Report on the Deliberation Results

September 8, 2009

Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau

Ministry of Health, Labour and Welfare

[Brand name]	Cervarix
[Non-proprietary name]	Recombinant Adsorbed Bivalent Human Papillomavirus-like Particle Vaccine (derived from *Trichoplusia ni* cell)
[Applicant]	GlaxoSmithKline K.K.
[Date of application]	September 26, 2007

[Results of deliberation]

In the meeting held on August 31, 2009, the Second Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

In addition, the following conclusions were reached: the product is classified as a biological product, the re-examination period is 8 years, and the drug substance and the drug product are both classified as powerful drugs.

Review Report

August 20, 2009
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Cervarix
[Non-proprietary name]	Recombinant Adsorbed Bivalent Human Papillomavirus-like Particle Vaccine (derived from *Trichoplusia ni* cell)
[Applicant]	GlaxoSmithKline K.K.
[Date of application]	September 26, 2007
[Dosage form/strength]	Injections, each syringe being filled with a 0.5 mL suspension (one-dose volume) containing HPV-16 L1 VLP 20 μg and HPV-18 L1 VLP 20 μg as active ingredients
[Application classification]	Prescription drug (1) Drug with a new active ingredient
[Items warranting special mention]	· Priority review · Minimum Requirements for Biological Products (draft) "Recombinant Adsorbed Bivalent Human Papillomavirus-like Particle Vaccine (derived from *Trichoplusia ni* cell)" has been submitted.
[Reviewing office]	Office of Biologics II

Review Results

August 20, 2009

[Brand name]	Cervarix
[Non-proprietary name]	Recombinant Adsorbed Bivalent Human Papillomavirus-like Particle Vaccine (derived from *Trichoplusia ni* cell)
[Applicant]	GlaxoSmithKline K.K.
[Date of application]	September 26, 2007

[Results of review]

It is concluded that the submitted data have demonstrated the efficacy and safety of the product for prevention of cervical cancer (squamous-cell carcinoma and adenocarcinoma) and its precursor lesions caused by infection with human papillomavirus (HPV) types 16 and 18 .

In regard to the efficacy, the clinical studies conducted in foreign countries have shown the preventive effect of the product against cervical intraepithelial neoplasia grades 2 and 3 (CIN2+) caused by infection with HPV types 16 and 18. In addition, in the Japanese clinical studies, a certain level of preventive effect against persistent infection and an increase in the serum antibody titer have been confirmed. Therefore, it is concluded that vaccination of this product has the efficacy as described in the indication shown below.

As for the safety, the data including post-marketing safety data in foreign countries suggest that there are no particular problems which would preclude approval of the product application. However, considering that this product contains a new adjuvant ingredient and that it is the first recombinant drug product in Japan produced using insect cells as the protein-expressing cells, it is necessary that the safety-related information be continuously collected from the post-marketing surveillance, etc., and provided appropriately.

As a result of its regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product may be approved for the following indication and dosage and administration.

[Indication]

Prevention of cervical cancer (squamous-cell carcinoma and adenocarcinoma) and its precursor lesions (cervical intraepithelial neoplasia [CIN] grades 2 and 3) caused by infection with human papillomavirus (HPV) types 16 and 18.

[Dosage and administration]

This vaccine should be administered in females 10 years or older as a single 0.5 mL injection by the intramuscular route into the deltoid region of the upper arm. The primary vaccination course consists of 3 doses according to the following schedule: 0, 1, and 6 months.

Review Report (1)

June 22, 2009

I. Product Submitted for Registration

[Brand name]	Cervarix
[Non-proprietary name]	Recombinant Adsorbed Bivalent Human Papillomavirus Vaccine (derived from [redacted] cells) (tentative)
[Applicant]	GlaxoSmithKline K.K.
[Date of application]	September 26, 2007
[Dosage form/strength]	Injections, each syringe being filled with a 0.5 mL suspension (one-dose volume) containing HPV-16 L1 VLP 20 μg and HPV-18 L1 VLP 20 μg as active ingredients
[Proposed indications]	Prevention of cervical cancer (squamous-cell carcinoma and adenocarcinoma) by protecting against the following precursor lesions and infections caused by oncogenic human papillomavirus (HPV) types 16 and 18: ・Cervical intraepithelial neoplasia (CIN) grades 2 and 3 ・Cervical intraepithelial neoplasia (CIN) grade 1 ・Abnormal cytology (atypical squamous cells of undetermined significance [ASC-US], low-grade squamous intraepithelial lesions [LSIL] and high-grade squamous intraepithelial lesions [HSIL]) ・Persistent infection ・Incident infection Cervarix has also shown efficacy against persistent infection caused by oncogenic HPV types, in addition to HPV-16 and HPV-18, inclusive of phylogenetically-related types HPV-31and HPV-45.
[Proposed dosage and administration]	This vaccine should be administered in females 10 years or older as a single 0.5 mL injection by the intramuscular route into the deltoid region of the upper arm. The primary vaccination course consists of 3 doses according to the following schedule: 0, 1, and 6 months.
[Items warranting special mention]	Priority Review Minimum Requirements for Biological Products (draft) "Recombinant Adsorbed Bivalent Human Papillomavirus-like Particle Vaccine (derived from [redacted] cells) (tentative)" has been submitted. .

II. Summary of the Submitted Data and Outline of the Review by Pharmaceuticals and Medical Devices Agency

For this application, the data submitted by the applicant and the applicant's responses to the inquiries made by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined below.

1. Origin or history of discovery and usage conditions in foreign countries

Cervical cancer is the most frequently occurring cancer next to breast cancer among the women over the world. It is reported that human papilloma virus (HPV) infection is the major cause of cervical cancer (*N Engl J Med.* 2003;348(6):518-27, NHS Cancer Screening Programmes. *The Aetiology of Cervical Cancer.* 2005;1-80). HPV is a double stranded circular DNA virus of about 8k base pairs that belong to papillomavirus family. Up to present, 100 or more genotypes of HPV have been reported. At least 30 types HPV are verified to infect the genital mucosa and it is reported that HPV infection is widely observed among the sexually matured adults. Though some reports indicate that most of HPV infections are transient and are eliminated by natural immunity (*Lancet.* 2004;364 (9446):1678-83, *J Infect Dis.* 2005;191 (11):1808-16), the details of infection including its molecular base are unknown. About 16 types of HPV are known as carcinogenic HPV and the persistent HPV infection is known to increase the onset risk of cervical cancer (*N Engl J Med.* 1998; 338(7):423-8, *J Pediatr.* 1998;132(2):277-84, *Virology* .2005;337(1):76-84, *J Natl Cancer.* 2005;97(14):1072-9). Two types of HPV, HPV-16 and HPV-18, have been detected at a high prevalence as the carcinogenic HPV. It is reported that HPV-16 or HPV-18 have been detected in about 70% of cervical cancer cases over the world (76% in North America) (*Br J Cancer.* 2003; 88:63-73, *Int J Cancer.*2004;111:278-285, *Int J Cancer.* 2007;121:621-632).

According to the demographic statistics in 2005 in Japan, the mortality of cervical cancer is 3.8 persons per 100,000 and the tendency of increase is recently reported in the women in their 20s to 30s (Cancer Information Center of National Cancer Center, 2007). The HPV infection rate among patients with cervical cancer and cervical dysplasia in Japan is close to 100%, which is similar to those reported in foreign countries. According to the report by Miura, et. al., the HPV type most frequently detected among cervical cancer cases is HPV-16, followed by HPV-18, HPV-52 and HPV-58 in this order. HPV-16 is also the most frequently detected among cases with high grade squamous intraepithelial lesion (HSIL), followed by HPV-52, HPV-58 and HPV-51 in this order. Though it was reported that the HPV-16 and HPV-18 detection frequency in cervical cancer cases was 58.5% in Japan (*Int J Cancer.* 2006;119:2713-5), the sample collection method or test method, etc. are not consistent. Accordingly, the national level result based on a prescribed assessment method has not been obtained up to present.

When a patient is diagnosed to have cervical cancer, radical treatments including hysterectomy and radiotherapy, etc. are basically conducted in Japan as well as abroad. As a result, conservation of uterus becomes difficult in many cases. Currently, the uterine cancer screening is the main prophylactic measure against cervical cancer in Japan. The target age was changed from 30 years old or older to 20 years old or older in 2004. However, it is reported that the percentage of examinees is early 10% (Ministry of Health, Labour and Welfare, Outline of the project report on community and elderly health 2004) or under 30% (*Journal of Clinical & Experimental Medicine.* 2008;224:669-80). In other words, the cervical cancer screening is not fully utilized as the secondary prevention measure. Considering that the estimated morbidity and mortality of cervical cancer are on the increase among the women especially in their 30s, it is necessary to strengthen the cervical cancer preventive measures.

Cervarix (the "HPV Vaccine") is an HPV vaccine developed by GSK Biologicals (Belgium) in affiliation with [redacted] (USA) under the indication of prevention of cervical cancer attributable to carcinogenic HPV. Each dose (0.5 mL) contains 20 µg of HPV-16L1 protein and 20 µg of HPV-18L1 protein that are formed as virus-like particles as active ingredients and are added with AS04 adjuvant consisting of aluminum hydroxide (500 µg as aluminum) and 50µg of 3-deacylated-4'-monophosphoryllipid A (MPL). L1 VLP, used as the antigen, induces its specific antibody. MPL is known to contribute to the activation of natural immune response. The addition of adjuvant that contains MPL is intended to provide high antibody titer continuously and to induce specific cellular immunity.

This HPV Vaccine is approved in 95 countries as a vaccine to prevent cervical cancer (as of March 9, 2009), and the quantity to take care of about 6,800,000 doses of vaccine has cumulatively been supplied over the world up to May 2009. In addition to the approval on this HPV Vaccine given in Australia in May 2007, the approval on this HPV Vaccine was granted in September 2007 in EU as the vaccine to prevent cervical precancer lesions and cervical cancer attributable to HPV-16 and HPV-18. The application was filed in March 2007 in USA and the application is under review as of June 2009.

Along with the approvals granted abroad on HPV vaccines, the medical and social interest requesting clinical use of HPV vaccine as a measure to prevent cervical cancer has been increased in Japan after June 2006, and the Japan Society of Obstetrics and Gynecology submitted "A request for early approval of cervical cancer (HPV) vaccine" on October 23, 2006. While clinical studies of this HPV Vaccine were going on in Japan, the Ministry of Health, Labour and Welfare (MHLW) instructed the applicant to file the application for marketing approval of this HPV Vaccine on the basis of overseas clinical study results, etc. to promptly and appropriately proceed

with the review of this HPV Vaccine. In response to this instruction, in September 2007 the applicant prepared the clinical data package based on overseas clinical study results and filed an application for marketing approval on the condition that the results of clinical studies currently going on in Japan (Study HPV-032 [phase II double blind comparative study in females aged 20-25 years] and Study HPV-046 [phase III open study in females aged 10-15 years]) are submitted as soon as they are available. This product was filed as a "kit product" in accordance with "Handling of injection kit product, etc. combined with diluent, etc." (PAB/SERD Notification No. 98 of the Second Evaluation and Registration Division, Pharmaceutical Affairs Bureau, MHLW, dated March 12, 1986). On January 9, 2008 after product application, this HPV Vaccine was designated as the "priority review product" in view of the seriousness of cervical cancer against which this HPV Vaccine is expected to have a preventive effect and the usefulness as the preventive measure, etc. The clinical study report on Study HPV-046 that had been under way at the time of filing was submitted on [redacted], 20[redacted] while the clinical study report on Interim Analysis II of Study HPV-032 was submitted in [redacted] 20[redacted]. It is reported that the final clinical study report will be submitted by end-[redacted], 20[redacted].

