RESEARCH PAPER

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Comparison of the immunogenicity and safety of *Cervarix*™ and *Gardasil*® human papillomavirus (HPV) cervical cancer vaccines in healthy women aged 18–45 years

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Abbreviations: AEs, adverse events; ANOVA, analysis of variance; AS04, Adjuvant System 04; ATP, according-to-protocol; BEVS, baculovirus expression vector system; CI, confidence interval; CVS, cervicovaginal secretion; DNA, deoxyribonucleic acid; ED_{50}, effective dose producing 50% response; ELISA, enzyme-linked immunosorbent assay; EU, ELISA units; GM, geometric means; GMTs, geometric mean titers; GSK, GlaxoSmithKline; HPV, human papillomavirus; IgG, immunoglobulin G; LiPA, line probe assay; LPS, lipopolysaccharide; MedDRA, Medical Dictionary for Regulatory Activities; MPL, monophosphoryl lipid A; MSCs, medically significant conditions; NOAD, new onset of autoimmune disease; NOCD, new onset of chronic disease; PBNA, pseudovirion-based neutralization assay; PCR, polymerase chain reaction; SAEs, serious adverse events; SeAP, secreted alkaline phosphatase gene; VLPs, virus-like particles

This observer-blind study compared the prophylactic human papillomavirus (HPV) vaccines, *Cervarix*™ (GlaxoSmithKline) and *Gardasil*® (Merck), by assessing immunogenicity and safety through one month after completion of the three-dose vaccination course. Women (n = 1106) were stratified by age (18–26, 27–35, 36–45 years) and randomized (1:1) to receive *Cervarix*™ (Months 0, 1, 6) or *Gardasil*® (Months 0, 2, 6). At Month 7 after first vaccination, all women in the according-to-protocol cohort who were seronegative/DNA negative before vaccination for the HPV type analyzed had seroconverted for HPV-16 and HPV-18 serum neutralizing antibodies, as measured by pseudovirion-based neutralization assay (PBNA), except for two women aged 27–35 years in the *Gardasil*® group who did not seroconvert for HPV-18 (98%). Geometric mean titers of serum neutralizing antibodies ranged from 2.3–4.8-fold higher for HPV-16 and 6.8–9.1-fold higher for HPV-18 after vaccination with *Cervarix*™ compared with *Gardasil*®, across all age strata. In the total vaccinated cohort (all women who received at least one vaccine dose, regardless of their serological and DNA status prior to vaccination), *Cervarix*™ induced significantly higher serum neutralizing antibody titers in all age strata ($p < 0.0001$). Positivity rates for anti-HPV-16 and -18 neutralizing antibodies in cervicovaginal secretions and circulating HPV-16 and -18 specific memory B-cell frequencies were also higher after vaccination with *Cervarix*™ compared with *Gardasil*®. Both vaccines were generally well tolerated. The incidence of unsolicited adverse events was comparable between vaccinated groups. The incidence of solicited symptoms was generally higher after *Cervarix*™, injection site reactions being most common. However, compliance rates with the three-dose schedules were similarly high (≥ 84%) for both vaccines. Although the importance of differences in magnitude of immune response between these vaccines is unknown, they may represent determinants of duration of protection against HPV-16/18. Long-term studies evaluating duration of efficacy after vaccination are needed for both vaccines.

Introduction

Persistent infection with an oncogenic human papillomavirus (HPV) type is a necessary cause of cervical cancer,[1-3] the second most common cause of cancer death among women worldwide.[3,4] Globally, HPV-16 and -18 are the predominant oncogenic types, cumulatively accounting for over 70% of all invasive cervical cancer cases.[5,6]

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HPV, which exhibits a specific tropism either for the squamous epithelium of the skin or mucosal sites, evades local immune responses and does not cause viremia or systemic infection. Despite low or undetectable antibody levels following natural infection, [7] HPV type-specific immunity is stimulated by natural infection; however, it should be noted that immune responses after natural infection are not always protective against reinfection and whether natural immunity can be lifelong is unknown.[7,8]

Two prophylactic HPV vaccines have recently been licensed in many countries. Both use virus-like particles (VLPs) comprised of recombinant L1 capsid proteins of individual HPV types to prevent HPV-16 and -18 cervical precancerous lesions and cancers. *Cervarix*™ contains HPV-16 and -18 VLPs produced in *Trichoplusia ni* Rix4446 cell substrate using a baculovirus expression vector system (BEVS) and formulated with the proprietary immunostimulatory Adjuvant System 04 [AS04; comprised of 3-O-desacyl-4'-monophosphoryl lipid A (MPL) and aluminum hydroxide salt].[9-11] *Gardasil*® contains HPV-16 and -18 VLPs produced in the yeast *Saccharomyces cerevisiae* and formulated with amorphous aluminum hydroxyphosphate sulfate salt.[12-14] In addition, *Gardasil*® contains VLPs from non-oncogenic types HPV-6 and -11, which are implicated in 75–90% of genital warts.[12-15] For both vaccines, protection against infection with oncogenic types HPV-16 and HPV-18 and associated precancerous lesions has been demonstrated in randomized clinical trials (for reviews see references 16–18). Protection has been demonstrated for at least 6.4 years post-vaccination for *Cervarix*™ [19-22] and at least 5 years for *Gardasil*®.[23-25]

Since women may be at risk for acquisition of HPV infection for as long as they are sexually active, vaccination needs to induce long-term protective efficacy. Serum neutralizing antibodies, which are known to transudate to the site of infection, are generally presumed to constitute the major basis of protection against HPV infection for prophylactic vaccines.[8,26-29] Induction of HPV-specific memory B-cells that are able to replenish the pool of antibody-secreting cells is important for long-term maintenance of vaccine-induced protection.[27]

Given the length of time usually required for development of cervical pre-cancer (several years) and invasive cancer (typically 10 years or longer from incident HPV infection)[2,3,30,31] outcomes and the high levels of efficacy observed with both licensed HPV vaccines in pre-licensure studies, any differences in clinical efficacy associated with waning protection (should they be present) may not become apparent for many years. In addition, direct comparison of the available clinical trial data for the two vaccines across different studies is not feasible given the absence of an established serological correlate of protection and differences in study design and methodology used to evaluate HPV-16/18 specific efficacy endpoints and immune responses. For *Cervarix*™, vaccine-induced antibody responses have primarily been measured by conventional enzyme-linked immunosorbent assay (ELISA), which measures neutralizing and non-neutralizing antibodies, or pseudovirion-based neutralization assay (PBNA), which measures a range of functional neutralizing antibodies.[19,20] For *Gardasil*®, immunogenicity has mainly been evaluated by competitive radioimmunoassay[32] or competitive Luminex-based immunoassay.[33,34] Furthermore, direct comparison of the levels of immune response to HPV-16 and HPV-18 cannot be performed even when the same measurement system (e.g., ELISA) is used.

Public health authorities are currently evaluating which vaccine to use when implementing HPV vaccination programs. Duration of vaccine-induced protection and the likelihood of requiring booster vaccination are important in cost-benefit analyses. This randomized, observer-blind study compared the two vaccines in a single, well-defined population of healthy women aged 18–45 years, using identical methodology for assessment of immunogenicity and safety. *Cervarix*™ and *Gardasil*® were administered according to their recommended three-dose vaccination schedules (Months 0, 1, 6 and Months 0, 2, 6, respectively). The age range of 18–45 years was chosen to enable full characterization of the immune response to vaccination, which included collection of cervicovaginal secretion (CVS) samples for assessment of mucosal HPV antibody levels. This age range also provides stringent conditions for comparison of the two vaccines, as immune response to vaccination decreases with increasing age. In this study, neutralizing antibody levels induced by the two vaccines were evaluated using PBNA[35] in order to objectively compare functional immune responses using an unbiased assay.

Results

Study population. A total of 1106 women were enrolled and vaccinated; 553 in each group. Of these, 37.7% were aged 18–26 years, 32.2% were aged 27–35 years and 30.1% were aged 36–45 years (it was not considered ethical for females younger than 18 years of age to be enrolled in the study due to the speculum exam required for CVS sampling). Slightly more women were enrolled in the 18–26 year age group to ensure that the study was sufficiently powered for the primary objective. Four hundred and fifty-nine women in the *Cervarix*™ group and 461 women in the *Gardasil*® group completed the study to Month 7 (83.0% and 83.4% of those enrolled in the two groups, respectively). Reasons for withdrawal from the study did not differ between groups (**Fig. 1**). In the total vaccinated cohort (TVC, all subjects who received at least one vaccine dose), key demographic characteristics of women who did not complete the study through Month 7 were comparable to those of women who did continue. The according-to-protocol (ATP) cohort for immunogenicity assessment comprised 370 women in the *Cervarix*™ group and 364 in the *Gardasil*® group. Reasons for exclusion from the ATP cohort were balanced between groups (**Fig. 1**). Key demographic characteristics for this cohort are shown in **Table 1**.

