Original Article

Safety and Immunogenicity of a 9-Valent Human Papillomavirus Vaccine Administered to 9- to 15-Year-Old Japanese Girls

Satoshi Iwata^{1*}, Shinya Murata², Shi Rong Han², Akira Wakana², Miyuki Sawata², and Yoshiyuki Tanaka²

¹*Keio University School of Medicine, Tokyo 160-8582; and*

²*MSD KK, Tokyo 102-8667, Japan*

SUMMARY: A 9-valent human papillomavirus (HPV 6/11/16/18/31/33/45/52/58) virus-like particle vaccine (9vHPV) has been proven highly efficacious in preventing anogenital diseases related to HPV, in a pivotal phase III study for women aged 16–26 years. Here, we report the results of an open-label phase III study conducted to bridge the gap between the findings in women aged 16–26 years and Japanese girls aged 9–15 years. All subjects ($n = 100$) received a 3-dose regimen of 9vHPV vaccine on day 1 and at months 2 and 6. Anti-HPV serological assays were performed on day 1 and at months 7, 12, 24, and 30. At month 7 (4 weeks after the third dose), 100% of the subjects exhibited seroconversion for each type of HPV. Increases in geometric mean of the titers for anti-HPV 6/11/16/18/31/33/45/52/58 in the subjects were similar to those in Japanese women aged 16–26 years in a previous phase III study. Persistence of the anti-HPV response was observed for 2 years after administration of the third dose. In addition, administration of the 9vHPV vaccine was generally well-tolerated in Japanese girls.

INTRODUCTION

Human papillomavirus (HPV) infection causes benign and malignant dysplastic diseases, localized primarily in the anogenital area, including cervical cancers and pre-cancers (Cervical Intraepithelial Neoplasia grades 1, 2, and 3) (1,2), anal (3,4), vulvar (3,5), and vaginal cancers (3,6) and their precursors, genital warts (7,8), lesions in the aerodigestive tract, including oropharyngeal cancers (9), and recurrent respiratory papillomatosis (10). GARDASILTM 9 (9vHPV vaccine; Merck & Co., Inc., Kenilworth, NJ, USA) is a prophylactic 9-valent HPV (types 6, 11, 16, 18, 31, 33, 45, 52, and 58) L1 virus-like particle (VLP) vaccine that is composed of VLPs of the 4 HPV types (types 6, 11, 16, and 18) represented in GARDASILTM/SILGARDTM (4vHPV vaccine; Merck & Co., Inc.) and the VLPs of 5 additional high risk HPV types (types 31, 33, 45, 52, and 58) (11,12). This vaccine offers potentially significant prophylactic coverage for cancer, in addition to that already provided by the 4vHPV vaccine, with an increase in global prophylactic coverage for cervical cancer from approximately 70% to 90% (2). In Japanese women, administering this vaccine can potentially increase prophylactic coverage for cervical cancer from approximately 50–60% to 80–84% (13,14).

A pivotal study in young women aged 16–26 years demonstrated that the 9vHPV vaccine is highly efficacious in preventing anogenital diseases associated with vaccine types of HPV (15). Virgins were not included in the study because of low exposure to HPV, and due to difficulties involved in collecting genital samples from sexually naïve girls. Thus, we conducted a study

to bridge the gap between the findings in young women aged 16–26 years and non-Japanese aged 9–15 years (the ideal population for receiving prophylactic HPV vaccination), by demonstrating non-inferior immunogenicity (16). In this study, we evaluated the immunogenicity and safety of the 9vHPV vaccine in Japanese girls aged 9–15 years.

METHODS

Study design: This was an open-label study to evaluate the safety, tolerability, and immunogenicity of the 9vHPV vaccine. This study was conducted in accordance with the principles of good clinical practices, and was approved by the appropriate Institutional review boards and Japanese regulatory agency. Written informed consent and assent were obtained from a parent/legal guardian and the subject, respectively. Approximately 100 healthy Japanese girls aged 9–15 years were administered a standard 3-dose regimen (day 1, month 2, and month 6) of 9vHPV vaccine. Collection of medical history and physical examinations were performed on day 1 for all subjects. Vital signs were taken prior to administration of each dose of the vaccine. Subjects were required to be generally healthy and sexually naïve at enrollment, and through month 7. Subjects were excluded if they had a known allergy to any vaccine component, had a history of severe allergic reaction, thrombocytopenia, immunodeficiency, immunosuppressive therapy or the HPV vaccine.

