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Article

Immunogenicity and safety of an *Escherichia coli*-produced human papillomavirus (types 6/11/16/18/31/33/45/52/58) L1 virus-like-particle vaccine: a phase 2 double-blind, randomized, controlled trial

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ABSTRACT

The *Escherichia coli*-produced human papillomavirus (HPV) 16/18 bivalent vaccine (Cecolin) has received prequalification by the World Health Organization based on its high efficacy and good safety profile. We aimed to evaluate the immunogenicity and safety of the second-generation nonavalent HPV 6/11/16/18/31/33/45/52/58 vaccine (Cecolin 9) through the randomized, blinded phase 2 clinical trial. Eligible healthy women aged 18–45 years were randomly (1:1) allocated to receive three doses of 1.0 mL (270 μg) of Cecolin 9 or placebo with a 0-1-6-month schedule. The primary endpoint was the seroconversion rate and geometric mean titer of neutralizing antibodies (nAbs) one month after the full vaccination course (month 7). The secondary endpoint was the safety profile including solicited adverse reactions occurring within 7 d, adverse events (AEs) occurring within 30 d after each dose, and serious adverse events (SAEs) occurring during the 7-month follow-up period. In total, 627 volunteers were enrolled and randomly assigned to Cecolin 9 (n = 313) or placebo (n = 314) group in Jiangsu Province, China. Almost all participants in the per-protocol set for immunogenicity (PPS-I) seroconverted for nAbs against all the nine HPV types at month 7, while two failed to seroconvert for HPV 11 and one did not seroconvert for HPV 52. The incidence rates of total AEs in the Cecolin 9 and placebo groups were 80.8% and 72.9%, respectively, with the majority of them being mild and recovering shortly. None of the SAEs were considered related to vaccination. In conclusion, the *E. coli*-produced 9-valent HPV (9vHPV) vaccine candidate was well tolerated and immunogenic, which warrants further efficacy studies in larger populations.

1. Introduction

Premalignant and malignant lesions of the cervix and other sites related to the human papillomavirus (HPV) infection have imposed a substantial disease burden worldwide, especially in developing countries [1]. Currently, 13 HPV types are defined as high-risk and cause approximately 604,127 new cases and 341,831 deaths from cervical cancer annually [2,3]. It is estimated that more than

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88% of new cervical cancer cases occur in low- and middle-income countries that lack sufficient medical resources. China stands as one of the developing countries with the heaviest disease burden of cervical cancer, reporting approximately 109,741 new cases and 59,060 deaths annually [4]. In addition, more than 90% of genital warts are associated with HPV 6 and 11 infection, which is one of the most common sexually transmitted diseases [5].

Prophylactic HPV vaccination is considered one of the most effective interventions to prevent cervical cancer and other related diseases mainly by inducing HPV type-specific neutralizing antibodies. Six prophylactic HPV vaccines are currently available and administered in many countries worldwide, and all of them are prepared from the purified L1 structural protein that self-assembles to form virus-like particles (VLPs) [6,7]. Five of the six HPV vaccines are first-generation HPV vaccines and include only the two most important high-risk types (HPV 16 and 18), causing approximately 70% of cervical cancers [3]. The remaining vaccine (a 9vHPV vaccine, Gardasil 9, MSD, Wilson, USA) is a second-generation HPV vaccine that contains five additional high-risk HPV types (31, 33, 45, 52, and 58) which increases the overall prevention of cervical cancer (from 70% to 90%) [3]. However, owing to the insufficient supply and relatively high price, Gardasil 9 has only been introduced into approximately 31 countries, with the vast majority being high-income countries (https://view-hub.org/vaccine/hpv/data). Low and middle-income countries, which account for 88.1% of cervical cancer cases worldwide and are in more urgent need of 9vHPV vaccines, face considerable accessibility barriers [3]. With the World Health Organization (WHO) targeting cervical cancer elimination, more solutions are expected to address this issue [8].

Among the six available prophylactic HPV vaccines, an *Escherichia coli* (*E. coli*)-produced HPV 16/18 bivalent vaccine (Cecolin, Xiamen Innovax, Xiamen, China) demonstrated efficacy of 100.0% (95% confidential intervals (CI): 67.2%–100.0%) against high-grade genital lesions and a reassuring safety profile in a phase 3 clinical trial, which has been licensed in China in 2019 and prequalified by WHO in 2021 [9]. With the advantages of low cost and high production capacity, this *E. coli*-produced vaccine has great potential to alleviate supply constraints and improve the affordability of HPV vaccines [10–12]. Based on the same *E. coli* expression system, a second-generation nonavalent HPV 6/11/16/18/31/33/45/52/58 vaccine (Cecolin 9, Xiamen Innovax) was developed, and its safety and immunogenicity were preliminarily revealed in an open-label phase 1 clinical trial [13]. To further evaluate the immunogenicity and safety of this 9vHPV vaccine candidate, a single-center, double-blind, randomized, and placebo-controlled phase 2 clinical trial was conducted in an expanded female population aged 18–45 years old.