2. Data relating to quality

2.A Summary of the submitted data

This HPV Vaccine is a pre-filled syringe preparation for injection containing, as the active ingredients, virus-like particles (VLP), which are assembly of C-terminally truncated L1 proteins of human papillomavirus (HPV) type 16 and type 18 manufactured by gene recombinant technology, together with an adjuvant system consisting of 3-deacylated-4'- monophosphoryl lipid A (MPL) and aluminum hydroxide.

2.A.(1) Drug substance

2.A.(1).1) Manufacturing method

The manufacturing method consists of (i) preparation of the inocula using the recombinant baculovirus seed and cell bank, (ii) preparation of L1 harvests and extraction/purification of L1 proteins. As for [redacted] cells, the [redacted] master cell bank (MCB) and the working cell bank (WCB) have been established, and as for the baculovirus seed, the seed/lot system of master seed (MS) and working seed (WS) has been established for each of the HPV-16 L1 and HPV-18 L1 gene-encoding recombinant baculoviruses. HPV-16 L1 VLP and HPV-18 L1 VLP are separately prepared using similar operating procedures, except that the same [redacted] cell bank system is used for both HPV-16 L1 VLP and HPV-18 L1 VLP. The manufacturing method is outlined below.

a. Cell bank system

The [redacted] cell bank being the parent cell line for [redacted] cells was started from the cell line derived from eggs of *Trichoplusia ni* ([redacted]) ([redacted]), which was established after repeating subculture more than [redacted] times and supplied to [redacted]. The [redacted] cell line was thereafter established as a pre-master cell bank by GSK Biologicals through cloning. This cell line can be subjected to suspension culture in a serum-free culture medium.

The MCB was prepared by repeating subculture of the pre-master bank in shake flasks [redacted] times (to passage [redacted]). The cells have been cryopreserved with liquid nitrogen after dispensing. No quality control test is planned to conduct during the storage period.

The WCB was prepared by repeating subculture of the MCB in shake flasks [redacted] times (to passage [redacted]). The cells have been cryopreserved after dispensing in the same way as the MCB. The following rule has been established for WCB renewal: the WCB should be renewed with the same procedures as used for initial WCB preparation, and the renewed WCB should be subjected to the same quality control tests as those used for initial preparation (described later) and furthermore to the tests related to cell line stability, virus content after infection with baculovirus, and production level and quality of the target recombinant protein, for the purpose of confirming conformity to the specifications.

The quality control tests of the [redacted] cell bank have been specified according to the guidelines for mammal cell lines (quality of biopharmaceuticals: “Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin” [ICH-Q5A], quality of biopharmaceuticals: “Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products” [ICH-Q5D] and “Requirements for the Use of Animal Cells as *in Vitro* Substrates for the Production of Biologicals” [WHO Technical Report Series No. 878, 1998 – Appendix 1]). As the quality control tests at the time of preparing the cell bank, the following tests were performed for both the MCB and WCB: identity (isoenzyme analysis), purity (DNA fingerprinting), sterility (membrane filtration, direct inoculation), mycoplasma (culture method, DNA-staining method), spiroplasma (culture method, DNA-staining method), adventitious viruses (test by adding cell lysate to 4 kinds of living cells, i.e., cercopithecus kidney cell [Vero cells], human diploid cells [MRC-5 cells], BHK-21 cells, [redacted] cells). For the MCB, the following tests were performed additionally: identity (morphological test by light and electron microscopy), adventitious viruses (inoculation of cell lysate to adult mice, suckling mice, guinea-pigs, embryonated eggs), retroviruses (transmission electron microscopy, PCR-enhanced reverse transcriptase [PERT] assay after infection to

[redacted] cells). According to the results of these tests, it was confirmed that both the MCB and WCB conform to the specifications in terms of all the items tested.

The characteristics and safety of the cells subjected to [redacted] passages exceeding the number of subcultures used in manufacturing (approximately [redacted] passages) (EPC) were analyzed by the following tests: identity (morphological test by light and electron microscopy, isoenzyme analysis), purity (DNA fingerprinting), adventitious viruses (test by adding cell lysate to 4 kinds of living cells, i.e., Vero cells, MRC-5 cells, BHK-21 cells, [redacted] cells, cocultivation tests with the 4 kinds of cells, inoculation of cell lysates and living cells to adult mice, suckling mice, guinea-pigs, embryonated eggs), retroviruses (transmission electron microscopy, PERT assay after infection to [redacted] cells). Conformity was established in terms of all the tests. The applicant explains that, from the results of the identity test (isoenzyme analysis), purity test (DNA fingerprinting) and analyses of population doubling level (PDL), virus content after infection with baculovirus, and production level of the target recombinant protein, it was shown that the cell line was stable after [redacted] passages up to at least [redacted] passages.

The stability of WCB during the storage period has been evaluated by checking the mean population doubling level once a year. As of [redacted] 20[redacted], 5-year stability has been confirmed in liquid nitrogen (-196 °C), and the applicant explains that it intends to continue the stability evaluation in future.

The following characterization analyses were performed, though not specified as the cell bank quality control test items.

Since [redacted] cells are derived from insects, the following tests were experimentally performed to check the presence/absence of any insect virus and the insect-mediated arbovirus which is known to infect mammals including humans: (i) detection of cytopathic effect and haemagglutination using [redacted] cells and [redacted] cells (MCB and EPC), (ii) PCR detection of *Trichoplusia ni*-infecting insect virus and [redacted]-derived insect virus in co-culture with insect cells for detection (MCB and EPC), (iii) PCR detection of *Trichoplusia ni*-infecting insect virus in co-culture with insect cells for detection (MCB and EPC), and (iv) detection of viral RNAs by *in vivo* cell labelling in presence of [redacted] (MCB, WBC and EPC). As a result, no virus was detected in any tests, and it was concluded that neither the current MCB and WCB nor EPC are contaminated with any exogenous insect virus or arbovirus.

In order to investigate the possible tumor-forming capacity of [redacted] cells, *in vitro* growth capacity in a soft agar culture medium and *in vivo* tumor formation after implantation to nude

mice were examined. As a result, colony formation in the soft agar culture medium was seen but tumor formation in nude mice was not seen, so it was concluded that [redacted] cells have no tumor-forming capacity.

When the fine structure of [redacted] cells was analyzed by electron microscopy, a cluster of subspherical particle-like structures with a high electron density about [redacted] nm in size was detected in the cytoplasm (hereinafter referred to as "cytoplasmic cluster of particle-like structures"), which were investigated for characteristics and natures. As a result, the applicant drew the following conclusions for the cytoplasmic cluster of particle-like structures: (i) the frequency of appearance in the cytoplasm is low (< [redacted]%), but elimination by cloning is not possible, (ii) appearance in the nucleus has never been detected so far, (iii) no increase is seen even when cultured under stress, and no replicative capability is observed, (iv) there is no capacity to infect mammal animal cells, insect cells or animal bodies, (v) since no pathogens such as insect virus have been detected so far from the screening of the cell bank, it is unlikely to be insect virus and (vi) the product safety is not affected when considering the virus clearance ability of the manufacturing processes (described later).

b. Recombinant baculovirus seed/lot system

The recombinant baculovirus encoding the HPV-16 L1 truncated gene ([redacted]) was prepared using a recombinant baculovirus transfer vector ([redacted]) and a protease-deficient nucleopolyhedrovirus vector [redacted]([redacted]) and is controlled by the 2-stage seed/lot system of master seed (MS) and working seed (WS). The methods to establish and control the seed/lot system are shown below.

In the [redacted], a gene encoding the HPV-16 L1 protein consisting of [redacted] amino acids where C-terminal [redacted] residues were truncated has been cloned, and the sequence is the same as the base sequence of DNA isolated from the condyloma acuminatum specimen of the German Cancer Research Center ([redacted] sequence). [redacted] has a polyhedrin promoter and the [redacted] sequence downstream to it between the sequences homologous to the baculovirus genome, and a target sequence can be introduced to the baculovirus vector by homologous recombination.

[redacted] was prepared by transfection of [redacted] and [redacted] to [redacted] cells using the calcium phosphate co-precipitation method, i.e., by homologous recombination between these vectors, followed by separation through plaque purification to yield pre-master seed. The base sequences of the truncated HPV-16 L1 gene region and 3' and 5' -flanking region were determined in [redacted], and it was confirmed that the sequence encoded by [redacted] was

retained. When [redacted] was infected to [redacted] cells, HPV-16 L1 protein was produced, and it was confirmed that (i) molecular weight of about [redacted] kDa (SDS-PAGE), (ii) VLP formation, and (iii) reactivity with a monoclonal antibody recognizing HPV-16 L1 VLP specifically ([redacted] mAb). The pre-master seed was confirmed negative for any RNA variants of bacterium, yeast, mycoplasma and insect flock house virus (FHV).

HPV-16 L1 master seed (hereinafter referred to as "MS") was obtained as follows. From [redacted] MCB ([redacted] passages), the cells were amplified up to [redacted] passages, and after the pre-master seed was infected so that the multiplicity of infection (MOI) was [redacted], the cells were cultured for [redacted] days and centrifuged. The MS was obtained as the supernatant, added with glycerol so that the final concentration was [redacted] vol%, dispensed into [redacted] containers ([redacted] mL) and cryopreserved below -70°C. The amount of MS is sufficient for at least [redacted]-year use.

HPV-16 L1 working seed (hereinafter referred to as "WS") was prepared in the same way as MS except for infecting the MS to the cells so that MOI was [redacted] and cryopreserved in the same way. For WS renewal, the following rule has been established: the WS should be renewed with the same procedures as those used in the initial preparation, and the renewed WS should be subjected to the same quality control tests as those used initially and in addition, the tests on genetic stability of seed, baculovirus content, production level of the target L1 protein, and quality shall be performed for the purpose of confirming conformity to the specifications. The control [redacted] cells were prepared in parallel with preparation of the MS and WS and used in the quality control tests (described later) of MS and WS.

The quality control tests of the baculovirus seed and the relevant control cells have been specified based on the Committee for Medicinal Products for Human Use (CHMP) Scientific Advice in March 2005 according to the guidelines for mammal cell lines (mentioned before) and European Pharmacopoeia 2.6.16 "Tests for extraneous agents in viral vaccines for human use" .

For both the MS and WS, the following tests were performed: identity (HPV-16 L1 gene type [PCR]), sterility (membrane filtration method), mycoplasma (culture method, DNA-staining method), spiroplasma (culture method, DNA-staining method), *Mycobacterium tuberculosis* (culture method), adventitious viruses (tests by adding cell lysate to 4 kinds of cells, i.e., Vero cells, MRC-5 cells, BHK-21 cells, [redacted] cells) and baculovirus content (titration). For the WS, furthermore the following tests were performed: adventitious viruses (inoculation of cell lysate to adult mice, suckling mice, guinea-pigs) and retroviruses (PERT assay after infection to [redacted] cells). The results of these tests clarified that (i) the introduced gene is maintained completely, (ii) the infection potency of baculovirus is maintained, and (iii) the seed is not contaminated with any

adventitious pathogen. Also, for the control cells, the following tests were performed: microscopic observation (cell degeneration during the culture period or presence of adventitious haemagglutinating viruses after the culture), identity (isoenzyme analysis), mycoplasma (culture method, DNA-staining method) and adventitious viruses (test by adding cell lysate to 4 kinds of cells, i.e., Vero cells, MRC-5 cells, BHK-21 cells, [redacted] cells). None of the results of these tests suggested contamination with any adventitious pathogen.

In addition, the following items were investigated as biological natures and genetic characteristics.

For the WS and control cells, detection of [redacted] RNA variant (direct detection, detection after inoculation to [redacted] cells), detection of any insect virus and detection of insect-mediated arbovirus (detection of a cytopathic effect or haemagglutinating effect using [redacted] cells and [redacted] cells, PCR detection of *Trichoplusia ni*-infecting insect virus in co-culture with insect cells for detection) were attempted, and none of these viruses were detected.

