Immunogenicity and safety of an *E. coli*-produced bivalent human papillomavirus (type 16 and 18) vaccine: A randomized controlled phase 2 clinical trial

Ting Wu [a,1], Yue-Mei Hu [b,*,1], Juan Li [c,1], Kai Chu [b], Shou-Jie Huang [a], Hui Zhao [c], Zhong-Ze Wang [d], Chang-Lin Yang [d], Han-Min Jiang [d], Yi-Jun Wang [d], Zhi-Jie Lin [e], Hui-Rong Pan [e], Wei Sheng [a], Fei-Xue Wei [a], Shao-Wei Li [a,f], Ying Wang [g], Feng-Cai Zhu [b], Chang-Gui Li [c], Jun Zhang [a,*], Ning-Shao Xia [a]

[a] State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics, National Institute of Diagnostics and Vaccine Development in Infectious Diseases, Collaborative Innovation Center of Biological Products, School of Public Health, Xiamen University, Xiamen, China
[b] Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, Jiangsu Province, China
[c] National Institute for Food and Drug Control, Beijing, China
[d] Dongtai Center for Disease Control and Prevention, Dongtai, Jiangsu Province, China
[e] Xiamen Innovax Biotech Company, Ltd, Xiamen, China
[f] School of Life Science, Xiamen University, Xiamen, China
[g] National Center for Biotechnology Development, Beijing, China

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ABSTRACT

Background: This study aimed to investigate the dosage, immunogenicity and safety profile of a novel human papillomavirus (HPV) types 16 and 18 bivalent vaccine produced by *E. coli*.
Methods: This randomized, double-blinded, controlled phase 2 trial enrolled women aged 18–25 years in China. Totally 1600 eligible participants were randomized to receive 90 μg, 60 μg, or 30 μg of the recombinant HPV 16/18 bivalent vaccine or the control hepatitis B vaccine on a 0, 1 and 6 month schedule. The designated doses are the combined micrograms of HPV16 and 18 VLPs with dose ratio of 2:1. The immunogenicity of the vaccines was assessed by measuring anti-HPV 16 and 18 neutralizing antibodies and total IgG antibodies. Safety of the vaccine was assessed.
Results: All but one of the seronegative participants who received 3 doses of the HPV vaccines sero-converted at month 7 for anti-HPV 16/18 neutralizing antibodies and IgG antibodies. For HPV 16, the geometric mean titers (GMTs) of the neutralizing antibodies were similar between the 60 μg (GMT = 10,548) and 90 μg (GMT = 12,505) HPV vaccine groups and were significantly higher than those in the 30 μg (GMT = 7596) group. For HPV 18, the GMTs of the neutralizing antibodies were similar among the 3 groups. The HPV vaccine was well tolerated. No vaccine-associated serious adverse events were identified.
Conclusion: The prokaryotic-expressed HPV vaccine is safe and immunogenic in women aged 18–25 years. The 60 μg dosage formulation was selected for further investigation for efficacy.
    Clinical trials registration: NCT01356823.

1. Introduction

Cervical cancer is the third leading cause of cancer in women worldwide [1]. Approximately 70% of cervical cancer cases are associated with infection with human papillomavirus (HPV) type 16 or 18. A safe and efficacious vaccine that can protect against HPV infection is an important tool to control these threats [2].

Three human papillomavirus (HPV) vaccines, Gardasil® (HPV 6/11/16/18 quadrivalent vaccine) [3,4], Cervarix® (HPV 16/18 bivalent vaccine) [5,6] and more recently Gardasil®9 (HPV 6/11/16/18/31/33/45/52/58 9-valent vaccine) [7], which are composed of virus-like particles (VLPs) produced in eukaryotic cells [8], have been shown to be safe and efficacious and have been marketed [9]. Unfortunately, despite many calls for the inclusion of the HPV vaccine in national immunization programs, the high cost of the vaccines has hindered their wider use in developing countries. Hence, additional safe and efficacious HPV vaccine sources are desirable to lower the acquisition cost, particularly for developing countries.

Recently, another HPV 16/18 bivalent vaccine candidate, Cecolin®, composed of the VLP antigen produced in *E. coli*, was shown to be safe and highly immunogenic in animals and was well tolerated in 38 adult women in a phase 1 clinical trial [10]. The present study was a double-blinded, randomized, controlled phase 2 clinical trial to assess the safety and immunogenicity of Cecolin®.