Serum neutralizing antibody responses. Seropositivity rates and geometric mean titers (GMTs) for HPV-16 and HPV-18 antibodies, measured by PBNA in women in the ATP cohort for immunogenicity who were seronegative and deoxyribonucleic acid (DNA) negative prior to vaccination for the HPV antigen under analysis, are shown by age stratification in **Table 2**. One month after completion of the three-dose vaccination course (Month 7), all women in both vaccine groups had seroconverted for HPV-16 and HPV-18, except for two women aged 27–35 years in the *Gardasil*® group who did not seroconvert for HPV-18.

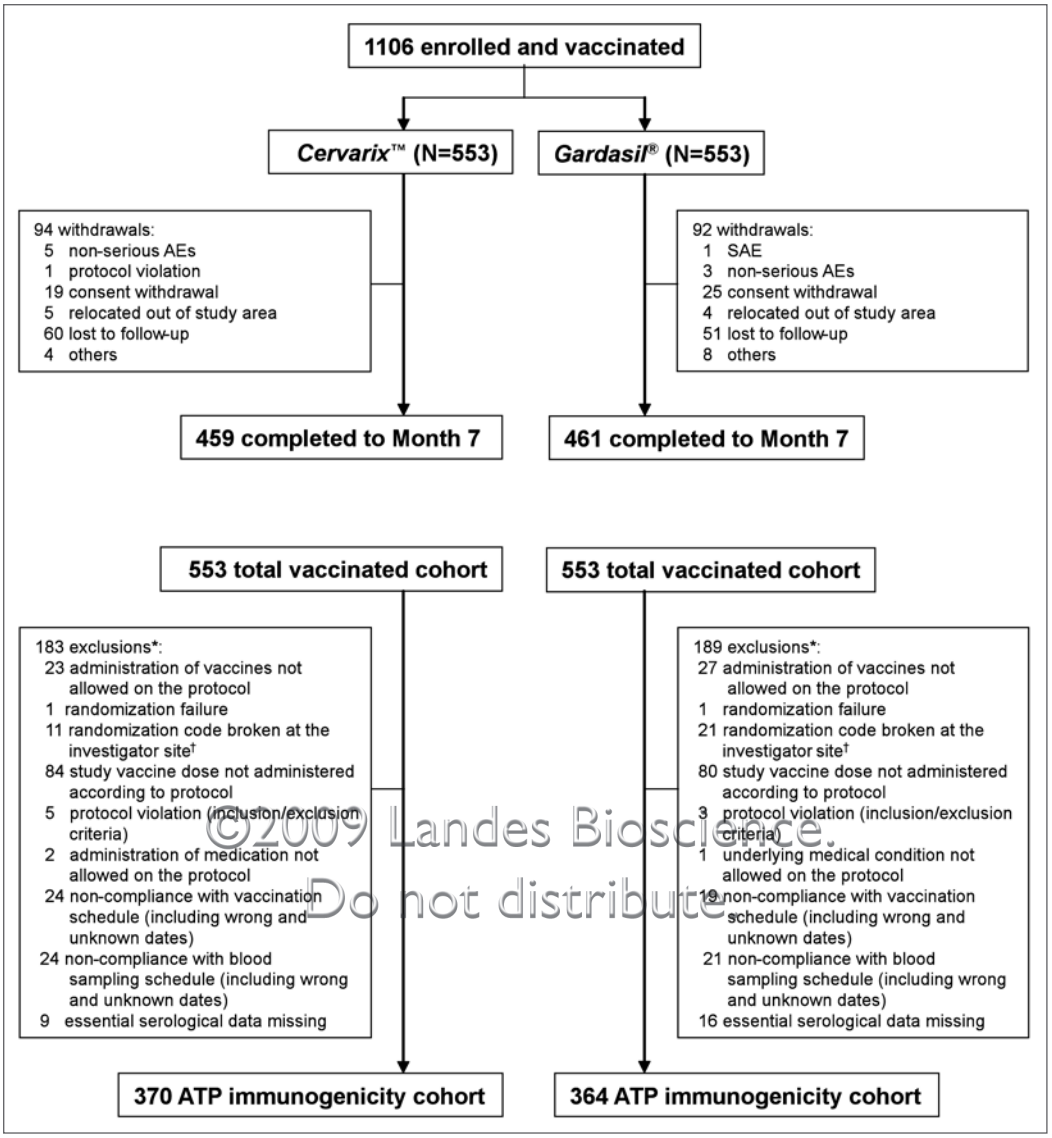

Figure 1. Subject disposition. ATP, according to protocol. * Women may have been excluded for more than one reason, but were only counted for the primary reason for exclusion.† The randomization code was broken at the investigator site for 34 women, two of whom already had a previous elimination code. The most common reason for the randomization code being broken was a technical problem with the randomization system (24 women). Primary and secondary between-group comparisons to assess non-inferiority were performed in the according-to-protocol (ATP) cohort on women who were HPV seronegative and HPV DNA negative (by PCR) prior to vaccination for the antigen under analysis (ATP seronegative/DNA negative cohort). Analysis of superiority and reactogenicity/safety was performed in the total vaccinated cohort on all women regardless of their serological and DNA status prior to vaccination.

For all age groups combined, neutralizing antibody GMTs measured by PBNA in women in the total vaccinated cohort who had cleared natural infection (i.e., seropositive and DNA negative at Month 0 for the HPV antigen under analysis) were 180.1 ED_{50} (effective dose producing 50% response) [95% confidence interval (CI): 153.3, 211.4] for HPV-16 and 137.3 ED_{50} [95% CI: 112.2, 168.0] for HPV-18. For both vaccines, neutralizing antibody GMTs at Month 7 in women in the ATP cohort for immunogenicity who were seronegative and DNA negative prior to vaccination for the HPV antigen under analysis (**Table 2**) were well above those associated with natural infection. Non-inferiority of HPV-16 and -18 immune responses of *Cervarix*™ versus *Gardasil*® was shown in all three age groups for both HPV-16 and HPV-18 (**Table 2**). Anti-HPV-16 and -18 neutralizing antibody GMTs at Month 7 were 3.7- and 7.3-fold higher, respectively, in the *Cervarix*™ group than in the *Gardasil*® group in women aged 18–26 years (**Table 2**). Compared with *Gardasil*®, anti-HPV-16 and -18 GMTs with *Cervarix*™ were 4.8- and 9.1-fold higher in women aged 27–35 years and 2.3- and 6.8-fold higher in women aged 36–45 years, respectively (**Table 2**).

The higher HPV-16 and -18 antibody titers in the *Cervarix*™ group are also illustrated by reverse cumulative distribution curves for women aged 18–26 years in the ATP seronegative/DNA negative cohort (**Fig. 2**). For HPV-16, the median titer of

Table 1. Summary of demographic characteristics (ATP cohort for immunogenicity)

Characteristic	18–26 years		27–35 years		36–45 years		Total	
	Cervarix™ (N = 132)	*Gardasil*® (N = 137)	*Cervarix*™ (N = 117)	*Gardasil*® (N = 116)	*Cervarix*™ (N = 121)	*Gardasil*® (N = 111)	*Cervarix*™ (N = 370)	*Gardasil*® (N = 364)
Age (years)								
Mean	22.1	22.3	30.2	30.5	40.5	39.8	30.7	30.2
SD	2.25	2.44	2.57	2.57	2.70	2.86	8.02	7.67
Ethnic origin (%)								
Caucasian	84.8	86.1	81.2	80.2	86.8	87.4	84.3	84.6
African American	5.3	5.8	9.4	6.0	7.4	3.6	7.3	5.2
Asian	1.5	1.5	1.7	2.6	0.8	1.8	1.4	1.9
Other	8.4	6.6	7.7	11.2	5.0	7.2	7.0	8.3
No. of women seronegative and DNA negative at baseline, n (%)								
HPV-16	104 (78.8)	103 (75.2)	90 (76.9)	85 (73.3)	96 (79.3)	83 (74.8)	290 (78.4)	271 (74.5)
HPV-18	118 (89.4)	131 (95.6)	102 (87.2)	101 (87.1)	110 (90.9)	91 (82.0)	330 (89.2)	323 (88.7)
No. of women seropositive and DNA negative at baseline, n (%)								
HPV-16	13 (9.8)	18 (13.1)	17 (14.5)	25 (21.6)	22 (18.2)	27 (24.3)	52 (14.1)	70 (19.2)
HPV-18	8 (6.1)	3 (2.2)	11 (9.4)	12 (10.3)	9 (7.4)	19 (17.1)	28 (7.6)	34 (9.3)
No. of women seropositive and DNA positive at baseline, n (%)								
HPV-16	10 (7.6)	9 (6.6)	2 (1.7)	2 (1.7)	1 (0.8)	0 (0.0)	13 (3.5)	11 (3.0)
HPV-18	1 (0.8)	2 (1.5)	1 (0.9)	2 (1.7)	0 (0.0)	1 (0.9)	2 (0.5)	5 (1.4)
No. of women DNA negative for HR HPV at baseline, n (%)	84 (63.6)	96 (70.1)	88 (75.2)	83 (71.6)	105 (86.8)	100 (90.1)	277 (74.9)	279 (76.6)

SD, standard deviation; HR HPV, high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. For ethnic origin, other includes American Indian, Alaskan native, native Hawaiian or other Pacific Islander, Arabic or North African heritage. No racial, ethnic or HPV differences existed between the two groups at the p = 0.05 level. HPV serostatus and HPV DNA status (assessed by pseudovirion-based neutralization assay and PCR, respectively) prior to vaccination for the antigen under analysis

serum neutralizing antibodies was 34899 ED_{50} in the *Cervarix*™ group versus 10924 ED_{50} in the *Gardasil*® group. More than 85% of women who received *Cervarix*™ had HPV-16 antibody titers above the median titer for *Gardasil*®. Less than 10% of women vaccinated with *Gardasil*® had HPV-16 antibody titers above the median titer for *Cervarix*™. For HPV-18, the median antibody titer was 14482 ED_{50} in the *Cervarix*™ group versus 2266 ED_{50} in the *Gardasil*® group. More than 95% of women who received *Cervarix*™ had HPV-18 antibody titers above the median titer for *Gardasil*®. Less than 10% of women vaccinated with *Gardasil*® had HPV-18 antibody titers above the median titer for *Cervarix*™. Similar trends were observed in women aged 27–35 and 36–45 years (**Fig. 2**).

