

Immunogenicity and Safety of the AS04-adjuvanted Human Papillomavirus-16/18 Cervical Cancer Vaccine in Malaysian Women Aged 18–35 years: A Randomized Controlled Trial

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SUMMARY

Introduction: Cervical cancer is the third most common cancer in women worldwide. The HPV-16/18 AS04-adjuvanted vaccine (Cervarix®) has previously been shown to be highly immunogenic with a clinically acceptable safety profile. This phase IIIb, double-blind, randomized (1:1) and placebo controlled trial (NCT00345878) was designed to evaluate the vaccine immunogenicity against HPV-16 and HPV-18 as well as its safety and reactogenicity in Malaysian women.

Methods: Healthy women aged 18–35 years received intramuscularly three doses of either the vaccine (HPV group) or aluminium hydroxide (ALU group) at 0, 1, and 6 months. Antibody titers were measured by an enzyme-linked immunosorbent assay (ELISA).

Results: A total of 271 eligible subjects were enrolled and 266 subjects completed the study. Initially seronegative subjects in the HPV group showed 100% seroconversion one month post-dose-3 for anti HPV-16 and anti-HPV-18 antibodies with geometric mean titers of 11107.5 (95% CI: 9727.3–12683.4) EL.U/mL and 4273.5 (95% CI: 3771.8–4841.9) EL.U/mL, respectively. Over 96% of subjects in both groups received all three vaccine doses. Solicited local (pain) and general symptoms (myalgia, fatigue, arthralgia and headache) were commonly reported in both HPV and ALU groups. Eight serious adverse events were reported throughout the study (five in the HPV group; three in the ALU group), all considered by investigators to be unrelated to vaccination.

Conclusion: The HPV-16/18 AS04-adjuvanted vaccine was immunogenic and generally well tolerated in Malaysian women aged 18–35 years.

KEY WORDS:

AS04-adjuvanted, Cervarix, cervical cancer, HPV-16, HPV-18, human papillomavirus, immunogenicity, seropositive, vaccine

INTRODUCTION

Cervical cancer is the third leading cause of cancer among women in the world, with more than 85% of the global

burden occurring in developing countries¹. In Malaysia, cervical cancer is the third most common cancer among women, constituting 8.4% of all women cancers². The incidence of cervical cancer in Malaysia is higher than in other Asian and Western countries³. Estimates from Malaysia in 2010 indicate that 2126 women are diagnosed and 631 die from cervical cancer every year⁴. An age standardized incidence rate of 17.9 and mortality rate of 5.6 per 100,000 women per year has been reported in Malaysia¹.

Persistent infection with oncogenic human papillomavirus (HPV) types is an established necessary cause for cervical cancer development^{5,6}. HPV-16 and HPV-18 combined account for approximately 70% of all invasive cervical cancer (ICC) cases worldwide, including in Malaysia⁶. The other notable oncogenic HPV types in cervical cancer identified in Malaysia are HPV-31, -51, -52, -56, -58 and -66⁷. Of the different cancer cases reported by morphology in Malaysia, prevalence of squamous cell carcinoma (71.7%) was much higher than that reported for adenocarcinoma (18.9%)⁸.

It has been demonstrated that HPV infection can be prevented by early immunization in adolescent and young women, an approach that is considered as the most promising for primary prevention against cervical cancer. HPV vaccination has been implemented for girls aged 9–13 years in many countries as part of their public health policy, with some variation in target age range. A recommended catch-up vaccination for young women up to 26 years of age is also being implemented⁹. Secondary prevention involves early identification of pre-cancerous lesions by cytology-based Papanicolaou (Pap) smear screening, and the treatment of such lesions can help in preventing cervical cancer. Cervical screening in combination with immunization of adolescents and young women may be an effective strategy for the prevention of cervical cancer.

The HPV-16/18 AS04-adjuvanted cervical cancer vaccine (Cervarix®, GlaxoSmithKline, Rixensart, Belgium) is licensed in more than 120 countries globally, including Europe, US, India, Japan, Korea and Malaysia, and also received WHO prequalification to combat cervical cancer in developing nations¹⁰. In Malaysia, the AS04-adjuvanted cervical cancer

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vaccine was launched in 2008. The vaccine was shown to be highly efficacious and immunogenic with a clinically acceptable profile in global studies¹¹⁻¹⁶. However, the vaccine has never been tested in an exclusively Malaysian population. This randomized study was specifically conducted in healthy Malaysian women aged 18–35 years, to evaluate antibody response against HPV-16 and HPV-18 as well as the safety and reactogenicity of the vaccine following each dose.