2. Methods

2.1. Study design and participants

This randomized, double-blinded, controlled, single-center phase 2 trial was conducted between May and December, 2011, in Dongtai County, Jiangsu Province, China. Healthy young women aged 18–25 years were enrolled (detailed inclusion and exclusion criteria are described in the Appendix Text S1–S3). All the participants came from local villages and were recruited through advertising. The participants were required to use effective contraception during the trial.

The study was designed by Xiamen University and the Jiangsu Provincial Center for Disease Control and Prevention (JSCDC). The study staff at the JSCDC was responsible for data collection. Serial sera collected from the participants were independently tested by the National Institute for Food and Drug Control (NIFDC, Beijing, China). Before initiation of the study, it was approved by the Ethics Committee of the JSCDC. Written informed consent was obtained from all the participants. The trial was undertaken by JSCDC in accordance with the Declaration of Helsinki and Good Clinical Practice (ClinicalTrials.gov: NCT01356823).

2.2. Vaccines

The studied bivalent HPV vaccine, Cecolin® (Xiamen Innovax Biotech Co. Ltd., Xiamen, China), was prepared as previously described [10]. It is a mixture of two recombinant HPV type—specific VLPs consisting of the L1 major capsid proteins of HPV 16 and 18 expressed in *E. coli*. The two L1 VLP types were separately absorbed to a hydroxyl aluminum adjuvant and then mixed together. Three preparations of bivalent HPV types 16 and 18 were used. The dose ratio of type 16 and type 18 was maintained at 2:1. The three formulations were: 30 μg (containing 20 μg of type 16 and 10 μg of type 18), 60 μg (containing 40 μg of type 16 and 20 μg of type 18) and 90 μg (containing 60 μg of type 16 and 30 μg of type 18), all suspended in 0.5 mL of buffered saline and 208 μg of aluminum adjuvant. The control vaccine was a licensed hepatitis B (HepB) vaccine (Shenzhen Biokangtai, Shenzhen, China) containing

10 μg of the hepatitis B surface antigen absorbed to 240 μg of aluminum adjuvant in 0.5 mL volume. All the investigational vaccines and the control vaccines were repackaged under Good Manufacturing Practice conditions for identical appearance but were labeled with different vaccine numbers.

2.3. Randomization and masking

The randomization schedule was computer generated using a blocking factor of 20. The participants were randomly allocated in a 1:1:1:1 ratio to the 30 μg, 60 μg, or 90 μg groups of the HPV 16/18 vaccine or the control HBV vaccine. The control and HPV vaccine formulations had identical packaging with blindly labeled sequential codes. The individuals involved in the randomization and masking did not participate in any other part of the trial. All the participants and investigators were masked to the treatment allocation.

2.4. Procedures

The vaccine was given by intramuscular injection at day 0, month 1 (±10 d), and month 6 (±30 d). After vaccination, all the participants remained at the vaccination site and were observed for 30 min for immediate adverse reactions. They were then visited or called by the investigators at 6 h, 24 h, 48 h, 72 h, 7 d, 14 d and 28 d after each dose. Any observed or reported adverse events (AEs) that occurred within one month after each vaccination were recorded by the participants on safety diary cards, using a four-grade scale of symptom intensity under the guidance of investigators. Serious adverse events (SAEs) were recorded throughout the study. Serum samples were collected at 0 m before the first vaccination and at 7 m for all the participants. Reasons for drop out were listed in Table S1 in Appendix.

2.5. Antibody detection

Both the pseudovirion-based neutralization assay (PBNA) and the *E. coli*-expressed HPV L1 VLP-based ELISA (eVLP-based ELISA) were employed to measure the HPV 16/18 neutralizing antibodies and IgG antibodies as previously described [11]. In brief, for the PBNA, the serum samples were 2-fold serially diluted and then cultured with HPV pseudovirions, after which the mixtures were transferred to 293FT cell monolayers and incubated. A positive sample was defined as one that caused a 50% reduction in green fluorescent protein (GFP) expression compared with the negative control, and the neutralizing titers were defined as the highest dilution of the positive samples. The neutralizing titers of the negative samples were set at 1:10, which is half of the starting serum dilution. For the eVLP-based ELISA, each well of the 96-well microtiter plates was coated with HPV 16 VLPs or HPV 18 VLPs, then the 2-fold serially diluted serum samples were added. The optical density (OD) was read at 450/620 nm. The titers were calculated based on a diluted sample with an OD within the working range of the standard curve as previously described [11].

The serum samples at 0 m were qualitatively tested with the PBNA assay. For samples collected at month 7, only sera from the first 600 participants were titrated with the PBNA assay. All the collected serum samples were titrated by a VLP-based ELISA.