Using the HPV-16 L1 recombinant baculovirus samples taken from the MS, intra-manufacturing harvests and post-production harvests (harvests obtained from [redacted] passages of the [redacted] cells exceeding the cell age in the normal manufacturing), the base sequence of the part including the HPV-16 L1 translation site was determined, and it was demonstrated that the L1 sequence is consistent with the theoretical sequence. The DNA extracted from each of such samples was subjected to Southern blot analysis after [redacted]. In all the samples, the band was detected at the position expected from the theoretical sequence. From these results, the applicant explains that the L1-inserted sequence and the baculovirus backbone sequence are maintained during the manufacturing steps and even when subcultured after the manufacturing steps.

The applicant explains that, as for the seed/lot stability, the virus infection potency of the WS is being determined every year, and the stability has been confirmed for at least [redacted] months as of [redacted] 20[redacted].

c. Inocula production

The HPV-16 L1 inocula is manufactured as follows.

After thawing, the [redacted] WCB cells are subcultured [redacted] times in [redacted]-mL shake flasks and [redacted] in [redacted]-mL shake flasks (pre-culture process) and then cultured in a sterile bioreactor ([redacted] L) for [redacted] day under conditions of controlled cell density, dissolved oxygen level and temperature. At the end of culture, the HPV-16 L1 baculovirus WS is added at MOI of [redacted], followed by

additional [redacted]-day culture under controlled dissolved oxygen level and temperature (infection process). Thereafter, glycerol is added to the cell/baculovirus suspension so that the final concentration is [redacted] vol%, and the resulting fluid is dispensed into [redacted] containers and cryopreserved below -70ºC as HPV-16 L1 inocula.

Among the manufacturing processes, control values have been set in the cell-thawing process (removal of cryopreservation agent), the processes of culture in shake flasks and bioreactors, and the baculovirus infection process (virus inoculation and [redacted] culture in bioreactors). For the baculovirus WS-adding process, an in-process control test ([redacted]) has been set.

The HPV-18 L1 inocula is manufactured in the same way as the HPV-16 L1 inocula except for infecting the HPV-18 L1 baculovirus WS to [redacted] WCB.

For the HPV-16 L1 inocula and HPV-18 L1 inocula, the shelf life has been set as [redacted] months as of [redacted] 20[redacted] based on the results of determining the baculovirus potency and L1 antigen production capacity of the lots manufactured so far (HPV-16, 1 development lot and 3 commercial lots; HPV-18, 2 development lots and 3 commercial lots).

d. L1 harvest

The HPV-16 L1 harvest is manufactured as follows.

After thawing, the [redacted] WCB cells are subcultured [redacted] times in [redacted]-mL shake flasks, and [redacted] in [redacted]-mL shake flasks, cultured in a sterile bioreactor ([redacted] L) for [redacted] days, and then in a bioreactor ([redacted] L) for [redacted] hours as a pre-culture process. The cell suspension obtained is transferred into a [redacted]-L bioreactor, and the volume is adjusted to [redacted] L. After culturing for [redacted] hours, the HPV-16 L1 inoculation seed is added at MOI of [redacted]. Thereafter, the agitation speed is reduced and oxygen supply is stopped for [redacted] hours (baculovirus adsorption step). After adding the [redacted] / [redacted] together with the fresh culture medium and making the volume [redacted] L, oxygen supply is resumed and the agitation speed is increased. After culturing for [redacted] days, the [redacted] / [redacted] is added again and the culture is continued for additional [redacted] days to express HPV-16 L1 in the cytoplasm. The infected cell suspension is retrieved as the HPV-16 L1 harvest.

Among the manufacturing processes, control values have been set in the cell-thawing process (removal of cryopreservation agent), all the culture processes and the baculovirus infection process. For the HPV-16 L1 inocula addition process and the viral adsorption process, an in-process control test ([redacted]) has been set.

The HPV-18 L1 harvest is manufactured in the same way as the HPV-16 L1 harvest except for using the different inoculation seed.

e. Extraction and purification of L1 protein

The cell suspension is centrifuged, and the infected cells are retrieved from the bioreactor and re-suspended in the buffer for extraction to release the L1 protein expressed in the cytoplasm. Thereafter, the cross-flow (TFF) filtration (μm) is performed to remove cell fragments (clarification).

The HPV-16 L1 protein is purified as follows:

i) Anion exchange chromatography step 1 ([ ] column)
ii) Anion exchange chromatography step 2 ([ ] column)
iii) Affinity chromatography ([ ] column)
iv) Nano filtration process (filter)
v) Ultrafiltration process
vi) Anion exchange chromatography step 3 ([ ] column)
vii) Sterilization filtration process (sterilization filter)

The major objective of each process is as follows: i)-iii) removal of impurities such as protein, DNA, etc.; iv) clearance of viruses; v) removal of (hereinafter abbreviated into ,) and adjustment of pH and electroconductivity (these procedures result in self-assembly into virus-like particles [VLP]); vi) removal of impurities (protein); and vii) sterilization. While is present at a certain concentration during the procedures of i) - iv), the folding to VLP is suppressed and the L1 protein is kept in the state of capsomere (pentamer, etc.).

The sterile HPV-16 L1 VLP purified bulk thus obtained is immediately filled in a steel container dedicated for manufacturing the monovalent adsorbed bulk [see "2.A.(2).2) Manufacturing method"] and stored in the temperature range of 18.0-25.0°C or 2-8° C (maximum 3 days).

The purification process is performed in a closed system using sterile buffers and solutions containing no pyrogen with operating the columns and pumps. All the filters and chromatographic solvents are dedicated for the HPV-16 L1 protein. The purification process is performed at .

Among the manufacturing processes, control values have been set for the centrifugation process, process to extract L1 protein from the buffer purification, and processes i) - iii) and vi), and filter specifications have been set for each of purification process iv), v) and vii), and an in-process control test (content) is prescribed for purification process v).

The HPV-18 L1 purified bulk is manufactured in the same way as the HPV-16 L1 purified bulk except for using [redacted] column in the purification process of vi).

f. Control of critical steps and critical intermediates

Among the drug substance manufacturing processes, the inocula manufacturing, L1 harvest manufacturing and L1 protein purification are handled as the critical steps.

The inocula and L1-harvests are handled as the critical process intermediates. As the quality control tests, in addition to the tests to detect adventitious viruses, bacteria and fungi, the other tests are performed to confirm that the inocula, recombinant baculoviruses in the inocula and the control cells maintain the original characteristics and gene structures. In the adventitious virus test, since the target cell can propagate baculovirus, the baculovirus-reactive [redacted] antiserum is used to neutralize the infection potency of baculovirus in advance.

The following quality control tests have been set for the control cells (the cells as well as the cell lysate) used for manufacturing the inocula and the inocula themselves.

For the control cells, the following tests have been set: identity (isozyme analysis) and microscopic observation (cell degeneration or adventitious haemagglutinating viruses); and for the cell lysate, mycoplasma (culture method, DNA-staining method) and adventitious viruses (Vero cells, MRC-5 cells, BHK-21 cells, [redacted] cells). For the inocula, the following tests have been set: identity (HPV-16/18 L1 gene type [PCR]), sterility (membrane filtration method), mycoplasma (culture method, DNA-staining method), *Mycobacterium tuberculosis* (culture method), adventitious viruses (Vero cells, MRC-5 cells, BHK-21 cells, [redacted] cells) and baculovirus content assay (infection potency).

The following quality control tests have been set for the control cells (the cells as well as the cell lysate) used for manufacturing the L1 harvests and the L1 harvests themselves.

For the control cells, the following tests have been set: identity (isozyme analysis) and microscopic observation (cell degeneration or adventitious haemagglutinating viruses); and for the cell lysate, mycoplasma (culture method) and adventitious viruses (Vero cells, MRC-5 cells, BHK-21 cells, [redacted] cells). For the L1 harvests, the following tests have been set: sterility (membrane filtration method), mycoplasma (culture method), *Mycrobacterium tuberculosis* (culture method) and adventitious viruses (Vero cells, MRC-5 cells, BHK-21 cells, [redacted] cells).

In addition to the storage periods of the [redacted] WCB, WS, inocula and sterile purified bulks, the shelf life of materials used in the purification processes and the storage periods of culture media are also handled as control items.

g. Safety evaluation of adventitious agents

The following table shows the quality control tests performed during the drug substance-manufacturing processes for the purpose of bacterium/mycoplasma/fungus detection.

Table 1. Quality control tests for the detection of bacteria, mycoplasma and fungi conducted during the manufacture of the HPV vaccine

Tests	Analytical Reference	MCB & WCB	HPV-16 or HPV-18 MS & WS	HPV-16 or HPV-18 MS & WS control cells	HPV-16 or HPV-18 inocula	HPV-16 or HPV-18 inocula control cells	HPV-16 or HPV-18 L1 harvest	HPV-16 or HPV-18 L1 harvest control cells
Sterility testing (bacteria and fungi)								
TSB	JP	×	×		×		×	
FTM		×	×		×		×	
Mycoplasma testing								
by DNA staining	EP	×	×	×	×	×		
by cell culture		×	×	×	×	×	×	×
Spiroplasma testing								
by DNA staining	EP	×	×					
by cell culture		×	×					
Mycobacterium tuberculosis testing								
by cell culture	EP		×		×		×	

JP: Japanese Pharmacopoeia
EP: European Pharmacopoeia

Also, the viral removal capacity of the entire purification processes (including clearance of baculovirus) was evaluated according to the PMSB/ELD Notification No. 329 dated February 22, 2000 (ICH Q5A) and the EU guidelines. The results are shown in Table 2. The baculovirus used in the viral clearance test is the same as the virus used in manufacturing L1 VLP except for not encoding the L1 gene.

Table 2. Viral clearance study results

Virus	X-MuLV		baculovirus		FHV		SV-40	
	RNA virus with envelope		DNA virus with envelope		RNA virus with no envelope		DNA virus with no envelope	
L1 Purification Process	HPV-16	HPV-18	HPV-16	HPV-18	HPV-16	HPV-18	HPV-16	HPV-18
Anion exchange chromatography step 1 ([redacted])								
New resin	≥[redacted]	≥[redacted]	≥[redacted]	≥[redacted]	[redacted]	[redacted]	≥[redacted]	[redacted]*
Old resin	≥[redacted]	≥[redacted]	≥[redacted]	≥[redacted]	[redacted]	[redacted]	≥[redacted]	≥[redacted]
Affinity chromatography step ([redacted])								
New resin	≥[redacted]	≥[redacted]	≥[redacted]	≥[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Old resin	≥[redacted]	≥[redacted]	≥[redacted]	≥[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Nanometric filtration step								
Run 1	≥[redacted]	≥[redacted]	≥[redacted]	≥[redacted]	≥[redacted]	≥[redacted]	≥[redacted] ≥[redacted]	≥[redacted]
Run 2	≥[redacted]	≥[redacted]	≥[redacted]	≥[redacted]	≥[redacted]	≥[redacted]	[redacted]* ≥[redacted]	≥[redacted]
Total	-	-	(≥15.9)	(≥19.7)	(≥13.3)	(≥12.1)	(≥14.7)	(≥13.5)

Log_{10} reduction factors (mean values) are written to two significant digits and their standard deviations are omitted.
*: these standard deviations are >0.5.