Serum samples were obtained on day 1 and at months 7, 12, 24, and 30 from all subjects for anti-HPV 6, anti-HPV 11, anti-HPV 16, anti-HPV 18, anti-HPV 31, anti-HPV 33, anti-HPV 45, anti-HPV 52, and anti-HPV 58 antibody testing by the 9-Valent competitive Luminex Immunoassay (cLIA) (17). A subject was defined to be anti-HPV 6, anti-HPV 11, anti-HPV 16, anti-HPV 18, anti-HPV 31, anti-HPV 33, anti-HPV 45, anti-HPV 52, and anti-HPV 58 seropositive if the anti-HPV serum cLIA level was ≥ 30 , ≥ 16 , ≥ 20 , ≥ 24 , ≥ 10 , ≥ 8 , ≥ 8 ,

Received July 15, 2016. Accepted October 11, 2016.

J-STAGE Advance Publication December 22, 2016.

DOI:10.7883/yoken.JJID.2016.299

*Corresponding author: Mailing address: 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. Tel: +81-3-3353-1211, Fax: +81-5363-3711, E-mail: siwata@a8.keio.jp

≥ 8 , or ≥ 8 mMU/mL, respectively. Serostatus cutoffs were determined from 11 different cutoff values, by assessing the percentage of positive samples for each cutoff value, using 352 samples. Prior to testing, the 352 samples were classified into panels according to their potential for being true positives or true negatives, based on clinical history, PCR status, or seropositivity in prior cLIA testing. Based on percentage of positive titers for those samples that were likely to be positive, the appropriate serostatus cutoffs were selected from 11 cutoff values for each HPV type. Antibody titers for each individual HPV type were determined using type-specific monoclonal antibodies, hence it was not possible to directly compare assay results across HPV types.

Subjects were monitored for safety and tolerability throughout the study. All subjects received a Vaccination Report Card (VRC) on day 1 and at months 2 and 6 of the study. On the VRC, the parent/guardian of the subject was asked to record the subject's oral temperature every evening for 4 days after each dose of the vaccine, for the purpose of identifying febrile events. Also, after each dose of the vaccine, and for a total of 15 days including the day of vaccination, the parent/guardian of the subject was asked to record on the VRC, whether the subject had any injection-site or systemic adverse events (AEs), concomitant medications, and concomitant vaccinations.

The probability of observing at least one specific AE in this study was dependent on the number of subjects vaccinated, and the underlying percentage of subjects who experienced a specific AE in the study population. If the underlying incidence of a specific AE was 1%, there would be a 63% chance of observing at least one specific AE among 100 subjects. If no AEs were observed among the 100 subjects, this study would indicate with 80% confidence, that the underlying percentage of subjects who experienced an AE was $< 1.6\%$.

In this study, we enrolled 100 subjects for receiving 9vHPV vaccine, and estimated the seroconversion percentage with a 95% confidence interval, and a half-width of approximately 5 percentage points. This was calculated after assuming an approximately 5% dropout and/or protocol violation rate. The calculation was based on a well-defined binomial method proposed by Clopper and Pearson (18). This study allowed estimation of geometric mean titers (GMTs) among subjects receiving 9vHPV vaccine with a 95% confidence interval, and a half-width of 0.24 (ln mMU/mL) on the natural log scale (equivalent to 1.27-fold on the original scale). The accuracy of this method was dependent on: i) an approximately 5% dropout rate and/or protocol violation rate; and ii) a SD of the natural-log-transformed titers of 1.2 ln (mMU/mL).

Study objectives: The primary study objectives were: i) to evaluate the safety and tolerability of 9vHPV vaccine when administered to 9- to 15-year-old girls; and ii) to estimate the immune response of subjects to the various vaccine HPV types (6, 11, 16, 18, 31, 33, 45, 52, and 58) at 4 weeks post dose-3, using seroconversion rates. For i), all subjects who received at least 1 vaccine, and had follow-up data were included in the analysis of safety. The AEs of interest in this study included injection site AEs/vaccine-related AEs which were recorded on the VRC from day 1 through day 5 following any

vaccination administered, and elevated body temperatures from day 1 to day 5 following any vaccination administered in the study. For ii), with respect to the subjects' anti-HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 seroconversion percentages obtained during month 7, the primary hypothesis was evaluated by computing point estimates and constructing a 95% CI. No multiplicity adjustments were planned, as no formal statistical testing was performed. To be included in the immunogenicity analyses, subjects had to meet the following criteria: i) test seronegative on day 1; ii) receive all 3 doses of the study vaccine, within accepted time intervals; and iii) have a serological test result at month 7, within the accepted time intervals.