2.6. Statistical methods

The sample size of each group was based on the guidelines of the Chinese Food and Drug Administration (CFDA), which required approximately 300 per-protocol participants for assessing the safety profile of a novel vaccine [12]. Assuming that 25% of the participants would drop out, we chose a sample size of 400 per
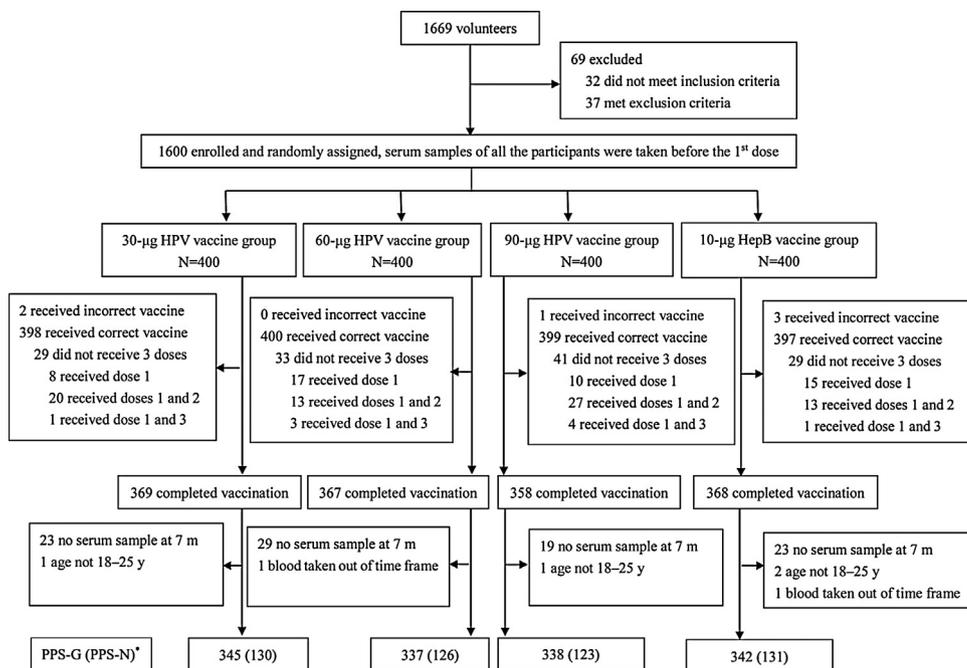
Fig. 1. *Trial profile.* A total of 1600 eligible female volunteers 18–25 years of age were enrolled and randomly assigned to the investigational HPV vaccine or the control hepatitis B vaccine group. PPS-G: per-protocol set of anti-HPV IgG analysis; PPS-N: per-protocol set of anti-HPV neutralizing antibody analysis. The PPS cohorts consisted of women who received all three doses of the HPV vaccine or the control vaccine, had corresponding antibody data for 0-m and 7-m serum samples obtained during the protocol-specified time frames, and committed no important violations of the protocol. All the serum samples collected from the 1600 women were tested for IgG antibodies. All the baseline serum samples were qualitatively tested by the PBNA assay, whereas for the serum samples collected at month 7, only the serum samples from the participants assigned vaccine codes from 1001 to 1600 (i.e., the first 600 eligible participants) were titrated for neutralizing antibodies.

treatment group. A per-protocol sample size of 300 produces a two-sided 95% confidence interval with a width equal to 0.1 when the response rate is 80% and provides a power of 0.9 to detect the unequivalence of two dosage group when the true ratio of the means is 1.0, the coefficient of variation on the original, unlogged scale is 1.0, and the equivalence limits of the mean ratio are 0.8 and 1.25.

Statistical comparisons were made using two-sided tests with an alpha value of 0.05. The immune response rates and mean titers were summarized with 95% confidential intervals (CIs) by the Clopper–Pearson method or Student's t distribution, respectively. The statistical analyses were performed by an independent statistician using SAS (version 9.2).

Participants who received vaccine with incorrect code by mistake during the trial were excluded from any analysis sets before unblinding, and their safety data were separately assessed. The safety-analysis cohort included all the remaining participants who received at least one dose and whose safety data were recorded. Primary immunogenicity analyses were conducted in the per-protocol set (PPS), which consisted of the women who received all three doses of the HPV vaccine or the control vaccines, had antibody data for 0 m and 7 m serum samples obtained during the protocol-specified time frames, and committed no important violations of the protocol. The intention-to-treat (ITT) immunogenicity cohort included those who received at least one dose and for whom the antibody data for 0 m and 7 m serum samples were available. Seroconversion was defined as an increase in antibody titers of at least fourfold in an individual's paired sera.

