

Original Article

Safety and immunogenicity of human papillomavirus-16/18 AS04-adjuvanted vaccine in healthy Chinese females aged 15 to 45 years: a phase I trial

Feng-Cai Zhu¹, Chang-Gui Li², Hong-Xing Pan¹, Yi-Ju Zhang¹, Dan Bi³, Hai-Wen Tang⁴ and Sanjoy Datta³

Abstract

Globally, about 70% of cervical cancers are associated with human papillomavirus (HPV)-16 or HPV-18 infection. A meta-analysis of epidemiologic studies in China showed that HPV was present in 98% of cervical cancer samples. The HPV-16/18 AS04-adjuvanted vaccine *Cervarix*[®]* has shown a high level of protection against HPV-16/18 infections and associated cervical lesions. This phase I trial (NCT00549900) assessed the safety, tolerability, and immunogenicity of the vaccine in Chinese. Thirty healthy Chinese females, aged 15 to 45 years with a median age of 29.5 years, received three doses of *Cervarix*[®] in Months 0, 1, and 6. Safety was assessed via recording solicited local and systemic symptoms within 7 days and unsolicited symptoms within 30 days after each vaccination. Serious adverse events, new onset of chronic diseases, and other medically significant conditions were recorded throughout this trial. As an exploratory objective, HPV-16/18 antibody titers were determined by enzyme-linked immunosorbent assay in serum samples collected in Months 0 and 7. Pain at the injection site was the most frequently reported local symptom. Two subjects reported medically significant adverse events. Both cases were assessed as unrelated to vaccination by the investigator. In Month 7, 100% seroconversion was observed for both anti-HPV-16 and anti-HPV-18 with high geometric mean antibody titers. HPV-16/18 AS04-adjuvanted vaccine, evaluated for the first time in Chinese females, was generally well tolerated and immunogenic, as previously shown in global studies.

Key words Cervical cancer, Chinese females, human papillomavirus, HPV-16/18 AS04-adjuvanted vaccine, phase I trial

Cervical cancer is the second most common cause of cancer death among women globally^[1]. Epidemiologic studies, coupled with detection of human papillomavirus (HPV) DNA in up to 99.7% of cervical cancers from all geographic areas, convincingly demonstrate that oncogenic HPV infection is the causative agent of

cervical cancer^[2]. This has been confirmed in China in a recently published meta-analysis^[3] as well as in a centralized, systematic HPV prevalence survey in tissue specimens from seven different geographic regions in China^[4].

Approximately 30 to 40 HPV types infect the human genital tract^[5], at least 15 of which are considered oncogenic and associated with cervical cancer^[6]. Globally, about 70% of all cases of cervical cancer are associated with HPV-16 and -18, followed by HPV-45, -31, -33, and -52^[6]. A generally similar distribution has been demonstrated in China, with some variations^[3,4,7]. The most common HPV type found in Chinese women with squamous cell carcinoma was HPV-16 (76.7%), followed by HPV-18 (7.8%), HPV-31 (3.2%), HPV-52 (2.2%), and HPV-58 (2.2%); the most common types found in

Authors' Affiliations: ¹Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, Jiangsu 210009, P. R. China; ²National Institutes for Food and Drug Control, Beijing 100050, P. R. China; ³GlaxoSmithKline Biologicals, B-1300 Wavre, Belgium; ⁴GlaxoSmithKline, Shanghai 200001, P. R. China.

Corresponding Author: Feng-Cai Zhu, Jiangsu Provincial Center for Disease Control and Prevention, No. 172 Jiangsu Road, Nanjing, Jiangsu 210009, P. R. China. Tel: +86-25-83354770; Fax: +86-25-83759409; Email: jszfc@vip.sina.com.

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Chinese women with adenocarcinoma were HPV-16 (35.3%) and HPV-18 (29.4%)^[4].