MATERIALS AND METHODS

Study participants and procedures

Malaysian women were recruited for this phase IIIb, double-blind, placebo-controlled trial (NCT00345878) conducted between September 2006 and December 2007 at two study centers in Malaysia (University Malaya Medical Centre, Kuala Lumpur and Putrajaya Hospital, Putrajaya). Healthy women aged 18–35 years with a negative urine pregnancy test and who were willing to use effective contraception during the vaccination period were eligible for inclusion. Exclusion criteria included previous vaccination against HPV, use of any investigational drug/vaccine other than the study vaccine, chronic use of immunosuppressants, history of allergy to vaccine compounds, history of chronic conditions requiring treatment, like cancer or autoimmune diseases, and acute disease at the time of enrolment.

All subjects provided written informed consent prior to the conduct and performance of study related procedures. The study was conducted in accordance with Good Clinical Practice and complied with all applicable regulatory requirements including the Declaration of Helsinki. The informed consent form, study protocol and amendments were approved by both investigational and national institutional review boards.

Subjects received three doses of either HPV-16/18 AS04 adjuvanted vaccine (HPV group) or 500 µg aluminium hydroxide as a placebo (ALU group), both administered intramuscularly according to a 0, 1 and 6 months schedule. The HPV-16/18 AS04-adjuvanted vaccine contained 20 µg each of HPV-16 and -18 L1 virus-like particles (VLP) formulated with the proprietary immunostimulatory AS04 adjuvant system (50 µg 3-O-desacyl-4'-monophosphoryl lipid A [MPL] adsorbed on 500 µg aluminium hydroxide). The vaccine was developed and manufactured by GlaxoSmithKline Vaccines in Rixensart, Belgium.

Randomization sequence and treatment allocation

The randomization of supplies was performed at GlaxoSmithKline Vaccines, Rixensart using a standard SAS (Statistical Analysis System) program. A randomization blocking scheme (1:1 ratio) was used to ascertain that the balance between treatments was maintained. Random allocation of subjects into two groups was performed using an internet based randomization system (SBIR) at the investigator site. The randomization algorithm used a minimization procedure.

The enrolment of subjects was age-stratified (18–25 and 26–35 years) to ensure that approximately equal numbers of subjects were enrolled in each age stratum. All subjects,

investigators, and study personnel directly involved in the study conducted were blinded throughout the study with respect to the individual subject treatment allocation.

Immunogenicity assessment

Blood samples were collected before the first vaccination and a month after the third dose to evaluate the antibody response against HPV-16 and HPV-18 using enzyme linked immunosorbent assay (ELISA)¹²⁻²². Anti-HPV-16 and anti-HPV-18 seropositivity was defined as an anti-HPV-16 antibody titer ≥ 8 ELISA units/milliliter (EL.U/mL) and an anti-HPV-18 antibody titer ≥ 7 EL.U/mL, respectively. These assay cut offs were established from previous calculations using two HPV-negative groups in MedImmune (Gaithersburg, MD, USA) studies MICP-057 and MICP-058.

Reactogenicity and safety assessment

Solicited local adverse events (AE; injection site pain, redness and swelling) and general AEs (fever, headache, fatigue, gastrointestinal symptoms, myalgia, arthralgia, rash and urticaria) during the 7-day follow-up period after each vaccination were self-recorded by all subjects using a diary card; post vaccination reactions (urticaria or rash that appeared within 30 minutes of each vaccine dose) were documented by the investigator.

Unsolicited AEs were recorded for a 30-day follow-up period after each vaccination by the subjects. Serious adverse events (SAEs), new onset chronic diseases (NOCDs) such as autoimmune diseases, asthma, Type I diabetes mellitus and allergies, medically significant conditions (MSCs) and pregnancy/pregnancy outcomes were recorded throughout the study period. An event was considered to be an NOCD if it had not been recorded in the subjects' medical history (i.e. new onset). MSCs were defined as AEs prompting emergency room or physician visits that are not related to common diseases and are not routine visits for physical examination or vaccination, or SAEs unrelated to common diseases.

