RESEARCH PAPER

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Comparative humoral and cellular immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and HPV-6/11/16/18 vaccine in healthy women aged 18–45 years: Follow-up through Month 48 in a Phase III randomized study

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Abbreviations: ANOVA, analysis of variance; AS04, Adjuvant System containing 3-O-desacyl-4'-monophosphoryl lipid A (MPL; 50 μg) adsorbed on aluminum salt (500 μg $Al(OH)_3$); ATP, according-to-protocol; CI, confidence interval; CMI, cell-mediated immune; CVS, cervicovaginal secretion; ED_{50}, effective dose producing 50% response; ELISA, enzyme-linked immunosorbent assay; GM, geometric mean; GMR, geometric mean (titer) ratio; GMT, geometric mean titer; HPA, Health Protection Agency; HPV, human papillomavirus; IgG, immunoglobulin G; MSC, medically significant condition; nAb(s), neutralizing antibody(ies); NOAD, new onset autoimmune disease; NOCD, new onset chronic disease; PBMC, peripheral blood mononuclear cells; PBNA, pseudovirion-based neutralization assay; SAE, serious adverse event; TVC, total vaccinated cohort; VLP, virus-like particle.

We previously reported higher anti-HPV-16 and -18 immune responses induced by HPV-16/18 vaccine compared with HPV-6/11/16/18 vaccine at Month 7 (one month after completion of full vaccination series) in women aged 18–45 y in an observer-blind study NCT00423046; the differences of immune response magnitudes were maintained up to Month 24. Here we report follow-up data through Month 48. At Month 48, in according-to-protocol cohort for immunogenicity (seronegative and DNA-negative for HPV type analyzed at baseline), geometric mean titers of serum neutralizing antibodies were 2.0- to 5.2-fold higher (HPV-16) and 8.6- to 12.8-fold higher (HPV-18) in HPV-16/18 vaccine group than in HPV-6/11/16/18 vaccine group. The majority of women in both vaccine groups remained seropositive for HPV-16. The same trend was observed for HPV-18 in HPV-16/18 vaccine group; however, seropositivity rates in HPV-6/11/16/18 vaccine group decreased considerably, particularly in the older age groups. In the total vaccinated cohort (regardless of baseline serological and HPV-DNA status), anti-HPV-16 and -18 neutralizing antibody levels induced by HPV-16/18 vaccine were higher than those induced by HPV-6/11/16/18 vaccine. CD4+ T-cell response for HPV-16 and HPV-18 was higher in HPV-16/18 vaccine group than in HPV-6/11/16/18 vaccine group. Memory B-cell responses appeared similar between vaccine groups. Both vaccines were generally well tolerated. Overall, the higher immune response observed with the HPV-16/18 vaccine was maintained up to Month 48. A head-to-head study incorporating clinical endpoints would be required to confirm whether the observed differences in immune response between the vaccines influence the duration of protection they provided.

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Introduction

Persistent infection with an oncogenic human papillomavirus (HPV) is a necessary cause of invasive cervical cancer.[1] HPV infections are frequently acquired soon after initiation of sexual activity,[2,3] women remain at risk while they are sexually active.[4] Therefore, long-term protection against HPV infection is required to reduce the prevalence and burden of what is the second most common cancer in women worldwide.[5]

HPV-16 and HPV-18 are responsible for around 70% of cervical cancer cases globally.[6] Prophylactic HPV vaccines designed to induce protection against both HPV-16 and HPV-18 are currently licensed in over 130 countries. *Cervarix*® (GlaxoSmithKline Vaccines) is a HPV-16/18 AS04-adjuvanted vaccine containing HPV-16 and -18 virus-like particles (VLPs) assembled from the L1 major capsid proteins of HPV-16 and HPV-18. It is formulated with a proprietary immunostimulatory Adjuvant System (AS04) containing 3-O-desacyl-4'-monophosphoryl lipid A (50 μg MPL) adsorbed on aluminum salt (500 μg Al $(OH)_3$).[7] The HPV-16/18 vaccine has shown efficacy against incident and persistent HPV-16/18 infection and cervical intraepithelial neoplasia grade 2+ associated with HPV-16/18 for up to 6.4 y.[8-10] *Gardasil*® (Merck and Co., Inc..) is a HPV-6/11/16/18 vaccine containing L1 VLPs for HPV types 6, 11, 16 and 18 and is formulated with amorphous aluminum hydroxyphosphate sulfate adjuvant. To date, efficacy of the HPV-6/11/16/18 vaccine has been demonstrated through 5 y of follow-up.[11]

Immunogenicity and safety of the HPV-16/18 and HPV-6/11/16/18 vaccines have been compared in a randomized, observer-blind study in healthy women aged 18–45 y (study HPV-010; NCT00423046). The primary objective was analyzed at Month 7, where we showed that geometric mean titers (GMTs) of serum anti-HPV-16 and anti-HPV-18 neutralizing antibodies (nAbs), determined by a pseudovirion-based neutralization assay (PBNA), elicited by the HPV-16/18 vaccine were significantly higher than those elicited by the HPV-6/11/16/18 vaccine.[12] These differences were maintained up to Month 24,[13] and CD4+ T-cell responses for HPV-16 and HPV-18 were consistently higher with the HPV-16/18 vaccine. We now report extended follow-up of this study cohort up to Month 48.

Results

Study population

Between January and April 2007, 1,106 women were enrolled, randomized into 2 vaccine groups (n = 553 each) and received one or more vaccine dose (total vaccinated cohort [TVC]). Among them, 524 women consented to participate in the extended follow-up and attended the Month 48 visit (259 in the HPV-16/18 vaccine group and 265 in the HPV-6/11/16/18 vaccine group). The according-to-protocol (ATP) cohort for immunogenicity (all subjects who met all eligibility criteria, received 3 vaccine doses and complied with study procedures) comprised 421 women (205 women in the HPV-16/18 vaccine group and 216 in the HPV-6/11/16/18 vaccine group). In the Month 48 immunogenicity analysis, the number of subjects excluded and the reasons for exclusion were similar between the vaccine groups (**Fig. 1**). In the TVC, the characteristics of subjects who attended the Month 48 visit were comparable between vaccine groups (mean age 31 y in both vaccine groups at study entry, 86% vs. 82% Caucasian in the HPV-16/18 and HPV-6/11/16/18 vaccine groups, respectively).