In all of the 3 processes evaluated, [redacted] exists in the solution at a certain concentration. In the viral clearance test using the eluent of anion exchange chromatography step 1 ([redacted]), the inactivating effect of [redacted] (18-hour contact) was as follows: X-MuLV ≥[redacted] (HPV-16) and≥ [redacted] (HPV-18); baculovirus, [redacted] (HPV-16) and [redacted] (HPV-18); FHV, [redacted] (HPV-16) and [redacted] (HPV-18); and SV-40, [redacted] (HPV-16) and [redacted] (HPV-18). Including this inactivating effect of [redacted] itself, the applicant explained that the L1 VLP purification processes (from the [redacted] process to the nano filtration process) have a sufficient virus removing capacity.

h. Removal of impurities

As for the impurities derived from the insect cell-baculovirus expression system, the efficiency to remove the DNA and host cell protein (HCP) was evaluated with [redacted] commercial lots. The DNA was removed by the anion exchange chromatography step 1 and the amount of DNA contained per mg protein was close to the quantitation limit. The remaining DNA was further removed by the anion exchange chromatography step 2 and the affinity chromatography process to less than the quantitation limit and did not increase again to the quantitation limit or higher in the subsequent processes. As for the efficiency to remove the HCP, the removal rate in the purification processes exceeded [redacted] log 10.

The removal rates of infectious recombinant baculovirus and [redacted] that are raw materials used in the manufacturing process were investigated. The results of [redacted] development lots and [redacted] commercial lots indicated that the recombinant baculovirus was removed in the extraction process and clarification process in the very early stage of purification and efficiently removed by the anion exchange chromatography step 1, resulting in no detection in the subsequent processes. For detection, the [redacted] method against [redacted] cells was used. As for [redacted], the results of [redacted] commercial lots indicated that the [redacted] concentration was intentionally maintained at a certain level until the nano filtration process, but a large amount was removed in the subsequent ultrafiltration process so that the concentration of [redacted] in the sterile purified bulk is below or close to the quantification limit.

i. Management of raw materials of human or animal origin

Any raw materials of human or animal origin are not used in the processes after the preparation of [redacted] cell banks (MCB, WCB) to the establishment of HPV-16 L1 and HPV-18 L1 seed/lot (MS, WS) and the sterilization and purification of the bulk.

j. Manufacturing process development (comparability)

In the course of development, after the lots used in the phase I and IIa clinical studies were manufactured, the manufacturing methods were changed because of transfer of manufacturing technology, etc., and the lots used in the phase IIb and III clinical studies were manufactured after the change, followed by establishment of the commercial production processes.

The purified bulk has been evaluated and compared between lots in terms of physicochemical properties (quality control test results for the purified bulk at release, electrophoresis profile, primary and secondary structures of L1, structure of virus-like particle [VLP], impurity amounts), immunological properties (reactivity between the antigens and the monoclonal antibody or human polyclonal antibody [serum of vaccine receiver] recognizing the stereostructure, immunity-inducing capacity of the formulated antigens in humans and mice) and stability. Thus, the lot consistency has been confirmed.

2.A.(1).2) Characterization analysis

The L1 VLP manufactured at the commercial production scale ([redacted] L) was evaluated for the following physicochemical properties: (i) electrophoresis pattern of L1 protein (SDS-PAGE under reductive conditions [Coomassie staining and silver staining], Western blot method using L1 VLP-specific antibody, capillary electrophoresis), (ii) molecular weight (mass spectrometry [ES-MS]), (iii) L1 protein primary structure (amino acid analysis, peptide mapping, N-terminal and C-terminal sequence analysis), (iv) L1 protein secondary structure (Fourier transform infrared spectroscopy [FTIR], circular dichroism spectrometry [CD], differential scanning calorimetry), (v) VLP structure and size (size exclusion chromatography [SEC], electron microscopy, disc centrifuge sedimentation [DCS]). As a result of DCS, the particle size distributed mainly around [redacted] nm (more than [redacted] % of the particles were in the range of [redacted] nm, the rest in the range of [redacted] nm) in the case of HPV-16 and around [redacted] nm (more than [redacted] % of the particles were in the range of [redacted] nm, the rest in the range of [redacted] nm) in the case of HPV-18. The mean number of L1 molecules in each VLP was calculated to be [redacted] based on the results of high-performance size exclusion chromatography and [redacted] method ([redacted]). The numbers were close to [redacted] being the theoretical number on arranging the HPV capsid proteins neatly as a regular icosahedra, suggesting the formation of virus-like particles together with the electron microscope image. Also, the following items were evaluated as immunological properties: (i) affinity between VLP and monoclonal antibody against L1 capsid protein ([redacted]scopy), (ii) L1 VLP potency (ELISA), (iii) interaction between L1 VLP and polyclonal antibody (ELISA), (iv) immunogenicity of L1 VLP in mice under

coexistence of AS04 adjuvant (determination of anti-L1 VLP antibody titer [ELISA], production amounts of [redacted] and [redacted]).

As impurities, DNA, host cell protein (HCP), infectious recombinant baculovirus and [redacted] were quantitatively determined using commercial lots. Each testing method was validated, and all the determination results were less than each quantitation limit as mentioned in the section of "2.A.(1).1) h. Removal of impurities". The amount of DNA, HCP and [redacted] contained in each vaccine dose was calculated to be < [redacted] pg, < [redacted] ng and [redacted] μg (note that the maximum daily acceptable amount of [redacted] is [redacted] mg), respectively, based on the actual lot analysis results. The applicant explains that these amounts are considered sufficiently safe taking into account the number of vaccination in clinical use.

2.A.(1).3) Specifications and test methods

As the specifications and test methods of the drug substance, i.e., L1 VLP purified bulk, the following tests have been set: identity (ELISA), purity (SDS-PAGE, Coomassie staining), endotoxin (kinetic colorimetry), protein content assay (nitrogen assay), L1 VLP potency (ELISA).

2.A.(1).4) Reference material

The L1 VLP purified bulk which was manufactured in the past and used in the clinical development is currently used as the reference material for potency testing of L1 VLP purified bulk. The reference material has been confirmed to conform to the release specifications through the following tests: identity (ELISA), purity (SDS-PAGE, Coomassie staining), sterility (membrane filtration method), endotoxin (kinetic colorimetry) and protein content assay (nitrogen assay).

To establish a new reference material in the future, it is stipulated as follows: The candidate reference material shall be confirmed to conform to the current release specifications of L1 VLP purified bulk, and the protein content (nitrogen assay) and L1 VLP potency (ELISA) shall be evaluated in the tests performed as [redacted] separate runs. An assay internal control and at least [redacted] commercial lot of L1 VLP purified bulk shall be tested using both the old and the new reference materials, and when the difference in the obtained test results is not > [redacted]%, the new reference material can be regarded as validated.

2.A.(1).5) Stability

Two lots of L1 VLP purified bulk manufactured at the commercial production scale were subjected to stability tests (dedicated sterile stainless steel containers, 25±1°C / 3 days or 2-8°C /

10 days, and also at 25±1°C/10 days or 2 - 8 °C/3 days for HPV-18). The appearance, protein content (nitrogen assay), purity (SDS-PAGE), potency (L1 protein / total protein), VLP structure (electron microscopy), VLP particle size (disc centrifuge sedimentation) and L1 protein electrophoresis pattern (Western blot method) were evaluated.

When stored for [redacted] days, no changes were recognized, but when stored at [redacted]°C for [redacted] days, the HPV-18 L1 VLP purified bulk showed a tendency toward a change of VLP structure and protein aggregation, so the storage method and shelf life have been set as [redacted] days at [redacted]°C or [redacted]°C.

2.A.(2) Drug product

2.A.(2).1) Formulation, container closure system

The single-dose portion (0.5 mL) of this drug product contains 20 μg each of HPV-16 L1 VLP and HPV-18 L1 VLP as antigen proteins and AS04 (aluminum hydroxide [500 μg as aluminium] and MPL [50 μg]) as an adjuvant. In addition, tonicity agents, buffering agents and solvents are contained. To assure the one-dose portion, a prefilled syringe is used.

The syringe and attached needle are used only for this drug product. The syringe is made of silicon-coated glass, conforming to the Test for Glass Containers of the Japanese Pharmacopoeia. The needle conforms to JIS T3209 except for the section of “15. Display, 15. 2 Secondary package”.

The formulation was changed once during the clinical development period. The amount of [redacted] was changed on preparing the [redacted], but the contents of [redacted] and [redacted] in the final drug product were not changed. The comparability between the drug products before and after this change was confirmed by (i) the quality control test results, (ii) the characterization analysis results, (iii) the stability test results and (iv) the results of comparing immune responses to formulated antigens in mice and humans.

2.A.(2).2) Manufacturing method

The drug product-manufacturing method consists of (i) the process of adsorbing the HPV-16 and HPV-18 L1 VLP antigens to aluminum hydroxide (L1 VLP-adsorbed monovalent bulk [AMB] manufacturing process), (ii) the MPL liquid bulk manufacturing process, (iii) the process of adsorbing the MPL liquid bulk to aluminum hydroxide (MPL-adsorbed bulk manufacturing process), (iv) the final bulk manufacturing process, (v) the filling process and (vi) the packaging process. Among these, the MPL liquid bulk manufacturing process is handled as the critical process step.

Each L1 VLP purified bulk is added with aluminum hydroxide ([redacted] μg of aluminum ion per [redacted] μg of L1 VLP), followed by stirring at [redacted], to prepare each adsorbed monovalent bulk (L1 VLP AMB). Meanwhile, MPL is dispersed in water [redacted], [redacted] and then [redacted]. It is [redacted] and subjected to sterilization filtration to obtain the MPL liquid bulk. The MPL liquid bulk is added with aluminum hydroxide suspension ([redacted] mg of aluminum ion per [redacted] μg of MPL) for adsorbing to aluminum hydroxide to prepare the MPL adsorbed bulk. Each L1 VLP AMB and the MPL adsorbed bulk are added with the aluminum hydroxide suspension followed by stirring and pH adjustment to obtain the final bulk. The final bulk is filled into glass syringes and sealed under sterile conditions (final container), followed by labeling and storage.

In the drug product manufacturing processes, no human-derived raw materials are used. The MPL is of bacterial origin, and the casamino acids used for manufacturing MPL are derived from bovine milk (New Zealand, Australia) [see "2.A.(2).5) Novel excipients"].

For each L1 VLP AMB, the MPL liquid bulk and the MPL adsorpted bulk, quality control tests are performed. Also, in the MPL liquid bulk manufacturing process, the process is monitored to evaluate the process consistency and an in-process control test ([redacted]) is performed. In the MPL adsorbed bulk manufacturing process and the AMB manufacturing process, no in-process control tests are performed.

For each L1 VLP AMB, identity (ELISA), sterility (membrane filtration method), protein content (nitrogen assay) and completeness of adsorption to aluminium component (nitrogen assay) are evaluated, and the analysis results of [redacted] commercial batches have been submitted. For the MPL liquid bulk, descriptions (visual observation), MPL congener distribution ([redacted], [redacted], [redacted] and [redacted] [HPLC]), MPL content (GC), sterility (membrane filtration method) and particle size ([redacted] spectroscopy) are evaluated, and the analysis results of [redacted] commercial batches have been submitted. For the MPL adsorbed bulk, description (visual observation), pH, completeness of adsorption to aluminium component – unbound MPL (GC) and sterility (membrane filtration method) are evaluated, and the analysis results of [redacted] commercial batches have been submitted. For the MPL liquid bulk, the amount of [redacted] produced from a [redacted] cell line ([redacted]) stimulated with the MPL solution is determined as one of its characteristics, and the applicant explains that all of the [redacted] commercial batches showed the similar results.

2.A.(2).3) Specifications and test methods

For the drug product (final bulk), the sterility test (membrane filtration method) has been set. For the drug product (final container), the following specifications and test methods have been set:

description (visual observation), identity (HPV-16 L1 VLP [ELISA]), identity (HPV-18 L1 VLP [ELISA]), sterility (membrane filtration method), abnormal toxicity, pH, extractable volume, insoluble foreign matter, protein content, *in vitro* HPV-16 L1 VLP potency, i*n vitro* HPV-18 L1 VLP potency, aluminum content, MPL content, completeness of adsorption to aluminium component – unbound MPL, completeness of adsorption to aluminium component – unbound HPV-16 L1 VLP, and completeness of adsorption to aluminium component – unbound HPV-18 L1 VLP. As the lot analysis results, the results of 3 final bulk lots (commercial lots), 3 final container product lots (syringe-filled product, commercial lots) and final container product lots (development lots) have been submitted.