The high concordance of HPV infections with the incidence of cervical cancer underscores the importance of prophylactic vaccination against this disease, especially in rural areas where implementation of cytological screening schemes may exert a significant burden on healthcare systems^[8]. An analysis of a 2002 World Health Organization survey found that in China there was only a 23% effective screening coverage, defined as the proportion of women aged 25 to 64 years who reported having had a pelvic examination and Pap smear in the preceding 3 years^[9]. Therefore, an effective vaccine designed to prevent cervical cancer has public health relevance and should protect against multiple oncogenic HPV types, including at least HPV-16 and -18.

The prophylactic HPV vaccine, *Cervarix*[®]* (GlaxoSmithKline Biologicals, Belgium), has been licensed in over 110 countries. It is a bivalent vaccine, containing HPV-16 and -18 L1 protein virus-like particles (VLPs), formulated with an adjuvant system AS04 (aluminium hydroxide [Al(OH)₃] combined with 3-*O*-desacyl-4'-monophosphoryl lipid A). Over 40 000 subjects have been vaccinated in clinical trials of this vaccine, and a recent integrated safety and efficacy analysis of 11 trials showed that the vaccine was generally well tolerated in over 16 000 females (\geq 10 years old) who received at least one dose^[10]. The importance of the adjuvant system to generate and maintain an enhanced immune response, compared with a similar VLP vaccine adjuvanted with Al(OH)₃ alone, has been demonstrated in humans^[11,12]. Furthermore, the HPV-16/18 AS04-adjuvanted vaccine has shown high efficacy against HPV-16/18 infections and associated cervical neoplasia^[13], with long-term efficacy demonstrated up to 7.3 years^[14]. This vaccine also affords cross-protection against some other oncogenic HPV types (-31, -33, and -45) not included in the current vaccine, but phylogenetically related to HPV-16 and -18^[13].

Currently, there are no HPV vaccines licensed in China. As required by the State Food and Drug Administration regulations, the present study (the first HPV L1-VLP vaccine trial in mainland China) was undertaken to evaluate the safety and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine in healthy Chinese females, prior to larger safety, immunogenicity, and efficacy studies.

Subjects and Methods

Study population

Healthy females aged 15 to 45 years (inclusive), of Chinese origin and residing in China, were enrolled into

the study in December 2007. Written informed consent was obtained from the subjects. For subjects below the legal age of consent (i.e. <18 years old), written informed consent was obtained from the subject and her parent or legally acceptable representative. The trial was conducted according to the Good Clinical Practice, in compliance with the Declaration of Helsinki, and following approval by the Ethics Review Committees from appropriate centers according to local rules and regulations.

Subjects were required to undergo a negative urine pregnancy test before inclusion and prior to each vaccination. Subjects of childbearing potential were required to be abstinent or to use adequate contraception for 30 days prior to vaccination and for 2 months after the last vaccination. Other exclusion criteria included chronic administration of immunosuppressant/immune-modifying drugs and taking any other investigational products.

Study design

The phase I, open-labeled trial was performed in a single center in Jiangsu Province (China) in which subjects were vaccinated with the HPV-16/18 AS04-adjuvanted vaccine *Cervarix*[®]. The vaccine (0.5 mL in individual pre-filled syringes) was administered intramuscularly into the deltoid muscle of the non-dominant arm in Months 0, 1, and 6 (one dose at each visit). The subjects were observed closely for at least 30 min after each dosing, with medical treatment readily available in case of a rare anaphylactic reaction. Four visits were planned per subject, scheduled in Months 0, 1, 6, and 7.

Safety and reactogenicity

Safety and reactogenicity in the total vaccinated cohort were the primary endpoints of the study. Diary cards were used by the participants to record the occurrence of solicited local and systemic symptoms within 7 days and unsolicited events within 30 days following each vaccination. Serious adverse events, pregnancies and their outcomes, new onset of chronic diseases and medically significant conditions were evaluated. Medically significant conditions were defined as (serious) adverse events unrelated to common diseases (including upper respiratory infections, sinusitis, pharyngitis, gastroenteritis, urinary tract infections, cervicovaginal yeast infections, menstrual cycle abnormalities, and injury), or unrelated to routine physician visits.