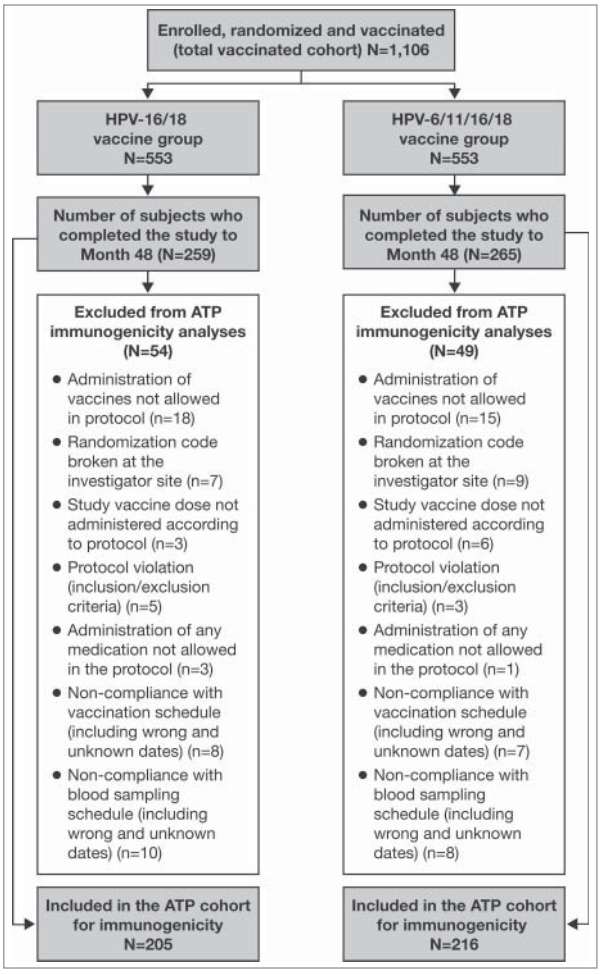

Figure 1. Subject disposition ATP, according-to-protocol. The ATP cohort for immunogenicity included all evaluable subjects who received 3 vaccine doses (i.e., those meeting all eligibility criteria and complying with the procedures defined in the protocol) for whom data concerning immunogenicity endpoint 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 the time point under analysis.

Antibody responses in serum

Table 1 shows the GMTs and seropositivity rates of anti-HPV-16 and -18 nAbs in women in the ATP cohort for immunogenicity at each time point who were seronegative and DNA-negative for the HPV type analyzed before vaccination, measured by PBNA. At Month 48, anti-HPV-16 nAb GMTs were 5.2-fold higher in the HPV-16/18 vaccine group than in the HPV-6/11/16/18 vaccine group in women aged 18–26 y In the same age

Table 1. Seropositivity rates, GMTs and GMT ratios for serum anti-HPV-16 and anti-HPV-18 type-specific neutralizing antibodies measured by PBNA at Months 7, 12, 18, 24, 36 and 48 (ATP cohort for immunogenicity, seronegative and DNA-negative for the HPV type analyzed prior to vaccination)

		HPV-16/18 vaccine			HPV-6/11/16/18 vaccine			
Antigen	Month	N	% SP [95% CI]	GMT [95% CI]	N	% SP [95% CI]	GMT [95% CI]	GMT Ratio*
A	18–26 years							
HPV-16	7	104	100 [96.5, 100]	36792 [29266, 46254]	103	100 [96.5, 100]	10053 [8136, 12422]	3.7 [2.6, 5.2]
	12	101	100 [96.4, 100]	14525 [11070, 19058]	99	100 [96.3, 100]	3265 [2545, 4190]	4.5 [3.1, 6.4]
	18	100	100 [96.4, 100]	6000 [4681, 7691]	91	100 [96.0, 100]	1183 [883, 1585]	5.1 [3.5, 7.4]
	24	97	100 [96.3, 100]	5184 [4015, 6694]	89	97.8 [92.1, 99.7]	894 [672, 1188]	5.8 [4.0, 8.5]
	36	60	100 [94.0, 100]	3845 [2804, 5272]	62	98.4 [91.3, 100]	653 [460, 927]	5.9 [3.7, 9.4]
	48	54	100 [93.4, 100]	3901 [2745, 5543]	57	98.2 [90.6, 100]	750 [505, 1115]	5.2 [3.1, 8.8]
HPV-18	7	118	100 [96.9, 100]	16487 [13383, 20310]	131	100 [97.2, 100]	2258 [1809, 2818]	7.3 [5.1, 10.4]
	12	112	100 [96.8, 100]	4472 [3528, 5669]	127	96.1 [91.1, 98.7]	596 [469, 757]	7.5 [5.4, 10.5]
	18	109	100 [96.7, 100]	2256 [1762, 2890]	114	93.0 [86.6, 96.9]	249 [195, 318]	9.1 [6.4, 12.8]
	24	106	100 [96.6, 100]	1652 [1296, 2105]	109	84.4 [76.2, 90.6]	175 [133, 231]	9.4 [6.5, 13.6]
	36	64	100 [94.4, 100]	1594 [1177, 2158]	76	78.9 [68.1, 87.5]	128 [92.6, 177]	12.5 [8.0, 19.5]
	48	59	100 [93.9, 100]	1711 [1180, 2482]	70	81.4 [70.3, 89.7]	139 [98.7, 196]	12.3 [7.5, 20.3]
B	27–35 years							
HPV-16	7	90	100 [96.0, 100]	23908 [18913, 30222]	85	100 [95.8, 100]	4958 [3896, 6311]	4.8 [3.3, 7.1]
	12	91	100 [96.0, 100]	7419 [5592, 9843]	85	98.8 [93.6, 100]	1756 [1290, 2390]	4.2 [2.8, 6.4]
	18	87	100 [95.8, 100]	2908 [2229, 3793]	83	98.8 [93.5, 100]	690 [506, 941]	4.2 [2.8, 6.3]
	24	84	100 [95.7, 100]	2269 [1766, 2916]	79	97.5 [91.2, 99.7]	619 [447, 856]	3.7 [2.5, 5.5]
	36	63	100 [94.3, 100]	1898 [1419, 2538]	49	100 [92.7, 100]	502 [347, 726]	3.8 [2.4, 6.0]
	48	54	100 [93.4, 100]	2046 [1469, 2850]	51	96.1 [86.5, 99.5]	678 [433, 1060]	3.0 [1.8, 5.2]
HPV-18	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]
	12	105	99.0 [94.8, 100]	2266 [1765, 2910]	102	90.2 [82.7, 95.2]	280 [209, 376]	8.1 [5.5, 11.8]
	18	101	100 [96.4, 100]	1302 [1011, 1677]	99	74.7 [65.0, 82.9]	133 [101, 176]	9.8 [6.7, 14.2]
	24	98	100 [96.3, 100]	1028 [801, 1320]	94	72.3 [62.2, 81.1]	116 [87.4, 155]	8.9 [6.1, 12.9]
	36	75	100 [95.2, 100]	943 [713, 1247]	61	70.5 [57.4, 81.5]	102 [69.6, 149]	9.3 [5.9, 14.6]
	48	66	100 [94.6, 100]	982 [741, 1302]	59	57.6 [44.1, 70.4]	76.9 [52.7, 112]	12.8 [8.1, 20.2]
C	36–45 years							
HPV-16	7	96	100 [96.2, 100]	17302 [13605, 22002]	83	100 [95.7, 100]	7634 [5916, 9853]	2.3 [1.5, 3.4]
	12	89	100 [95.9, 100]	7110 [5386, 9386]	83	100 [95.7, 100]	2678 [1987, 3610]	2.7 [1.8, 4.0]
	18	90	100 [96.0, 100]	2344 [1808, 3039]	82	100 [95.6, 100]	995 [733, 1350]	2.4 [1.6, 3.5]
	24	87	100 [95.8, 100]	2059 [1592, 2661]	80	100 [95.5, 100]	875 [637, 1201]	2.4 [1.6, 3.5]
	36	61	100 [94.1, 100]	1794 [1278, 2519]	57	100 [93.7, 100]	824 [567, 1196]	2.2 [1.3, 3.6]
	48	51	98.0 [89.6, 100]	2081 [1378, 3144]	54	98.1 [90.1, 100]	1019 [645, 1608]	2.0 [1.1, 3.8]
HPV-18	7	110	100 [96.7, 100]	9846 [7835, 12372]	91	100 [96.0, 100]	1439 [1105, 1873]	6.8 [4.6, 10.2]
	12	104	100 [96.5, 100]	3032 [2321, 3962]	91	98.9 [94.0, 100]	434 [325, 579]	7.0 [4.7, 10.3]
	18	103	99.0 [94.7, 100]	1427 [1084, 1878]	91	86.8 [78.1, 93.0]	182 [137, 241]	7.9 [5.3, 11.6]
	24	100	99.0 [94.6, 100]	1040 [786, 1377]	88	77.3 [67.1, 85.5]	136 [99.0, 186]	7.7 [5.0, 11.6]
	36	71	97.2 [90.2, 99.7]	904 [625, 1306]	61	73.8 [60.9, 84.2]	103 [74.6, 143]	8.8 [5.3, 14.4]
	48	61	96.7 [88.7, 99.6]	785 [529, 1164]	61	72.1 [59.2, 82.9]	91.5 [67.0, 125]	8.6 [5.2, 14.1]