2.A.(2).4) Reference material

For the *in vitro* potency test, one final container product lot is used as the reference material as of [redacted] 20[redacted], and the analysis results of this lot have been submitted. To newly establish a new reference material, it is stipulated as follows: The candidate reference material shall be confirmed to conform to the current specifications of final container product and shall be determined for potency more than [redacted] times; and when the difference between the old reference material and the new reference material is less than the variation observed in the validation of the testing method, the new reference material can be regarded as validated. As of [redacted] 20[redacted], there is no established shelf life for the current reference material.

2.A.(2).5) Novel excipients

As novel excipients, this drug product contains MPL and aluminum hydroxide (both are adjuvant ingredients) [see "2.B.(8) Novel excipients"].

MPL is a non-toxic derivative of lipid A originating from lipopolysaccharide (LPS) of Gram-negative bacteria and is added as an immunostimulant. *Salmonella minnesota* Strain R595 is cultured, and LPS is extracted from the bacteria with [redacted] and [redacted]/[redacted]. The extract is [redacted] and [redacted] to obtain crude MPL, which is purified by anion exchange chromatography, and the [redacted] MPL is obtained by using a [redacted]. The [redacted] MPL is converted into a [redacted] ([redacted]) salt and freeze-dried to obtain [redacted] MPL (powder).

Concerning the impurities derived from the MPL manufacturing process (LPS and [redacted] [[redacted]]), the applicant explains that all the MPL lots were subjected to pyrogen test in rabbits and more than 140 lots of [redacted] MPL were subjected to multiple tests including characterization using [redacted], which demonstrated that these impurities could be removed efficiently in the manufacturing processes. In addition, the applicant explains that the results of liquid chromatography for multiple process intermediates show that [redacted] is detected in

the crude MPL at a content of about [redacted]% yet [redacted] can be efficiently removed in the subsequent purification process (ion exchange chromatography).

As the specifications and test methods of MPL, the following tests have been set: description, identity (thin-layer chromatography), hexosamine, phosphate, congener distribution ratio, triethylamine, water content, free fatty acids, microbial limit, pyrogen and residual solvents. It was confirmed from the lot analysis results that the [redacted] lots for clinical study and commercial production conformed to the specifications. In a long-term testing (2-8°C), 60-month stability was confirmed. The lot fulfilling the 4 additional tests other than the quality control tests above is used as the reference material.

2.A.(2).6) Stability

For stability evaluation of the final container products manufactured in the production scale, a long-term testing (2-8°C) was conducted using 3 lots for clinical trial and 3 commercial lots. The stability during the transport (37±1°C and 2-8°C, 25±1°C and 2-8°C) was also investigated. Since this drug product is packaged in a paper box and is to be stored at 2-8°C, normally in a dark place in a refrigerator or a cold box, a photostability test was judged unnecessary and thus not performed.

As for the long-term testing, results of 36-month storage of 3 lots for clinical trial and 24-month storage of 3 commercial lots were obtained (the lots have been tested for 48 months as of April 2009). The test items in the long-term testing were descriptions (visual observation), identity (HPV-16 L1 VLP [ELISA]), identity (HPV-18 L1 VLP [ELISA]), sterility (membrane filtration method), abnormal toxicity, pH, extractable volume, protein content, aluminum content, HPV-16 L1 VLP potency (mice), HPV-18 L1 VLP potency (mice), osmolality, completeness of adsorption to aluminium component – unbound HPV-16 L1 VLP, completeness of adsorption to aluminium component – unbound HPV-18 L1 VLP, completeness of adsorption to aluminium component – unbound MPL, MPL content, HPV-16 L1 VLP potency (ELISA), HPV-18 L1 VLP potency (ELISA), Western blotting, SDS-PAGE (Coomassie staining and silver staining), HPV-16 L1 VLP potency (ELISA, after aluminum adsorption), HPV-18 L1 VLP potency (ELISA, after aluminum adsorption), completeness of adsorption to aluminium component – unbound HPV-16 and HPV-18 L1 VLP, and container closure integrity. It was confirmed that results of each test either complied with the acceptance criteria or remained stable without change.

For evaluation of transport stability of the final container products, the stability tests were performed under the following conditions (number of lots) using the lots for clinical trial.

・Seven-day storage at 37±1°C followed by 36-month storage at 2-8°C (3 lots)
・Thirty-six-month storage at 2-8°C followed by 7 day storage at 37±1°C (3 lots)
・Eleven-month storage at 2-8°C followed by 1-month storage at 25±1°C and 24-month storage at 2-8°C (2 lots)

The tests for transport stability were descriptions (visual observation), sterility, pH, HPV-16 L1 VLP potency (mice), HPV-18 L1 VLP potency (mice), completeness of adsorption to aluminium component – unbound MPL, completeness of adsorption to aluminium component – unbound HPV-16 L1 VLP, completeness of adsorption to aluminium component – unbound HPV-18 L1 VLP, HPV-16 L1 VLP potency (ELISA), HPV-18 L1 VLP potency (ELISA), HPV-16 L1 VLP potency (ELISA, after aluminum adsorption), HPV-18 L1 VLP potency (ELISA, after aluminum adsorption), Western blotting, SDS-PAGE (Coomassie staining and silver staining), and completeness of adsorption to aluminium component – unbound HPV-16 and HPV-18 L1 VLP. It was confirmed that the results of each test either complied with the acceptance criteria or remained stable without change.

2.B. Outline of the review by PMDA

2.B.(1) Cytoplasmic cluster of particle-like structures observed in [redacted] cells

Since it was explained in the documents submitted at filing of the new drug application that the origin of the cytoplasmic cluster of particle-like structures in [redacted] cells observed by electron microscopic analysis was unclear, PMDA asked the applicant to provide new findings, if obtained after filing of the application. The applicant replied that no particular new findings had been obtained.

PMDA considers as follows:

When considering the investigation of cell bank conducted up to present, reduction of contamination risk of the drug product expected from the manufacturing process, and information on the safety of this vaccine accumulated up to present, etc., it is not justified at present to hold approval on this vaccine because of the presence of the particle-like structures in host cells. However, since the origin, behaviour, etc. of these structures are still unknown, the applicant should conduct further data collection and analysis, for example, by using isolated structures (if technically possible), and provide the information appropriately. In this regard, PMDA is now asking the applicant to clarify the policy to deal with the matter.

2.B.(2) Evaluation of viral clearance

Since [redacted] inactivates virus and a certain amount of [redacted] is present in the solution in the 3 processes (anion exchange chromatography step 1, affinity chromatography process and nano filtration process) evaluated for reduction of virus infection potency, PMDA asked the applicant if viral clearance capacity of the said purification processes had been evaluated properly.

The applicant responded as follows:

Since [redacted] demonstrates virus inactivation, it may not be appropriate to assess the viral clearance capacity in the purification processes as the sum of clearance index values of each process. However, the capacity has been evaluated by spiking model viruses into in-process samples and comparing the samples that have undergone the purification process with untreated samples, and the method has been considered to enable accurate evaluation of the viral clearance capacity in each process. Among the purification processes, at least the nano filtration process and anion exchange chromatography step 1 are effective in clearing virus. Furthermore, it was found that inactivation by [redacted] and affinity chromatography process are effective for enveloped viruses.

PMDA accepted the above response and judged that the said purification processes can effectively inactivate and remove virus.

2.B.(3) Product-related substances and product-related impurities

Since the analysis results of product-related substances and product-related impurities of the drug substance (L1 VLP purified bulk) were not submitted, PMDA asked the applicant for explanation.

The applicant responded as follows:

All bands other than the main band are treated as impurities in the purity test (SAS-PAGE, CB staining). The detected impurities are L1-related proteins and are not derived from cells of expression system. Furthermore, it is technically difficult to isolate the detected impurities. On these basis, further analysis of individual impurities regarding their structures and activities has not been performed, nor have the impurities been determined as to whether they are product-related substances or product-related impurities.

PMDA considers as follows:

Though the information related to impurities should be collected as much as possible, PMDA does not request to present the results of further analysis at present because no safety problem definitely related to impurities has been found in the clinical use of the drug product marketed

under the control of prescribed specifications and test methods. However, it is necessary that the applicant should try to maintain the constant quality under consistent manufacturing process control and at the same time to provide appropriate information when new findings are obtained in the future in line with the progress of technology.

2.B.(4) Consistency of MPL manufacturing process

The applicant explained that the consistency of MPL manufacturing process was confirmed on the basis of quality control data on [redacted] concentrated harvest lots, and [redacted] lots each of [redacted], crude MPL, [redacted] MPL and [redacted] MPL as well as [redacted] lots of MPL.

PMDA considers as follows:

In reviewing the data submitted, the measured values of control tests in each MPL-manufacturing process do not considerably vary among the lots. Since MPL is a non-toxic derivative of lipid A derived from lipopolysaccharides (LPS), it is necessary that MPL should be manufactured continuously under rigid manufacturing process control.

2.B.(5) Control of MPL liquid bulk manufacturing process

The manufacturing process of MPL liquid bulk is prescribed as a critical process step among the drug product manufacturing processes. PMDA asked the applicant to explain the reason for setting this process as important and the control practiced in the said process.

The applicant explained as follows:

The MPL liquid bulk manufacturing process has been set as a critical process step because the quality of this vaccine may be directly influenced by the degree of MPL [redacted] performed in the said process. An in-process control test ([redacted]) is also performed to control the said process.

PMDA is currently checking with the applicant on other control tests of the said process.

2.B.(6) Abnormal toxicity

At filing of the application, abnormal toxicity test was set in the specifications and test methods of the drug product (final container) and it was explained that only the first [redacted] commercial lots would be subjected to this test. PMDA asked the applicant to provide the test results which had ever been performed and also asked if this test had been performed in accordance with the general tests specified in the Minimum Requirements for Biological Products (MRBP) in Japan.

The applicant provided the test results in the [redacted] clinical development lots, the first [redacted] commercial lots, and [redacted] lots manufactured at the manufacturing site where the drug products for Japan and US had been manufactured, and explained that all batches met the specification, thereby confirming the consistency of manufacturing process. As for test methods, it was explained that the test was set in accordance with US 21CFR610.11.

PMDA asked the applicant to set abnormal toxicity test in the specifications and test methods of this drug product in accordance with the general tests specified in the MRBP for the following reasons.

- Only the results of [redacted]+[redacted] commercial lots were obtained in the abnormal toxicity test and it is considered difficult to sufficiently confirm the consistency of manufacturing process explained by the applicant on the basis of these data.
- The observation point (day) after administration of a drug and the assessment criteria/method prescribed in the test method used in Japan are different from those in the US (More specifically, changes in body weight immediately after vaccination [Days 1-2], which is considered to serve as an index for toxicity, are checked in Japan but not in the US. Furthermore, in Japan, a body weight decrease during the observation period is compared with that of a control group and the acceptance criteria is that there's no statistically significant difference. More information thus collected would be useful in capturing the characteristics of drug product.)
- Other test methods would be acceptable if it allows more rational quality control. However, unless a more convincing substitute method is presented, the abnormal toxicity test stipulated in MRBP is considered appropriate for this drug product since the quality of many biological products has been established in Japan by utilizing the MRBP test .

The applicant responded that they would set the abnormal toxicity test in accordance with the general tests specified in the MRBP in the specifications and test methods based on the comment from PMDA.

2.B.(7) Shelf life of the drug product

In the document additionally submitted during the review process, it was explained that two different types of raw materials would be used to manufacture a plunger stopper and a tip cap of a syringe. PMDA asked the applicant to explain the background and if stability test data had been obtained on each container closure system made of respective raw materials.