Analysis of antibody kinetics before dose three (Month 6) showed that anti-HPV-18 antibody levels were already higher in the *Cervarix*™ group than in the *Gardasil*® group after two vaccine doses; the lower limit of the two-sided 97.6% CI for the GMT ratio was >1 in all age groups (**Table 2**). No differences in anti-HPV-16 GMTs were seen between the two vaccine groups prior to dose three (**Table 2**).

Superiority testing performed on the total vaccinated cohort (irrespective of HPV serostatus and HPV DNA status prior to vaccination) confirmed the neutralizing antibody levels induced by *Cervarix*™ to be significantly higher than that induced by *Gardasil*® for each antigen in all age groups ($p < 0.0001$) (**Table 3**).

Antibody levels in cervicovaginal secretions. Positivity rates for anti-HPV-16 and -18 antibodies in CVS measured by PBNA at Month 7 in the ATP cohort for immunogenicity are shown in **Table 4A**. CVS neutralizing antibody positivity rates were higher in the *Cervarix*™ group than in the *Gardasil*® group for both HPV-16 [81.3% (95% CI: 67.4, 91.1) versus 50.9% (95% CI: 37.3, 64.4)] and HPV-18 [33.3% (95% CI: 20.4, 48.4) versus 8.8% (95% CI: 2.9, 19.3)]. The use of the PBNA to measure antibody levels in CVS samples is associated with methodological challenges that may potentially reduce the sensitivity of this assay, e.g. presence of inhibitors, timing relative to menstrual cycle, sample dilution. All CVS samples were also tested by ELISA to assess positivity rates (**Table 4B**). For both *Cervarix*™ and *Gardasil*®, positivity rates for anti-HPV-16 and anti-HPV-18 antibodies in CVS were higher when measured by ELISA versus PBNA.

Figure 3 shows scatter plots, for HPV-16 and HPV-18, of the ratios between HPV-specific antibody titers (measured either by PBNA or VLP-specific ELISA) and total immunoglobulin G (IgG) content in serum versus CVS at Month 7 in the ATP cohort for immunogenicity. For each vaccine, geometric means (GM) of the ratios between HPV-specific neutralizing antibodies

Table 2. Seropositivity rates, GMTs and GMT ratios for HPV-16 and HPV-18 serum neutralizing antibodies measured by pseudovirion-based neutralization assay at Months 6 and 7 (ATP cohort for immunogenicity, seronegative and DNA negative prior to vaccination)

A 18–26 years

			Cervarix™			*Gardasil*®			
Antigen	Month	N	% SP [95% CI]	GMT [95% CI]	N	% SP [95% CI]	GMT [95% CI]	GMT ratio	97.6% CI
HPV-16	6	104	100 [96.5, 100]	1628 [1304, 2032]	102	99.0 [94.7, 100]	1592 [1204, 2106]	1.0	0.7, 1.5
	7	104	100 [96.5, 100]	36792 [29266, 46254]	103	100 [96.5, 100]	10053 [8136, 12422]	3.7	2.6, 5.2
HPV-18	6	118	99.2 [95.4, 100]	686 [549, 858]	130	93.1 [87.3, 96.8]	234 [187, 294]	2.9	2.0, 4.2
	7	118	100 [96.9, 100]	16487 [13384, 20310]	131	100 [97.2, 100]	2258 [1809, 2818]	7.3	5.1, 10.4

B 27–35 years

			Cervarix™			*Gardasil*®			
Antigen	Month	N	% SP [95% CI]	GMT [95% CI]	N	% SP [95% CI]	GMT [95% CI]	GMT ratio	97.6% CI
HPV-16	6	90	100 [96.0,100]	1263 [893, 1787]	84	98.8 [93.5, 100]	1014 [738, 1394]	1.2	0.7, 2.1
	7	90	100 [96.0,100]	23908 [18913, 30222]	85	100 [95.8, 100]	4958 [3896, 6311]	4.8	3.3, 7.1
HPV-18	6	102	97.1 [91.6, 99.4]	429 [326, 564]	100	84.0 [75.3, 90.6]	176 [133, 233]	2.4	1.6, 3.8
	7	102	100 [96.4, 100]	9502 [7519, 12008]	101	98.0 [93.0, 99.8]	1043 [790,1378]	9.1	6.0, 13.8

C 36–45 years

			Cervarix™			*Gardasil*®			
Antigen	Month	N	% SP [95% CI]	GMT [95% CI]	N	% SP [95% CI]	GMT [95% CI]	GMT ratio	97.6% CI
HPV-16	6	96	99.9 [94.3, 100]	1730 [1215, 2463]	81	100 [95.5, 100]	1917 [1361, 2698]	0.9	0.5, 1.6
	7	96	100 [96.2, 100]	17302 [13605, 22002]	83	100 [95.7, 100]	7634 [5916, 9853]	2.3	1.5, 3.4
HPV-18	6	110	97.3 [92.2, 99.4]	619 [447, 857]	89	87.6 [79.0, 93.7]	169 [127, 224]	3.7	2.2, 6.1
	7	110	100 [96.7, 100]	9846 [7835, 12372]	91	100 [96.0, 100]	1439 [1105, 1873]	6.8	4.6, 10.2

GMT, geometric mean antibody titer; SP, seropositivity (defined as neutralizing antibody titer ≥ 40 ED_{50}). GMT ratio = *Cervarix*™ GMT divided by *Gardasil*® GMT at Month 6 and Month 7 computed using an ANOVA model on the log_{10} transformation of the titers in each age cohort. The ATP cohort for immunogenicity included all evaluable subjects who received three vaccine doses (i.e. those meeting all eligibility criteria, complying with the procedures defined in the protocol) for whom data concerning immunogenicity endpoint measures were available. This included subjects for whom assay results were available for antibodies against at least one study vaccine antigen (HPV-16 or HPV-18) at Month 7.

(measured by PBNA) and total IgG were generally similar for serum and CVS samples, for both HPV-16 and HPV-18, indicating that a similar proportion of HPV-specific neutralizing antibodies transudates from serum to CVS for both vaccines. GM ratios in serum and CVS were approximately ≥ three-fold higher with *Cervarix*™ than with *Gardasil*®, regardless of the assay used for CVS measurements (PBNA or ELISA). Since the mechanism of transudation of serum antibodies into the CVS is expected to be the same regardless of the vaccine eliciting the immune response, overall Pearson correlation coefficients were calculated for each antigen using data for *Cervarix*™ and *Gardasil*® combined. For both HPV-16 and -18, a linear relationship (**Fig. 3**) was found for ratios of HPV-specific antibodies/total IgG between serum and CVS, irrespective of whether CVS measurements were performed by PBNA or ELISA.

Memory B-cell responses. In women with no detectable B-cell response prior to vaccination, the proportion of responders (defined as women with detectable memory B-cell response at Month 7) for HPV-16 was similar in the two groups [89.8% (95% CI: 79.2, 96.2) in the *Cervarix*™ group and 94.3% (95% CI: 84.3, 98.8) in the *Gardasil*® group; p=0.5]. The proportion of responders for HPV-18 was higher in the *Cervarix*™ group than in the *Gardasil*® group [88.7% (95% CI: 78.1, 95.3) versus 66.1% (95% CI: 52.6, 77.9), respectively; p = 0.0041]. At Month 7, the frequency of antigen-specific memory B-cells in responders was 2.7-fold higher in the *Cervarix*™ group than in the *Gardasil*® group for both HPV-16 and HPV-18 (p < 0.0001 for both antigens) (**Fig. 4**).