CI, confidence interval; GMT, geometric mean titer; N, number of subjects with available results; PBNA, pseudovirion-based neutralization assay; SP, seropositivity (defined as neutralizing antibody titer $\geq$40 ED_{50} [effective dose producing 50% response]).

Month 7–Month 48 data are presented for the ATP cohort for immunogenicity corresponding to the time point under analysis.

*GMT ratio, GMT in the HPV-16/18 group divided by GMT in the HPV-6/11/16/18 group; Month 7 GMT ratios are provided with 97.6% CI while Month 12–Month 48 GMT ratios are provided with 95% CI.

The ATP cohort for immunogenicity included all evaluable subjects who received 3 vaccine doses (i.e., those meeting all eligibility criteria and 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 the time point under analysis.

group, anti-HPV-18 nAb GMTs were 12.3-fold higher in the HPV-16/18 vaccine group than in the HPV-6/11/16/18 vaccine group. At the same time point, compared with the HPV-6/11/16/18 vaccine, anti-HPV-16 and -18 GMTs induced by the HPV-16/18 vaccine were 3.0- and 12.8-fold higher, respectively, in women aged 27–35 y and were 2.0- and 8.6-fold higher, respectively, in women aged 36–45 y (**Table 1**). At Month 48, in the ATP cohort for immunogenicity, the majority of subjects in both vaccine groups were still seropositive for HPV-16. The seropositivity rates for HPV-18 remained high with the HPV-16/18 vaccine, while they decreased considerably with the HPV-6/11/16/18 vaccine (18–26 years: 81.4 % [95% confidence interval (CI): 70.3, 89.7]; 27–35 years: 57.6% [95% CI: 44.1, 70.4]; 36–45 years: 72.1% [95% CI: 59.2, 82.9]). The GMTs and seropositivity rates of anti-HPV-16 and -18 nAbs, as assessed by PBNA, in women in the ATP cohort for

