
</table>

Combined analysis of the initial study, first follow-up study and current follow-up study in the population of women who were enrolled at Brazilian centres in the current follow-up study. TVC (women who were enrolled in the current study, had received at least one dose of study vaccine or placebo in the initial study and for whom endpoint measures were available).

Vaccine efficacy against these abnormalities associated with any oncogenic HPV type was also observed (Table 3). No women who received the HPV-16/18 vaccine experienced a CIN event associated with HPV-16 or HPV-18 up to 7.3 years, and vaccine efficacy was 100% for both CIN1+ and CIN2+ (Table 3). Vaccine efficacy against CIN1+ associated with any oncogenic HPV type was also high (69%; Table 3). Few women in either group experienced a CIN2+ event associated with any oncogenic HPV type (Table 3). Few cytohistological abnormalities associated with HPV-31 or HPV-45 were observed in the vaccine and placebo group.

3.3. Immunogenicity

All women were seropositive for anti-HPV-16 and anti-HPV-18 IgG antibodies at the time of the current analysis (Fig. 3). Antibody levels reached a plateau approximately 18 months after first vaccination and were sustained at a high level thereafter (Fig. 3). After up to 7.3 years following first vaccination, anti-HPV-16 and anti-HPV-18 antibody levels were ≥13-fold and ≥11-fold higher, respectively, than levels following natural infection seen in a phase III efficacy study [15]. Similar results were seen for neutralising antibodies measured by PBN.A in a subset of 45 women (Fig. 4). All women remained seropositive for anti-HPV-16 functional antibodies and ≥96% for anti-HPV-18 functional antibodies. The kinetics of neutralising antibodies were comparable with those of IgG antibodies, and levels also remained several folds higher than those following natural infection in a previous study [10] (Fig. 4).

3.4. Safety

The safety profile of the vaccine group was similar to that of the placebo group (Table 4). Medically significant AEs occurred in 8.1% of women in the vaccine group and 6.2% of women in the placebo group during the first year of follow-up in the current study. Four women in the vaccine group and five in the placebo group experienced a SAE, none of which were considered by the investigators to

Table 4
Number of women reporting medically significant adverse events, serious adverse events, new onset chronic diseases and new onset autoimmune diseases (TVC).

<table>
<thead>
<tr>
<th></th>
<th>HPV-16/18 vaccine</th>
<th>Placebo</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medically significant adverse events, n (%)</td>
<td>18 (8.1)</td>
<td>13 (6.2)</td>
<td></td>
</tr>
<tr>
<td>Serious adverse events, n (%)</td>
<td>4 (1.8)</td>
<td>5 (2.4)</td>
<td></td>
</tr>
<tr>
<td>NOCD, n (%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>NOAD, n (%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown for the first year of the current follow-up study. TVC (women who were enrolled in the current study, had received at least one dose of study vaccine or placebo in the initial study and for whom endpoint measures were available). NOCD: new onset chronic disease; NOAD: new onset autoimmune disease.
be related to the study vaccine or placebo. No women experienced an NOCD or NOAD.

A total of 41 women became pregnant, with 35 normal infants born (19 in the vaccine group and 16 in the placebo group). Two pregnancies were ongoing at the time of the analysis and there were four abnormal outcomes (ectopic pregnancy, elective termination, missed abortion and still birth); as the study blind is still in place, it is not possible to state whether these occurred in women in the vaccine or placebo group.

4. Discussion

The HPV-16/18 AS04-adjuvanted vaccine continues to offer sustained protection up to 7.3 years after first vaccination, as shown by the absence of HPV-16/18 infection or cytohistological lesions associated with HPV-16/18 during the first year of the current follow-up study. In the combined analysis of the preceding and current studies, high vaccine efficacy was achieved against HPV-16/18 infection and cytohistological lesions and we also continue to observe evidence of cross-protection against non-vaccine HPV types.

Vaccine efficacy was associated with high and persistent levels of IgG and neutralising antibodies against HPV-16 and HPV-18. The observation of high neutralising antibody levels is important, as these antibodies are likely to be a part of the immune response forming the basis of protection against HPV infection [22]. A correlation between the IgG ELISA assay and the PBNA which directly measures functional antibodies has been demonstrated previously [20]. This is illustrated by the similar antibody kinetics observed for IgG and neutralising antibodies by which levels reached a plateau after 18 months following first vaccination, with no apparent waning of levels up to 7.3 years. Antibody levels were several folds higher for both IgG and neutralising antibodies than those following natural infection seen in other studies [15,10]. Women who have cleared a natural infection can become re-infected with the same HPV type [23–25]; it is therefore important that vaccines against HPV provide sustained higher levels of antibodies than produced following natural infection. The antibody kinetics demonstrated in the current study indicate production of long-lived plasma cells and memory B-cells that replenish the plasma cell pool. A statistical modelling study based on data from women who received three vaccine doses during the initial study, with data up to 6.4 years post-vaccination, predicts that anti-HPV-16 and anti-HPV-18 antibody levels will remain several folds higher than those associated with natural infection for at least 20 years [26]. Therefore, in view of the long-term immunogenicity data from our study, we anticipate that efficacy will be extended beyond these projections.

The AS04 Adjuvant System is likely to be key in the sustained immunogenicity of the HPV-16/18 vaccine. In a study comparing...
the HPV-16/18 AS04-adjuvanted vaccine with the licensed HPV-6/11/16/18 vaccine that contains aluminium as an adjuvant, a greater immune response, including higher antibody titres and a higher frequency of antigen-specific memory B-cells and CD4+ T-cells, was achieved with the HPV-16/18 AS04-adjuvanted vaccine [10,11]. In an investigation of the administration of a fourth dose of the HPV-16/18 AS04-adjuvanted vaccine given approximately 7 years after first vaccination, a strong anamnestic response was induced [27].