The secondary study objectives were: i) to demonstrate that at 4 weeks post dose-3, administration of 9vHPV vaccine induces non-inferior GMTs in girls aged 9–15 years, compared with those in Japanese women aged 16–26 years. The GMTs were compared for anti-HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58; ii) to describe the persistence of serum antibody titers for the various HPV types 24 months following the third dose of 9vHPV vaccine; and iii) to estimate the immune response of subjects to the HPV types (6, 11, 16, 18, 31, 33, 45, 52, and 58) at 4 weeks post dose-3, using GMTs. The secondary hypothesis was evaluated by comparing GMTs obtained from 9- to 15-year-old girls during month 7, with the GMTs in 16- to 26-year old Japanese young women. The GMTs were compared for anti-HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58. A 95% confidence interval was constructed for the ratio of GMTs (All Japanese women who received the 9vHPV vaccine, and met the per-protocol immunogenicity criteria mentioned in Protocol 001 were included). The statistical criteria required that the lower bound of the two-sided 95% confidence interval for the GMT ratio (9- to 15-year-old girls/16- to 26-year-old women) be greater than 0.5 for each HPV type. Thus, no multiplicity adjustment was necessary.

Study vaccine: 9vHPV vaccine is a sterile suspension requiring intramuscular administration. The deltoid muscle of the non-dominant arm was the preferred site of vaccination in this study. On day 1 and at months 2 and 6, subjects received 9vHPV vaccine in the form of a 0.5 mL intramuscular injection. Each 0.5 mL dose contained approximately 30 μ g of HPV Type 6 L1 VLP, 40 μ g of HPV Type 11 L1 protein, 60 μ g of HPV Type 16 L1 protein, 40 μ g of HPV Type 18 L1 protein, 20 μ g of HPV Type 31 L1 protein, 20 μ g of HPV Type 33 L1 protein, 20 μ g of HPV Type 45 L1 protein, 20 μ g of HPV Type 52 L1 protein, and 20 μ g of HPV Type 58 L1 protein, and approximately 500 μ g of aluminum hydroxyphosphate sulfate.

RESULTS

Study population: All enrolled subjects ($n = 100$) were Japanese women, with an average age of 11.7 years (Table 1). There were slightly more 9- to 12-year-olds (64%) than 13- to 15-year-olds (36%). Approximately 40% of the study population reported a pre-study medical condition. The most common medical condition was seasonal allergy. Forty-four percent of the subjects had received prior medications. The most common

Table 1. Subject characteristics

	Subject <i>n</i>	(%)
Subjects in population	100	
Gender		
Female	100	(100.0)
Age (yr)		
9 to 12	64	(64.0)
13 to 15	36	(36.0)
Mean	11.7	
SD	1.7	
Median	12.0	
Range	9 to 15	
Race		
Japanese	100	(100.0)
Past medical history		
No	60	(60.0)
Yes	40	(40.0)
Prior medication (within 3 days prior to the any vaccination)		
No	56	(56.0)
Yes	44	(44.0)

Table 2. Seroconversion rates for each of HPV types (per-protocol immunogenicity population¹⁾)