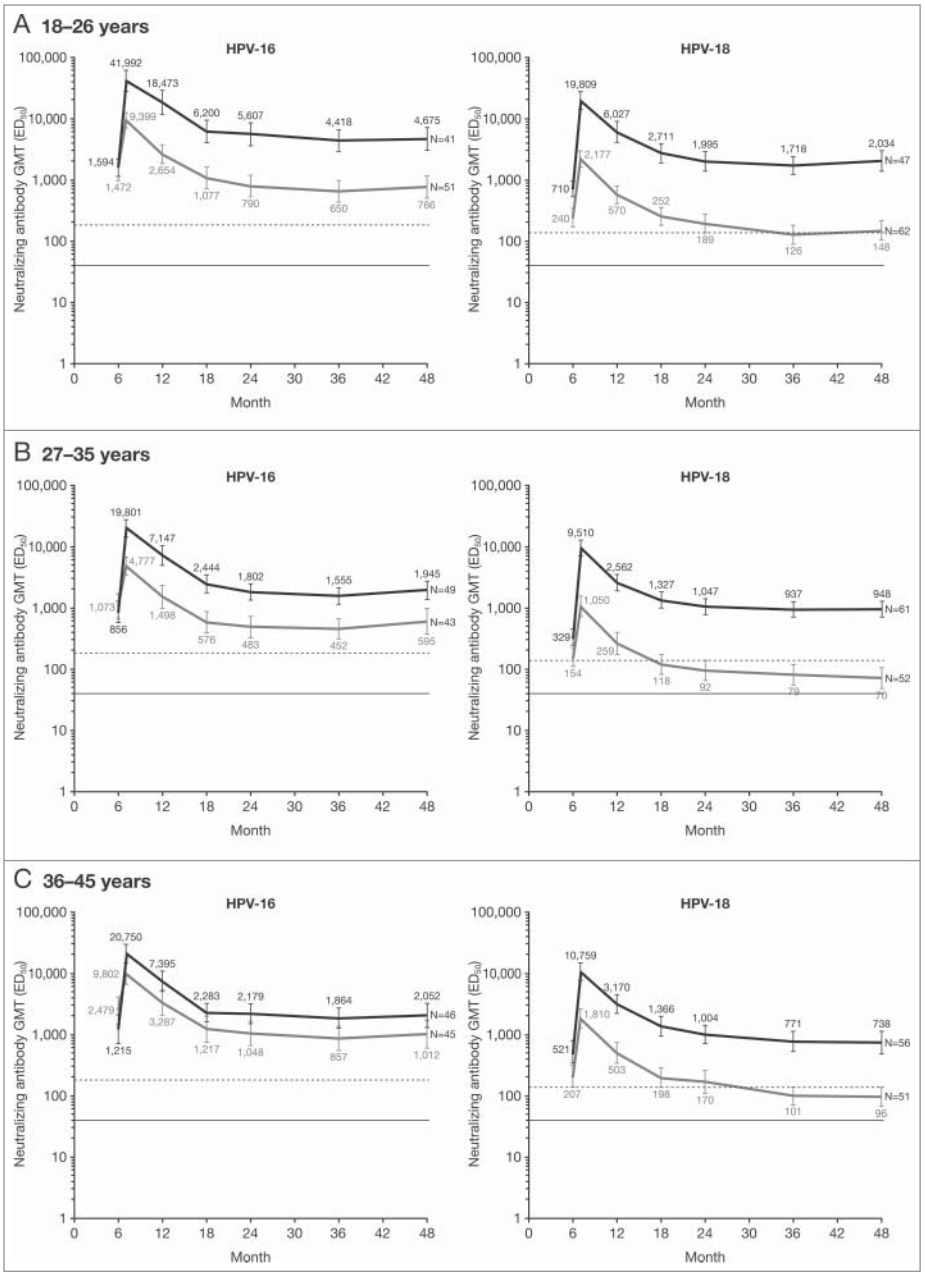

Figure 2. GMTs for serum anti-HPV-16 and anti-HPV-18 type-specific neutralizing antibodies at Months 6, 7, 12, 18, 24, 36 and 48 (PBNA, ATP kinetic cohort; seronegative and DNA-negative for the HPV type analyzed prior to vaccination) Black lines, Human Papillomavirus Types 16 and 18 Vaccine (Recombinant, AS04-adjuvanted, adsorbed) (*Cervarix*®); gray lines, Human Papillomavirus Types 6, 11, 16 and 18 Vaccine, Recombinant (*Gardasil*®). Error bars denote 95% confidence intervals of geometric mean titers (GMTs). Dashed line, neutralizing antibody GMTs measured by pseudovirion-based neutralization assay (PBNA) in women in the total vaccinated cohort of the HPV-010 study who had cleared natural infection prior to vaccination (i.e., those who were seropositive and DNA-negative at Month 0): 180.1 ED_{50} for HPV-16 and 137.3 ED_{50} for HPV-18.[12] Solid line, PBNA limit of detection (40 ED_{50}). ED_{50}, effective dose producing 50% response. The according-to-protocol (ATP) kinetic cohort is a sub-cohort of the ATP cohort for immunogenicity (seronegative and DNA-negative at baseline) that included all subjects without any elimination codes and with valid results available for the HPV type(s) and the assay considered in the analysis for each time point.

immunogenicity (irrespective of serostatus and DNA status prior to vaccination) are shown in Supplementary **Table 1**. Exploratory analyses performed in the TVC (irrespective of serostatus and DNA status prior to vaccination) showed that anti-HPV-16 and -18 nAb levels induced by the HPV-16/18 vaccine appeared to be higher than those induced by the HPV-6/11/16/18 vaccine across all age groups and at all time points up to Month 48 ($p < 0.0085$).

In ATP subjects seronegative and DNA-negative for the HPV type analyzed at baseline and with valid results available at each time point (ATP kinetic cohort), the kinetics of the antibody responses induced by both vaccines showed a similar pattern. Anti-HPV-16 and -18 nAb titers peaked at Month 7, slowly declined and then plateau from Month 18 onwards (**Fig. 2**). In all age groups, the HPV-16/18 vaccine induced higher plateauing levels of anti-HPV-16 and -18 nAbs than did the HPV-6/11/16/18 vaccine. This was particularly evident for anti-HPV-18 neutralizing antibodies, where plateau levels induced by the HPV-16/18 vaccine remained markedly higher than the level associated with natural infection through to Month 48, while plateau levels of anti-HPV-18 nAbs induced by the HPV-6/11/16/18 vaccine were close to or below the level associated with natural infection (**Fig. 2**).

Vaccine-induced HPV type-specific serum immunoglobulin G (IgG) antibody responses measured by enzyme-linked immunosorbent assay (ELISA) corroborated the PBNA results. The kinetics of antibody response assessed by ELISA followed a similar trend to those assessed by PBNA; the plateauing levels of anti-HPV-16 and -18 antibodies induced by the HPV-16/18 vaccine appeared higher than those induced by the HPV-6/11/16/18 vaccine in all age groups (Supplementary **Fig. 1**).

Antibody response in cervicovaginal secretions

HPV-specific antibody responses in cervicovaginal secretion (CVS) samples were evaluated in a subset of women. Due to the limited CVS samples available, the results are presented for all 3 age groups combined in the TVC. At Month 48, anti-HPV-16 positivity rates in CVS were 72.1% (95% CI: 56.3, 84.7) in the HPV-16/18 vaccine group and 54.3% (95% CI: 39.0, 69.1) in the HPV-6/11/16/18 vaccine

Table 2. Positivity rates and GMTs of anti-HPV-16 and anti-HPV-18 type-specific IgG antibodies measured in cervicovaginal secretions by ELISA at Months 0, 7, 12, 18, 24, 36 and 48 (TVC, irrespective of serostatus and DNA status for the HPV type analyzed prior to vaccination)

			HPV-16/18 vaccine			HPV-6/11/16/18 vaccine			
Antigen	**Month**	**N**	**n**	**% P [95% CI]**	**GMT* [95% CI]**	**N**	**n**	**% P [95% CI]**	**GMT* [95% CI]**
HPV-16	0	57	2	3.5 [0.4, 12.1]	15.6 [0.8, 321.3]	64	0	0 [0.0,0.0]	– [–, –]
	7	65	62	95.4 [87.1, 99.0]	200.4 [141.6, 283.6]	82	74	90.2 [81.7, 95.7]	92.9 [68.1, 126.5]
	12	67	57	85.1 [74.3, 92.6]	121.1 [88.6, 165.5]	66	52	78.8 [67.0, 87.9]	59.0 [43.5, 79.9]
	18	51	42	82.4 [69.1, 91.6]	102.1 [70.6, 147.8]	65	41	63.1 [50.2, 74.7]	68.8 [47.7, 99.1]
	24	54	42	77.8 [64.4, 88.0]	86.9 [60.7, 124.6]	61	34	55.7 [42.4, 68.5]	43.4 [27.8, 67.5]
	36	60	43	71.7 [58.6, 82.5]	57.7 [41.5, 80.4]	62	42	67.7 [54.7, 79.1]	51.5 [36.2, 73.2]
	48	43	31	72.1 [56.3, 84.7]	41.6 [29.3, 59.1]	46	25	54.3 [39.0, 69.1]	72.8 [38.5, 137.8]
HPV-18	0	57	3	5.3 [1.1, 14.6]	18.9 [8.8, 40.6]	64	4	6.3 [1.7, 15.2]	11.4 [1.4, 96.4]
	7	65	60	92.3 [83.0, 97.5]	96.7 [73.1, 128.0]	82	57	69.5 [58.4, 79.2]	38.1 [28.0, 51.8]
	12	67	54	80.6 [69.1, 89.2]	57.6 [43.2, 76.7]	67	35	52.2 [39.7, 64.6]	36.4 [24.9, 53.1]
	18	51	37	72.5 [58.3, 84.1]	40.2 [25.7, 62.9]	65	22	33.8 [22.6, 46.6]	28.7 [16.5, 49.8]
	24	54	38	70.4 [56.4, 82.0]	49.0 [33.3, 72.0]	61	22	36.1 [24.2, 49.4]	20.8 [11.7, 36.8]
	36	60	41	68.3 [55.0, 79.7]	27.5 [20.3, 37.2]	62	23	37.1 [25.2, 50.3]	30.6 [17.8, 52.7]
	48	43	24	55.8 [39.9, 70.9]	24.1 [16.0, 36.2]	46	18	39.1 [25.1, 54.6]	26.1 [13.4, 50.9]