2. Materials and methods

2.1. Study design and participants

This randomized, double-blind, and placebo-controlled phase 2 clinical trial was conducted at the Centre for Disease Control and Prevention (CDC) of Dongtai (Jiangsu Province, China) from June 2019 to February 2020. Healthy female volunteers aged 18–45 years were recruited through local village health centers and signed written informed consent forms if they agreed to participate. Each eligible participant needed to meet all inclusion criteria and not had any one of the following: (1) an axillary body temperature over 37.0 ℃; (2) were currently pregnant or lactating; (3) had received any other experimental drug or vaccine within 30 d before vaccination; (4) had received immune globulin and/or blood preparations within 3 months, inactivated vaccines within 14 d, or live vaccines within 21 d before receiving the study vaccine; (5) had any preexisting severe, acute or chronic disease; (6) had a severe anaphylaxis history for any vaccine ingredient; or (7) had any other condition that, judged by the investigator, could prevent the participant from complying with this protocol or signing their informed consent.

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and the 1964 *Helsinki Declaration* and its later amendments. This study was approved by the Ethics Committee of Jiangsu Provincial CDC (JSJK2019-A001). All the participants signed written informed consent forms before enrollment. This study has been registered at ClinicalTrials.gov (NCT03935204).

2.2. Randomization and masking

Age-stratified block randomization with a block size of eight was used in this trial. The randomization codes were computer-generated by an independent statistician. Allocation concealment was performed by a preconfigured interactive web response system (IWRS, Taimei Technology, Jiaxing, China).

All eligible participants were first stratified by age (18–26 (defined as ≥18 and <27 years), 27–36 (≥27 and <37 years), and 37–45 years (≥37 and ≤45 years)) and 1:1 randomly assigned to the 9vHPV vaccine group or placebo group based on a unique randomization code. The vaccine code for the corresponding group was assigned by the IWRS before each vaccination. None of the participants or personnel at the study site, including the research staff responsible for operating the randomization system, were aware of the group assignment.

Furthermore, several methods were incorporated to maintain blinding. Firstly, the test vaccines and control vaccines with identical appearance and packaging underwent masking prior to transportation, neither investigators nor participants could distinguish between them. Secondly, any behavior of unblinding, including emergency unblinding in specific situations, was exclusively carried out through the IWRS and would be duly recorded by the system. Finally, the laboratory operators responsible for the antibody testing remained completely independent and had no access to any information on the characteristics and allocation of the participants.

2.3. Procedures

Based on the same *E. coli*-expression system as Cecolin, Cecolin 9 is formulated with aluminum adjuvant and L1 VLPs of seven high-risk genotypes (HPV 16, 18, 31, 33, 45, 52, and 58) and two low-risk genotypes (HPV 6 and 11). Each dose of Cecolin 9 contained 30 μg of HPV 6, 40 μg of HPV 11, 60 μg of HPV 16, 40 μg of HPV 18, and 20 μg each of HPV 31, 33, 45, 52, and 58 VLPs, absorbed into 0.42 mg of aluminum hydroxide adjuvant suspended in 1.0 mL of phosphate-buffered saline. The placebo was composed of aluminum hydroxide adjuvant and diluent without any HPV L1 VLP. All test vaccines and placebo were produced by Xiamen Innovax, under satisfactory good manufacturing practice conditions according to the requirements of the National Medical Products Administration of China (NMPA). Both the test vaccines and the placebo were 1.0 mL sterile suspensions, supplied in vials of the same volume, and appearance, and were indistinguishable.

All eligible participants were randomly assigned to receive three doses of 9vHPV vaccine or placebo intramuscularly in the upper arm deltoid muscle according to a 0-1-6-month schedule. Following vaccination, participants were monitored for 30 min to record any immediate adverse events on paper diary cards, including injection-site and systemic adverse events. Serious

adverse events (SAEs) were collected throughout the study by a combination of spontaneous participant reports and regular follow-up. The reported adverse events were graded according to the Guidelines for Adverse Event Classification Standards for Clinical Trials of Preventive Vaccines issued by the NMPA in 2019, and the causal relationship between adverse events and vaccination was determined by the trained investigators.