3. Results

3.1. Baseline demographic characteristics

We enrolled 1600 women aged 18–25 years who were randomly assigned to receive 30 μg, 60 μg, or 90 μg of the HPV vaccine or the

control HBV vaccine, with 400 women in each group (Fig. 1). Six participants erroneously received the incorrect vaccine in the second or third dose and were excluded from further analyses. None of the women reported serious adverse events or adverse events worse than grade 2. Totally 91.4% of the enrolled participants received all the 3 doses per protocol, the rates of drop-out were similar among the 4 groups. None of the recorded reasons for drop-out was associated with adverse events.

The baseline demographic characteristics of each group are summarized in Table 1. The mean age of the participants was 21.9 ± 2.2 years. The baseline seropositive rates of HPV 16 neutralizing antibodies, HPV 18 neutralizing antibodies, HPV 16 IgG antibodies and HPV 18 IgG antibodies were 7.8%, 2.5%, 24.3% and 9.5%, respectively.

3.2. Immunogenicity

Assessment of the immunogenicity of the bivalent HPV vaccine was based primarily on the neutralizing antibody response to the corresponding types in the baseline seronegative participants. Compared with the 0.8% seroconversion rate of neutralizing antibodies in the control group, 100% of the participants who received three doses of the HPV vaccine produced neutralizing antibodies against HPV 16, with GMTs of approximately 10,000 (range 160 to 163,840) (Table 2). Except for one participant in the 30 μg group, all the participants who received three doses of the HPV vaccine were seroconverted for neutralizing antibodies against HPV 18, with similar GMTs of approximately 7,500 (range 160 to 81,920) for the three dosage groups. Although the GMTs of antibodies against type 18 in the three vaccine groups were similar, the GMTs of antibodies against type 16 in the 30 μg group were marginally lower than those in the 60 μg and 90 μg groups (Table 2).

The distribution of the neutralizing antibody titers is plotted in Fig. 2. The vaccination induced strong neutralizing antibody

Table 1
Baseline characteristics of the participants.

	30 μg HPV vaccine group	60 μg HPV vaccine group	90 μg HPV vaccine group	Control group	Total
Number of participants	400	400	400	400	1600
Mean age (SD)	22.0 (2.2)	22.0 (2.1)	21.9 (2.2)	21.9 (2.2)	21.9 (2.2)
HPV16 IgG +ve (rate, %)	99 (24.8)	80 (20.0)	110 (27.5)	99 (24.8)	388 (24.3)
GMT (95% CI)	43.4 (37.5,50.3)	45.4 (37.2,55,3)	40.1 (35.3,45.7)	45.8 (38.6,54.4)	43.4 (40.2,47.0)
HPV18 IgG +ve (rate, %)	44 (11.0)	34 (8.5)	34 (8.5)	40 (10.0)	152 (9.5)
GMT (95% CI)	45.5 (36.2,57.2)	52.0 (39.1,69.2)	56.9 (41.8,77.3)	55.5 (42.2,73.1)	52.0 (45.5,59.3)
HPV 16/18 IgG double +ve (rate, %)	23 (5.8)	14 (3.5)	17 (4.3)	16 (4.0)	70 (4.4)
HPV 16/18 IgG double −ve (rate, %)	280 (70.0)	300 (75.0)	273 (68.3)	277 (69.3)	1130 (70.6)
Participants who were assigned to quantitative testing for neutralizing antibodies					
Number	150	150	150	150	600
Age (SD)	22.1 (2.2)	21.9 (2.1)	21.5 (2.2)	21.6 (2.2)	21.8 (2.2)
HPV16 neutralizing Ab +ve (rate, %)	9 (6.0)	9 (6.0)	16 (10.7)	13 (8.7)	47 (7.8)
GMT (95% CI)	80.0 (37.7,170.1)	63.5 (22.6,178.2)	35.1 (4.4,363,1)	75.8 (40.5,142.0)	57.0 (41.0,79.1)
HPV18 neutralizing Ab +ve (rate, %)	3 (2.0)	4 (2.7)	5 (3.3)	3 (2.0)	15 (2.5)
GMT (95% CI)	63.5 (1.8,2287.8)	40.0 (4.4,363.1)	46.0 (9.9,214.2)	160.0 (160.0,160.0)	52.8 (28.6,97.3)
HPV 16/18 neutralizing Ab double +ve (rate, %)	1 (0.6)	2 (1.3)	1 (0.6)	3 (2.0)	7 (1.2)
HPV 16/18 neutralizing Ab double −ve (rate, %)	139 (92.7)	139 (92.7)	130 (86.7)	137 (91.3)	545 (90.8)