Reactogenicity and safety. No difference in compliance (i.e., the percentage of women in each group receiving all three vaccine doses) was seen between the two groups (84.6% in the *Cervarix*™ group and 84.4% in the *Gardasil*® group). The percentage of women reporting at least one solicited local or general symptom within seven days after any vaccine dose was higher in the *Cervarix*™ group than in the *Gardasil*® group [95.1% (95% CI: 92.8, 96.7) versus 85.1% (95% CI: 81.8, 88.1), respectively]. The percentages of women reporting specific solicited local symptoms at least once within seven days after any vaccine dose are shown in **Table 5**. Injection site pain was the most frequent solicited local symptom in both groups, reported by 92.9% [95% CI: 90.4, 95.0] of women who received *Cervarix*™ and 71.6% [95% CI: 67.5, 75.4] of women who received *Gardasil*®. This was of Grade

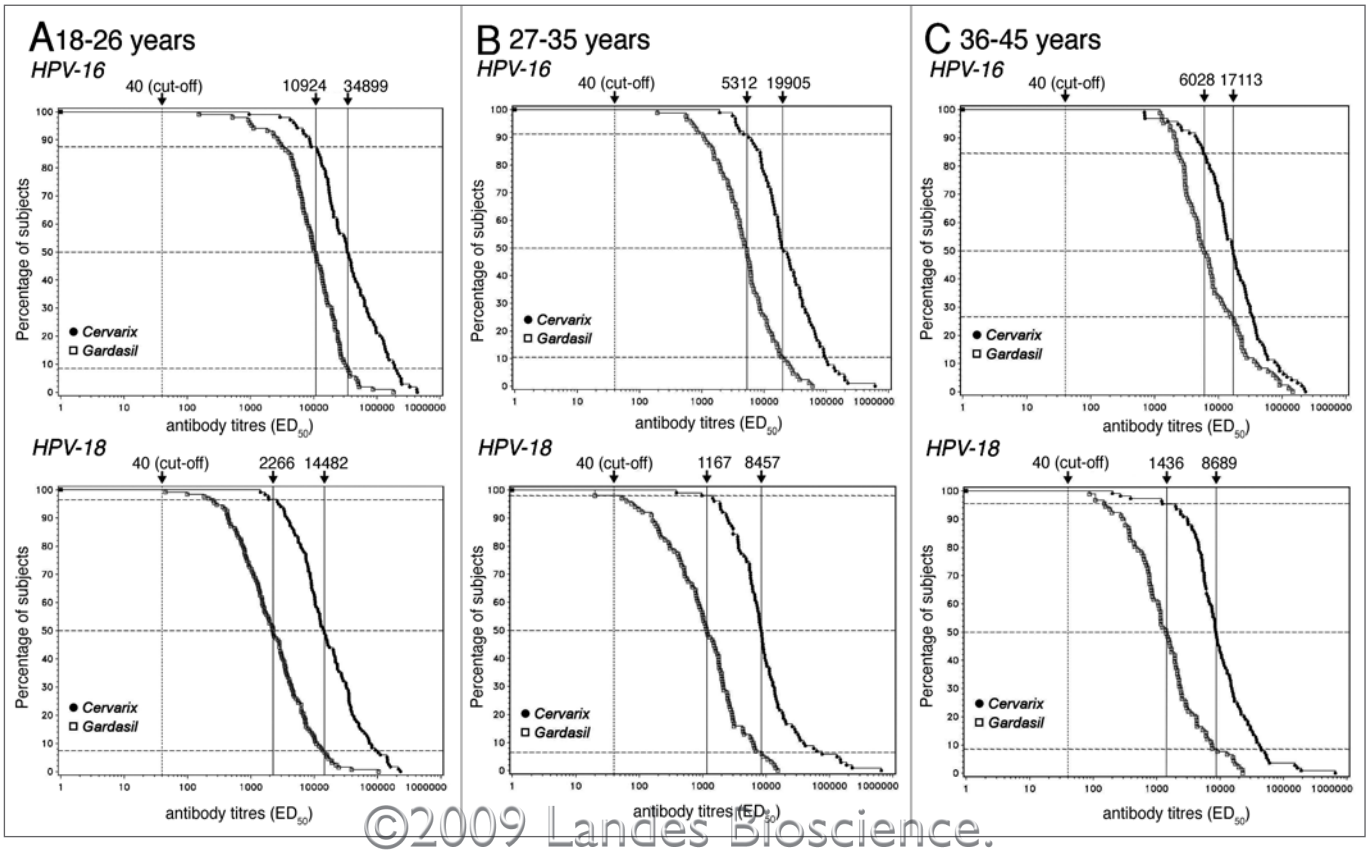

Figure 2. Reverse cumulative distribution curves of HPV-16 and HPV-18 serum neutralizing antibodies measured by pseudovirion-based neutralization assay at Month 7 (ATP cohort for immunogenicity, seronegative and DNA-negative prior to vaccination). Solid vertical lines represent median values. For example, for women aged 18–26 years (A), the median titer of serum anti-HPV-16 neutralizing antibodies was 34899 ED_{50} in the *Cervarix*™ group versus 10924 ED_{50} in the *Gardasil*® group. More than 85% of women (see upper horizontal dashed line) aged 18–26 years who received *Cervarix*™ had anti-HPV-16 antibody titers above the median titer for *Gardasil*®. Less than 10% of women (see lower horizontal dashed line) aged 18–26 years vaccinated with *Gardasil*® had anti-HPV-16 antibody titers above the median titer for *Cervarix*™. For HPV-18, the median antibody titer was 14482 ED_{50} in the *Cervarix*™ group versus 2266 ED_{50} in the *Gardasil*® group. More than 95% of women who received *Cervarix*™ had HPV-18 antibody titers above the median titer for *Gardasil*®. Less than 10% of women vaccinated with *Gardasil*® had HPV-18 antibody titers above the median titer for Cervarix™. Similar trends were observed in women aged 27–35 years (B) and 36–45 (C) years.

3 severity in 17.4% [95% CI: 14.2, 20.9] and 3.4% [95% CI: 2.0, 5.4] of women in the *Cervarix*™ and *Gardasil*® groups, respectively. Redness and swelling were also reported more frequently in the *Cervarix*™ group than the *Gardasil*® group (**Table 5**). All solicited local symptoms were transient (mean duration ≤ 3.3 days) and resolved spontaneously without sequelae. In both groups, rates of solicited local symptoms were similar after each dose, with no consistent pattern of increase with subsequent doses.

The percentages of women reporting specific solicited general symptoms at least once within seven days after any vaccine dose are shown in **Table 6**. Fatigue and myalgia were more frequently reported after administration of *Cervarix*™ compared with *Gardasil*®. Most solicited general symptoms were transient

Table 3. Superiority assessment in terms of GMTs for HPV-16 and HPV-18 serum neutralizing antibodies measured by pseudovirion-based neutralization assay at Month 7 (total vaccinated cohort, irrespective of HPV serostatus and HPV DNA status prior to vaccination)

		Cervarix™		*Gardasil*®				
Age (years)	HPV type	N	GMT	N	GMT	GMT ratio	97.6% CI	ANOVA p-value*
18–26	16	167	31715	168	8682	3.7	2.7, 5.0	<0.0001
	18	167	13732	168	1886	7.3	5.2, 10.2	<0.0001
27–35	16	146	25134	148	7322	3.4	2.4, 5.0	<0.0001
	18	146	9390	148	1178	8.0	5.5, 11.6	<0.0001
36–45	16	143	21874	143	9828	2.2	1.6, 3.1	<0.0001
	18	143	9760	143	1709	5.7	4.0, 8.1	<0.0001

GMT, geometric mean antibody titer. * p-value refers to superiority testing; superiority of *Cervarix*™ demonstrated if $p \leq 0.024$

Table 4. Cervicovaginal secretion antibody titers: positivity rates at Month 7 for anti-HPV-16 and anti-HPV-18 antibodies measured by (A) pseudovirion-based neutralization assay (PBNA) and (B) enzyme-linked immunosorbent assay (ELISA) [ATP cohort for immunogenicity, irrespective of HPV serostatus and HPV DNA status prior to vaccination]

A PBNA							
			Cervarix™			*Gardasil*®	
Antigen	Timing	N	n	% P [95% CI]	N	n	% P [95% CI]
HPV-16	Baseline	24	3	12.5 [2.7, 32.4]	36	5	13.9 [4.7, 29.5]
	Month 7	48	39	81.3 [67.4, 91.1]	57	29	50.9 [37.3, 64.4]
HPV-18	Baseline	24	1	4.2 [0.1, 21.1]	36	2	5.6 [0.7, 18.7]
	Month 7	48	16	33.3 [20.4, 48.4]	57	5	8.8 [2.9, 19.3]
B ELISA							
			Cervarix™			*Gardasil*®	
Antigen	Timing	N	n	% P [95% CI]	N	n	% P [95% CI]
HPV-16	Baseline	24	0	0.0 [0.0, 14.2]	36	0	0.0 [0.0, 9.7]
	Month 7	48	46	95.8 [85.7, 99.5]	57	51	89.5 [78.5, 96.0]
HPV-18	Baseline	24	1	4.2 [0.1, 21.1]	36	3	8.3 [1.8, 22.5]
	Month 7	48	43	89.6 [77.3, 96.5]	57	40	70.2 [56.6, 81.6]

In (A), P = positivity for neutralizing antibodies (defined as a CVS dilution greater than or equal to the assay threshold of 40 ED_{50} for each antigen with both vaccines). In (B), P = positivity for VLP-specific IgG antibodies (defined as an antibody titer ≥ 0.58 EU/mL for HPV-16 and ≥ 0.35 EU/mL for HPV-18).N = number of subjects with available results with Hemastix® ≤ 80 erythrocytes/μL in CVS. A higher proportion of CVS samples collected at baseline had blood contamination when compared with those collected at Month 7, likely due to the additional collection of cervical samples for HPV DNA testing at the baseline visit. Blood contamination is an unpredictable, random phenomenon of this collection method and could be due to many factors: infection, lesion, (HPV-related or not), menstruation, or from the sampling method itself (i.e. microabrasion and microscopic lesions caused by the sampling device).