Assay (cLIA)	Time point	9vHPV vaccine (<i>N</i> = 100)		
		<i>n</i>	<i>m</i>	Percent ²⁾ (95% CI)
Anti-HPV 6	day 1	97	0	0.0 (0.0, 3.7)
	mo 07	97	97	100 (96.3, 100)
	mo 12	97	96	99.0 (94.4, 100)
	mo 24	97	95	97.9 (92.7, 99.7)
	mo 30	94	94	100 (96.2, 100)
Anti-HPV 11	day 1	97	0	0.0 (0.0, 3.7)
	mo 07	97	97	100 (96.3, 100)
	mo 12	97	97	100 (96.3, 100)
	mo 24	97	97	100 (96.3, 100)
	mo 30	97	97	100 (96.3, 100)
Anti-HPV 16	day 1	99	0	0.0 (0.0, 3.7)
	mo 07	99	99	100 (96.3, 100)
	mo 12	99	99	100 (96.3, 100)
	mo 24	99	99	100 (96.3, 100)
	mo 30	99	99	100 (96.3, 100)
Anti-HPV 18	day 1	98	0	0.0 (0.0, 3.7)
	mo 07	98	98	100 (96.3, 100)
	mo 12	98	98	100 (96.3, 100)
	mo 24	98	97	99.0 (94.4, 100)
	mo 30	98	97	99.0 (94.4, 100)
Anti-HPV 31	day 1	97	0	0.0 (0.0, 3.7)
	mo 07	97	97	100 (96.3, 100)
	mo 12	97	97	100 (96.3, 100)
	mo 24	97	97	100 (96.3, 100)
	mo 30	97	97	100 (96.3, 100)
Anti-HPV 33	day 1	98	0	0.0 (0.0, 3.7)
	mo 07	98	98	100 (96.3, 100)
	mo 12	98	98	100 (96.3, 100)
	mo 24	98	98	100 (96.3, 100)
	mo 30	98	98	100 (96.3, 100)
Anti-HPV 45	day 1	99	0	0.0 (0.0, 3.7)
	mo 07	99	99	100 (96.3, 100)
	mo 12	99	99	100 (96.3, 100)
	mo 24	99	99	100 (96.3, 100)
	mo 30	99	98	99.9 (94.5, 100)
Anti-HPV 52	day 1	99	0	0.0 (0.0, 3.7)
	mo 07	99	99	100 (96.3, 100)
	mo 12	99	99	100 (96.3, 100)
	mo 24	99	99	100 (96.3, 100)
	mo 30	99	99	100 (96.3, 100)
Anti-HPV 58	day 1	95	0	0.0 (0.0, 3.8)
	mo 07	95	95	100 (96.2, 100)
	mo 12	95	95	100 (96.2, 100)
	mo 24	95	95	100 (96.2, 100)
	mo 30	95	95	100 (96.2, 100)

¹⁾ The per-protocol immunogenicity population includes all subjects who were not general protocol violators, received all 3 vaccinations within acceptable day ranges, were seronegative at day 1 for the relevant HPV type(s), and had a month 7 serum sample collected within an acceptable day range.

²⁾ Percent represents proportion of subjects with anti-HPV serum levels $\geq$ 30, 16, 20, 24, 10, 8, 8, 8, and 8 mMU/mL for HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58, respectively.

N, Number of subjects randomized to the respective vaccination group who received at least 1 injection.

n, Number of subjects contributing to the analysis.

m, Number of subjects seropositive to the relevant HPV type(s).

Table 3. GMTs for each of the HPV types (per-protocol immunogenicity population¹⁾)

Assay (cLIA)	Time point	9vHPV vaccine (<i>N</i> = 100)		
		<i>n</i>	GMT (mMU/mL)	95% CI
Anti-HPV 6	day 1	97	< 16	(< 16, < 16)
	mo 07	97	1,836.5	(1,597.7, 2,111.0)
	mo 12	97	657.7	(562.8, 768.6)
	mo 24	97	323.4	(272.2, 384.3)
	mo 30	94	313.2	(266.6, 368.0)
Anti-HPV 11	day 1	97	< 6	(< 6, < 6)
	mo 07	97	1,331.3	(1,135.0, 1,561.4)
	mo 12	97	493.6	(417.3, 583.8)
	mo 24	97	243.5	(206.4, 287.3)
	mo 30	97	196.6	(164.9, 234.5)
Anti-HPV 16	day 1	99	< 12	(< 12, < 12)
	mo 07	99	6,823.6	(5,907.9, 7,881.2)
	mo 12	99	2,424.6	(2,067.1, 2,844.0)
	mo 24	99	1,229.4	(1,032.6, 1,463.6)
	mo 30	99	1,001.9	(823.6, 1,218.7)
Anti-HPV 18	day 1	98	< 8	(< 8, < 8)
	mo 07	98	2,159.9	(1,803.8, 2,586.3)
	mo 12	98	692.0	(572.7, 836.2)
	mo 24	98	302.3	(244.8, 373.3)
	mo 30	98	268.2	(214.8, 334.7)
Anti-HPV 31	day 1	97	< 4	(< 4, < 4)
	mo 07	97	2,052.5	(1,735.9, 2,426.8)
	mo 12	97	721.7	(610.7, 853.0)
	mo 24	97	365.6	(304.3, 439.3)
	mo 30	97	305.8	(253.2, 369.4)
Anti-HPV 33	day 1	98	< 4	(< 4, < 4)
	mo 07	98	994.8	(857.2, 1,154.4)
	mo 12	98	356.2	(307.5, 412.5)
	mo 24	98	184.1	(157.2, 215.6)
	mo 30	98	142.9	(120.5, 169.5)
Anti-HPV 45	day 1	99	< 3	(< 3, < 3)
	mo 07	99	811.0	(679.7, 967.6)
	mo 12	99	262.0	(216.5, 317.1)
	mo 24	99	129.0	(107.0, 155.5)
	mo 30	99	101.3	(82.0, 125.0)
Anti-HPV 52	day 1	99	< 3	(< 3, < 3)
	mo 07	99	1,069.1	(908.4, 1,258.4)
	mo 12	99	392.0	(330.4, 465.2)
	mo 24	99	196.7	(166.5, 232.4)
	mo 30	99	157.6	(131.5, 188.9)
Anti-HPV 58	day 1	95	< 4	(< 4, < 4)
	mo 07	95	1,488.2	(1,285.0, 1,723.4)
	mo 12	95	646.5	(555.6, 752.2)
	mo 24	95	312.9	(264.9, 369.6)
	mo 30	95	272.9	(227.2, 327.7)