Blood samples

In Months 0 and 7, blood samples were collected

from all subjects to determine HPV antibody response and evaluate biochemical and hematologic parameters, respectively.

Analysis of immunogenicity

The immunogenicity against HPV-16 and -18 was an exploratory endpoint of the trial and was primarily analyzed on the according-to-protocol cohort. Antibodies to HPV-16 and -18 VLPs were quantified at the National Institute for Food and Drug Control by direct enzyme-linked immunosorbent assay^[12].

Statistical analysis

Descriptive statistics were applied to analyze safety, reactogenicity, and immunogenicity data, with 95% confidence interval (CI) estimated when appropriate.

Results

A total of 30 females, aged 15 to 45 years with a median age of 29.5 years, were enrolled in the study. All participants were of Chinese heritage, residing in China and all received the HPV vaccine. One subject withdrew during the study period (between December 2007 and July 2008) due to a serious adverse event and 29 subjects completed the study.

Safety and reactogenicity

For the 30 subjects who underwent at least one vaccination, 26 (86.7%) reported solicited local symptoms and 13 (43.3%) reported solicited systemic symptoms

within 7 days after vaccination. Pain at injection site (65.2% of doses) was the most frequently reported solicited local symptom, followed by redness (34.8% of doses) and swelling (20.2% of doses). Grade 3 pain was reported after 4.5% of doses. None of the subjects reported grade 3 redness or swelling (above 50 mm diameter) (Table 1). Fatigue (19.1% of doses) was the most commonly reported solicited systemic symptom, followed by headache (13.5% of doses) and myalgia (11.2% of doses). Incidences of grade 3 solicited systemic symptoms were low and did not exceed 2.2% of doses for individual symptoms (Table 2). As per protocol, all solicited local symptoms were considered as causally related to vaccination while the incidences of vaccine-related solicited general symptoms were low (after 16.9%, 12.4%, 11.2%, 4.5%, and 4.5% of doses for fatigue, headache, myalgia, arthralgia, and gastrointestinal symptoms, respectively). No subjects reported urticaria/rash within 30 min after vaccination.

Within 30 days after vaccination, 4 unsolicited adverse events (aphthous stomatitis, chest discomfort, injection site hematoma, and upper respiratory tract infection) were reported by 4 subjects. The injection site hematoma was assessed as causally related to the vaccination. Two medically significant adverse events including one serious adverse event (as classified by Medical Dictionary for Regulatory Activities) were reported during the entire study period: chest discomfort in one subject who required a physician visit; left breast cancer in another subject who required hospitalization and withdrew from the study. Both adverse events were assessed as unrelated to vaccination.

No clinically relevant differences were observed in biochemical and hematologic parameters assessed in subjects between Month 0 and Month 7.

Table 1. Solicited local symptoms reported by the 30 females within 7 days after vaccination with human papillomavirus-16/18 AS04-adjuvanted vaccine

Symptom Type	HPV vaccination						Overall/subject		Overall/dose		
	Dose 1		Dose 2		Dose 3		n*	% (95% CI)	n†	% (95% CI)	
	n*	% (95% CI)	n*	% (95% CI)	n†	% (95% CI)					
Pain	All	26	86.7 (69.3–96.2)	19	63.3 (43.9–80.1)	13	44.8 (26.4–64.3)	26	86.7 (69.3–96.2)	58	65.2 (54.3–75.0)
	Grade 3	1	3.3 (0.1–17.2)	2	6.7 (0.8–22.1)	1	3.4 (0.1–17.8)	4	13.3 (3.8–30.7)	4	4.5 (1.2–11.1)
Redness	All	12	40.0 (22.7–59.4)	9	30.0 (14.7–49.4)	10	34.5 (17.9–54.3)	16	53.3 (34.3–71.7)	31	34.8 (25.0–45.7)
Swelling	All	5	16.7 (5.6–34.7)	6	20.0 (7.7–38.6)	7	24.1 (10.3–43.5)	9	30.0 (14.7–49.4)	18	20.2 (12.4–30.1)

CI = confidence interval; n/% = number/percentage of doses followed by at least one symptom.