(mean duration ≤ 2.7 days) and of mild or moderate severity, with few Grade 3 symptoms reported in either group (≤ 5.7% [95% CI: 3.9, 8.0] in the *Cervarix*™ group and ≤ 3.8% [95% CI: 2.3, 5.8] in the *Gardasil*® group). In both groups, the overall incidence of solicited general symptoms was highest after the first vaccine dose compared with subsequent doses.

The proportion of women reporting at least one unsolicited symptom within 30 days after any vaccine dose was 42.5% (95% CI: 38.3, 46.7) in the *Cervarix*™ group and 36.5% (95% CI: 32.5, 40.7) in the *Gardasil*® group. Rates of medically significant conditions (MSCs) were 29.7% [95% CI: 25.9, 33.7] and 26.8% [95% CI: 23.1, 30.7] in the *Cervarix*™ and *Gardasil*® groups, respectively. Rates of all individual unsolicited symptoms and MSCs were low and comparable between groups. New onset of chronic disease (NOCD) events were observed in 14 women in the *Cervarix*™ group and 13 women in the *Gardasil*® group. The most frequent were depression, hypertension, and hypothyroidism. Four NOCD cases were considered to be new onset of autoimmune disease (NOAD) events. Serious adverse events (SAEs) were reported by six women in the *Cervarix*™ group and seven women in the *Gardasil*® group, two of which were considered possibly related to vaccination (one grand mal convulsion which occurred one day after administration of the third dose of *Cervarix*™ and one spontaneous abortion which occurred 47 days after the first dose of *Gardasil*®). It is important to reiterate that decisions relating adverse events (AEs) to vaccination were based on the judgment of the investigator at the study site reporting the event. Withdrawals due to AEs were infrequent (five women in the *Cervarix*™ group and four women in the *Gardasil*® group).

Nineteen pregnancies were reported (10 in the *Cervarix*™ group and nine in the *Gardasil*® group). There were four normal infants (two in each group), two spontaneous abortions (one in each group), four elective terminations (three in the *Cervarix*™ group; one in the *Gardasil*® group), two subjects lost to follow-up (one in each group), and five ongoing pregnancies (three in the *Cervarix*™ group, two in the *Gardasil*® group) at the time of this analysis. There was also one missed abortion and one premature birth in the *Gardasil*® group.

Discussion

HPV-16 and HPV-18 are responsible for approximately 54% and 17% of invasive cervical cancer cases worldwide, respectively.[5] Prophylactic HPV vaccines are expected to reduce the burden of cervical cancer, a major cause of cancer death among women. Based on the natural history of HPV infection and the high efficacy observed for both licensed vaccines in pre-licensure studies, any differences in clinical efficacy associated with waning protection between prophylactic HPV vaccines, if they exist, are unlikely to become apparent for many years.

Currently, no serologic correlate has been defined for protection afforded by HPV vaccines. Preclinical data suggest that transudation of neutralizing antibodies to the site of infection constitutes the primary mechanism for protection against HPV-16/18 infection following vaccination with HPV L1 VLPs.[26,36-41] One preclinical study suggested that protection against HPV-11 infection may be predicted based on the concentration of VLP-specific IgG antibodies induced by immunization with purified HPV-11 virions.[36] Furthermore, clinical experience with other

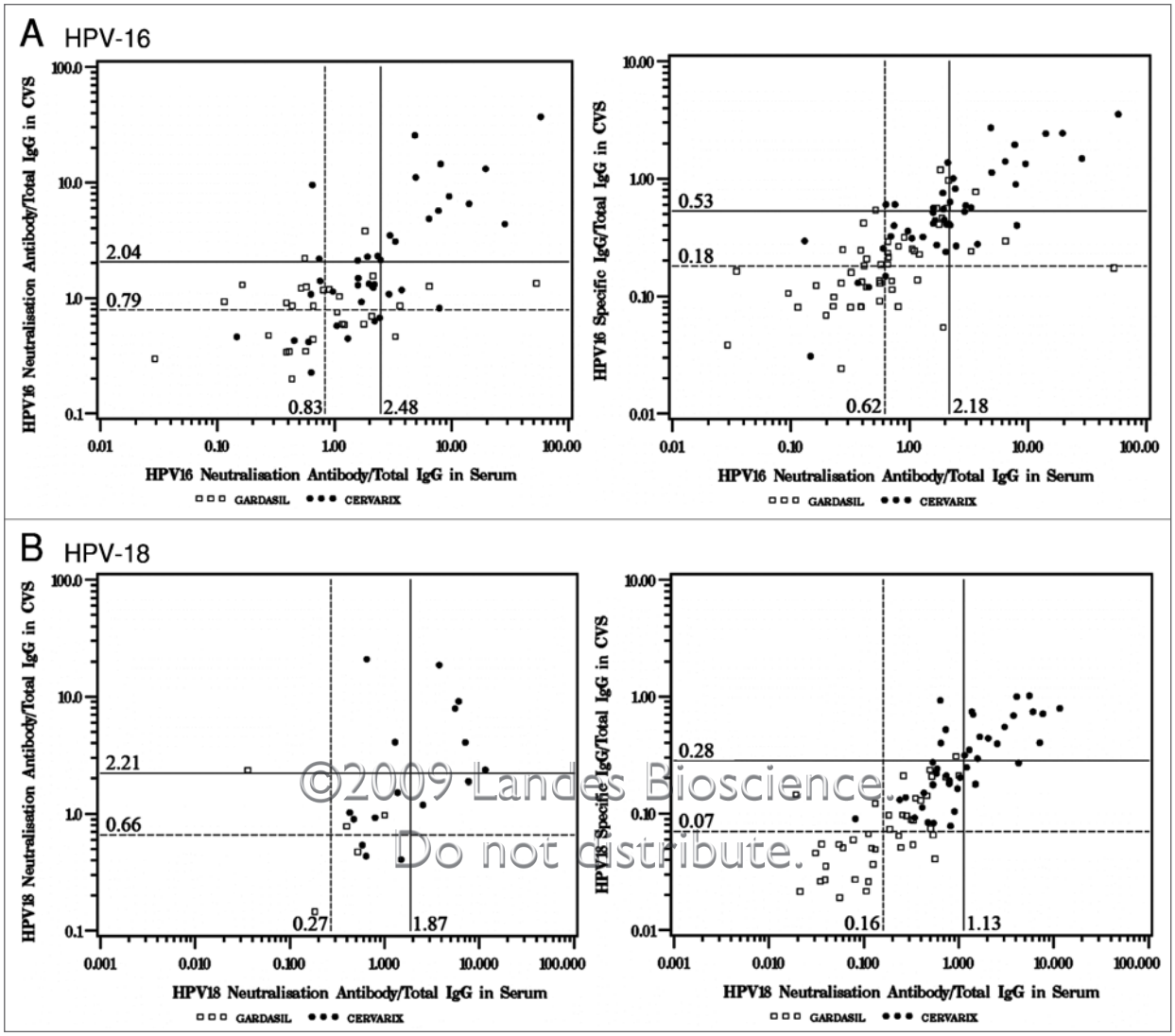

Figure 3. Scatter plots for (A) HPV-16 and (B) HPV-18 showing ratios between HPV-specific antibody titers and total IgG content in serum and cervicovaginal secretion (CVS) samples at Month 7 (ATP cohort for immunogenicity). In (A), the overall Pearson correlation coefficients [r] (*Cervarix*™ and *Gardasil*® data combined) are: *Left panel*; r = 0.64 (N = 68). *Right panel*; r=0.74 (N = 96). In (B), the overall Pearson correlation coefficients (*Cervarix*™ and *Gardasil*®data combined) are: *Left panel*; r = 0.46 (N = 21). *Right panel*; r=0.83 (N = 82). Only samples that tested positive for the HPV antigen under analysis in both serum and CVS (i.e., double-positive samples) were analyzed. Solid and dotted lines (vertical = serum, horizontal = CVS) represent the geometric means of the ratios between HPV-specific antibody titers and total IgG content of samples for *Cervarix*™ and *Gardasil*®, respectively. HPV-16/18 neutralizing antibody levels (both panels) were measured by pseudovirion-based neutralization assay. HPV-16/18 specific IgG levels (panels at right only) were measured by VLP-specific ELISA. The total IgG concentration of each sample was measured using an ELISA developed and validated in-house by GSK.

vaccines, such as those against pertussis and hepatitis A, suggests that the magnitude of the humoral response together with the induction of antigen-specific memory B-cells are important determinants of duration of protection.[42-48] This may not always be the case, since loss of detectable antibodies following vaccination with hepatitis B vaccine has rarely been associated with breakthrough cases of clinical hepatitis B disease in the presence of hepatitis B virus exposure, as measured by hepatitis B core antibody ELISA.[49,50] However, the relevance of this observation to HPV disease is unknown; unlike hepatitis B virus, which is a blood-borne infection, HPV does not cause viremia or systemic infection but exhibits a specific tropism either for the squamous epithelium of the skin or mucosal sites.