Serum samples of all the participants were collected at baseline (day 0) and one month after the third vaccination (month 7) to evaluate the immunogenicity of Cecolin 9. In addition, women from one town with an expected sample size of approximately 200 were preselected as the immunogenicity subset to evaluate the immune response after the second dose of vaccine, whose serum samples were additionally collected at month 2 (one month after the second vaccination). HPV 6/11/16/18/31/33/45/52/58 type-specific neutralizing antibodies (nAbs) and IgG antibodies were measured by the China National Institute for Food and Drug Control (NIFDC), with a modified triple-color pseudovirion-based neutralization assay (Triple-color PBNA) and an *E. coli*-produced HPV VLP-based enzyme-linked immunosorbent assay (ELISA), respectively.

For Triple-color PBNA, a detailed method had been described previously [13]. Briefly, after culturing the serially diluted serum sample replicates with nine types of pseudovirions and incubating with 293FT cell suspension, the 384-well microtiter plates were scanned by a high-throughput cell imaging microplate detection system. The neutralization titers of seropositive samples were determined by identifying the highest serum dilution at which the percent inhibition of infection exceeded 50%. The defined cutoff titers for nAbs against HPV 6/11/16/18/31/33/45/52/58 were as follows: 177, 97, 85, 100, 77, 112, 148, 84, and 125. For the VLP-based ELISA, a detailed procedure has also been previously reported [13]. The standard reference curve was established using a series of dilutions from a reference serum pool obtained from recipients of the HPV vaccine. Anti-HPV IgG antibody levels were further quantified using various references that are traceable to the WHO international standards for HPV 16 and 18, NIFDC national standards for HPV 6, 33, 45, 52, and 58, and Xiamen Innovax reference standards for HPV 11 and 31. The established cutoffs for HPV 6/11/16/18/31/33/45/52/58 IgG antibody levels were defined as follows: 8.1 U/mL, 6.4 U/mL, 3.0 U/mL, 2.1 U/mL, 281.0 U/mL, 6.6 U/mL, 171.0 U/mL, 94.0 U/mL, and 78.5 U/mL, correspondingly. For both tests, the antibody level of seronegative samples was artificially defined as half of the cutoffs.

2.4. Outcomes

The primary endpoint of this phase 2 trial was the seroconversion rate and antibody level of the nAbs against HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 at month 7. The secondary endpoints were local and systemic adverse events within 30 d after each vaccination; and serious adverse events throughout the observation period. The exploratory endpoints included the seroconversion rate and antibodies level of the nAbs and IgG antibodies after the second dose.

2.5. Statistical analysis

The sample size for this trial was primarily estimated based on the assumption of a 90% seroconversion rate of each HPV type-specific antibody. Assuming an approximate baseline seroprevalence of 15% for any HPV type, alongside a dropout rate of 15%, at least 572 participants were deemed necessary for enrollment. In line with the Technical Guidelines for Clinical Trials of Vaccines released by the NMPA of China (2004), at least 600 healthy participants aged 18–45 years needed to be included to ensure that the primary endpoint and safety endpoint were met, with equal allocation between the two groups.

The full analysis set included women who received at least one dose of vaccine. Immunogenicity of neutralizing antibodies was analyzed primarily in the per-protocol set for immunogenicity (PPS-I) of each HPV type, which included participants who met the following requirements: (1) received three doses of the test or control vaccine; (2) had no violation of the protocol; (3) had antibody data available at month 7; and (4) were baseline seronegative for nAbs against the corresponding HPV type. The per-protocol subset for immunogenicity (PPS-I-sub) was evaluated for immune response after the second dose, with an additional requirement of available antibody data at month 2 based on the PPS-I. Immunogenicity analysis for IgG antibody was conducted based on the serostatus of IgG antibody at baseline. Moreover, participants who had received at least one dose and had undergone a minimum of one valid safety follow-up were included in the safety analysis set.

Seroconversion was defined as at least a fourfold increase in antibody titers over baseline. The neutralizing antibody level was characterized by the geometric mean titer (GMT), which was defined as the highest dilution capable of inhibiting 50% of fluorescent protein expression (ID50). IgG antibody level was characterized by geometric mean concentration (GMC), which was quantified by employing various reference standards in ELISA. The GMT for nAbs or GMC of IgG with 95% CI was calculated based on the Student's t distribution of the log-transformed values. The difference in seroconversion rates and antibody levels across age groups was evaluated by the $\chi 2$ test and ANOVA respectively. Adverse events were coded using the Medical Dictionary for Regulatory Activities (version 23.0) developed by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. The number and proportion of participants experiencing at least one adverse event following vaccinations were delineated by groups, and the $\chi 2$ test and Fisher's exact test were used. All statistical analyses were performed by SAS 9.4. The P value for evaluating the immunogenicity comparison between the 9vHPV vaccine group and the placebo group was one-sided with an α value of 0.025, while all other analyses were conducted using a two-sided value of 0.05.