CI, confidence interval; GMT, geometric mean titer; N, number of subjects with available results; n, number of subjects with an antibody titer ≥ the limit of quantification; P, positivity (defined as antibody titer ≥0.58 ELISA units/mL for HPV-16 and ≥0.35 ELISA units/mL for HPV-18); TVC, total vaccinated cohort. Month 7–Month 48 data are presented for the TVC corresponding to the time point under analysis.
*GMTs were calculated on positive subjects (n values) because data for all subjects in the subset did not follow a normal distribution. Dashes (–) indicate where there were insufficient values (i.e., n≤ 1) to calculate GMTs.

group. Anti-HPV-18 positivity rates in CVS at Month 48 were 55.8% (95% CI: 39.9, 70.9) in the HPV-16/18 vaccine group and 39.1% (95% CI: 25.1, 54.6) in the HPV-6/11/16/18 vaccine group. GMTs of anti-HPV-16 and -18 IgG antibodies measured in CVS, calculated in the positive subjects, are given in **Table 2**.

At Month 48, the Pearson correlation coefficients (r) between HPV-specific antibody titers in CVS and serum were: for HPV-16, 0.88 in the HPV-16/18 vaccine group and 0.82 in the HPV-6/11/16/18 vaccine group; for HPV-18, 0.47 in the HPV-16/18 vaccine group and 0.66 in the HPV-6/11/16/18 vaccine group.

CD4+ T-cell responses

At Month 48, in all 3 age groups combined, the proportion of responders (women with ≥500 HPV type-specific memory CD4+ T-cells/million cells) appeared to be higher in the HPV-

Table 3. Proportion of responders and geometric means for (a) HPV-16- and (b) HPV-18 type-specific CD4+ T-cell responses at Months 7, 12, 18, 24, 36 and 48 (ATP cohort for immunogenicity; seronegative, DNA-negative and HPV type-specific CD4+ T-cell negative prior to vaccination)

		Positivity rates							GM					
		HPV-16/18 vaccine			HPV-6/11/16/18 vaccine				HPV-16/18 vaccine		HPV-6/11/16/18 vaccine			
Antigen	**Month**	**N**	**n**	**% P**	**N**	**n**	**% P**	**p-value***	**N**	**GM (95% CI)**	**N**	**GM (95% CI)**	**GMR (95% CI)**	**p-value†**
HPV-16	7	41	36	87.8	33	21	63.6	0.0245	41	1080 (853, 1369)	33	665 (511, 865)	1.63 (1.14, 2.32)	0.0029
	12	34	27	79.4	27	13	48.2	0.0151	34	794 (630, 1001)	27	448 (345, 580)	1.77 (1.25, 2.51)	0.0008
	18	40	37	92.5	25	10	40.0	< 0.0001	40	1149 (898, 1471)	25	397 (291, 543)	2.89 (1.94, 4.31)	< 0.0001
	24	33	30	90.9	20	12	60.0	0.0128	33	1070 (837, 1367)	20	441 (322, 605)	2.42 (1.63, 3.61)	< 0.0001
	36	20	16	80.0	15	6	40.0	0.0322	20	627 (292, 1346)	15	285 (118, 688)	2.20 (0.69, 7.07)	0.0020
	48	13	12	92.3	14	5	35.7	0.0044	13	732 (314, 1707)	14	498 (220, 1127)	1.47 (0.45, 4.76)	0.0057
HPV-18	7	43	34	79.1	40	23	57.5	0.0571	43	907 (668, 1232)	40	507 (370, 697)	1.79 (1.15, 2.78)	0.0102
	12	38	26	68.4	32	11	34.4	0.0078	38	461 (285, 747)	32	315 (186, 533)	1.46 (0.72, 2.99)	0.0131
	18	42	33	78.6	33	14	42.4	0.0018	42	842 (572, 1240)	33	314 (203, 486)	2.68 (1.50, 4.81)	0.0006
	24	35	26	74.3	25	10	40.0	0.0152	35	694 (488, 987)	25	294 (194, 446)	2.36 (1.37, 4.08)	0.0003
	36	22	12	54.6	17	5	29.4	0.1930	22	352 (189, 653)	17	243 (120, 492)	1.45 (0.57, 3.70)	0.0210
	48	14	11	78.6	15	5	33.3	0.0253	14	740 (415, 1319)	15	257 (147, 449)	2.88 (1.29, 6.44)	0.0088

ATP, according-to-protocol; CI, confidence interval; GM, geometric mean (calculated from responder [defined as subjects with detectable HPV type-specific memory CD4+ T-cells, i.e., > 500 HPV-specific CD4+ T-cells/million CD4+ T-cells] and non-responder data); GMR, geometric mean ratio; N, number of subjects with available results; n, number of positive subjects; P, positivity (defined as > 500 HPV-specific CD4+ T-cells/million CD4+ T-cells).
Month 7–Month 48 data are presented for the ATP cohort for immunogenicity corresponding to the time point under analysis.
*For the comparison of proportions of responders, p-values were calculated using Fisher's exact test. †For statistical assessment of CD4+ T-cell GM ratios, p-values were computed using a Kruskal-Wallis model.