No incident or persistent infection with HPV-16/18 and no cytohistological lesions associated with HPV-16/18 were seen in the vaccine group during the first year of the current follow-up study. Only two cases of incident infection with HPV-16/18 and no cases of persistent HPV-16/18 infection or cytological lesions associated with vaccine types were seen in the placebo group. The sample size of the current study (~450), which includes only women from Brazilian centres, is more limited than in the preceding studies which also included centres in North America, and therefore fewer infections were seen. In addition, women in the study have now reached an age when they are more likely to be in a stable relationship and therefore less likely to acquire an infection. Several studies have shown that peak levels of HPV infection occur in women aged under 25 years, and prevalence decreases with increasing age [28–30].

Women were censored from the analysis of vaccine efficacy if they had reached an endpoint in a previous analysis of the preceding studies. Because of the high efficacy of the vaccine, more women were censored from the placebo group than the vaccine group, leading to disproportionally fewer women in the placebo group remaining at risk. Therefore, the combined analysis of the preceding studies and the current follow-up study, which was not affected by censoring, provides important information. Study procedures, laboratory methodology and endpoint collection were similar in all three studies to allow a combined analysis.

In the combined analysis, vaccine efficacy against infection with HPV-16/18 was high: 94.5% (82.9, 98.9) against incident infection, 100% (79.5, 100) against 6-month persistent infection, and 100% (55.7, 100) against 12-month persistent infection. Persistent
oncogenic HPV infection is a necessary cause of cervical cancer development [31]. It thus provides a marker for the risk of pre-cancerous lesions [32,33], and is a useful and important endpoint in HPV vaccine trials. Vaccine efficacy against ≥ASC-US and ≥LSIL associated with HPV-16/18 was 96.7% (79.9, 99.9) and 92.2% (62.8, 99.9), respectively. A diagnosis of ≥ASC-US usually leads to a referral for colposcopy, and a reduction in such procedures through vaccination would provide significant patient and cost benefits over and above prevention of cervical cancer.

There were a small number of cases of CIN1+ or CIN2+ associated with HPV-16/18, again due to the limited sample size of this study, with all cases observed occurring in the placebo group (7 CIN1+ cases and 3 CIN2+ cases). The vaccine efficacy against CIN1+ in the combined analysis was 100% (34.4, 100). In the larger population including the Brazilian and North American study centres, the analysis of the preceding studies up to 6.4 years demonstrated vaccine efficacy against CIN1+ and CIN2+ of 100%, with much narrower confidence intervals [14].

The vaccine has been previously shown to offer cross-protection against infection and cytohistological lesions associated with various combinations of non-vaccine oncogenic types, including HPV-31 and HPV-45 individually [14,16]. In the present study, there was evidence of cross-protection against non-vaccine HPV types: vaccine efficacy against >ASC-US, >LSIL and CIN1+ associated with any oncogenic type was 40.5% (12.9, 59.7), 53.9% (25.3, 72.2) and 59.0 (18.5, 89.9), respectively. Few cases were observed of incident or persistent infection or cytohistological lesions associated with HPV-31 and HPV-45, the two types most closely related to HPV-16 and HPV-18, respectively, and the most frequent types associated with cervical cancer after HPV-16 and HPV-18 [34]. Vaccine efficacy against incident infection with HPV-31 and HPV-45, respectively, was 41.1% (40.1, 76.3) and 72.2% (20.5, 92.0). A full assessment of cross-protection requires a large sample size and/or a high accumulation of endpoints over a long follow-up period.

The study demonstrated the continuing long-term favourable safety profile of the vaccine, similar to placebo. Vaccine safety has been previously demonstrated in a pooled analysis of almost 30,000 participants in phase II and III trials, including the initial and first follow-up studies [17]. An integrated analysis of AS04-adjuvanted vaccines in over 68,000 participants showed no evidence of an increase in the relative risk of autoimmune disorders associated with the Adjuvant System [18]. During the current long-term observation, no cases of new onset of chronic diseases or autoimmune disorders were observed.

Given the fact that the potential benefits of vaccination are now well accepted, we carefully considered the ethics of the study with respect to maintaining the inclusion of a placebo group. Alongside ethical approval, we believe that this was justified because at the time of enrolment into the current follow-up study, women were above the maximum age at which an HPV vaccine is licensed for use in Brazil, and would therefore have been unable to receive vaccination under normal circumstances. Women also had continuous access to gynaecological care and screening for cervical lesions during the trial. During the course of the follow-up studies, women were informed that a licensed vaccine had become commercially available in their country and that they were able to discontinue their participation in the studies to receive it at any point.

In conclusion, the study showed that the HPV-16/18 AS04-adjuvanted vaccine induces a strong immune response, with high and sustained levels of IgG and neutralising antibodies against HPV-16 and HPV-18 up to 7.3 years, and has a favourable safety profile. No cases of infection with HPV-16/18 and no cases of cytohistological lesions associated with HPV-16/18 were observed during the first year of the current study. High vaccine efficacy against infection and cytohistological lesions associated with HPV-16/18 has been observed in a combined analysis of the preceding and current studies, and there continues to be evidence of vaccine-induced cross-protection against non-vaccine oncogenic HPV types, as previously observed [14,16]. The study is the longest follow-up to date of a licensed vaccine containing the two most frequently observed oncogenic HPV types, HPV-16 and HPV-18, and will continue for a further 2 years.

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**References**