3. Results

3.1. Characteristics of study participants

From June 15 to 19, 2019, 670 volunteers from four towns underwent eligibility screening, of whom 627 healthy women aged 18 to 45 years were enrolled and randomly allocated, with 313 (49.9%) assigned to the 9vHPV vaccine group and 314 (50.1%) to the placebo group (Fig. 1). Twenty participants did not complete the full course of vaccination. In total, 303 (96.8%) in the 9vHPV vaccine group and 301 (95.9%) in the placebo group were included in the PPS-I cohort. The age of the two groups (mean (standard deviation): 31.5 (7.2) years vs. 31.7 (7.2) years) was similar. Baseline seropositive rates and titers of naturally induced neutralizing antibodies against each HPV type were comparable between the two study groups (Table 1 and Table S1 online).

A total of 229 participants were included in the immunogenicity subset to evaluate the immune response after two doses administered at months 0 and 1, with 114 (49.8%) assigned to the 9vHPV vaccine group and 115 (50.2%) to the placebo group (Fig. 1). Among them, five participants did not complete the full course of vaccination; nine participants did not donate serum samples at all of the 3 time points, months 0, 2, and 7. In total, 220 participants were

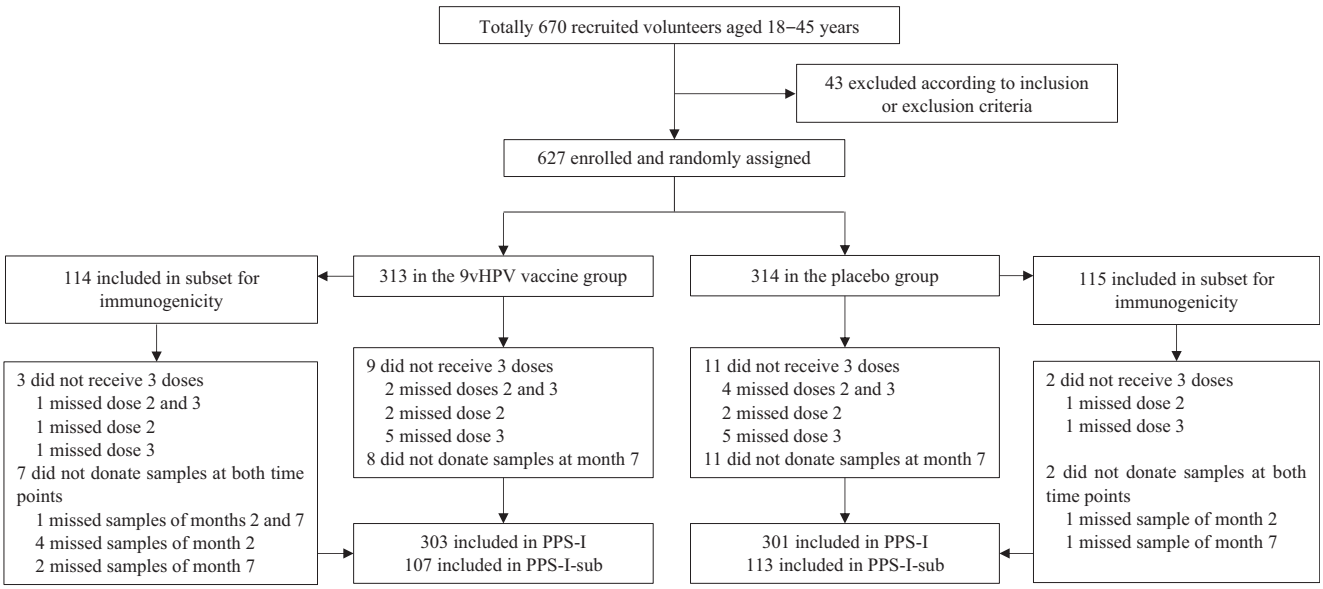

Fig. 1. Flow chart of the study. PPS-I included women who received 3 doses and donated serum samples at month 7 within predefined time windows, with no violation of the protocol, and were baseline seronegative for nAbs or IgG antibodies for the corresponding HPV type; PPS-I-sub included women from the PPS-I who donated serum samples at month 2 within predefined time windows; 9vHPV: 9-valent human papillomavirus vaccine; HPV: human papillomavirus; nAbs: neutralizing antibodies; PPS-I: per-protocol set for immunogenicity; PPS-I-sub: per-protocol subset for immunogenicity.

Table 1
Baseline characteristics of the participants.