¹⁾ The per-protocol immunogenicity population includes all subjects who were not general protocol violators, received all 3 vaccinations within acceptable day ranges, and had a month 7 serum sample collected within an acceptable day range.

N, Number of subjects who received at least 1 injection.

n, Number of subjects contributing to the analysis.

CI, confidence interval; cLIA, competitive luminex immunoassay; GMT, geometric mean titer; mMU, milli merck units.

prior therapy received by the subjects was anti-histamine therapy, used systemically (18%). At enrollment, serum antibody titers for one or more types of HPV (6, 11, 16, 18, 31, 33, 45, 52, or 58) that were greater than the pre-defined seropositive threshold values (indicative of previous exposure to the respective HPV types) were detected in 10% (10 of 100) of the subjects. Overall, 99% (99/100) of subjects enrolled in the study received all 3 doses and completed the follow-up tests. A single subject withdrew consent following dose-1.

Immunogenicity: The seroconversion rate for each of the 9 HPV types (Types 6, 11, 16, 18, 31, 33, 45, 52, and 58) 1 month after dose-3 (month 7) was 100%, and this high seroconversion rate was maintained for 2 years after the third vaccination (month 30) (Table 2). Analytical results indicating the anti-HPV GMTs for each of the HPV types (Types 6, 11, 16, 18, 31, 33, 45, 52,

9vHPV Vaccine in Japanese Girls

Table 4. Comparison of GMTs for each HPV type (month 7): comparison between 9- to 15-year-old Japanese girls (P008) and 16- to 26-year-old Japanese women (P001) (per-protocol immunogenicity population¹⁾)

Old Japanese women (1001) (per protocol immunogenically population)						
Assay (cLIA)	Comparison group				Estimated fold difference group A / group B ²⁾ (95% CI)	p-value for non-inferiority ²⁾
	Subjects aged 9 to 15 (comparison group A) (N = 100)		Subjects aged 16 to 26 (comparison group B) (N = 127)			
	n	Estimated GMT ²⁾ (mMU/mL)	n	Estimated GMT ²⁾ (mMU/mL)		
Anti-HPV 6	97	1,836.5	93	839.7	2.19 (1.78, 2.68)	< 0.001
Anti-HPV 11	97	1,331.3	93	611.1	2.18 (1.75, 2.72)	< 0.001
Anti-HPV 16	99	6,823.6	96	2,672.7	2.55 (2.09, 3.13)	< 0.001
Anti-HPV 18	98	2,159.9	99	688.7	3.14 (2.43, 4.05)	< 0.001
Anti-HPV 31	97	2,052.5	104	672.3	3.05 (2.43, 3.84)	< 0.001
Anti-HPV 33	98	994.8	109	398.3	2.50 (2.04, 3.05)	< 0.001
Anti-HPV 45	99	811.0	111	258.5	3.14 (2.45, 4.01)	< 0.001
Anti-HPV 52	99	1,069.1	98	306.3	3.49 (2.78, 4.38)	< 0.001
Anti-HPV 58	95	1,488.2	95	459.6	3.24 (2.65, 3.96)	< 0.001