AEs were graded by the investigator as either: mild (Grade 1), an AE that was easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities; moderate (Grade 2), an AE that was sufficiently discomforting to interfere with normal daily activities; or, severe (Grade 3), an AE that prevented normal daily activities (e.g., attendance at work/school and necessitating the administration of corrective therapy). Causality was assessed by individual investigator's clinical judgement.

Statistical analyses

The primary analysis of immunogenicity was performed on the according-to-protocol (ATP) cohort, which included all evaluable subjects (those meeting all eligibility criteria, complying with protocol defined procedures, without elimination code during the study) for whom immunogenicity data were available. The primary analysis of safety was performed on the total vaccinated cohort (TVC). This cohort included all subjects with at least one documented vaccine dose administration. A sample size of 120 evaluable subjects in the HPV group was required to demonstrate, with at least 92% power, that the seroconversion rates obtained for HPV-16 and -18 one month post-dose-3 were not less than 90% (assuming a

seroconversion rate of 98%). Power calculation was based on a one-sided exact test for one binomial population using nQuery 4.0, $\alpha=2.5\%$.

Seroconversion/seropositivity rates for anti-HPV-16 and anti-HPV-18 antibodies were calculated with their exact 95% confidence interval (CI). Geometric Mean Titers (GMTs) with 95% CI was also tabulated. Seroconversion was defined as the appearance of antibodies (i.e. antibody titer \geq cut-off value) in the serum of subjects seronegative before vaccination. GMT calculations were performed by taking the anti-log of the mean of the log antibody titers transformations. Antibody titers below the assay cut-off were given an arbitrary value of half the cut-off for GMT calculation in all subjects. Statistical analyses were performed using SAS 9.1 and Proc StatXact (version 7.0) software on Windows XP.

RESULTS

Study population and demographic characteristics

A total of 271 eligible subjects (135 in the HPV group and 136 in the ALU group) were enrolled, of which 266 subjects (131 in the HPV group and 135 in the ALU group) completed the study (Figure 1). The mean age of subjects (TVC) was 24.9 ± 4.02 years. The subjects were predominantly Asian: 63.1% of subjects had South East Asian heritage, 28.4% had East Asian heritage, 5.9% had Central/South Asian heritage and 2.6% of subjects belonged to other races that included Eurasian, Kadazan, Iban, Rungus or Bidayuh races.

Immunogenicity

Baseline serostatus (seropositive/seronegative for HPV-16 and/or HPV-18) was similar in both groups, 83.1% of subjects in the HPV group and 82.1% of subjects in the ALU group were seronegative for both HPV-16 and -18 antibodies before vaccination (TVC) (Table I).

In the ATP cohort for immunogenicity, all initially seronegative subjects in the HPV group seroconverted one month post-dose-3 for anti-HPV-16 and anti-HPV-18 antibodies. All initially seropositive subjects remained seropositive for anti-HPV-16 and/or -18 antigens one month post-dose-3 in the HPV group (Table II and Table III).

The anti-HPV-16 and anti-HPV-18 antibody GMTs in the HPV group (initially seronegative subjects) were 11107.5 EL.U/mL (95% CI: 9727.3–12683.4) and 4273.5 EL.U/mL (95% CI: 3771.8–4841.9) one month post-dose-3. Subjects seropositive at baseline in the HPV group had GMTs of 11342.5 EL.U/mL (95% CI: 7240.5–17768.6) for anti-HPV-16 antibodies and 4203.2 EL.U/mL (95% CI: 2578.7–6851.1) for anti-HPV-18 antibodies one month post-dose-3 (Table II and Table III).

Safety

Solicited local and general adverse events

During the 7-day post-vaccination follow-up period, pain at injection site was the most frequently reported solicited local symptom in both groups (overall/dose) (Figure 2). Incidence of all Grade 3 local symptoms reported did not exceed 3.3% of doses in the HPV group while no Grade 3 symptoms were reported in the ALU group (overall/dose). The local symptoms

were mostly transient with the mean duration ranging from 2.8–2.9 days in the HPV group and 2.1–2.5 days in the ALU group.