* Number of subjects with at least one documented dose was 30. † Number of subjects with at least one documented dose was 29, as one subject withdrawn after the second visit due to a serious adverse event. ‡ Number of documented doses was 89.

Symptom severity was graded on a scale of 0-3; grade 3 solicited symptoms were defined as any symptom that prevented normal daily activities, redness or swelling >50 mm in diameter, fever >39°C, or urticaria that distributed on at least four areas. No grade 3 redness and swelling were reported.

Table 2. Solicited systemic symptoms reported by the 30 females within 7 days after during the 7-day post-vaccination with human papillomavirus-16/18 AS04-adjuvanted vaccine

Symptom	Type	HPV vaccination						Overall/subject		Overall/dose	
		Dose 1		Dose 2		Dose 3		n [#]	% (95% CI)	n [#]	% (95% CI)
		n [#]	% (95% CI)	n [#]	% (95% CI)	n [#]	% (95% CI)				
Arthralgia	All	2	6.7 (0.8–22.1)	2	6.7 (0.8–22.1)	0	0.0 (0.0–11.9)	2	6.7 (0.8–22.1)	4	4.5 (1.2–11.1)
	Grade 3	0	0.0 (0.0–11.6)	1	3.3 (0.1–17.2)	0	0.0 (0.0–11.9)	1	3.3 (0.1–17.2)	1	1.1 (0.0–6.1)
Fatigue	All	6	20.0 (7.7–38.6)	7	23.3 (9.9–42.3)	4	13.8 (3.9–31.7)	10	33.3 (17.3–52.8)	17	19.1 (11.5–28.8)
Fever	All	1	3.3 (0.1–17.2)	1	3.3 (0.1–17.2)	0	0.0 (0.0–11.9)	2	6.7 (0.8–22.1)	2	2.2 (0.3–7.9)
Gastro-intestinal	All	1	3.3 (0.1–17.2)	3	10.0 (2.1–26.5)	0	0.0 (0.0–11.9)	4	13.3 (3.8–30.7)	4	4.5 (1.2–11.1)
Headache	All	6	20.0 (7.7–38.6)	4	13.3 (3.8–30.7)	2	6.9 (0.8–22.8)	8	26.7 (12.3–45.9)	12	13.5 (7.2–22.4)
	Grade 3	0	0.0 (0.0–11.6)	1	3.3 (0.1–17.2)	0	0.0 (0.0–11.9)	1	3.3 (0.1–17.2)	1	1.1 (0.0–6.1)
Myalgia	All	5	16.7 (5.6–34.7)	3	10.0 (2.1–26.5)	2	6.9 (0.8–22.8)	7	23.3 (9.9–42.3)	10	11.2 (5.5–19.7)
	Grade 3	1	3.3 (0.1–17.2)	1	3.3 (0.1–17.2)	0	0.0 (0.0–11.9)	1	3.3 (0.1–17.2)	2	2.2 (0.3–7.9)
Rash	All	0	0.0 (0.0–11.6)	0	0.0 (0.0–11.6)	1	3.4 (0.1–17.8)	1	3.3 (0.1–17.2)	1	1.1 (0.0–6.1)

Footnotes as in Table 1. No cases of grade 3 fatigue, fever, gastrointestinal reaction, and rash and no cases of urticaria were reported.