SD: standard deviation; GMC: geometric mean concentration; GMT: geometric mean titer; +ve: positive; −ve: negative. Only positive samples were used to calculate baseline GMC or GMT.

responses for both types in both seronegative and seropositive participants (Fig. 2A and B). The reverse accumulation curve of the neutralizing antibodies showed that almost all the participants in the HPV vaccine groups produced high neutralizing antibody titers higher than 1280 (Fig. 2C and D). With respect to the antibody response to type 16, the titers of the participants in the 30 μg group seemed lower than those in the 60 μg and 90 μg groups (Fig. 2C). However, the antibody response titers to type 18 were similar for the three dosage groups (Fig. 2D).

The IgG antibody responses were also assessed by a VLP-based ELISA. The findings were quite similar to those for the neutralizing antibodies (Table 3, Fig. S1 in the Appendix). All the participants who received three doses of the HPV vaccine were seroconverted for IgG antibodies against both HPV types, and the GMTs of antibodies against both types induced by the 30 μg dose were lower than those with the higher doses.

The preexisting type-specific antibodies had no or minimally significant effect on the vaccine-induced antibody levels (Table S2 in Appendix).

3.3. Safety

The administration of three injections of the HPV 16/18 bivalent vaccine was well tolerated at all three dose levels (Table 4). The frequency of local, systemic and unsolicited adverse events was similar in the participants receiving the 30, 60, or 90 μg dose or the control vaccine. Almost all the adverse events were mild or moderate (<grade 3). Pain at the injection site was the most common local adverse event in any of the vaccine groups as well as in the control group, with a similar rate of approximately 20% (Table 4). The second most common local reaction was induration, at a much lower rate of 3–5%. The most common systemic adverse event was pyrexia, with a similar rate of 37–39% in all the groups. The rates of headache and fatigue were both approximately 7–9%. The frequencies of other systemic events were all less than 5%.

Three participants, all in 90 μg vaccine group, reported solicited adverse events of grade 3. Two of them reported an induration with a diameter of approximately 40 mm; they recovered within 5 days without treatment. The third participant reported the highest

Table 2
Neutralizing antibody responses at month 7 in participants who received all three vaccine doses and were seronegative for neutralizing antibodies against the corresponding HPV types at entry.

		30 μg HPV vaccine group	60 μg HPV vaccine group	90 μg HPV vaccine group	Control group
HPV-16	n	123	117	111	120
	No. +ve	123	117	111	1
	Seroconversion rate (95%CI) (%)	100 (97.1, 100)	100 (96.9, 100)	100 (96.7, 100)	0.8 (0, 2.5)
	Minimal titer	160	640	1280	–
	Maximal titer	163,840	163,840	163,840	160
	GMT (95%CI)	7596 (6,395, 9,024)	10,548 (9,034, 12,315)	12,505 (10,532, 14,848)	160 (N/A)
HPV-18	n	127	123	121	130
	No. +ve	126	123	121	0
	Seroconversion rate (95%CI) (%)	99 (97.7, 100)	100 (97.1, 100)	100 (97, 100)	0 (0, 2.8)
	Minimal titer	320	160	160	–
	Maximal titer	81,920	40,960	40,960	–
	GMT (95%CI)	6875 (5595, 8448)	7261 (6043, 8725)	8097 (6911, 9485)	N/A (N/A)