The mean duration of solicited general symptoms ranged from 1.6–3.8 days in the HPV group and 1.5–2.7 days in the ALU group. Myalgia, fatigue, arthralgia and headache were the most commonly reported solicited general symptoms in both groups (overall/dose) with a higher reporting rate for myalgia and arthralgia in the HPV group (Figure 3). Grade 3 general symptoms reported were rare in both groups (not exceeding 1% of doses in the HPV group). Grade 3 symptoms were not reported in the ALU group except gastrointestinal symptoms which were reported after 0.2% of doses. One subject reported urticaria or rash (ALU group) within 30-minutes post first placebo dose.

Compliance with the three-dose vaccination course was high in both the HPV group (96.3%) and in the ALU group (97.8%) (TVC).

Unsolicited AEs, MSCs, NOCDs, pregnancy and SAEs.

Unsolicited AEs (classified by MedDRA Primary System Organ Class and Preferred Term) were reported in 22.2% (N=135) and 26.5% (N=136) of subjects in the HPV and ALU groups, respectively. Infections and infestations were the most frequently reported unsolicited symptoms for 10.4% of subjects in the HPV group and 15.4% of subjects in the ALU group. No differences in the incidence of any individual unsolicited symptoms were identified between the two groups.

Six Grade 3 unsolicited AEs were reported by six subjects: two in the HPV group and four in the ALU group. The Grade 3 unsolicited events in the HPV group were cases of keratitis and upper respiratory tract infection while those in the ALU group were tonsillitis, migraine and dysmenorrhoea.

At least one medically significant AE (classified by MedDRA Primary System Organ Class and Preferred Term) was reported by 7.4% (10/135) of subjects in the HPV group and 8.1% of subjects (11/136) in the ALU group. One subject (0.7%) in the HPV group reported a NOCD in the study (allergic rhinitis). Eight SAEs were reported during the study (five in the HPV group were cholelithiasis, chronic tonsillitis, dengue fever, nasal turbinate hypertrophy and abortion missed and three in the ALU group were dengue fever, overdose and intervertebral disc protrusion). None of these were considered to be related to vaccination by the investigator. One withdrawal due to non-serious AEs (throat and vagina burning sensation) was reported, considered unrelated to vaccination by the investigator.

Four pregnancies were reported during the study (two in each group). Both subjects in the ALU group delivered healthy babies. Of the two subjects in the HPV group, one opted for elective abortion after 12 weeks of pregnancy owing to personal reasons and the other reported a missed abortion that was categorized as an SAE.

Table I: Baseline serological status (Total vaccinated cohort)

		HPV Group (N=135)	ALU Group (N=136)
HPV-16 IgG	HPV-18 IgG	n (%)	n (%)
Seropositive	Seropositive	7 (5.4)	3 (2.2)
Seropositive	Seronegative	6 (4.6)	10 (7.5)
Seronegative	Seropositive	9 (6.9)	11 (8.2)
Seronegative	Seronegative	108 (83.1)	110 (82.1)
Seropositive	MISSING	1 (-)	0 (-)
Seronegative	MISSING	4 (-)	2 (-)

ALU Group: subjects who received aluminium hydroxide

HPV Group: subjects who received the HPV-16/18 LI VLP AS04-adjuvanted vaccine

N: number of subjects in each group

n (%): number (percentage) of subjects in the considered category

Table II: Immune response to HPV-16 (ATP cohort for immunogenicity)

Group	Pre-vaccination status	Timing	N	≥8 EL.U/mL % (95% CI)	GMT (95% CI)
HPV	Seronegative	Pre-vaccination	112	0.0 (0.0–3.2)	4.0 (4.0–4.0)
		One month post-dose-3	112	100 (96.8–100)	11107.5 (9727.3–12683.4)
	Seropositive	Pre-vaccination	14	100 (76.8–100)	35.4 (11.0–113.6)
		One month post-dose-3	14	100 (76.8–100)	11342.5 (7240.5–17768.6)
	Total	Pre-vaccination	126	11.1 (6.2–17.9)	5.1 (4.3–6.0)
		One month post-dose-3	126	100 (97.1–100)	11133.3 (9817.6–12625.3)
ALU	Seronegative	Pre-vaccination	117	0.0 (0.0–3.1)	4.0 (4.0–4.0)
		One month post-dose-3	115	3.5 (1.0–8.7)	4.4 (4.0–4.8)
	Seropositive	Pre-vaccination	12	100 (73.5–100)	18.8 (11.2–31.5)
		One month post-dose-3	12	58.3 (27.7–84.8)	10.6 (5.4–20.8)
	Total	Pre-vaccination	129	9.3 (4.9–15.7)	4.6 (4.2–5.0)
		One month post-dose-3	127	8.7 (4.4–15.0)	4.7 (4.2–5.3)