	Total (n = 627)	9vHPV vaccine group (n = 313)	Placebo group (n = 314)	P value
Age (years), mean ± SD	31.6 ± 7.2	31.5 ± 7.2	31.7 ± 7.2	0.9708
18–26 years	23.1 ± 2.1	23.0 ± 2.1	23.1 ± 2.1	0.8797
27–36 years	31.5 ± 3.0	31.4 ± 3.0	31.7 ± 2.9	0.8674
37–45 years	39.7 ± 2.0	39.7 ± 2.0	39.7 ± 1.9	0.6175
Seropositivity of nAb, n (%)				
HPV6	24 (3.8)	13 (4.2)	11 (3.5)	0.6714
HPV11	5 (0.8)	3 (1.0)	2 (0.6)	0.6860
HPV16	28 (4.5)	12 (3.8)	16 (5.1)	0.4444
HPV18	9 (1.4)	5 (1.6)	4 (1.3)	0.7519
HPV31	29 (4.6)	14 (4.5)	15 (4.8)	0.8561
HPV33	37 (5.9)	19 (6.1)	18 (5.7)	0.8576
HPV45	4 (0.6)	2 (0.6)	2 (0.6)	1.0000
HPV52	20 (3.2)	9 (2.9)	11 (3.5)	0.6547
HPV58	41 (6.5)	20 (6.4)	21 (6.7)	0.8800

9vHPV: 9-valent human papillomavirus vaccine; HPV: human papillomavirus; nAb: neutralizing antibody; SD: standard deviation.

included in the PPS-I-sub cohort, with 107 and 113 in the 9vHPV vaccine and placebo groups, respectively.

3.2. Immunogenicity

Seroconversion rates and GMTs of nAbs among the participants in the PPS-I who received the full course of vaccination and were baseline seronegative for relative HPV type are summarized in Table 2. Almost all participants seroconverted for nAbs against all nine HPV types at month 7, while two failed to seroconvert for HPV 11 and one did not seroconvert for HPV 52, whereas the seroconversion rates ranged from 0% (95% CI: 0%, 0%) to 1.1% (95% CI: 0.2%, 3.1%) among placebo recipients at month 7. The GMTs of nAb ranged from 1553.9 (95% CI: 1410.6, 1711.7) for HPV 11 to 18,370.8 (95% CI: 16,563.5, 20,375.4) for HPV 33, and the nAbs levels induced in the age groups of 18–26, 27–36 and 37–45 years were comparable (P > 0.05) (Fig. S1a online). Among the women who received 3 doses of Cecolin 9 and were seropositive at baseline, nearly all participants seroconverted for neutralizing antibodies at month 7, while two failed to seroconvert for HPV 58 (Table S2 online).

The immune responses at one month after the first two doses of the 9vHPV vaccine administered at months 0 and 1 were assessed in the PPS-I-sub cohort. As depicted in Fig. 2, the GMTs of nAbs of all nine HPV types exhibited an obvious rise after two doses of the vaccine with a one-month interval, and another sharp surge of nAbs titers was shown at one month after the third dose administered at month 6.

Similar outcomes were also demonstrated in terms of IgG antibody data, which are shown in Tables S1–S5, Figs. S1b, S2 and S3b (online).

3.3. Safety and tolerability

All 627 enrolled participants had received at least one dose of vaccine and were included in the safety analysis set. As shown in Table 3, approximately 80.8% of participants in the 9v HPV vaccine group reported at least one adverse event within 30 d following vaccinations, which is significantly higher than the placebo group

Table 2
Anti-HPV seroconversion and geometrical mean titers of nAbs for HPV types 6/11/16/18/31/33/45/52/58 at month 7 in women aged 18–45 years (PPS-I population).

HPV type	9vHPV vaccine group			Placebo group		
	n/*N*	Seroconversion, % (95% CI)	GMT (95% CI)	*n*/*N*	Seroconversion, % (95% CI)	GMT (95% CI)
HPV6	290/290	100 (98.7, 100)	15787.4 (14357.2, 17360.1)	2/291	0.7 (0.1, 2.5)	90.9 (88.6, 93.2)
HPV11	298/300	99.3 (98.4, 99.9)	1553.9 (1410.6, 1711.7)	0/299	0 (0)	48.7 (48.4, 49.0)
HPV16	292/292	100 (98.7, 100)	13431.1 (12137.8, 14862.3)	2/285	0.7 (0.1, 2.5)	43.3 (42.5, 44.2)
HPV18	298/298	100 (98.8, 100)	3551.2 (3217.2, 3920.0)	1/297	0.3 (0.0, 1.9)	50.4 (49.8, 51.1)
HPV31	289/289	100 (98.7, 100)	9292.6 (8308.7, 10392.9)	0/286	0 (0)	39.1 (38.5, 39.7)
HPV33	284/284	100 (98.7, 100)	18370.8 (16563.5, 20375.4)	3/283	1.1 (0.2, 3.1)	57.7 (56.4, 59.2)
HPV45	302/302	100 (98.8, 100)	6840.7 (6197.8, 7550.3)	0/299	0 (0)	74.2 (73.8, 74.5)
HPV52	294/295	99.7 (98.1, 100)	1925.6 (1748.6, 2120.4)	2/290	0.7 (0.1, 2.5)	42.7 (42.0, 43.4)
HPV58	284/284	100 (98.7, 100)	6512.9 (5830.8, 7274.9)	3/281	1.1 (0.2, 3.1)	64.0 (62.6, 65.4)