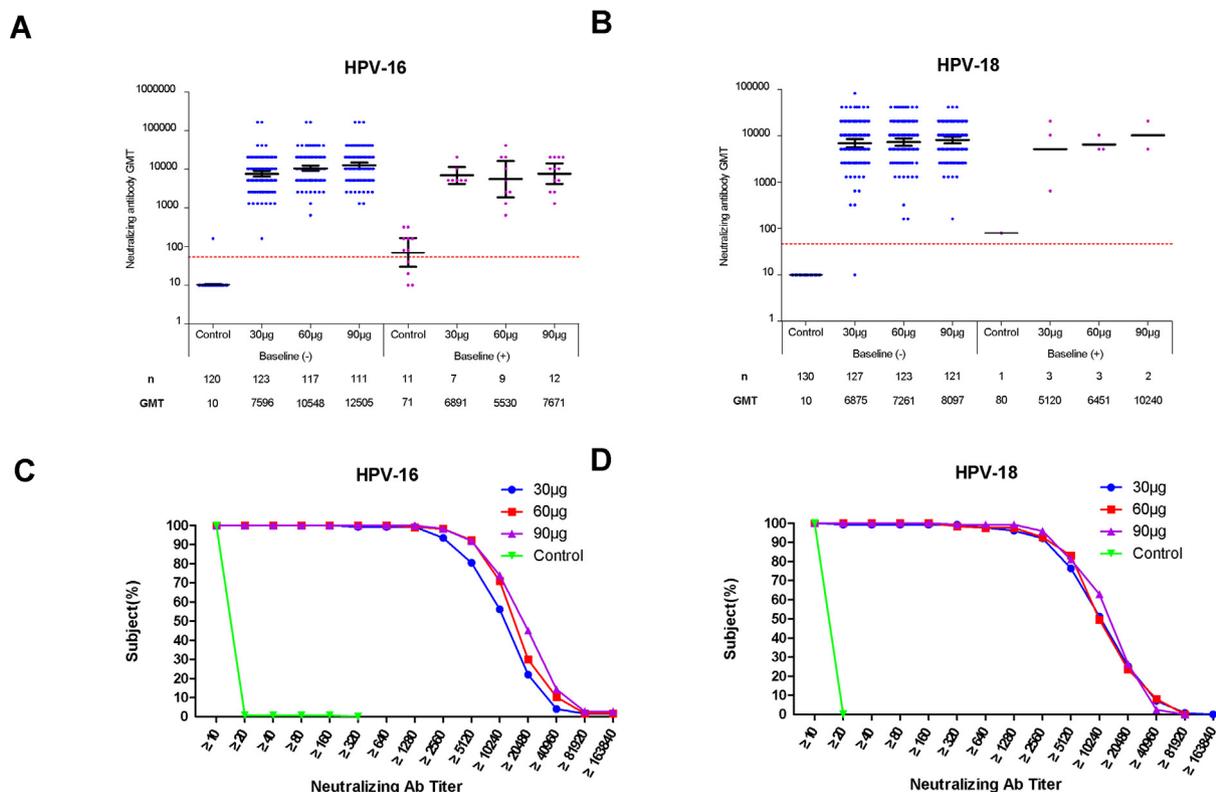GMT: geometric mean titer; CI: confidence interval; N/A: not applicable.

Fig. 2. HPV 16/18 neutralizing antibody levels in vaccine recipients in the three groups at one month after receiving all three doses (month 7). Only data for the participants in the per-protocol set (PPS) are included here. GMT: geometric mean titer. Pre (−): women who were seronegative at entry; Pre (+): women who were seropositive at entry; A. HPV 16; B. HPV 18. The dashed lines indicate the GMTs of the baseline seropositive participants (natural infection-induced antibodies). The reverse cumulative distribution curve of the GMTs of the neutralizing antibodies in the participants in the PPS cohort is shown in C (HPV 16) and D (HPV 18).

subaxillary body temperature of 39.1 °C on day 5 after the second dose and completely recovered 6 days later following treatment. There was no notable difference in the reported solicited adverse events in the vaccine recipients who were IgG seropositive at entry compared with the IgG seronegative vaccine recipients in the same group. None of the fourteen reported serious adverse events (SAEs) was related to the vaccination (detailed in the Appendix, Table S3).

4. Discussion

This is the first systemic report of the immunogenicity and safety of an *E. coli*-expressed HPV vaccine in a large cohort including women aged 18–25. All three formulations of the *E. coli*-expressed bivalent HPV (types 16 and 18) vaccines showed good safety profiles and robust immunogenicity in young women.

The neutralizing antibody was selected as the primary immunogenicity biomarker for several reasons: (1) serum neutralizing antibodies are thought to be the major basis of protection afforded by HPV vaccines [13–17]; (2) the PBNA is well validated in many laboratories and measures a range of neutralizing antibodies [18];

and (3) the cell line and pseudovirus genome used in the PBNA is not used in the production of either the studied vaccine or the three commercialized vaccines, making the PBNA unbiased to all the HPV vaccines [19]. Nearly all the participants vaccinated with the tested vaccine produced neutralizing antibodies for both HPV types 16 and 18, and the mean titers at one month after the third dose were approximately 100 times greater than the titers in individuals who had preexisting antibodies, which were most likely obtained through natural infection (Table 2). The mean level of neutralizing antibodies against HPV 16 in the 30 μg vaccine group was marginally lower than those in the 60 μg and 90 μg groups, and the levels of neutralizing antibodies against type 18 in the three vaccine groups were similar.