ALU: subjects who received aluminium hydroxide

GMT (95% CI): geometric mean antibody titers calculated on all subjects with 95% confidence interval

HPV: subjects who received the HPV-16/18 LI VLP AS04-adjuvanted vaccine

N: number of subjects with pre-vaccination results available in each group

Seronegative: subjects with antibody titer <8 EL.U/mL prior to vaccination.

Seropositive: subjects with antibody titer ≥8 EL.U/mL prior to vaccination.

% (95%CI): percentage of subjects with concentration within specified range; with exact 95% confidence interval

Table III: Immune response to HPV-18 (ATP cohort for immunogenicity)

Group	Pre-vaccination status	Timing	N	≥7 EL.U/mL % (95% CI)	GMT (95% CI)
HPV	Seronegative	Pre-vaccination	106	0.0 (0.0–3.4)	3.5 (3.5–3.5)
		One month post-dose-3	106	100 (96.6–100)	4273.5 (3771.8–4841.9)
	Seropositive	Pre-vaccination	16	100 (79.4–100)	15.8 (11.1–22.6)
		One month post-dose-3	16	100 (79.4–100)	4203.2 (2578.7–6851.1)
	Total	Pre-vaccination	122	13.1 (7.7–20.4)	4.3 (3.9–4.7)
		One month post-dose-3	122	100 (97.0–100)	4264.2 (3771.6–4821.2)
ALU	Seronegative	Pre-vaccination	113	0.0 (0.0–3.2)	3.5 (3.5–3.5)
		One month post-dose-3	113	1.8 (0.2–6.2)	3.6 (3.4–3.9)
	Seropositive	Pre-vaccination	14	100 (76.8–100)	20.1 (14.5–27.9)
		One month post-dose-3	14	85.7 (57.2–98.2)	14.6 (9.1–23.2)
	Total	Pre-vaccination	127	11.0 (6.2–17.8)	4.2 (3.8–4.7)
		One month post-dose-3	127	11.0 (6.2–17.8)	4.2 (3.8–4.7)

ALU: Subjects who received aluminium hydroxide

GMT (95% CI) : Geometric mean antibody titers calculated on all subjects with 95% confidence interval

HPV: Subjects who received the HPV-16/18 LI VLP AS04-adjuvanted vaccine

N: Number of subjects with pre-vaccination results available in each group

Seronegative: Subjects with antibody titer <7 EL.U/mL prior to vaccination.

Seropositive: Subjects with antibody titer ≥7 EL.U/mL prior to vaccination.

% (95%CI): Percentage of subjects with concentration within specified range; with exact 95% confidence interval

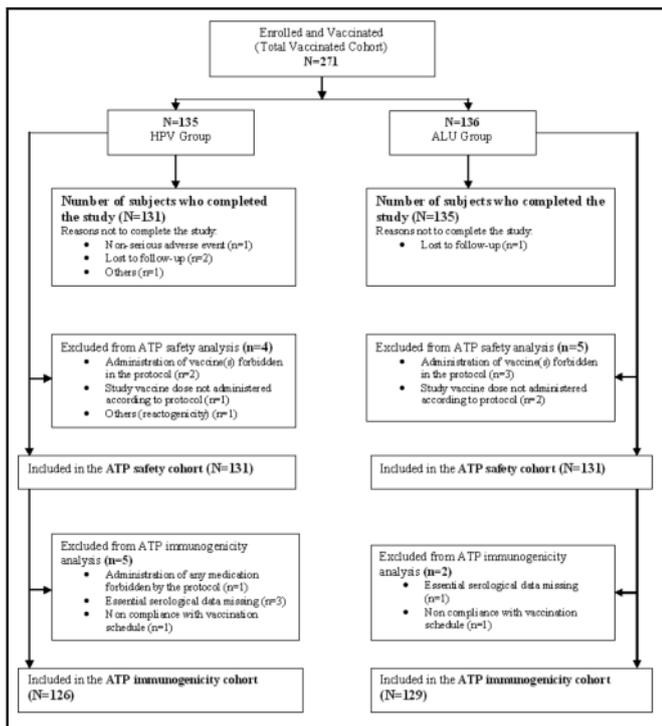


Fig. 1 : Study Participants' Flowchart.