Immunogenicity

Of the 29 subjects who completed the study, 24 were negative for both anti HPV-16 and -18 antibodies at baseline, 4 were negative for anti HPV-16 but positive for anti HPV-18, 1 was positive for anti HPV-16 and negative for anti HPV-18. Pre-vaccination geometric mean titers were 4.1 ELISA units per millilitre (EU/mL) (95% CI, 3.9 to 4.4 EU/mL) for anti HPV-16 and 4.4 EU/mL (95% CI, 3.4 to 5.5 EU/mL) for anti HPV-18. In Month 7, 100% seroconversion and seropositivity were observed for both anti HPV-16 and -18. Geometric mean titers for initially seronegative subjects were 6230.5 EU/mL (95% CI, 4755.1 to 8163.7 EU/mL) for anti-HPV-16 and 2411.1 EU/mL (95% CI, 1734.0 to 3352.7 EU/mL) for anti-HPV-18.

Discussion

The present phase I trial is the first HPV vaccine clinical trial ever conducted in mainland China in preparation for larger safety, immunogenicity, and efficacy studies, in line with local criteria for vaccine licensure by the State Food and Drug Administration. The HPV-16/18 AS04-adjuvanted vaccine was generally well tolerated in the participants; injection site reactions (pain, redness, and swelling) were the most frequently reported vaccine-related adverse events. We recognize

that this was an open study conducted at a single center and that the sample size was small. However, findings are consistent with the results of an integrated safety analysis of 11 phase II/III trials in a multi-ethnic (including women of Chinese origin) and geographically diverse cohort of approximately 30 000 females aged 10 to 72 years, including over 16 000 who received at least one dose of this HPV vaccine^[10].

The present study also found 100% seropositivity for anti-HPV-16 and -18 in all subjects who completed the study. Additionally, antibody levels observed were in the same range as levels found in a previous trial in a larger cohort in Hong Kong^[15] and in other populations^[16,17], thus indicating a lack of ethnic difference in antibody responses to this vaccine. In a long-term follow-up study, at up to 7.3 years after first vaccination with the HPV-16/18 AS04-adjuvanted vaccine, 100% of women remained seropositive with sustained levels of anti HPV-16 \geq 13-fold and anti HPV-18 \geq 11-fold higher respectively than those associated with natural infection^[14]. This long-term seropositivity, combined with the documented impact of HPV-16/18 vaccination on the reduction of virological and histological outcomes associated with HPV-16/18 infection^[13,18], and data showing substantial cross-protection against several non-vaccine oncogenic HPV types^[13], suggest that the HPV-16/18 AS04-adjuvanted vaccine has the potential to (possibly considerably) reduce the burden of cervical cancer worldwide.

Approximately 83% of worldwide cases of cervical

cancer occur in developing countries, where, due to infrastructure systems, both the cost-effectiveness and the feasibility of screening strategies have been under significant debate^[1]. In China, 30% of women aged 25 to 64 years have never had a pelvic examination and only 23% have had an effective screening coverage, in line with the difficulties in implementing screening programs in a country with a large rural population. In such settings, vaccination has been considered to have the potential to save many lives^[19] and prophylactic vaccination of younger women may provide significant advantages over screening as it has fewer logistical requirements^[9].

In conclusion, results of the present indicate that the HPV-16/18 AS04-adjuvanted vaccine appears to be well tolerated and immunogenic in Chinese females aged 15 to 45 years. Based on these favorable results, a large (6000 subjects) double-blind, randomized, controlled phase III trial (NCT00779766) is currently ongoing in China to further evaluate the efficacy, immunogenicity, and safety of this vaccine.

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Conflict of interest

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf and declare that Drs Feng-Cai Zhu, Chang-Gui Li, Hong-Xing Pan, and Yi-Ju Zhang have received research grants for the study from GlaxoSmithKline Biologicals; Dan Bi, Sanjoy Datta, and Hai-Wen Tang are employees of GlaxoSmithKline Biologicals and have been awarded share options.

* *Cervarix*[®] is a registered trademark of the GlaxoSmithKline group of companies.

† *Gardasil*[®] is a registered trademark of Merck & Co., Inc.

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