The use of the labor-intensive PBNA in large clinical trials is challenging. Hence, a fast and highly reproducible eVLP-based ELISA is desirable to be used as an alternative to the PBNA [20]. The eVLP-based ELISA measures the IgG antibodies that bind to the *E. coli*-expressed L1 VLP antigen, the same as the vaccine antigen, fixed to a solid surface, and irrespective of their neutralizing nature [21]. In participants with or without preexisting type-specific

Table 3
IgG antibody responses at month 7 in participants who received all three vaccine doses and were seronegative for IgG antibodies against the corresponding HPV types at entry.

		30 μg HPV vaccine group	60 μg HPV vaccine group	90 μg HPV vaccine group	Control group
HPV-16	*n*	257	272	253	256
	No. +ve	257	272	253	4
	Seroconversion rate (95%CI) (%)	100 (98.6, 100)	100 (98.7, 100)	100 (98.6, 100)	1.6 (0.4, 3.5)
	GMT (95%CI)	5882 (5374, 6438)	7977 (7321, 8693)	9145 (8383, 9976)	11 (11,12)
HPV-18	*N*	312	307	313	310
	No. +ve	312	307	313	6
	Seroconversion rate (95%CI) (%)	100 (98.8, 100)	100 (98.8, 100)	100 (98.8, 100)	2 (0.4, 3.5)
	GMT (95%CI)	4720 (4305, 5176)	5184 (4788, 5612)	5657 (5203, 6151)	10 (10, 11)

IgG antibody was detected by VLP-based ELISA. GMT: geometric mean titer; CI: confidence interval.

Table 4
Adverse events in the participants.

Group	30 μg HPV vaccine group (n = 398)		60 μg HPV vaccine group (n = 400)		90 μg HPV vaccine group (n = 399)		Control group (n = 397)		P
	Cases	%	Cases	%	Cases	%	Cases	%	
Solicited local adverse events within 7 days after each vaccination									
Local AE	99	24.9	91	22.8	92	23.1	77	19.4	0.313
Grade 3	0	0	0	0	2	0.5	0	0	0.187
Pain	83	20.9	75	18.8	83	20.8	63	15.9	0.235
Grade 3	0	0	0	0	0	0	0	0	/
Erythema	12	3.0	16	4.0	11	2.8	10	2.5	0.651
Grade 3	0	0	0	0	0	0	0	0	/
Swelling	5	1.3	8	2.0	8	2.0	7	1.8	0.865
Grade 3	0	0	0	0	1	0.3	0	0	0.749
Induration	16	4.0	20	5.0	13	3.3	11	2.8	0.377
Grade 3	0	0	0	0	2	0.5	0	0	0.187
Rash	4	1.0	6	1.5	4	1.0	4	1.0	0.932
Grade 3	0	0	0	0	0	0	0	0	/
Pruritus	12	3.0	19	4.8	10	2.5	12	3.0	0.305
Grade 3	0	0	0	0	0	0	0	0	/
Solicited systemic adverse events within 7 days after each vaccination									
Systemic AE	197	49.5	199	49.8	184	46.1	189	47.6	0.705
Grade 3	0	0	0	0	1	0.3	0	0	0.749
Pyrexia	154	38.7	156	39.0	151	37.8	154	38.8	0.988
Grade 3	0	0	0	0	1	0.3	0	0	0.749
Headache	35	8.8	35	8.8	35	8.8	27	6.8	0.679
Grade 3	0	0	0	0	0	0	0	0	/
Fatigue	38	9.6	29	7.3	26	6.5	31	7.8	0.428
Grade 3	0	0	0	0	0	0	0	0	/
Diarrhea	21	5.3	22	5.5	18	4.5	20	5.0	0.931
Grade 3	0	0	0	0	0	0	0	0	/
Myalgia	22	5.5	23	5.8	16	4.0	22	5.5	0.675
Grade 3	0	0	0	0	0	0	0	0	/
Cough	17	4.3	17	4.3	16	4.0	10	2.5	0.524
Grade 3	0	0	0	0	0	0	0	0	/
Nausea/vomiting	11	2.8	16	4.0	9	2.3	15	3.8	0.455
Grade 3	0	0	0	0	0	0	0	0	/
Allergy	3	0.8	0	0.0	3	0.8	8	2.0	0.014
Grade 3	0	0	0	0	0	0	0	0	/
Dizziness	1	0.3	0	0.0	1	0.3	1	0.3	0.718
Grade 3	0	0	0	0	0	0	0	0	0.749
Unsolicited events within 30 days after each vaccination									
Any	148	37.2	134	33.5	140	35.1	140	35.3	0.755
Grade 3	3	0.8	1	0.3	0	0	2	0.5	0.251
Serious adverse events									
Any	4	1.0	1	0.3	6	1.5	3	0.8	0.272
Death	0	0	0	0	0	0	0	0	/
Related to vaccination	0	0	0	0	0	0	0	0	/