Table 4. Proportion of responders and geometric means for (a) HPV-16- and (b) HPV-18 type-specific memory B-cell responses at Months 7, 12, 18, 24, 36 and 48 (ATP cohort for immunogenicity; seronegative, DNA-negative and with no detectable HPV type-specific B-cells prior to vaccination)

		Positivity rates							GM					
		HPV-16/18 vaccine			HPV-6/11/16/18 vaccine				HPV-16/18 vaccine		HPV-6/11/16/18 vaccine			
Antigen	Month	N	n	% P	N	n	% P	p-value*	N	GM (95% CI)	N	GM (95% CI)	GMR (95% CI)	p-value†
HPV-16	7	51	46	90.2	39	36	92.3	1.0000	46	996 (713, 1391)	36	326 (224, 476)	3.05 (1.84, 5.05)	< 0.0001
	12	44	40	90.9	33	25	75.8	0.1109	40	305 (217, 428)	25	217 (142, 333)	1.40 (0.81, 2.42)	0.2183
	18	45	39	86.7	29	17	58.6	0.0112	39	255 (178, 367)	17	231 (133, 401)	1.10 (0.57, 2.14)	0.7649
	24	36	30	83.3	24	16	66.7	0.2122	30	312 (205, 475)	16	233 (131, 415)	1.34 (0.65, 2.73)	0.4174
	36	20	11	55.0	16	11	68.8	0.5007	11	366 (209, 639)	11	195 (111, 340)	1.88 (0.85, 4.14)	0.1106
	48	14	10	71.4	10	7	70.0	1.0000	10	310 (139, 688)	7	257 (99.1, 668)	1.20 (0.35, 4.18)	0.7546
HPV-18	7	53	48	90.6	52	34	65.4	0.0021	48	513 (373, 704)	34	163 (112, 238)	3.14 (1.92, 5.14)	< 0.0001
	12	47	38	80.9	44	17	38.6	< 0.0001	38	228 (158, 331)	17	117 (67.3, 205)	1.95 (1.00, 3.80)	0.0507
	18	47	35	74.5	42	19	45.2	0.0087	35	236 (160, 347)	19	79.5 (47.0, 135)	2.97 (1.54, 5.70)	0.0015
	24	38	29	76.3	34	18	52.9	0.0489	29	258 (171, 389)	18	102 (60.3, 171)	2.54 (1.31, 4.93)	0.0071
	36	22	12	54.6	21	8	38.1	0.3640	12	267 (123, 583)	8	131 (50.4, 340)	2.04 (0.60, 7.00)	0.2387
	48	17	11	64.7	15	9	60.0	1.0000	11	259 (127, 531)	9	117 (52.7, 258)	2.23 (0.76, 6.48)	0.1336

Month 7–Month 48 data are presented for the ATP cohort for immunogenicity corresponding to the time point under analysis.
ATP, according-to-protocol; CI, confidence interval; GM, geometric mean (calculated on responders only [defined as subjects with detectable HPV-type specific memory B-cells, i.e., >0 cell/million cells] as data were log-transformed for statistical analyses); GMR, geometric mean ratio; N, number of subjects with available results; n, number of positive subjects; P, positivity (defined as >0 cell/million cells).
*For the comparison of proportions of responders, p-values were calculated using Fisher's exact test. †For statistical assessment of memory B-cell GM ratios, p-values were calculated using an ANOVA model.

16/18 vaccine group than in the HPV-6/11/16/18 vaccine group for both HPV-16 (92% vs. 36%) and HPV-18 (79% vs. 33%) (**Table 3**). The geometric mean (GM) frequency of circulating antigen-specific CD4+ T-cells in all subjects (responders and non-responders) was 1.5-fold higher (95% CI: 0.5, 4.8) for HPV-16 and 2.9-fold higher (95% CI: 1.3, 6.4) for HPV-18, in the HPV-16/18 vaccine group compared with the HPV-6/11/16/18 vaccine group (**Table 3**).

Memory B-cell responses

At Month 48, in all 3 age groups combined, the proportion of memory B-cell responders (subjects with detectable HPV type-specific memory B-cells, i.e., >0 antigen-specific memory B-cell/million memory B-cells) in the HPV-16/18 and HPV-6/11/16/18 vaccine groups appeared similar for HPV-16 (71% vs. 70%) and HPV-18 (65% vs. 60%), respectively (**Table 4**). The mean frequency of circulating antigen-specific memory B-cells was not substantially different between vaccine groups for HPV-16 (GM ratio, 1.2 [95% CI: 0.4, 4.2]) and HPV-18 (GM ratio, 2.2 [95% CI: 0.8, 6.5]), as assessed in responders only (**Table 4**).

Safety

From Month 0 to Month 48, the proportions of subjects reporting serious adverse events (SAEs), new onset chronic diseases (NOCDs), new onset autoimmune diseases (NOADs) and medically significant conditions (MSCs, conditions prompting physician visits) in the TVC appeared to be similar between vaccine groups (**Table 5**). Up to Month 48, 102 SAEs were reported by 71 subjects; 2 SAEs (one per vaccine group) considered to be possibly related to vaccination and one fatal SAE (metastatic

Table 5. Safety outcomes up to Month 48; proportion of subjects reporting at least one event (TVC; irrespective of serostatus and DNA status prior to vaccination)

	Proportion of subjects with ≥ 1 event,% (95% CI)	
	HPV-16/18 vaccine (N = 553)	HPV-6/11/16/18 vaccine (N = 553)
Medically significant conditions	45.4 (41.2, 49.6)	39.1 (35.0, 43.3) 216]
New onset of chronic diseases*	6.0 (4.1, 8.3)	6.0 (4.1, 8.3)
New onset of autoimmune diseases‡	1.3 (0.5, 2.6)	2.2 (1.1, 3.8)
Serious adverse events	6.7 (4.8, 9.1)	6.1 (4.3, 8.5)

CI, confidence interval; TVC, total vaccinated cohort.
*All adverse events reported were compared with a pre-defined list of potential chronic diseases derived from the Medical Dictionary for Regulatory Activities; 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 GlaxoSmithKline physician.
‡New onset autoimmune diseases were identified from events categorized as new onset chronic diseases using a list detailing potential autoimmune events, which excluded allergy-related events or isolated signs and symptoms, plus events not considered to be autoimmune in origin.

renal cell carcinoma) were reported previously.[12,13] Overall, 66 subjects experienced NOCDs, 19 of these subjects reported NOCDs that were identified as NOADs. The most common NOCD/NOAD was hypothyroidism. Up to Month 48, 139 pregnancies were reported (HPV-16/18 vaccine group, n = 77; HPV-6/11/16/18 vaccine group, n = 62). In the HPV-16/18 and HPV-6/11/16/18 vaccine groups, respectively, 57 (74%) and 42 (68%) pregnancies resulted in a live infant with no apparent congenital anomalies. No infants were born with congenital anomalies in the HPV-16/18 vaccine group compared with 2 (3%) in the HPV-6/11/16/18 vaccine group. The number of spontaneous abortions (with no apparent congenital anomaly) appeared similar between vaccine groups (HPV-16/18 vaccine, 10 [13%]; HPV-6/11/16/18, 9 [15%]).