¹⁾The per-protocol immunogenicity population includes all subjects who were not general protocol violators, received all 3 vaccinations within acceptable day ranges, were seronegative at Day 1 for the relevant HPV-type(s), and had a Month 7 serum sample collected within an acceptable day range. In addition, subjects were required to be seronegative at Day 1 and (for 16- to 26-year-old women) PCR negative from Day 1 through Month 7 only for the HPV type being analyzed.

²⁾The estimated GMT, fold difference, associated confidence intervals, and p-value are based on an ANCOVA model with a response of the natural log of individual titers and fixed effects for comparison group.

The noninferiority criterion for endpoints reported in this table is defined as statistically less than 1.5-fold decrease in Group A compared to Group B. Noninferiority of GMT in Group A relative to Group B is demonstrated if the lower limit of the 95% CI for the fold difference is greater than 0.67.

N, Number of subjects randomized to the respective vaccination group who received at least 1 injection.

n, Number of subjects contributing to the analysis.

CI, confidence interval; GMT, geometric mean titer; mMU, milli merck units; cLIA, 9 valent competitive luminex immunoassay.

Table 5. Comparison of GMTs for each HPV type (month 7): comparison between 9- to 15-year-old Japanese girls (P008) and non-Japanese women (P002) (per-protocol immunogenicity population¹⁾)

women (1002) (per protocol minimally-genotyped population)						
Assay (cLIA)	Comparison group				Estimated fold difference group A / group B (95% CI)	p-value for non-inferiority ²⁾
	Japanese (comparison group A) (N = 100)		Non-Japanese (comparison group B) (N = 1,932)			
	n	Estimated GMT (mMU/mL)	n	Estimated GMT (mMU/mL)		
Anti-HPV 6	97	1,836.5	1,597	1,712.0	1.07 (0.90, 1.28)	< 0.001
Anti-HPV 11	97	1,331.3	1,597	1,278.7	1.04 (0.87, 1.25)	< 0.001
Anti-HPV 16	99	6,823.6	1,627	7,071.6	0.96 (0.81, 1.15)	< 0.001
Anti-HPV 18	98	2,159.9	1,641	2,081.2	1.04 (0.84, 1.28)	< 0.001
Anti-HPV 31	97	2,052.5	1,617	1,879.3	1.09 (0.89, 1.33)	< 0.001
Anti-HPV 33	98	994.8	1,637	944.1	1.05 (0.88, 1.26)	< 0.001
Anti-HPV 45	99	811.0	1,647	737.1	1.10 (0.88, 1.38)	< 0.001
Anti-HPV 52	99	1,069.1	1,642	970.5	1.10 (0.91, 1.33)	< 0.001
Anti-HPV 58	99	1,488.2	1,630	1,277.7	1.16 (0.97, 1.41)	< 0.001

¹⁾The per-protocol immunogenicity population includes all subjects who were not general protocol violators, received all 3 vaccinations within acceptable day ranges, were seronegative at day 1 for the relevant HPV type(s), and had a month 7 serum sample collected within an acceptable day range.

²⁾Non-inferiority for GMTs is defined as statistically less than a 2-fold decrease. The estimated GMT, fold difference, associated confidence intervals, and p-values are based on a statistical analysis model.

N, Number of subjects who received at least 1 injection.

n, Number of subjects contributing to the analysis.

CI, confidence interval; GMT, geometric mean titer; mMU, milli merck units; cLIA, 9 valent competitive luminex immunoassay.

and 58) at 1 month after dose-3 (month 7) are shown in Table 3. The GMTs for HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 were 1,836.5 mMU/mL, 1,331.3 mMU/mL, 6,823.6 mMU/mL, 2,159.9 mMU/mL, 2,052.5 mMU/mL, 994.8 mMU/mL, 811.0 mMU/mL, 1,069.1 mMU/mL, and 1,488.2 mMU/mL, respectively. The GMTs for all vaccine HPV types decreased through month 30 after reaching the peak at month 7, with relatively slow pace after month 24. At month 30, the GMTs for each of HPV types ranged from 101.3 to 1,001.9 mMU/mL above the relevant seroconversion cutoffs.