N: the number of participants in PPS-I of each HPV type; *n*: the number of participants who seroconverted (having 4 times or higher increase of antibody titers) for corresponding HPV type at month 7; PPS-I included women received 3 doses and donated serum samples at months 0 and 7 within predefined time windows, with no violation of the protocol and were baseline seronegative for nAbs for the corresponding HPV type; 9vHPV: 9-valent human papillomavirus vaccine; CI: confidence interval; GMT: geometric mean titer; HPV: human papillomavirus; nAbs: neutralizing antibodies; PPS-I: per-protocol set for immunogenicity.

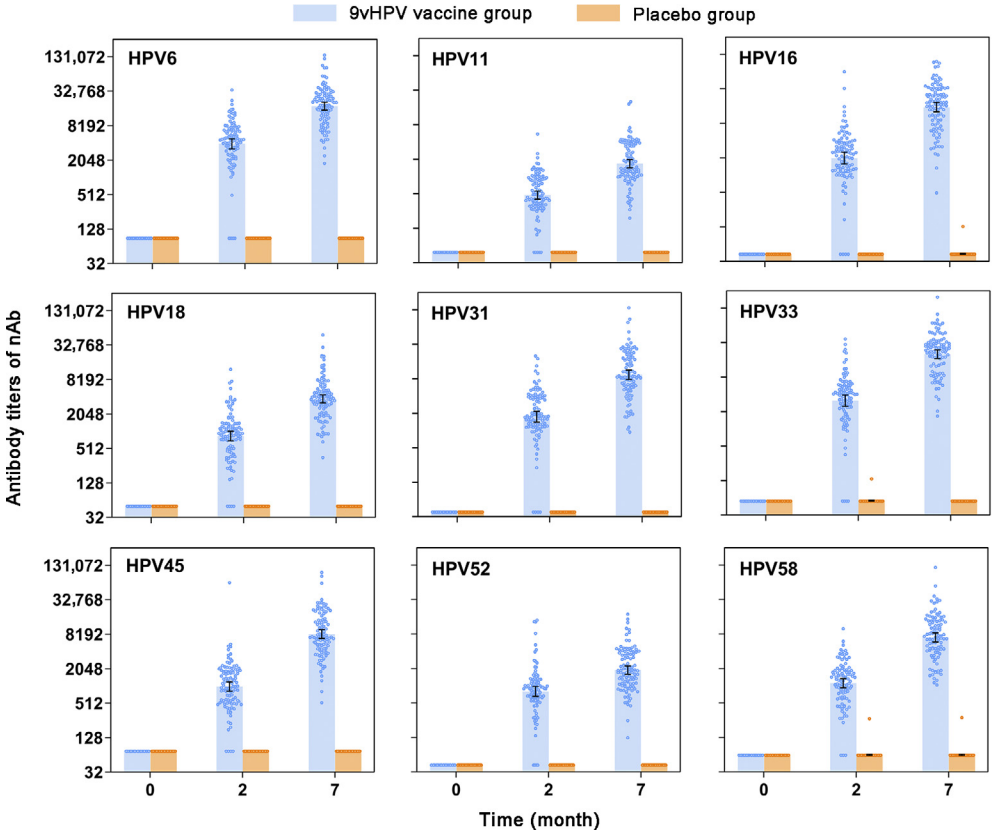

Fig. 2. Neutralizing antibodies titers at months 0, 2, 7 for HPV types 6/11/16/18/31/33/45/52/58 in PPS-I-sub. PPS-I-sub included women who received 3 doses and donated serum samples at months 0, 2, and 7 within predefined time windows, with no violation of the protocol, and were baseline seronegative for nAb for the corresponding HPV type; The titer of seronegative samples was artificially set as half of the cutoff value; The black lines indicate the GMTs and 95% CI; 9vHPV: 9-valent human papillomavirus vaccine; CI: confidence interval; GMTs: geometric mean titers; nAb: neutralizing antibody; HPV: human papillomavirus; PPS-I-sub: Per-protocol subset for immunogenicity.