antibodies, vaccine-induced type-specific antibody responses assessed by an ELISA were consistent with the PBNA results (Table S2 and Fig. S2 in the Appendix). Almost all the participants produced IgG antibodies and neutralizing antibodies, and the titers of both type-specific antibodies were highly correlated. Some other studies had also indicated the high correlation between the two tests when measuring antibodies in post-vaccination samples [20,22]. Hence, the correlation of the neutralizing antibody response and the IgG antibody response has been established, and the eVLP-based ELISA can be used as a surrogate for the PBNA in further research on the studied HPV vaccine.

The tested vaccine was well tolerated. The safety outcomes between the dose groups and the control group were generally similar. The adverse events were generally mild and self-limiting and did not affect overall compliance with the vaccination schedule. No serious adverse events were related to the vaccination. All the three recorded grade 3 solicited adverse events appeared in the highest-dose group, although the rate difference between the groups was not statistically significant.

Several HPV vaccines have demonstrated perfect efficacy against type-specific infection and related diseases [7,23–25];

however, the protective antibody levels have not been determined. Nonetheless, it is generally believed that the robust vaccine-induced antibody response is closely correlated with vaccine efficacy. Hence, to assess the protective potential of a novel vaccine, it would be ideal to conduct a head-to-head immunogenicity comparison with one of three commercialized HPV vaccines. Unfortunately, such a study cannot be done in China because neither of those vaccines is licensed in China. The lack of this comparison is the most notable limitation of the present study. To get some kinds of impression about the robust of antibody response of the tested vaccine, we compared our data for the 60 μg dose of Cecolin® with the published data from a head-to-head study of the two commercialized vaccines [19] (Table S4 in the Appendix), all of the serum samples were collected at one month post the full vaccination courses. To minimize the potential bias due to different methodologies in the different studies, the GMT ratios (GMRs) of the vaccine-induced antibodies to natural infection-induced antibodies were used for comparison. The results are consistent with those for the GMT data: the type-specific neutralizing antibody response is strongest for Cervarix®, followed by Cecolin® and Gardasil®. The difference in the antibody response among the three

HPV vaccines might reflect the different adjuvants used; Cervarix® employs a novel adjuvant AS04, which contains a Toll-like receptor 4 (TLR-4) agonist, whereas Gardasil® and Cecolin® use a traditional aluminum adjuvant.

Other limitations of the study included: (1) the lack of antibody data at months 1, 2 and 6, which makes it impossible to understand the post-vaccinated antibody dynamics after each injection; (2) two dose schedules were not included in the study arms; and (3) the study did not include younger girls, who would be the primary target population and benefit the most from an HPV vaccine. These limitations are expected to be overcome in further clinical trials of Cecolin®.

In conclusion, the desirable safety and immunogenicity of a novel HPV 16/18 bivalent vaccine based on *E. coli*-expressed L1 VLP antigens were demonstrated in healthy women aged 18–25 years, which encourages further efficacy trials of this vaccine and represents a crucial step toward a more affordable HPV vaccine. Based on the safety and immunogenicity data, a 60 μg dose formulation, containing 40 μg of HPV 16 L1 VLPs and 20 μg of HPV 18 L1 VLPs, is proposed for further efficacy trials. The correlation of the antibodies detected by the PBNA and by the VLP-based ELISA has been established for measuring vaccine-induced immunity; hence, the ELISA may be used in future studies to evaluate vaccine immunogenicity.

Authors' contributions

F-C Zhu, Y-M Hu, K Chu, and Y Wang coordinated the clinical aspects of the study; Z-Z Wang, C-L Yang, H-M Jiang, Y-J Wang collected the data; J Li, H Zhao, C-G Li, Z-J Lin, H-R Pan, W Sheng, F-X Wei tested the clinical samples; J. Zhang, T Wu, S-J Huang, S-W Li, N-S Xia planned and designed the study and interpreted the results. S-J Huang did the statistical analyses. All authors critically reviewed the different drafts of the manuscript and approved the final version.

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Conflict of interest statement

None.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.06.052

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