The results of comparison of GMTs for each age strata (9–15 years old and 16–26 years old) are shown in Table 4. The ratio of GMTs at 1 month post dose-3 (month 7)

among the 9- to 15-year-old girls, to the GMTs among the 16- to 26-year-old women for HPV Type 6, 11, 16, 18, 31, 33, 45, 52, and 58 were 2.19 (1.78, 2.68), 2.18 (1.75, 2.72), 2.55 (2.09, 3.13), 3.14 (2.43, 4.05), 3.05 (2.43, 3.84), 2.50 (2.04, 3.05), 3.14 (2.45, 4.01), 3.49 (2.78, 4.38), and 3.24 (2.65, 3.96), respectively. The lower bound of 95% CI was greater than 0.5, which is the predefined value according to the criterion of non-inferiority. Thus, the GMTs induced by 9vHPV vaccine administration to the 9- to 15-year-old girls were at least equal to those observed in the 16- to 26-year-old Japanese women (Protocol 001).

The GMTs for each of the HPV types at month 7 in this study (Protocol 008) and the Asia Ex-Japan study

Table 6. Adverse event summary

	Subject	
	9–15 year old <i>n</i>	Japanese girls (%)
Number of subjects in safety analysis population	100	
with adverse events	97	(97.0)
injection-site adverse events ¹⁾	95	(95.0)
Pain ²⁾	93	(93.0)
Mild	54	(54.0)
Moderate	36	(36.0)
Severe	3	(3.0)
Swelling ²⁾	42	(42.0)
Mild (0 to ≤2.5 cm)	28	(28.0)
Moderate (>2.5 to ≤5 cm)	4	(4.0)
Severe (>5 cm)	10	(10.0)
Erythema	33	(33.0)
Mild (0 to ≤2.5 cm)	28	(28.0)
Moderate (>2.5 to ≤5 cm)	5	(5.0)
Pruritus ²⁾	4	(4.0)
Mild	4	(4.0)
Haemorrhage ²⁾	3	(3.0)
Mild	28	(3.0)
Warmth ²⁾	3	(3.0)
Mild	1	(1.0)
Moderate	2	(2.0)
Systemic adverse events ³⁾	35	(35.0)
Vaccine related systemic adverse events ⁴⁾	14	(14.0)
Pyrexia	3	(3.0)
Abdominal pain	2	(2.0)
Nausea	2	(2.0)
Headache	2	(2.0)
Hypoaesthesia	2	(2.0)
Serious adverse events	0	(0.0)
Discontinued due to an adverse event ⁵⁾	0	(0.0)

¹⁾: Days 1–5 after any vaccination.

²⁾: Intensities of pain, pruritus, haemorrhage and warmth are defined as follows; mild is an awareness of sign or symptom but easily tolerated; moderate is discomfort enough to cause interference with usual activity; severe is incapacitating with inability to work or do usual activity.

³⁾: Days 1–15 after any vaccination.

⁴⁾: Determined by the investigator to be related to the vaccine.

⁵⁾: Study medication withdrawn.

(16) (Protocol 002) were comparable, and no substantial differences were found (Table 5).

Safety: The incidence of clinical AEs on days 1 through 15 following any vaccination was 97.0% (97/100 subjects) (Table 6). The incidence of vaccine-related clinical AEs was 96.0% (96/100 subjects). Injection-site AEs occurred in 95 subjects. The most common injection-site AE was pain at the site of injection (93%:93/100 subjects), the second most common injection-site AE was swelling at the site of injection (42%:42/100 subjects) and the third was erythema at the site of injection (33%:33/100 subjects). Other common injection-site AEs (incidence ≥ 2%) were pruritus, hemorrhage, and warmth. Severe injection-site AEs included pain (3.0%:3/100 subjects) and swelling (10%:10/100 subjects) at the site of injection. Overall, 12 out of 13 subjects with severe injection-site AE recovered within 8 days after onset of the AE. One subject with severe injection-site AE recovered in 21 days after onset of the AE. The rate of incidence of vaccine-related systemic AEs was 14.0% (14/100 subjects), and the systemic AEs that occurred in 2 or more subjects were pyrexia (3.0%: 3/100 subjects), abdominal pain, nausea, headache, and hypoaesthesia (2.0%, 2/100 subjects) in descending order

of prevalence. All systemic AEs were mild in intensity, with exceptions such as moderate tinnitus, pain in extremities, herpes zoster, and pyrexia (1/100 subjects). Tinnitus and pain of moderate intensity in the extremities were observed 2 days after the first vaccination, and the subjects recovered within 2 days after the onset of the AE. Pyrexia and herpes zoster were observed 9 and 10 days after the first vaccination respectively, and resolved in 5 and 17 days after onset, respectively. There were no serious AEs, deaths, or discontinuation due to an AE, or pregnancy among the subjects.