This study was undertaken to compare the immune response to the two prophylactic HPV vaccines, *Cervarix*™ and *Gardasil*®, using the same methodology for assessment of immune response and reactogenicity. Although inclusion of pre-teenage girls and young adolescents was considered, this was not ethical in this study involving administration of placebo and a speculum exam for CVS sampling. The exclusion of younger women is a weakness that is inherent to the study design; however, given that immune response in females aged <18 years has generally been as

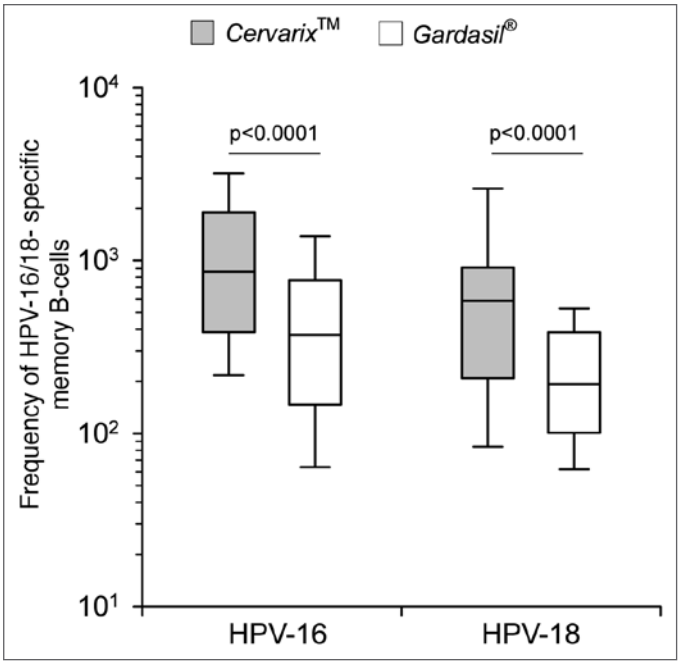

Figure 4. Frequency of HPV-16 and HPV-18 specific memory B-cells per million memory B-cells at Month 7 in responders (i.e., women with no detectable HPV type-specific B-cells prior to vaccination but with detectable HPV type-specific B-cells at Month 7) on a logarithmic scale (ATP cohort for immunogenicity). Whiskers represent 10th and 90th percentiles; p<0.0001 for *Cervarix*™ versus *Gardasil*® for both antigens.

Table 5. Percentages of women reporting specific solicited local symptoms at least once within seven days after any vaccine dose (total vaccinated cohort)

	% women [95% CI]	
Symptom	*Cervarix*™ (N = 524)	*Gardasil*® (N = 524)
Pain		
Any	92.9 [90.4, 95.0]	71.6 [67.5, 75.4]
Grade 3*	17.4 [14.2, 20.9]	3.4 [2.0, 5.4]
Redness		
Any	44.3 [40.0, 48.6]	25.6 [21.9, 29.5]
>50 mm	0.6 [0.1, 1.7]	0.0 [0.0, 0.7]
Swelling		
Any	36.5 [32.3, 40.7]	21.8 [18.3, 25.5]
>50 mm	1.0 [0.3, 2.2]	0.6 [0.1, 1.7]

N = number of women with at least one documented dose and diary card data available. * Grade 3 pain defined as pain preventing normal activity.

good or better than that in older females in studies of both vaccines,[11,12] the data collected from this study of women aged 18-45 years are useful despite the age-range limitation. The study was conducted observer-blind to enable the vaccines to be administered according to their recommended schedules, with placebo administered at Month 1 or 2 as necessary to maintain blinding. We have considered the possibility that administration of placebo at these different timepoints between the *Cervarix*™ and *Gardasil*® groups may have impacted the immune responses at Month 7. However, this is unlikely since administration of aluminum hydroxide in the absence of HPV VLPs one month before or after vaccination would not be expected to impact HPV-specific antibody levels.

Immunogenicity was primarily assessed by measurement of neutralizing antibody levels one month after completion of the three-dose vaccination course (Month 7) utilizing a PBNA performed by technicians blinded to treatment group assignment. The PBNA measures a range of functional antibodies and as such differs from a monoclonal antibody-based competitive ELISA, which is limited to the assessment of immune response to a single neutralizing epitope or a classical ELISA which measures neutralizing and non-neutralizing antibodies. In the PBNA, HPV pseudovirions are produced in human embryonic kidney cells. This cell line is not used in the production of either vaccine. As pseudovirions include both L1 and L2 capsid proteins with amino acid sequences that are unmodified from the sequences obtained from the independent laboratory which developed the assay,[35] they closely resemble the natural viral particles, making the PBNA unbiased to either vaccine. Published data suggest that no major differences exist between the L1 sequences of the pseudovirions and the VLPs present in *Cervarix*™ and *Gardasil*® (besides the C-terminal truncation of the *Cervarix*™ L1 sequences mentioned earlier and discussed later in this Discussion).

Results of this study showed that HPV-16 and -18 neutralizing antibody levels induced by *Cervarix*™ at Month 7 were higher than those induced by *Gardasil*® across all age strata ($p < 0.0001$ for each antigen in all age groups by analysis of variance [ANOVA]). Although *Gardasil*® contains a greater quantity of HPV-16 VLP than *Cervarix*™ (40 µg versus 20 µg, respectively), anti-HPV-16 neutralizing antibody levels were 2.3–4.8 fold higher in the *Cervarix*™ group one month after completion of the three-dose vaccination course. The difference in neutralizing antibody levels induced by the two vaccines was more pronounced for HPV-18, being 6.8–9.1-fold higher in the *Cervarix*™ group.

Antibody levels at the site of infection (i.e., in CVS) provide additional relevant information regarding protective immunological activity[8,51] and were also assessed in this study. CVS neutralizing antibody positivity rates were higher after vaccination with *Cervarix*™ when compared with *Gardasil*® for both HPV-16 and HPV-18. In line with serum antibody responses, *Cervarix*™ was found to induce higher GM ratios of HPV-specific response to total IgG content in CVS than *Gardasil*® for both HPV-16 and HPV-18, when measured either by PBNA or ELISA. This suggests that the higher levels of serum antibodies induced by *Cervarix*™ result in more antibodies transudating to the CVS and therefore more HPV-specific antibodies at the site of infection.

The ELISA used to measure anti-HPV-16 and -18 IgG antibody responses in CVS was based on the VLPs present in *Cervarix*™, thereby potentially biasing the results in favor of this vaccine. However, for both HPV-16 and HPV-18, when serum and CVS ratios of HPV-specific antibodies to total IgG were plotted, relationships were generally linear, regardless of whether CVS measurements were performed by PBNA or ELISA

Table 6. Percentages of women reporting specific solicited general symptoms at least once within seven days after any vaccine dose (total vaccinated cohort)

	% women [95% CI]	
Symptom	*Cervarix*™ (N = 526)	*Gardasil*® (N = 526)
Arthralgia		
Any	21.7 [18.2, 25.4]	15.4 [12.48, 18.8]
Grade 3	2.5 [1.3, 4.2]	0.6 [0.1, 1.7]
Fatigue		
Any	49.8 [45.5, 54.2]	39.8 [35.6, 44.1]
Grade 3	5.7 [3.9, 8.0]	2.3 [1.2, 4.0]
Fever		
≥37.5 °C	14.4 [11.6, 17.7]	11.0 [8.5, 14.0]
≥39.0 °C	0.4 [0.0, 1.4]	0.0 [0.0, 0.7]
Gastrointestinal		
Any	32.7 [28.7, 36.9]	26.5 [22.7, 30.5]
Grade 3	1.9 [0.9, 3.5]	2.3 [1.2, 4.0]
Headache		
Any	47.5 [43.2, 51.9]	41.9 [37.6, 46.3]
Grade 3	3.6 [2.2, 5.6]	3.8 [2.3, 5.8]
Myalgia		
Any	27.6 [23.8, 31.6]	19.6 [16.3, 23.3]
Grade 3	1.9 [0.9, 3.5]	1.5 [0.7, 3.0]
Rash		
Any	4.8 [3.1, 6.9]	3.4 [2.0, 5.4]
Grade 3	0.0 [0.0, 0.7]	0.2 [0.0, 1.1]
Urticaria		
Any	4.9 [3.3, 7.2]	4.0 [2.5, 6.0]
Grade 3*	0.2 [0.0, 1.1]	0.4 [0.0, 1.4]

N = number of women with at least one documented dose and diary card data available. Unless otherwise stated Grade 3 symptoms defined as preventing normal activity. * For urticaria, defined as rash distributed on at least four body areas.