Discussion

The higher anti-HPV-16 and -18 serum nAb levels induced by the HPV-16/18 vaccine versus the HPV-6/11/16/18 vaccine reported at Months 7 and 24[12,13] remained up to Month 48 after first dose. In the ATP cohort for immunogenicity, GMTs of anti-HPV-16 and -18 nAbs measured by PBNA remained several fold higher with the HPV-16/18 vaccine than the HPV-6/11/16/18 vaccine across all age groups and at all time points up to Month 48. The magnitudes of difference in GMTs at Month 48 between vaccines were comparable with those at all other time points.[13] As reported at Month 24,[13] the levels of vaccine-induced anti-HPV-16 and -18 antibodies peaked at Month 7, declined and then plateau from Month 18. In the ATP kinetic cohort, higher anti-HPV-16 nAb titers than those observed in women who had previously cleared their HPV infection (measured by PBNA in the TVC) were induced by both vaccines, through to Month 48.[12] However, vaccine-induced anti-HPV-18 nAb titers remained higher than those observed after natural infection in the HPV-16/18 vaccine group, while they declined to levels similar to or below those associated with previously cleared HPV infection in the HPV-6/11/16/18 vaccine group. In the ATP kinetic cohort at Month 48, higher ELISA antibody titers were detected with the HPV-16/18 vaccine compared with the HPV-6/11/16/18 vaccine for HPV-16 and HPV-18, across all age groups. Using exploratory analyses in the TVC, the cohort that is most representative of the general population, the HPV-16/18 vaccine demonstrated higher anti-HPV-16 and -18 serum nAbs at all measured time points through to Month 48 when compared with the serum nAb levels elicited by the HPV-6/11/16/18 vaccine. As an immune correlate of protection has not yet been defined, a head-to-head study incorporating clinical endpoints would be required to confirm whether the observed differences in immune response between the vaccines influence the duration of protection they provide.

The distinct immune responses between the HPV vaccines have been recently confirmed in an independent phase IV head-to-head study conducted by the Health Protection Agency (HPA) in England.[14] In this trial involving 12–15 y old girls, serum nAb responses to vaccine (HPV-16 and -18) and non-vaccine (HPV-31 and -45) types were found to be higher in the HPV-16/18 vaccine arm compared with the HPV-6/11/16/18 vaccine arm, up to 12 months after the first vaccination. Although the follow-up period drastically differs between the HPV-010 study (48 months) and the HPA trial, it appears that differences in the level of antibody responses remained consistent over time in each study. However, although nAbs are believed to play a significant role in mediating protection against HPV infection and disease, any attempt to extrapolate the observed differences in immunogenicity to clinical efficacy data should be treated with caution. Nonetheless, these independent data strengthen the current body of evidence that the HPV-16/18 vaccine has a higher immunogenicity profile compared with the HPV-6/11/16/18 vaccine.

The proportion of HPV-16-specific CD4+ T-cell responders was higher after the HPV-16/18 vaccine compared with the HPV-6/11/16/18 vaccine at all time points through to Month 48. Similarly, GMs of circulating HPV-16-specific CD4+ T-cells were higher after the HPV-16/18 vaccine at all time points. The proportion of subjects demonstrating an HPV-18-specific CD4+ T-cell response was higher after vaccination with the HPV-16/18 vaccine than after the HPV-6/11/16/18 vaccine at most time points (except Months 7 and 36). GMs of circulating HPV-18-specific CD4+ T-cells were higher after the HPV-16/18 vaccine at all time points. The observed enhanced CD4+ T cell response in the HPV-16/18 vaccine group is likely to be related to the ability of the MPL component of the AS04 adjuvant to enhance antigen presentation to CD4+ T cells, in turn resulting in increased differentiation of B cells into antibody-producing plasma cells and memory B cells.[7] The AS04-adjuvant was previously shown to induce an enhanced memory B-cell response compared with aluminum salt only formulations;[15] however, this was not a direct comparison to the HPV-6/11/16/18 vaccine formulation, and a direct comparison of the immune response induced by AS04 and amorphous aluminum hydroxyphosphate sulfate (AAHS) adjuvant has not been performed.

At all time points up to Month 24, the proportion of responders with detectable HPV-18-specific circulating memory B-cells was higher after vaccination with the HPV-16/18 vaccine than after the HPV-6/11/16/18 vaccine; however, from Month 36 through to Month 48, the proportion of responders with detectable HPV-16- and HPV-18-specific circulating memory B-cells was similar after each vaccine. At Month 48, GMs of HPV-16- and -18-specific circulating memory B-cells in responders were similar to the plateau levels reported from Month 12 and were comparable between vaccines.[13] We note that the B-cell ELISPOT assay is performed using peripheral blood samples and thus may be less relevant at the later time points, by which time memory B-cells might have migrated from the blood and into the different lymphatic compartments (e.g., spleen). It is still unclear precisely where memory B-cells usually reside preferentially and what the significance of their frequency is when measured in blood.[16] At Month 48, memory B-cell responses remain detectable and may account for the immune memory demonstrated in trials with the HPV-16/18 and HPV-6/11/16/18 vaccines.[17,18] Giving a fourth dose of the HPV-16/18 vaccine 6.8 y

after first vaccination has been shown to cause a rapid and considerable increase in antibody titer and in the GMs of T- and B-cells.[16] Similarly, a fourth dose of the HPV-6/11/16/18 vaccine given 5 y after first dose resulted in a rapid increase in anti-HPV antibody levels.[18]

High serum antibody titers have been associated with enhanced antibody concentrations in CVS, thus likely providing a first defense against HPV infection and subsequent disease.[19] At Months 7 and 24, a greater proportion of women who received HPV-16/18 vaccine had detectable HPV type-specific nAbs in CVS compared with those who received HPV-6/11/16/18 vaccine. This finding is consistent with the higher serological immune response observed with the HPV-16/18 vaccine (in the ATP cohort for immunogenicity).[12,13] At Month 48, serological immune response was higher with the HPV-16/18 vaccine than with the HPV-6/11/16/18 vaccine. At this time point, limited CVS samples were available so mucosal antibody levels were assessed in the TVC; positivity rates of anti-HPV-16 and -18 IgG antibodies in CVS appeared higher in the HPV-16/18 vaccine group compared with the HPV-6/11/16/18 vaccine group, while CVS antibody levels were of similar magnitude. Taken together, our results at Month 48 are in line with those observed at previous time points.

Antibody levels in serum and CVS are poorly correlated, especially for HPV-18. Any extrapolation on transudation rates from serum to CVS should be treated with caution as the relevance of CVS findings at Month 48 is limited by the sensitivity of the analyses, scores being close to the limit of detection, and the small number of samples analyzed at the later time points. Whether the CVS antibody titers observed in this study would be sufficient to offer protection at the site of infection is unknown, and an immune correlate of protection remains to be determined.