DISCUSSION

The immunogenicity and safety of a 3-dose regimen of 9vHPV vaccine was analyzed in Japanese girls aged 9–15 years, the primary target population for immunoprophylaxis as a means of preventing HPV-related anogenital diseases, including precancers and cancers. Seroconversion for all 9 HPV types was achieved in 100% of the study participants who received a 3-dose regimen of the 9vHPV vaccine. This seroconversion rate was at least equal to that observed in 16- to 26-year-old Japanese female subjects (Protocol 001). The GMTs obtained after administration of the 3-dose regimen of 9vHPV vaccine was higher in girls than in young women, and similar between Japanese and non-Japanese subjects (Protocol 002) aged 9–15 years. Most of the girls (≥ 99%) remained seropositive for 2 years after the third vaccination (month 30). Administration of the 3-dose regimen of 9vHPV vaccine to girls aged 9–15 years was found to be generally well-tolerated.

One of the limitations of this study is the lack of data related to efficacy of the vaccine in the age group under study. Direct evaluation of efficacy was not feasible, due to low exposure of the subjects to HPV, and the difficulties involved in collecting genital samples from sexually naïve girls. Therefore, previous findings related to efficacy of the vaccine in young women were extended to the subjects in this study, based on the demonstration of non-inferior immunogenicity between young women and girls. This approach has been previously used for administering 4vHPV vaccine, and is consistent with World Health Organization (WHO) guidelines (19). In addition, immune responses for HPV 6/11/16/18 elicited by the 9vHPV vaccine as well as by the 4vHPV vaccine were demonstrated to be similar among 9- to 15-year-old girls, in another study (V503-009) (20). This result also extends the findings related to efficacy of the 9vHPV vaccine to 9- to 15-year-old girls.

Follow-up studies were limited in duration. Studies of 4vHPV vaccine have not shown any evidence of waning of immunity in long-term cohorts (21,22), which suggests that 9vHPV vaccine may also offer long-term protection. On-going longer-term studies to determine the efficacy and immunogenicity of 9vHPV vaccine may provide information on the duration of protection offered by this vaccine.

Licensed HPV vaccines have been introduced in national immunization programs of more than 40 countries (23), including Japan. As of June 2016, the 9vHPV vaccine is licensed in over 30 countries under the name of Gardasil 9, and is already in use in national immunization programs of some of these countries. The 9vHPV

vaccine has the potential to provide additional benefits to women and girls in these countries, as compared to the existing vaccines, such as providing protection from 5 additional high-risk HPV types (31/33/45/52/58). The results of this study offers evidence for the potential benefits provided by the 9vHPV vaccine to the female population in Japan.

Acknowledgments The authors would like to thank: All the subjects who participated in this study and their parents or legal guardian. Jon E. Stek (Merck and Co., Inc., Kenilworth, NJ, USA) for his assistance in writing this manuscript. Investigators for this study: Toshiomi Kusano (Shinjuku kaijo Biru Clinic), Chihiro Morita (Nakameguro Atlas Clinic), and Yukari Utsugisawa (Women's Clinic LUNA ANNEX). This study was funded by MSD K.K. (sponsor). Although the sponsor formally reviewed a penultimate draft, the opinions expressed are those of the authorship and may not necessarily reflect those of the sponsor. All co-authors approved the final version of the manuscript.

Conflict of interest S Iwata received consulting fee from MSD K.K. as a consultant of this study. Outside the submitted work, S Iwata received grants and payment from GlaxoSmithKline, Japan Vaccine, Pfizer, Takeda Pharmaceutical, and MSD K.K. for lectures including services on speaker's bureaus and/or payment for manuscript preparation. S Iwata is the chairman of the vaccination promotion expert committee. All other authors were employees of MSD K.K. Employees may hold stock and/or stock options in the company.

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