Table 7. Composition of the study vaccines and administration schedules

	Cervarix™	*Gardasil*®
Antigens	20 µg HPV-16 VLP 20 µg HPV-18 VLP	40 µg HPV-16 VLP 20 µg HPV-18 VLP 20 µg HPV-6 VLP 40 µg HPV-11 VLP
Expression system	Baculovirus expression vector system in *Trichoplusia ni* Rix4446 cell substrate	*Saccharomyces cerevisiae* yeast
Adjuvant	AS04 [50 µg MPL and 500 µg Al(OH)3]	225 µg amorphous aluminum hydroxyphosphate sulfate
Administration schedule		
Month 0	Cervarix™	Gardasil®
Month 1	Cervarix™	Placebo [500 µg Al(OH)3]
Month 2	Placebo [500 µg $Al(OH)_3$]	Gardasil®
Month 6	Cervarix™	Gardasil®

MPL, 3-O-desacyl-4'-monophosphoryl lipid A

(**Fig. 3**). The magnitudes of the differences in GM ratios between *Cervarix*™ and *Gardasil*® were also similar when using CVS antibody titers generated by either PBNA or ELISA. This finding suggests that, despite using the VLPs present in *Cervarix*™ as the coating antigen, the ELISA does not appear to be biased in favor of *Cervarix*™.

Vaccine-induced anti-HPV-16 and -18 antibody levels peak at Month 7 (one month after completion of the vaccination course)[19-21,23,24] and they may be predictive of responses at later timepoints. Nonetheless, the serum and CVS data presented herein must be interpreted carefully, considering the short time period assessed and the small number of CVS samples analyzed. Pending results from follow-up at Months 12, 18, 24 and 48 will also be important. Furthermore, a limitation of this study is that the differences in immunogenicity observed between the two vaccines in women aged 18-45 years may not necessarily reflect differences observed in the target age range for vaccination (i.e. pre-teenage girls and young adolescents), where vaccine-induced immune response is generally higher for both vaccines.[11,12]

Exploratory analysis showed the frequency of antigen-specific memory B-cells one month after completion of vaccination to be 2.7-fold higher with *Cervarix*™ than with *Gardasil*® for both antigens. Memory B-cell frequencies were evaluated by B-cell ELISPOT assay, using the truncated form of the HPV-16 and -18 L1 VLPs present in *Cervarix*™ to detect strain-specific B-cells. As with the ELISA, we cannot completely rule out the possibility that use of the *Cervarix*™ construct in the B-cell ELISPOT assay introduced an element of bias. However, data are not expected to be significantly impacted by use of these truncated proteins, given their overall similarity of 93% with the full-length L1 protein sequences, as deduced from the published literature.

Experience with other vaccines indicates that memory B-cells are responsible for driving the rapid anamnestic antibody response that occurs after re-exposure to antigen. Little is known about the role of memory B-cells in protection against HPV; however, increased frequency of HPV-16/18 specific memory B-cells one month after the third vaccine dose may be an additional marker of both the longevity of specific immune responses and the durability of the vaccine-induced humoral response. Memory B-cells also play a role in replenishing the pool of plasma cells that maintain antibody levels in the absence of a pathogen.[44,46,52,53] This concept is supported by the observation of a positive correlation between the frequency of circulating antigen-specific memory B-cells and antigen-specific antibody production for various vaccine antigens[42,44,46] and by the plateauing of HPV vaccine-induced antibody levels to stable levels maintained for over 5 years post-vaccination with *Cervarix*™.[22]

The observed differences in immune response induced by the two vaccines could be due to differences in formulation, particularly with regard to adjuvant factors. Adjuvants have long been used to enhance the immune response to vaccine antigens.

Aluminum salts have been used successfully as vaccine adjuvants for more than 80 years and represent the conventional method of non-specific proinflammatory augmentation of an immune response.[54] Recently, novel adjuvant systems combining classical adjuvants (such as aluminum salts) and specific immunomodulatory molecules (such as MPL and AS04) have been developed to optimize vaccine-induced immune response.[55,56] MPL is derived from a chemical modification of the potent immunomodulator lipopolysaccharide (LPS) of *Salmonella minnesota*.[54,56,57] Humans are regularly exposed to LPS by natural exposure to bacteria, many of which contain LPS as a major component of the bacterial cell wall.[57] Studies with *Cervarix*™ and other novel vaccines [e.g. *FENdrix*™ (hepatitis B vaccine, GlaxoSmithKline Biologicals)] show antibody titers to be consistently higher and sustained over a longer period of time when adjuvanted with AS04 compared with vaccines adjuvanted with aluminum salts alone.[58-60] AS04-based formulations were also found to elicit an increased frequency of antigen-specific memory B-cells compared with aluminum salts alone.[58]

The reactogenicity profiles of both HPV vaccines in this study were consistent with results of previous clinical trials of *Cervarix*™ and *Gardasil*®.[11,12] The incidence of solicited symptoms was generally higher with *Cervarix*™, mainly with respect to local injection site reactions, which may be related to the use of AS04. However, these solicited local symptoms were transient, typically lasting no more than three days and resolving without sequelae. Furthermore, compliance with the three-dose vaccination schedule was high (≥ 84%) in both groups, indicative of clinically acceptable reactogenicity. The incidence of other adverse events (including unsolicited symptoms, MSCs, NOCDs, NOADs, SAEs and withdrawals due to AEs) was comparable between groups.

Data produced to date over at least 5 years of follow-up have shown *Cervarix*™ and *Gardasil*® to have very similar efficacy against virological and disease endpoints for HPV-16 and -18. In this first head-to-head trial of these two prophylactic HPV vaccines, serum neutralizing antibody titers, positivity rates and GM ratios for neutralizing antibodies in CVS, and the frequency of HPV-specific memory B-cells were higher at Month 7 after vaccination with *Cervarix*™ than with *Gardasil*®, in a broad age range of women. Although the clinical importance of these differences in immune response is unknown, they may represent determinants of duration of protection against HPV-16 and -18. Long-term follow-up studies evaluating the duration of immune response and efficacy in disease prevention for both vaccines are necessary to determine the clinical relevance, if any, of the immunological differences observed between vaccination with *Cervarix*™ and *Gardasil*®.

Materials and Methods

Study participants and ethics. Healthy women aged 18–45 years were eligible to participate. Participants were required to have an intact cervix (e.g., no history of cauterization or surgical treatment involving damage to the transformation zone of the cervix). A negative urine pregnancy test was required at study entry and prior to each vaccine dose. If of childbearing potential, participants were required to be abstinent or use adequate contraception for 30 days prior to vaccination and to agree to continue such precautions for two months after the final vaccine dose. Lifetime number of sexual partners was not a limiting factor for inclusion in the study. Women who had previously received any HPV vaccine or vaccine/product containing MPL or AS04 were excluded. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study design and all study materials were approved by the Institutional Review Board or equivalent at all participating centers. All women provided written informed consent to participate.

Study design and vaccines. This was a Phase III study conducted in 40 centers in the USA. Women were stratified by age (18–26, 27–35 and 36–45 years) and randomized (1:1 ratio in each age group) to receive 0.5 mL doses of either *Cervarix*™ or *Gardasil*® administered into the deltoid muscle of the non-dominant arm according to their recommended three-dose schedules (Months 0, 1, 6 or Months 0, 2, 6, respectively). The study was conducted in an observer-blind manner (i.e., vaccines were prepared and administered by qualified medical personnel not otherwise involved in the conduct of the study, with study personnel involved in the clinical evaluation of subjects and subjects themselves remaining blinded to treatment group). To maintain the blind, women received one dose of placebo [$Al(OH)_3$] at either Month 1 or 2 as appropriate. Vaccine composition and administration schedules are summarized in **Table 7**. In addition to providing protection against HPV-16 and HPV-18-associated endpoints, *Gardasil*® also contains HPV-type 6 and 11 VLPs and has been shown to offer protection against HPV-6 and -11 associated genital warts. Immunogenicity against HPV-6 and HPV-11 was not measured in this study since such protection is not offered by *Cervarix*™. In addition to the final analysis at Month 7 presented in this article, long-term follow-up of subjects through 48 months after first vaccination is ongoing in an extension phase of this study.

Blood, cervicovaginal secretion and cervical sampling. Blood samples for assessment of immune response were scheduled to be collected from all women at Months 0, 6 and 7. Additional samples were collected from a subset of women (planned for approximately 30% of the total number of enrolled women per age cohort at pre-selected sites) at Day 0 and Month 7 for further immunological assessment, including evaluation of HPV type-specific antibody levels in CVS and HPV type-specific memory B-cell responses. CVS samples were collected using sterile Merocel® Sponge Points (Medtronic, Jacksonville, FL) as described previously[61] and menstrual cycle duration and date of last menstrual period were recorded. In cases of menstruation/bleeding, the collection of samples was delayed until one day after cessation of menstrual flow. In addition, healthcare providers collected cervical samples from all participants prior to the first vaccination using a Cervex-Brush® (Rovers Medical Devices B.V., Oss, The Netherlands) or, an endocervical brush and spatula, placed in PreservCyt medium (Cytyc Corporation, Marlborough, MA). The collection method used was dependent upon physician preference and availability of the Cervex-Brush® and endocervical

brush at each study site. These cervical samples were used to assess baseline HPV DNA status by polymerase chain reaction (PCR), using SPF10 primers and a reverse hybridization line probe assay (LiPA) as previously described.[19] All testing was performed by laboratory technicians blinded to treatment group assignment.