The applicant replied as follows.

Stability studies of the drug products have been conducted with the plungers and tip caps using [redacted] or [redacted] as a raw material. In the early period after

marketing approval, the drug products made of these materials will be supplied. However, from the viewpoint of availability of raw materials, these will be switched to [redacted] and [redacted] as raw materials to manufacture plunger stoppers and tip caps, respectively. A stability study of the product using the new materials is being separately conducted (it will be continued up to 48 months), and as of [redacted] 20[redacted], the drug product has been confirmed stable for 9 months in the long-term testing.

PMDA considers it necessary to set the shelf life of the drug product with the container closure system using the new materials based on actual measurement because the influence of said change on the quality of drug product has not been fully explained. Since the detailed results, etc. of the stability studies are being checked, discussion on the shelf life of this product is provided in the Review Report (2).

2.B.(8) Novel excipients

2.B.(8).1) Treating MPL as an excipient

Since it is specified in the General Rules for Preparations of the Japanese Pharmacopoeia that "the excipients used must be non-toxic, harmless and pharmacologically inactive in the amount administered", PMDA asked the applicant to explain about the appropriateness of treating MPL contained in this vaccine as an excipient.

The applicant responded as follows:

It is considered that a pharmacological effect of a vaccine is to induce specific active immunization against infections. The effect of MPL on the immune system is nonspecific inflammatory effect and it has been reported that aluminium hydroxide, which is often used as an adjuvant, also has this effect (*Clin Exp Immunol.* 1980;39:426-34). As described above, MPL has only a nonspecific inflammatory effect but no induction effect of specific active immunization against infections, therefore MPL is considered to have no pharmacological effect as a vaccine. In addition, based on the results of nonclinical and clinical studies, it is considered that there are no safety concern in clinical use of this vaccine regarding addition of MPL as an adjuvant.

PMDA considers as follows:

Even though the target efficacy of this vaccine is not attained by MPL alone, MPL demonstrates pharmacological action on the living body. Therefore, in reviewing this application, PMDA decided to evaluate MPL not only as an excipient but also an active ingredient, i.e., including the assessment of the control method in the product manufacturing process [see "2.A.(2).2)

Manufacturing method," "2.A.(2).5) Novel excipients," "2.B.(4) Consistency of MPL manufacturing process," and "2.B.(5) Control of MPL liquid bulk manufacturing process"].

2.B.(8).2) Evaluation of a novel excipient

This drug product contains MPL as an adjuvant which is a novel excipient without precedent use of intramuscular administration. In Japan, aluminium hydroxide already has been used as an adjuvant in intramuscular administration, but the amount used in this proposed product exceeds the amount previously used in Japan.

a. Specifications and test methods and stability

PMDA judged that there was no particular problem in the specifications of aluminum hydroxide suspension. However, as a result of regulatory review, some specifications of MPL have been re-established with reference to the Japanese Pharmacopoeia, etc. As for the stability, PMDA judged that there was no particular problem based on the submitted data.

b. Safety

In view of the nature of adjuvant, it is clear that both MPL and aluminum hydroxide suspension demonstrate actions including local irritation, etc. Furthermore, it was suggested that such reaction would be slightly stronger than conventional adjuvants. However, since these actions are within the acceptable range for clinical use, it was judged that these substances may be clinically used. The local irritation, etc. by these excipients is considered unavoidable in the light of their intended use. However, it is considered problematic to approve these excipients as general additives and to allow their extensive use in other drugs. Therefore, it was judged appropriate to limit the use of these excipients as adjuvants only, while giving consideration to the balance with the benefit of the drug product.

c. Pharmacokinetics

The subnmitted studies on the pharmacokinetics of MPL were limited, but it was judged to cause no particular problem on the basis of test results obtained.

As described in the above, PMDA considered it appropriate to allow the use of MPL and aluminum hydroxide suspension as adjuvants up to their upper limit dosage proposed in this application, and judged that the usage should not be regarded as a general precedent.

3. Non-clinical data

3.(i) Outline of the results of pharmacological studies

3.(i).A Summary of the submitted data

The data submitted in this application includes results of pharmacology studies that investigated 3-deacylated-4'-monophosphoryllipid A (MPL) alone. In the primary pharmacodynamics studies, the influence of an lipopolysaccharides (LPS) derivative MPL on various factors related to the natural immunity was investigated on the basis of findings that LPS is a substance to induce potent natural immunity. Based on the result, the applicant explains that MPL plays an important role in enhancing the maturity of antigen-presenting cell and activating natural immunity, and that the involvement of TLR4 is suggested in the recognition. Furthermore, the applicant explains that the influence of MPL on the cardiovascular system and respiratory system was assessed in beagle dogs in a safety pharmacology study, and that the result indicated that MPL did not induce any undesirable pharmacological action on the respiratory and circulatory system.

3.(i).A.(1) Primary pharmacodynamics

As the studies to support the efficacy, 2 lines of studies were performed, i.e., on AS04 adjuvant [3.(i).A.(1).1)] and this vaccine [3.(i).A.(1).2) - 3.(i).A.(1).4)].

3.(i).A.(1).1) Evaluation of immunological characteristics of AS04 adjuvant

a. Effects on the production of inflammatory cytokines in human monocytes

The human peripheral blood monocytes (PBMC) were incubated at 37° for 6 hours in the presence of (i) 100 μg/mL of aluminum hydroxide, (ii) 3-deacylated-4'-monophosphoryllipid A (MPL) (0.1, 1 or 10 μg/mL) or (iii) AS04 (prepared by adsorption of MPL 0.1, 1 or 10 μg/mL to 1, 10 or 100 μg/mL of aluminum hydroxide, respectively), and the intracellular expression of TNF-α and IL-6 in CD14-positive monocytes was assessed by flow cytometry. The ratio of monocytes that produce TNF-α and IL-6 was similar to the background level in the presence of aluminum hydroxide alone. However, the production of cytokines was observed in the presence of AS04 as in the case of MPL. Accordingly, it was indicated that MPL and AS04 have similar monocyte-stimulating effects.

b. Effects on TNF-α production in human monocyte cell lines

Human monocyte cell lines U937 that were differentiated by phorbol myristate acetate (PMA) were incubated for 4 hours in the presence of (i) aluminum hydroxide (10, 30 or 100 μg/mL), (ii) MPL (1, 3 or 10 μg/mL) or (iii) AS04 (prepared by adsorption of MPL 1, 3 or 10 μg/mL to 10, 30 or 100 μg/mL of aluminum hydroxide, respectively), after which the TNF-α production in the culture supernatant was assessed by enzyme-linked immunosorbent assay (ELISA). The TNF-α production was at a very low level in the presence of aluminum hydroxide alone while the TNF-α

production of at a level and dose dependency observed in the presence of AS04 at various concentrations were similar to those in the presence of MPL. Based on the above data, it was indicated that AS04 demonstrates stimulation similar to that observed with MPL both in PBMC and human monocyte cell lines U937.

c. Effects of antigen presenting cells (APC) on CD40 expression *in vivo* in mouse

C57BL/6 mice (female, 4 per group) were intramuscularly administered (i) aluminum hydroxide (35 μg/mL), (ii) MPL (1.25, 2.5 or 5 μg/mL) or (iii) AS04 (aluminum hydroxide 8.75, 17.5 or 35 μg/ml added with MPL 1.25, 2.5 or 5 μg/mL, respectively), and the draining lymph nodes were collected after 24 hours. The CD40 expression in APC (CD11b positive cells and CD11c positive cells) present in the draining lymph nodes was determined by flow cytometry. CD40 expression was observed at all the doses in the MPL group and AS04 group (shown in (ii) and (iii) above), and the expression level was about 2 to 4 times as high as that of the group treated with aluminum hydroxide alone.

The pharmacological results on AS04 and MPL alone described in the above 3.(i).A.(1).1).a. to 3.(i).A.(1).1).c. have shown that MPL and AS04 enhance CD40 expression in APC and induce inflammatory cytokines, suggesting that they act as TLR4 agonist and have natural immunity activation effects, as with lipopolysaccharides (LPS) that is known as a strong inducer of natural immunity.

3.(i).A.(1).2) Examination of the contents of aluminum hydroxide, MPL and HPV antigen in this vaccine using mouse immune response as an index

a. Examination of the content of aluminum hydroxide (PIMS20[redacted]0134 study)

BALB/c mice (female, 15 per group) were intramuscularly administered 50 μL/injection of vaccines that contained MPL (5 μg), HPV-16/18 L1 VLP (2 μg each as the amount of antigen protein) and 3 different amounts of aluminum hydroxide (18, 26 or 50 μg), total two times including Day 1 and Day 21 of study. After 14 days from the second administration, the titers of specific antibodies to HPV-16 L1 VLP and HPV-18 L1 VLP in the serum (anti-HPV-16 antibody and anti-HPV-18 antibody, respectively) were determined by ELISA. Furthermore, mouse spleen cells were collected after14 days from the second administration and cultured for 96 hours in the presence of HPV-16 L1 or HPV-18 L1 antigen to determine the IFN-γ and IL-5 production levels in the culture supernatant by ELISA.

It was confirmed that the anti-HPV-16 antibody and anti-HPV-18 antibody titers in the group added with aluminum hydroxide 50 μg were [redacted] to [redacted] times higher ($p<0.05$) than those in the other groups added with 18 or 26 μg of aluminum hydroxide, and that the IFN-γ production level

in the group added with aluminum hydroxide 50 μg was also [redacted] to [redacted] times higher than that in the other groups. On the other hand, it was confirmed that IL-5 production levels were similar among the three groups added with aluminum hydroxide at different concentrations.

From the results, 50 μg of aluminum hydroxide was selected as the optimum dose.

b. Examination of MPL content (PIMS20[redacted]0755 study)

BALB/c mice (female, 10 per group) were administered 50 μl/injection of the vaccines that contained aluminum hydroxide (50 μg), HPV-16/18 L1 VLP (2 μg each as the amount of antigen protein) and 5 different amounts of MPL (0, 1.25, 2.5, 5 or 10 μg) in a manner similar to the section 3.(i).A.(1).2).a., and the titers of anti-HPV-16 and anti-HPV-18 antibodies in the serum and the ratios of IgG subtypes (IgG1, IgG2a and IgG2b) were determined by ELISA on the 14th day after the second administration. Furthermore, mouse spleen cells were collected after 14 days from the second administration and cultured for 96 hours in the presence of HPV-16 L1 or HPV-18 L1 to determine the IFN-γ and IL-5 production levels in the culture supernatant by cytometric bead array (CBA).

In the MPL-added groups, the anti-HPV-16 and anti-HPV-18 antibody titers increased to the levels [redacted]-[redacted] times higher than those in the no-MPL-added group ($p<0.0001$). As to the dose response of MPL, the anti-HPV-16 antibody titer in the MPL 2.5 μg group was [redacted] times higher than that in the 1.25 μg group ($p=0.0072$) but there was no difference from the 5 or 10 μg groups. On the other hand, no dose dependency was observed both in the anti-HPV-18 antibody titer and in the ratios of IgG subtypes of antibodies to both HPV genotypes, within the range of 1.25 μg-10 μg MPL examined.

The IFN-γ production level in the MPL 1.25 μg group for both antigens was about [redacted] times higher than that in the group treated with aluminum hydroxide alone and the level tended to increase (>[redacted] times) in the MPL 2.5, 5 and 10 μg groups. On the other hand, IL-5 production level for both types 16 and 18 was the highest in the aluminum hydroxide alone group while no change was observed between 1.25 and 5 μg of MPL and the production was decreased to about [redacted] at 10 μg of MPL.