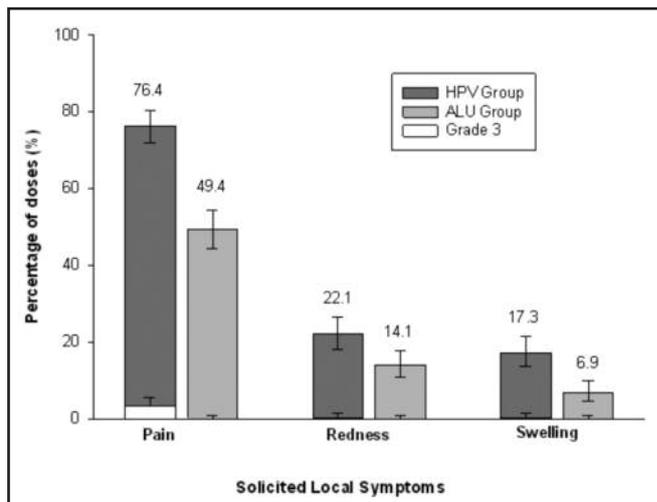


Fig. 2 : Incidence of solicited local symptoms overall/dose during the 7-day post-vaccination period (Total vaccinated cohort)

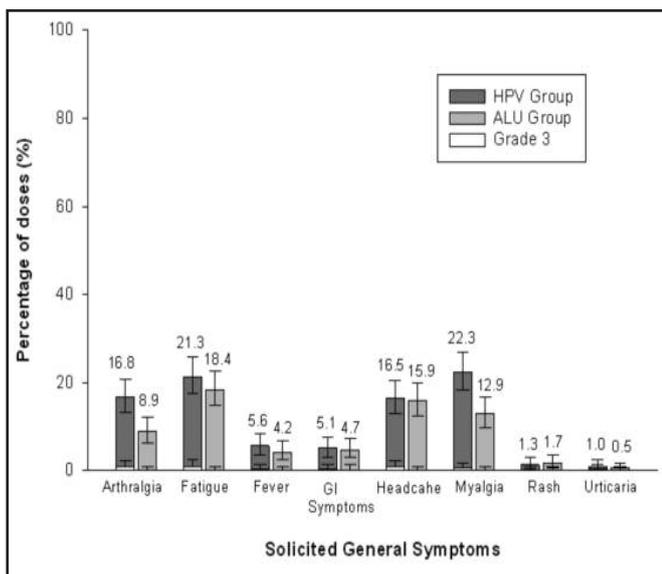


Fig. 3 : Incidence of solicited general symptoms overall/dose during the 7-day post-vaccination period (Total vaccinated cohort).

DISCUSSION

Worldwide, cervical cancer is the most common cancer type after breast cancer in women. In view of the cervical cancer disease burden in Malaysia, immunization of adolescents and young women as an important tool for significant reduction of HPV infection and cervical cancer burden in the region has been considered.

In the present study, the HPV-16/18 AS04-adjuvanted vaccine was found to be highly immunogenic in young and adult Malaysian women. All initially seronegative subjects who received HPV vaccine in the study seroconverted at one month post-dose-3, with high antibody titers achieved for both antigens. All initially seropositive subjects in the vaccine group not only remained seropositive for HPV-16 and/or HPV-18 antibodies one month post-dose-3 but also developed GMTs comparable to the seronegative study group. This is indicative of the fact that natural infection with HPV from prior exposure does not affect the immune response generated by the HPV-16/18 AS04-adjuvanted vaccine¹³⁻¹⁵. The immunogenicity results in this study are similar to that observed in studies conducted in countries from Asia and elsewhere¹³⁻¹⁷. The magnitudes of immune response (GMTs) achieved between different age strata (18–25 and 26–35) in the current study were comparable and also consistent with that of a large international phase III clinical study (Study HPV-008 [580299/008, NCT00122681]) across 14 countries (Australia, Belgium, Brazil, Canada, Finland, Germany, Italy, Mexico, the Philippines, Spain, Taiwan, Thailand, UK, and USA) in women aged 15–25 years¹⁸.