(72.9%, P = 0.0190). This difference was primarily attributable to a higher incidence of local adverse reactions, which were reported by 60.1% in the 9vHPV vaccine group compared with 28.7% in the placebo group (P < 0.0001). However, no significant difference was observed in the occurrence rate of systemic adverse reactions between the 9vHPV vaccine group and the placebo group (40.9% vs. 34.4%, P = 0.0930), and fever was the most commonly reported systemic adverse reaction in both groups (19.8%). Local adverse reactions, primarily pain at the injection site, occurred more frequently in the 9vHPV vaccine than in the placebo group (59.1% vs. 27.7%, P < 0.0001). The majority (97.6%) of adverse reactions were mild with a short duration, and the median time to recovery was 1.0 d. The incidence of grade 3 total adverse reactions was similar between the two groups (1.6% vs. 1.3%, P = 0.7519), and all grade 3 adverse events, regardless of their causal relationship with vaccination, are enumerated in Table S6 (online). A total of five participants reported serious adverse events throughout the study period, none of which were considered to be related to vaccination by the investigators (Table S7 online).

4. Discussion

In this study, the immunogenicity and safety of the candidate *E. coli*-produced nonavalent HPV vaccine were further evaluated

Table 3
Adverse events occurred within 30 d after each vaccination in women who received at least one dose of vaccine, *n* (%).

	9vHPV vaccine group (*n* = 313)	Placebo group (*n* = 314)	*P* value
Total adverse events	253 (80.8)	229 (72.9)	0.0190
Total adverse events ≥ grade 3	8 (2.6)	4 (1.3)	0.2414
Total adverse reactions	226 (72.2)	153 (48.7)	<0.0001
Total adverse reactions ≥ grade 3	5 (1.6)	4 (1.3)	0.7519
Local adverse reactions	188 (60.1)	90 (28.7)	<0.0001
Pain	185 (59.1)	87 (27.7)	< 0.0001
Itching	22 (7.0)	6 (1.9)	0.0019
Local adverse reactions ≥ grade 3	0 (0)	0 (0)	NA
Systemic adverse reactions	128 (40.9)	108 (34.4)	0.0930
Fever (≥37.3 ℃)	62 (19.8)	62 (19.8)	0.9842
Fatigue	42 (13.4)	29 (9.2)	0.0984
Headache	31 (9.9)	14 (4.5)	0.0083
Diarrhea	20 (6.4)	19 (6.1)	0.8606
Nausea	17 (5.4)	11 (3.5)	0.2425
Cough	17 (5.4)	8 (2.6)	0.0650
Muscle pain	16 (5.1)	10 (3.2)	0.2262
Dizziness	12 (3.8)	8 (2.6)	0.3595
Vomit	7 (2.2)	13 (4.1)	0.1750
Systemic adverse reactions ≥ grade 3	5 (1.6)	4 (1.3)	0.7519

9vHPV: 9-valent human papillomavirus vaccine; NA: not applicable.

in a larger number of healthy women aged 18–45 years old. The data indicate that the candidate vaccine was well tolerated and highly immunogenic against all nine HPV types contained in the vaccine formulation, which supports further pivotal phase 3 studies of vaccine efficacy against persistent infections, precancerous lesions, and cancers caused by the relevant HPV types.

Several studies have demonstrated that the primary mechanism by which HPV vaccines confer protection against HPV infection is through the production and enrichment of nAbs, although the specific threshold for protection has not yet been determined [14]. In this study, almost all participants included in the PPS-I seroconverted for nine HPV types within a month after receiving three doses of Cecolin 9, and produced neutralizing antibodies with tens or even hundreds folds higher than those induced by natural infection, which is consistent with findings from the previous phase 1 study. [13]. Although the three participants did not seroconvert for nAbs against HPV 11 or 52, all of them have positive IgG antibodies against the relative HPV types (HPV 11 or 52) post vaccination, indicating that the HPV 11 or 52 type-specific immune responses were induced in these three participants although with relatively low level. It was noted that the nAb titers of the rest HPV types in these three participants at month 7 were all lower than the average titer among all the nonavalent HPV vaccine receivers. Individual differences in efficiencies in presenting the vaccine antigens could be a contributing factor. No differences in antibody seroconversion rates or distribution of antibody levels were observed among the three age subgroups (18–26, 27–36, and 37–45 years) (Fig. S1 online).