In addition to these results, the results of other studies in mice were comprehensively evaluated, and, as the dose at which relatively high production level of IFN-γ and the relatively low production level of IL-5 would be obtained, 5 μg of MPL and a certain amount of aluminum hydroxide whose mixing ratio to MPL was 10:1 were selected. This mixing ratio of aluminum

hydroxide to MPL (10:1) corresponds to the optimal compounding ratio obtained in the clinical study of Fendrix (hepatitis B vaccine, not approved in Japan).

c. Examination of HPV-16/18 L1 VLP content (PIMS20[redacted]0028 study)

BALB/c mice (female, 12 per group) were administered 50 µL/injection of vaccine that contained 3 different doses of HPV-16/18 L1 VLP (0.6, 2 or 6 µg of each antigen as the amount of antigen protein) added with aluminum hydroxide (50 µg) or AS04 (aluminum hydroxide 50 µg + MPL 5 µg) in a manner similar to the section 3.(i).A.(1).2).a. After 14 days from the second administration, titers of anti-HPV-16 antibody and anti-HPV-18 antibody in the serum and the ratios of IgG subtypes (IgG1, IgG2a and IgG2b) were determined in a manner similar to the section 3.(i).A.(1).2).b. Furthermore, the amounts of IFN-γ and IL-5 production after re-stimulation of spleen cells by antigen were determined.

When the AS04-added group and the aluminum hydroxide-added group were compared, the titers of anti-HPV-16 antibody and anti-HPV-18 antibody in the former were significantly higher ([redacted] times; $p<0.0001$-0.0020) than those in the latter. As to the dose response related to HPV-16 L1 VLP and HPV-18 L1 VLP antigen amounts, the titers of anti-HPV-16 antibody and anti-HPV-18 antibody increased [redacted] to [redacted] times ($p=0.0012$-0.0020) in the AS04-added group as the amounts of HPV-16/18 L1 VLP antigens increased. However, the increase in antibody titers associated with the increased antigen amounts was less than [redacted] times in the group added with aluminum hydroxide alone. The ratios of IgG subtypes were not affected by the change in the antigen amount in any of the groups. As for the cytokine production, the IFN-γ production was lower ([redacted] times) in the group treated with 6 µg of each antigen added with AS04 in comparison with the levels in the other 2 dose groups but IL-5 production tended to be higher ([redacted] times) in this 6 µg group. On the other hand, the IFN-γ production was not influenced by the change in antigen content in the aluminum hydroxide-added group but the IL-5 production in the 0.6 µg group tended to be higher ([redacted] times) in comparison with the other groups.

Based on the above results, the contents of both HPV-16 L1 VLP and HPV-18 L1 VLP antigens were set at 2 µg each. At this level, the antigen treated groups showed high antibody-inducing activity and high IFN-γ production but low IL-5 production.

Based on the results of sections 3.(i).A.(1).2).a. to c., it was considered appropriate to set the composition of this vaccine (weight ratio of antigen: aluminum hydroxide: MPL) as 1:25:2.5.

3.(i).A.(1).3) Effects of addition of adjuvant on mouse immune response

a. Immunogenicity among 4 lots of formulations used in clinical studies (PIMS20[redacted]0026)

Four lots (2 lots added with AS04 and 2 lots added with aluminum hydroxide) for clinical studies whose composition was determined as in the section 3.(i).A.(1).2), was examined for immune responses in mice. BALB/c mice (female, 9 per group) were treated with 4 clinical lots of HPV-16/18 L1 VLP (2 μg each) vaccine added with aluminum hydroxide (50 μg) or AS04 (aluminum hydroxide 50 μg + MPL 5 μg) in a manner similar to the section 3.(i).A.(1).2).a., and the titers of anti-HPV-16 antibody and anti-HPV-18 antibody in the serum, the ratios of IgG subtypes (IgG1, IgG2a and Ig2b) and the IFN-γ and IL-5 production after re-stimulation of spleen cells by antigen were determined in a manner similar to the section 3.(i).A.(1).2).b.

In the AS04-added group, it was shown that, in comparison with the levels in aluminum hydroxide-added group, the titers of anti-HPV-16 antibody and anti-HPV-18 antibody increased [redacted] to [redacted] times ($p \leq 0.0001$), that the ratio of IgG2a antibody increased about [redacted]% and that the IFN-γ production increased [redacted] times while IL-5 production decreased [redacted] times. No significant difference was observed among these clinical lots that contained AS04 and those that contained aluminum hydroxide.

b. Comparison among nonadjuvant, aluminum hydroxide-added and AS04-added vaccines (PIMS20[redacted]0365 study)

The antigen-specific serum antibody induction and cytokine production enhancement by the HPV-16/18 L1 VLP vaccine were compared among those without addition of adjuvant, with the addition of aluminum hydroxide or AS04.

BALB/c mice (female, 12 per group) were administered HPV-16/18 L1 VLP (2 μg each) vaccines without adjuvant addition, or added with aluminum hydroxide (50 μg) or AS04 (aluminum hydroxide 50 μg + MPL 5 μg) in a manner similar to the section 3.(i).A.(1).2).a., and the titers of anti-HPV-16 antibody and anti-HPV-18 antibody in the serum were determined by ELISA after 14 days from the second administration. Furthermore, mouse spleen cells were collected in a manner similar to the section 3.(i).A.(1).2).a. and cultured for 48 hours in the presence of HPV-16 L1 or HPV-18 L1 antigen to determine the cytokines (IFN-γ, TNF-α, IL-2 and IL-5) production by CBA.

In the AS04-added group, it was observed that the titers of anti-HPV-16 antibody and anti-HPV-18 antibody increased [redacted] times ($p<0.0001$) in comparison with the levels in the no-adjuvanted group and [redacted] times ($p<0.0001$) with the aluminum hydroxide-added group. In the AS04-added vaccine group, moreover, it was observed that production levels of TNF-α and

antiviral cytokine IFN-γ were increased [redacted] to [redacted] times and [redacted] to [redacted] times higher than those in no adjuvant group, and [redacted] to [redacted] times and [redacted] to [redacted] times higher than those in the aluminum hydroxide-added vaccine group, respectively. On the other hand, IL-5 production in the AS04 added group was decreased to [redacted] to [redacted] and to [redacted] to [redacted] of those in the no adjuvant group and in the aluminum hydroxide added group, respectively.

c. Induction of immune memory B cells in the adjuvant added vaccine-treated group (PIMS20[redacted]0422 study)

Persistence of antibody titers and the frequency of appearance of immune memory B cells were compared after injection of HPV-16/18 L1 VLP vaccines without adjuvant, or added with aluminum hydroxide or AS04.

BALB/c mice (female, 12 animals/group) were treated with 50 μL/injection of the no-adjuvant, aluminum hydroxide (50 μg) added, or AS04 (aluminum hydroxide 50 μg + MPL 5 μg) added HPV-16/18 L1 VLP vaccines (2 μg each) in a similar manner to the section 3.(i).A.(1).2).a. The titers of anti-HPV-16 antibody and anti-HPV-18 antibody in the serum were determined by ELISA after 14 and 37 days from the second administration. B cells were collected from the spleens of mice (6 per group) after 37 days from the second administration and incubated for 5 days to calculate the frequency of appearance of immune memory B cells specific to HPV-16 L1 and HPV-18 L1 by determination of antigen-stimulated antibody producing cells and overall antibody producing cells by enzyme-linked immunospot (ELISPOT).

The titers of anti-HPV-16 antibody and anti-HPV-18 antibody in the AS04-added group increased [redacted] to [redacted] times and [redacted] to [redacted] times higher than the levels in the no-adjuvanted group ($p<0.05$) and aluminum hydroxide-added group ($p<0.05$), respectively. The antibody titers were stable in all the groups between 14 days and 37 days after the second administration. The frequency of appearance of immune memory B cells specific to HPV-16 L1 and HPV-18 L1 was close to the quantitation limit in the no-adjuvanted group but it was [redacted] to [redacted] times higher in the AS04-added group in comparison with the level in the aluminum hydroxide-added group.

These results indicated that the serum antibody titer was maintained at a high level in the mice administered with AS04-added vaccine until 37 days after the second administration, and that, at that time point, the appearance frequency of immune memory B cells was higher in these mice in comparison with that in the aluminum hydroxide-added group, suggesting that the mice vaccinated with AS04-added vaccine can respond to a booster injection of HPV-16 LI and HPV-18 L1 antigen (boostability).

d. Persistence of the antibody titer by adjuvant-added vaccines (PIMS20[redacted]0183 study)

The persistence of antibody titers (after 14, 36 and 91 days following the second injection), the frequency of appearance of immune memory B cells (after 91 days following the second injection) and cytokine production (after 91 days following the second injection) were compared between HPV-16/18 L1 VLP vaccine added with aluminum hydroxide and the vaccine added with AS04.

BALB/c mice (female, 48 per group) were treated with 50 μL/injection of HPV-16/18 L1 VLP (2 μg each) vaccines added with aluminum hydroxide (50 μg) or AS04 (aluminum hydroxide 50 μg + MPL 5 μg) in a manner similar to the section 3.(i).A.(1).2).a. The titers of anti-HPV-16 antibody and anti-HPV-18 antibody were determined after 14, 36 and 91 days from the second administration. Spleens were collected from mice (12 per group) after 91 days from the second administration to determine the appearance frequency of immune memory B cells specific to HPV-16 L1 and HPV-18 L1 in a manner similar to the section 3.(i).A.(1).3).c., and to determine the production of cytokines (IFN-γ and IL-5) after re-stimulation of spleen cells by antigen in a manner similar to the section 3.(i).A.(1).2).b.

The titer of anti-HPV-16 antibody was [redacted] times ($p<0.05$) higher after 14 and 36 days from the second administration and that of anti-HPV-18 antibody was [redacted] times ($p<0.05$) higher after 14, 36 and 91 days from the second administration in the serum of AS04-added group in comparison with the levels in the aluminum hydroxide-added group. As for the persistence of antibody titers in the serum, both the titers of anti-HPV-16 antibody and anti-HPV-18 antibody were stable both in the AS04-added group and aluminum hydroxide-added group between 14 days and 36 days after the second administration but the titers decreased to [redacted] levels after 91 days. As for the cytokine production in the spleen cells collected after 91 days from the second administration, the IFN-γ level was higher ([redacted] times) and IL-5 level was lower ([redacted] times) in the AS04-added group in comparison with those in the aluminum hydroxide-added group. At that time point, the frequency of appearance of immune memory B cells specific to HPV-16 L1 in the AS04-added group was similar (about [redacted] times) to that in the aluminum hydroxide-added group, but that of HPV-18 L1 was induced about [redacted] times higher compared to the aluminum hydroxide-added group.

The above results indicated that the AS04-added vaccine induces not only higher and more persistent increase in the antibody titer in the blood but also the production of IFN-γ that demonstrates anti-virus activity, in comparison with the vaccines added with aluminum hydroxide alone or with no adjuvant. Moreover, it was demonstrated that the AS04-added vaccine tended to

induce higher level of immune memory B cells even after 91 days from the second administration.

3.(i).A.(1).4) Effects of adjuvant added vaccines on immune response in rhesus monkeys (PIMS20[redacted]0121 study)

Rhesus monkeys (5 per group including 3 males and 2 females) were intramuscularly administered 3 times (Days 1, 28 and 84 of study) with 500 μl/injection of HPV-16/18 L1 VLP vaccines (20 μg each) without adjuvant addition, with aluminum hydroxide (500 μg), or AS04 (aluminum hydroxide 500 μg + MPL 50 μg). Titers of anti-HPV-16 antibody and anti-HPV-18 antibody in the serum and titers of antibodies to V5 (HPV-16 L1) and J4 (HPV-18 L1) that served as neutralizing epitopes were determined after 28 days of each administration. Moreover, the virus neutralizing antibodies were also determined after 28 days from the third administration.