Antibody extraction from cervicovaginal secretion samples. Antibody extraction from CVS samples was performed as described previously[61] except that only CVS samples showing ≤ 80 erythrocytes per µL [measured using the Hemastix® test (Siemens Medical Solutions Diagnostics Europe Ltd., Dublin, Ireland)] were retained for antibody assessment.

Pseudovirion-based neutralization assay. Serum and CVS anti-HPV-16 and -18 neutralizing antibody titers elicited by *Cervarix*™ or *Gardasil*® were measured using PBNA[35] at GlaxoSmithKline (GSK) laboratories where the methodology underwent further validation.[62] There is a high correlation between data generated with the PBNA, for both HPV-16 and -18, when testing is performed by GSK laboratories compared with the independent laboratory that developed the assay (the NCI).[62] Pseudovirions were produced in a manner that was independent of vaccine constructs by co-transfecting human embryonic kidney cells expressing SV40 T antigen (293TT) with plasmids coding for prototype HPV-16 or -18 L1 and L2 genes (codon-optimized for expression in human cell lines) and a secreted alkaline phosphatase gene (SeAP), as previously described.[62,63] The structures of the pseudovirions were as close as possible to those of the natural HPV-16 and -18 viral particles. Neutralizing titers were expressed as the serum dilution at which a 50% reduction in SeAP activity occurred, as compared with a control without serum (ED_{50}). For each antigen, positivity was defined as a sample (serum or CVS) dilution greater than or equal to the assay threshold of 40 ED_{50}.

Enzyme-linked immunosorbent assay. Quantitation of HPV-16 and HPV-18 antibodies in all CVS samples was also performed by ELISA using the purified type-specific recombinant VLPs present in *Cervarix*™ as coating antigen and adapted for CVS samples, as described previously.[61] IgG antibody titers (expressed as ELISA units [EU]/mL) were calculated by reference to standards using a four-parameters equation for each sample dilution, and the titer of each sample was calculated as the average of all titers within the proportional range of the reference curve. The final antibody titer was multiplied by the dilution factor obtained during the antibody extraction step. Positivity was defined as an antibody titer greater than or equal to the assay limit of quantitation (0.58 EU/mL for HPV-16 and 0.35 EU/mL for HPV-18).

Memory B-cell responses. Memory B-cell frequencies were evaluated using a previously described B-cell ELISPOT assay[58] which uses L1 VLP antigens present in *Cervarix*™ (truncated at the C-terminus). The B-cell ELISPOT assay quantitates HPV-specific memory B-cells after in vitro differentiation into antibody-secreting plasma cells. This assay was adapted from that developed by Crotty et al.,[52] and involves the incubation of peripheral blood mononuclear cells that are differentiated into antibody-secreting cells in nitrocellulose wells coated with either the antigen of interest (for the detection of antigen-specific memory B-cells) or anti-human Ig antibodies (for the detection of total memory B-cells). A conventional immunoenzymatic procedure[52] was applied to detect antibody/antigen spots enumerating total and specific antibody-secreting cells to evaluate the frequencies of antigen-specific memory B-cells within the total memory B-cell population.

Reactogenicity and safety. Subjects used diary cards to record the occurrence of solicited local symptoms (pain, redness and swelling at the injection site) and solicited general symptoms (i.e., systemic symptoms, specifically fever, headache, fatigue, gastrointestinal symptoms [such as, nausea, vomiting, diarrhea and/or abdominal pain], arthralgia, myalgia, rash and urticaria) for seven days after each vaccine dose. The severity of solicited symptoms was graded on a 0–3 scale. Grade 3 solicited symptoms were defined as pain that prevented normal activity, redness or swelling > 50 mm in diameter, fever > 39°C (axillary temperature), urticaria distributed on at least four body areas and, for other solicited symptoms, as preventing normal daily activity.

Unsolicited signs and symptoms were recorded for 30 days after each vaccine dose. SAEs, MSCs (defined as AEs prompting emergency room or physician visits that were not related to common diseases or SAEs that were not related to common diseases), NOCDs, pregnancy outcomes, and withdrawals due to AEs/SAEs were reported throughout the entire study period. Examples of common diseases not included in the definition of MSC were upper respiratory infections, sinusitis, pharyngitis, gastroenteritis, urinary tract infections, cervicovaginal yeast infections, menstrual cycle abnormalities and injury. Decisions relating adverse events to vaccination were based on the judgment of the investigator at the study site reporting the event. 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 pre-vaccination 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.

Statistical Analysis

Immunogenicity. The primary study objective was to compare the GMTs of HPV-16 and -18 serum neutralizing antibodies measured by PBNA at Month 7 after vaccination with either *Cervarix*™ or *Gardasil*® (i.e., one month after completion of the three-dose vaccination course) in women aged 18–26 years. Secondary objectives were to compare the immune response to HPV-16 and -18 induced by the two vaccines measured at Month 7 in serum by PBNA in women aged 27–35 and 36–45 years and in CVS by PBNA and ELISA in all age groups. To account for an interim analysis, the overall type I error of the study (5.0%) was split into 0.5% for the interim analysis and 4.8% for this final analysis (O'Brien-Fleming adjustment).[64] The type I error

of the final analysis (4.8%) was then split into 2.4% for each HPV antigen (Bonferroni adjustment). Two-sided 97.6% CIs of anti-HPV-16 and -18 GMT ratios (*Cervarix*™ GMT divided by *Gardasil*® GMT) at Month 7 were computed using an ANOVA model on the $\log_{10}$ transformation of the titers in each age cohort.

Primary and secondary between-group comparisons were performed to assess non-inferiority in terms of GMT ratios for HPV-16 and -18 neutralizing antibodies at Month 7 in each age group. These analyses were performed in the ATP cohort on women who were HPV seronegative and HPV DNA negative (by PCR) prior to vaccination for the antigen under analysis (ATP seronegative/DNA negative cohort). In order to achieve a global power of at least 89%, the sample size was calculated for the 18–26 year-old cohort to allow demonstration of non-inferiority for both antigens. Non-inferiority of *Cervarix*™ to *Gardasil*® was concluded if the lower limit of the two-sided 97.6% CI for the GMT ratio for a given antigen was greater than 0.5. If the lower limit of the two-sided 97.6% CI for the GMT ratio was greater than 1, the p-value associated with a test of superiority (ANOVA model) was calculated for that antigen on the total vaccinated cohort; i.e., all women, regardless of their serological and DNA status prior to vaccination, who received at least one dose of study vaccine.

For within-group assessments, positivity rates (i.e., the number of women with antibody titers greater than or equal to the assay cut-off) with exact 95% CIs and antibody GMTs with 95% CIs were calculated by vaccine group and age range.

HPV-16 and HPV-18 positivity rates in CVS measured by PBNA and ELISA were assessed at Month 7 in a subset of women in all age groups in both vaccine groups (ATP cohort for immunogenicity, irrespective of HPV serostatus and HPV DNA status prior to vaccination). In order to allow a valid evaluation of antibody levels in CVS, samples that were contaminated with blood (i.e., Hemastix® >80 erythrocytes/μL) were eliminated from the analysis.

To address the fluctuation of antibody levels in CVS during the menstrual cycle (as described by Nardelli-Haefliger and colleagues[51]) and enable comparison with antibody levels in serum, total IgG concentration was also measured using an ELISA developed and validated in-house by GSK. Anti-HPV-16 and anti-HPV-18 antibody titers were measured relative to total IgG concentration; ratios were calculated for anti-HPV-16 or anti-HPV-18 IgG titers divided by the total IgG concentration of each sample (CVS and serum). This ratio, expressed as EU/μg of total IgG, was used for comparison of vaccine-specific antibody titers between CVS and serum samples of vaccinated women.

Memory B-cell responses were assessed at Month 7 in a subset of women in all age groups in both vaccine groups. Primary between-group comparisons of memory B-cell responses were performed in the ATP cohort on women with no detectable B-cell response to the antigen prior to vaccination. As an exploratory analysis (type I error of 5% for both antigens), a Fisher's exact test was performed to compare proportion of responders (defined as women with detectable memory B-cell responses at Month 7) between the two groups for each antigen. In addition, ANOVA was performed on the $\log_{10}$ frequency of memory B-cells to compare the magnitude of frequencies in responders between groups.

Reactogenicity and safety. The primary analysis of reactogenicity and safety was performed on the total vaccinated cohort. The percentages of women reporting specific events (at least once within seven days after any vaccine dose for solicited symptoms) were tabulated with exact 95% CIs for each group. SAE information was collected from the time of randomization and continued throughout the entire study.

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Notes

Cervarix is a trade mark of the GlaxoSmithKline group of companies. *Gardasil* is a registered trade mark of Merck & Co Inc. FENdrix is a trade mark of the GlaxoSmithKline group of companies. SPF10 HPV LiPA25, version 1 and SPF10 HPV DEIA are manufactured by Labo Biomedical Products, Rijswijk, The Netherlands, based on licensed INNOGENETICS SPF10 technology.

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