The antibody levels induced are a valuable marker of probable vaccine efficacy and the duration of protection offered by the vaccine^{12,19}. Clinical trials in young women aged 15–25 years have shown that the immune response induced by the study vaccine persists up to 8.4 years post-vaccination at levels substantially higher than those induced by natural infection²⁰. Additional statistical modelling predicts that this benefit will extend for at least 20 years¹⁹. One limitation of the study design is that antibody specificity and affinity, important parameters in considering vaccine efficacy, were not assessed. Previous data have demonstrated that monoclonal antibodies against neutralizing epitopes bind with high affinity to L1 VLPs in the study vaccine²¹. In addition, an analysis comparing antibody response measured by direct ELISA or pseudovirion-based

neutralization assay (PBNA) showed a high sensitivity and correlation, suggesting that direct ELISA is an excellent surrogate for measuring neutralizing response against HPV 16 and -18²².

Although not evaluated in the current study, the HPV-16/18 AS04-adjuvanted vaccine has demonstrated protection up to 100% against pre-cancerous cervical lesions (CIN2+) attributed to HPV-16 and -18,^{17,18,23,24} and additional cross-protection against some non-vaccine oncogenic HPV types (HPV-31, -33, and -45)^{18,25}. The unique AS04-adjuvant used in the formulation of HPV-16/18 L1 VLP vaccine has been shown to play an important role in the induction of high and sustained antibody titers and induction of cell-mediated immunity²⁶.

The HPV-16/18 AS04-adjuvanted vaccine was generally well tolerated in Malaysian women and showed an acceptable safety profile consistent with previous Asian and global studies¹³⁻¹⁷. Compliance with complete vaccination course appeared to be unaffected by adverse events in both groups, with approximately 96% of women fully completing the vaccination schedule. Safety results from this study are consistent with other global studies^{14,25,25,27}.

Cancer of the cervix continues to pose a significant health concern in Malaysia. The Ministry of Health, Malaysia has taken significant steps in planning, organizing and evaluating a Pap smear screening program which was established in 1969²⁸. Malaysia relies on opportunistic screening delivery rather than an organized program to increase awareness of the disease²⁹. However, due to low screening uptake, there has been no reduction in the prevalence of cervical cancer noted to date. Considering that the burden of cervical cancer is a global public health problem, WHO recommended the inclusion of routine HPV vaccination in the national immunization programs³⁰. Countries like the Netherlands and UK have established national HPV immunization programs for adolescent girls,^{31,32} and a national immunization HPV immunization program for girls aged 13 years was implemented in Malaysia in early 2010³³. Vaccination coupled with proper education concerning HPV infection, increased disease awareness and information about the screening facilities, diagnosis and treatment of pre-cancerous lesions can lead to a possible reduction in HPV-related diseases and infections.

CONCLUSION

Vaccination against HPV in combination with screening, education and raising awareness, represents an important step towards reducing the burden of cervical cancer and other HPV-related diseases. This study demonstrated that the HPV-16/18 AS04-adjuvanted vaccine was highly immunogenic with a clinically acceptable profile in Malaysian women aged 18–35 years. These data support the need for HPV vaccination for the prevention of cervical cancer in young and adult women.

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Trademarks

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

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Conflict of Interest

The authors declare the following conflict of interest: Bhavyashree Gunapalaiah, Yee Leong Teoh, Hans L Bock, Dan Bi are/were employees of the GlaxoSmithKline group of companies, with GlaxoSmithKline Biologicals SA being sponsor of these studies. In addition to that, Dan Bi declares owning stock options. Whilst Bhavyashree Gunapalaiah, Yee-Leong Teoh and Hans L Bock were employees of GlaxoSmithKline during the study and much of the manuscript development, they have since left the company at the time of manuscript submission. Professor Boon Kiong Lim received honoraria/paid expert testimony/travel grants from GlaxoSmithKline Biologicals SA. The other authors have no conflict of interest.

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