In the position paper on human papillomavirus vaccines released by the WHO in 2022, the updated recommendation for immunization procedures is a two-dose schedule with a minimum interval of 6 months between doses for the primary target group from 9 years of age and for all other age groups for which HPV vaccines are authorized, and a single-dose schedule may be used off-label as an option for girls and boys aged 9–20 years [7]. Facing insufficient supply and unequal distribution of the HPV vaccine worldwide, reducing the dosage for each individual will enhance immunization coverage and promote accessibility of the HPV vaccine, especially in resource-constrained countries. In this research, immunogenicity in one month after two doses of the vaccine at day 0 and month 1 significantly increased with a high seroconversion rate (higher than 88.7% for all nine HPV types, as shown in Table S5 online). It indicates the potential of a two-dose Cecolin 9 vaccination, particularly when administered with a longer interval between doses, as evidence has shown that immunogenicity tends to be higher with longer intervals [15–17]. In addition, previous studies have shown that vaccine-induced antibody levels are inversely associated with age [18]. Therefore, the immunogenicity of the two-dose regimen may be underestimated in this study due to the lower level of vaccine-induced antibodies in adults (participants aged 18–45 years) compared with adolescents (the primary target population for single- or two-dose regimens) and the too short vaccination interval. On the other hand, increasing evidence suggests that even low titers of neutralizing antibodies can offer sufficient protection against HPV infection, potentially due to the complementary protection provided by other antibody functions [19]. Whilst the immunogenicity of 2 or 3 doses was superior to that of one dose, the efficacy was comparable, as reported in previous studies [20–22]. The efficacy of single or two doses of Cecolin 9 in adolescent girls and boys requires further investigation through efficacy studies or real-world studies in the future (ClinicalTrials.gov identifier: NCT05056402).

The safety of Cecolin 9 was further confirmed. The disparity in the occurrence of adverse reactions between the 9vHPV vaccine and the placebo group was primarily observed in local reactions at the injection site, particularly pain, which could be attributed to the higher VLP content of Cecolin 9. Therefore, it is conceivable that Cecolin 9 may have a higher incidence of adverse reactions as it contains an additional 7 types of HPV VLPs compared to Cecolin, the *E. coli*-produced bivalent HPV vaccine. A currently ongoing phase 3 study of Cecolin 9, utilizing a double-blind, randomized, and Cecolin-controlled design, may offer adequate safety evidence by directly comparing the two vaccines (ClinicalTrials.gov identifier: NCT04537156).

There are several limitations in our study. First, the immunization schedules of single dose or two doses with 6-month interval were not parallelly designed and evaluated in this study, as this study was initiated prior to the publication of the WHO recommendation. The strong immunogenicity observed in this study provides an impetus for further investigation into more vaccination schedules. Second, the comparison of the immunogenicity of HPV types 16 and 18 contained in Cecolin 9 and Cecolin was not conducted in this study, as Cecolin had not yet been approved until December 2019. The multi-center phase 3 study of Cecolin 9 is currently ongoing, in which the non-inferiority analysis of anti-HPV types 16 and 18 antibodies induced by Cecolin 9 compared with Cecolin has been set as one of the primary endpoints (ClinicalTrials.gov identifier: NCT04537156).

The insufficient supply and uneven distribution of HPV vaccines are one of the important barriers to the implementation of the WHO's strategy to accelerate the elimination of cervical cancer, which was proposed in 2018 [8]. Given the robustness and cost-effectiveness of the *E. coli* system, Cecolin 9 is believed to be a potentially important addition to the national immunization program, especially in countries with limited resources.

5. Conclusion

In the context of a rigorous randomized controlled trial, we demonstrated that a three-dose schedule of this *E. coli*-produced HPV 9-valent vaccine candidate was well tolerated and immunogenic in females aged 18–45 years, which warrants further efficacy studies against HPV infection and related lesions in larger populations.

Conflict of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Feng-Zhu Zheng and Qiu-Fen Zhang report being either current employees of Xiamen Innovax; Guang Sun report being current employees of and have stock options in Xiamen Innovax. All other authors declare that they have no conflict of interest.

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Author contributions

Yue-Mei Hu, Ya Zheng, Jun Zhang, Ting Wu, Shou-Jie Huang, and Ning-Shao Xia contributed to the study design. Kai Chu, Han-Min Jiang, Xia Zang, Dong-Lin Liu, Chang-Lin Yang, and Hong-Xing Pan contributed to the sample collection and participant follow-up. Zhao-Feng Bi, Ya-Fei Li, Ya-Hui Wang-Jiang, Qiu-Fen Zhang, and Guang Sun contributed to data interpretation; Li Zhang, Wei-Jin Huang, Feng-Zhu Zheng, Qi Chen, Jia-Li Quan, Xiao-Wen Hu, Xing-Cheng Huang, and Kong-Xin Zhu contributed to antibody-related detection or analysis. Zhao-Feng Bi, Yue Huang, Ying-Ying Su, Shou-Jie Huang, Ting Wu, and Jun Zhang were the core team for data analysis and manuscript preparation. Ting Wu, Yue Huang, Wei-Jin Huang, Jun Zhang, and Ning-Shao Xia were responsible for supervising the study. All authors critically reviewed the manuscript and approved the final version.

Appendix A. Supplementary materials

Supplementary materials to this article can be found online at https://doi.org/10.1016/j.scib.2023.09.020.

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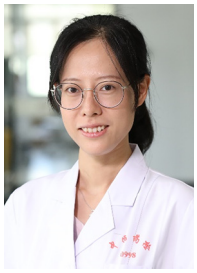

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