To induce optimal protection against HPV, adolescents are vaccinated prior to sexual debut; therefore, a limitation of this study is the exclusion of adolescents. However, as noted above, an independent head-to-head study has been performed in 12–15 y old girls and reported a broader and higher magnitude of serum nAb response with the HPV-16/18 vaccine when compared with the HPV-6/11/16/18 vaccine.[14] Further, a head-to-head study comparing the immunogenicity of the 2 vaccines when administered to 9–14 y old girls according to a 2-dose schedule is ongoing (NCT01462357). A separate study has already shown that, for the HPV-16/18 vaccine, a 2-dose schedule in 9–14 y old girls appears comparable to the standard 3-dose schedule in women aged 15–25 years, up to 4 y after the first dose.[20] Also a limitation of the present analysis was the lack of long term follow-up data on cross-reactivity against non-vaccine HPV types, such as HPV-31 and HPV-45—these data were available only up to Month 24, as previously reported.[21] We also note that our study was reliant on the accurate reporting of medical and sexual history.

Over the 48-month period of the trial, sample size decreased due to subject attrition and this led to a smaller subgroup for analysis of positivity rates and GMTs in CVS and for analysis of T-cell and B-cell responses. The relatively high drop-out rate is likely due to the subjects not wishing to participate in the extended follow-up; however, the drop-out rate is unlikely to affect the validity of results as demographic data at Month 7 and Month 48 are similar.

Tolerability was generally good for both vaccines, with clinically acceptable safety profiles for the HPV-16/18 vaccine and the HPV-6/11/16/18 vaccine up to Month 48. This is consistent with ongoing post-licensure vaccine monitoring data.[22] Between Months 24–48, no fatal SAEs or SAEs related to vaccination were reported.

Overall, the higher immune response previously observed with the HPV-16/18 vaccine compared with the HPV-6/11/16/18 vaccine was maintained up to Month 48. The higher immune response observed with the HPV-16/18 vaccine may be partially explained by the AS04 adjuvant. These findings may be of benefit to healthcare providers and public health officials in informing policy choices with regards to primary prevention of cervical cancer. Longer-term assessment and registries are required to ascertain if any clinical efficacy differences exist between the vaccines. Follow-up data to Month 60 are complete and are reported along with data modeling long-term persistence of antibody responses.[23]

Patients and Methods

Study design, immunogenicity and safety assessments

This long-term follow-up through Month 48 was conducted in 36 centers in the United States; ClinicalTrials.gov NCT00423046.

Study participants, ethics, study design and vaccine composition have previously been reported.[12,13] Briefly, women stratified by age (18–26, 27–35 and 36–45 years) were randomized (1:1 ratio in each age group) to receive 0.5 mL of either HPV-16/18 vaccine or HPV-6/11/16/18 vaccine according to their recommended 3-dose schedules (Months 0, 1, 6 or Months 0, 2 and 6, respectively). The treatment allocation at the investigator site was performed using a web-based central randomization system. The study was conducted in an observer-blind manner; to maintain the blind, women received one dose of placebo (aluminum hydroxide) at either Month 1 or 2, as appropriate. Follow-up data to Month 60 are complete and disclosed.[23]

Blood samples for assessment of serological humoral responses (measured by ELISA[24,25] and PBNA[25]) were collected at yearly visits during follow-up. At the preselected sites, peripheral blood mononuclear cell (PBMC) samples were obtained from a subset of women in all age groups and both vaccine groups for assessment of cell-mediated immune (CMI) responses. In addition, CVS samples were collected for assessment of mucosal HPV antibody response by ELISA. Anti-HPV nAbs were measured by PBNA using pseudovirions. Total HPV antibodies were measured by ELISA using the purified HPV type-specific recombinant VLPs. CD4+ T-cell responses were evaluated by *in vitro* stimulation with a pool of HPV peptides followed by quantification by cytokine flow cytometry.[13,26] Memory B-cells were evaluated by B-cell ELISPOT assay using L1 VLP antigens of the HPV-16/18 AS04-adjuvanted vaccine.[15] Methodology for

PBNA and PBMC isolation, antibody extraction from CVS samples, and immunological assays has been described previously.[13,26] In the absence of a serological correlate of protection, GMTs of anti-HPV-16 and -18 nAbs (measured by PBNA) induced by natural infection were used to evaluate vaccine-induced antibody responses. These antibody responses were defined as GMTs in women in the TVC who were DNA-negative but seropositive at Month 0 for the antigen under analysis, indicating clearance of natural infection.[12] For each antigen, positivity in the PBNA was defined as a serum dilution greater than or equal to the assay threshold of 40 ED_{50} (effective dose producing 50% response).

SAEs, NOCDs, NOADs, pregnancies, and other MSCs were recorded in the TVC throughout the study, as previously described.[12]

Statistical analysis

The objective of this follow-up analysis through Month 48 was to compare the serological nAb responses and CMI responses to HPV-16 and -18 induced by the 2 vaccines by means of descriptive and exploratory analyses. For nAb responses, GMT ratios with 2-sided 95% CI (GMT in HPV-16/18 vaccine group divided by GMT in the HPV-6/11/16/18 vaccine group) were calculated in the ATP cohort for immunogenicity (all subjects who received 3 vaccine doses and for whom data concerning immunogenicity endpoint measurements were available at Month 48; seronegative and DNA-negative at baseline for the HPV type analyzed). Exploratory analyses were performed on the total vaccinated cohort (TVC, all subjects who received $\geq$ 1 dose of vaccine; regardless of serostatus and DNA status at baseline) and the p-value associated with an analysis of variance (ANOVA) test was calculated to compare the 2 vaccine groups. In the exploratory analysis of CD4+ T-cell and memory B-cell responses, the proportion of responders in each vaccine group was compared using a Fisher's exact test. The GM ratio between vaccine groups was obtained using an ANOVA model on the $\log_{10}$-transformed frequencies. The ANOVA model included the vaccine group as fixed effect. The GM ratio and its 95% CI were derived as exponential transformation. For the statistical assessment of CD4+ T-cell GM ratios, p-values were computed using a Kruskal-Wallis model. For the statistical assessment of memory B-cell GM ratios, p-values were calculated using an ANOVA model. Additional objectives at Months 36 and 48 were to evaluate the response to HPV-16 and -18 induced by the 2 vaccines in serum and in CVS by ELISA.

Notes

Cervarix is a registered trade mark of the GlaxoSmithKline group of companies.

Gardasil is a registered trade mark of Merck & Co., Inc..

Disclosure of Potential Conflicts of Interest

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