In comparison with the aluminum hydroxide-added group, the titers of anti-HPV-16 antibody and anti-HPV-18 antibody increased [redacted]-[redacted] times (p=0.0130-0.0572) after the second and third administration in the AS04-added group. Moreover, the titers of antibodies specific to neutralizing epitopes V5 (HPV-16 L1) and J4 (HPV-18 L1) were [redacted]-[redacted] times (p=0.0200-0.0501) higher, and the neutralizing antibody titer was [redacted]-[redacted] times ($p<0.05$) higher than that in the aluminum hydroxide-added group.

It was confirmed that the AS04 added vaccine also showed excellent immunogenicity profile in monkeys.

3.(i).A.(2) Summary of the results of safety pharmacology study

3.(i).A.(2).1) Effects on the cardiovascular and respiratory systems (BVR 371/033059 study)

Wistar rats (male, 4 animals/group) were intramuscularly administered with a single 0.1 mL dose of the AS04 (aluminum hydroxide 500 μg + MPL 50 μg)-added HPV-16/18 L1 VLP (20 μg each) vaccines. The dose was determined to exceed 24 to 56 times higher than the expected clinical dose, and the cardiovascular parameters (blood pressure, heart rate and electrocardiogram) and respiratory parameters (respiration rate, tidal volume and minute ventilation) were continuously measured for 2 hours after the administration. The control group was intramuscularly administered with 0.1 mL of phosphate-buffered saline solution. The vaccine intramuscularly administered at a dose of 0.1 mL/animal did not exert any effects on the cardiovascular and respiratory parameters throughout the study period.

3.(i).B. Outline of the review by PMDA

3.(i).B. (1) Primary pharmacodynamics

In the primary phamrmacodynamics studies of the vaccine, changes of various factors such as titers of anti-HPV-16 antibody and anti-HPV-18 antibody in the serum, the appearance frequency of immune memory B cells, and cytokine levels (IFN-γ and IL-5) have been evaluated. PMDA requested the applicant to explain rationales and significance of the use of these parameters as the indices to support the efficacy of this vaccine.

The applicant answered as follows:

Since HPV has very high species-specificity, and different HPV genotypes have different tissue specificities and cause various lesions, it would be impossible to evaluate efficacy of this vaccine (protective effects against infection) directly by using some animal infection models. On the other hand, challenge studies of canine, rabbit and bovine-specific papilloma virus (PV) have shown that vaccines that induced strong and persistent serum antibody to PV L1 VLP could be very effective against PV infections and their associated diseases (*Proc Natl Acad Sci.*1995;92:11553-7, *Virology.* 1996;219:37-44). In the case of HPV infection, it is also strongly suggested that HPV VLP-containing vaccines are expected to serve as protection against various lesions induced by HPV. The proposed vaccine is an HPV VLP vaccine that contains HPV-16 L1 VLP and HPV-18 L1 VLP as active ingredients. In the development of this vaccine, the immunogenicity as a surrogate marker for efficacy was evaluated in mice and monkeys, and the titers of anti-HPV-16 antibody and anti-HPV-18 antibody were chosen as the primary endpoint of immunogenicity. As the secondary endpoints, the frequency of appearance of immune memory B cells were investigated as well as the inducibility of IFN-γ and IL-5 production in the spleen cells, which represents the inducibility of cellular immunity that influences the antibody titer persistence. In order to elucidate the characteristics of cellular immunity, it was considered useful to determine the ratios of IgG subtypes (IgG1, IgG2a and IgG2b). Based on the changes in the index values of immunogenicity, and from the results of the strong inducibility of anti-HPV-16 and anti-HPV-18 antibodies in the serum and the induction of immune memory B cells as well as the inducibility of IFN-γ production that has antiviral activity, 1:25:2.5 was judged appropriate as the composition of this vaccine (weight ratio of antigen: aluminum hydroxide: MPL). This composition was employed in the subsequent development.

PMDA accepted the above answer made by the applicant. In this regard, PMDA requested the applicant to explain the clinical significance of the increase in the titers of anti-HPV-16 and anti-HPV-18 L1 antibodies in the serum that served as the primary endpoint of immunogenicity.

The applicant answered as follows.

IgA antibody and IgG antibody have been found in the mucus of uterine cervix. It is widely known that the IgA antibody is produced in the uterine cervix, that the IgG antibody is derived from the serum and that leakage of serum IgG antibody into the mucus of uterine cervix is an important mechanism for protection against HPV infection in the uterine cervix (*10th ed USA McGraw Hill Lange Medical Immunology*. 2001;548-67). Nardelli-Haefliger et al. examined the HPV-16 L1 VLP IgG antibody level in the serum and the mucus of uterine cervix after administration of HPV-16 L1 VLP vaccine in female patients which had been taking oral contraceptive drugs and reported that the serum HPV-16 L1 VLP IgG antibody level in the serum was correlated well with that in the mucus of uterine cervix (*J Natl Cancer Inst*. 2003;95(15): 1128-37). From the results of these clinical and nonclinical studies, the applicant aimed at developing the vaccines capable of inducing strong and persistent production of anti-HPV-16 L1 VLP antibody and anti-HPV-18 L1 VLP antibody into serum and mucus of uterine cervix.

Based on the applicant's answer, PMDA understood that the increase in the titers of anti-HPV-16 and anti-HPV-18 antibodies in the serum is expected to increase the titers of these antibodies in the uterine cervix. However, since the relation between the increase in these serum antibody titers and the efficacy of this vaccine (infection-preventing effect) is unknown, it was decided to examine it in the review of clinical data.

3.(i).B.(2) Safety pharmacology studies

PMDA requested the applicant to explain the reason why the study concerning the central nervous system was considered to be possibly omitted among the safety pharmacology studies.

The applicant answered as follows.

In the single dose toxicity study in rabbits and the repeated dose toxicity studies in rabbits and rats, no abnormality of central nervous system was found in the observation of clinical signs in this vaccine group. Therefore, it was considered less possible that this vaccine might affect the central nervous system, and the study concerning central nervous system among the safety pharmacology studies were not performed. In the domestic and overseas phase I and II clinical studies, moreover, findings to suggest some influence on the central nervous system have not been observed.

PMDA accepted the above answer.

3.(ii) Summary of results of the pharmacokinetic studies

No studies in this category have been conducted.

3.(iii) Summary of results of the toxicology studies

3.(iii).A Summary of the submitted data

For the application for marketing authorization, the toxicology studies on this vaccine (HPV-16/18 L1 VLP AS04; containing an equivalent amount each of HPV-16 L1 VLP and HPV-18 L1 VLP on an antigenic protein basis), AS04 adjuvant and MPL have been conducted. MPL has been assessed as a new excipient.

3.(iii).A.(1) Single-dose toxicity studies (1513 study, 58678 study)

A single-dose toxicity study of this vaccine has not been conducted. However, single-dose toxicity was evaluated based on the reactions at the initial intramuscular administration of varied amount of HPV-16/18 L1 VLP AS04 with the highest dose of antigen being 120μg (containing 60μg each of HPV-16 L1 VLP and HPV-18 L1 VLP, the composition is the same in the following sections) in a repeat-dose toxicity study in rabbits. In the study, no changes in clinical signs including death due to a single-dose administration of this vaccine were observed (1513 study). In addition, after a single-dose administration of AS04 adjuvant alone (containing 50μg of MPL and 500μg of aluminium hydroxide) or 40μg of HPV-16/18 L1 VLP AS04 (containing 20μg each of HPV-16 L1 VLP and HPV-18 L1 VLP, the composition is the same in the following sections) in rabbits, no systemic effects were observed in either case. The AS04 adjuvant treatment group and the 40μg of HPV-16/18 L1 VLP AS04-treatment group showed equal incidence of bleeding, discolouration, inflammation and muscle fiber necrosis at the injection site, both of which were higher than that of the control group (saline) (58678 study).

3.(iii).A.(2) Repeat-dose toxicity studies

As a repeat-dose toxicity study of this vaccine, four studies in rabbits and one study in rats have been conducted.

3.(iii).A.(2).1) Four-time repeat-dose toxicity study in rabbits (1513 study)

NZW rabbits (18 females/group) were administered with 40μg (clinical dose [HD]: low-dose group) or 120μg (three times higher than the HD: high-dose group) of HPV-16/18 L1 VLP AS04 intramuscularly four times at a two-week interval, and were compared with those administered aluminium hydroxide (500μg) alone (aluminium hydroxide group). A 2-day or 28-day recovery period was set after the final administration, and the local and systemic toxicities and the adverse reaction profile were evaluated during the study period including the recovery periods.

During each period, clinical signs, dermal reactions at the injection site, body weight, food consumption, hematology, blood chemistry, necropsy, organ weight and histopathology were

evaluated and no changes other than dermal reactions at the injection site were observed. Several animals in each dose group, including the aluminium hydroxide group, exhibited erythema and/or edema at the injection site, but no dose-dependence was obeserved. In several animals of the vaccine high-dose and low-dose groups, moderate to severe erythema and/or edema were observed. In addition, one animal in the vaccine low-dose group exhibited transient limb impairment, but no histopathological changes indicating severe local effects were observed in this animal and the cause of the limb impairment was not identified. This finding occurred only in one animal in all studies. The inflammatory symptoms at the injection site were observed more extensively in the vaccine groups than in the control group, and the symptoms recovered earlier in the aluminium hydroxide group than in the vaccine groups.

3.(iii).A.(2).2) Four-time repeat-dose toxicity study in rabbits (1758 study)

In 1513 study, the blood collection site at the necropsy was different from that before administration and it was difficult to compare the test results correctly, therefore the retest was conducted to perform the hematological examination. The protocol was similar to that of 1513 study, giving animals saline, aluminium hydroxide or 120μg of HPV-16/18 L1 VLP AS04. The observation period was set for 16 days after the final administration and a hematological examination was performed.

No changes were observed in clinical signs, body weight and so on, and there were no death cases. Additionally, no abnormalities were observed in the hematological parameters and the bone marrow smear examination at the study termination.

3.(iii).A.(2).3) Four-time repeat-dose toxicity study in rabbits (58678 study)

A single-dose or repeat-dose (four times in total, 2, 4 and 8 weeks after the initial dosing) of 40μg of HPV-16/18 L1 VLP AS04 (vaccine group), the clinical dose (1HD) of AS04 adjuvant alone (AS04 group) or saline (control group) was given to NZW rabbits (females, 15/group) intramuscularly. The animals were necropsied three days after dosing in the single-dose administration and three days or 13 weeks after the final dosing in the repeat-dose administration.

There were no death during the study period and no effects associated with the administration of the vaccine were observed in clinical signs, skin reactions, body temperature, body weight, body weight gain, food consumption and ophthalmology. The antibody titers against the vaccine antigen in the serum were determined (ELISA) and the production of the antigen was confirmed in all of the animals given the vaccine four times. Immediately after a single-dose administration or a four-time repeat-dose administration in the vaccine group and the AS04 group, local changes such as mild to moderate localized degeneration, necrosis or regeneration of muscle fiber and

mild to moderate subacute inflammation were observed. After a single-dose administration, no apparent differences in the degree and incidence of the local reactions at the injection site were observed between the vaccine group and the AS04 group. However, immediately after the fourth administration in the repeat-dose study, the degree and incidence of the local reactions in the vaccine group were slightly higher than those in the AS04 group. Principal local changes 13 weeks after the final dosing were mild regeneration of muscle fiber, suggesting that the animals were on the course of recovery. In hematology, the vaccine group and the AS04 group showed high neutrophil and fibrinogen levels, which were considered to be due to the inflammatory changes at the injection site. In the final examination 13 weeks after the fourth administration, the vaccine group showed statistically-significant low platelet count (36% of the value in the control group). But no findings suggesting low platelet count were observed in the bone marrow and the reticuloendothelial organs/tissues, and no changes were observed in the hematological parameters other than platelet count and clinical signs. Though variations between the dosing groups were occasionally found in some blood chemistry parameters, those variations were not considered to be associated with administration since those variations did not show consistency and were within the range of the baseline values. In addition, no toxicological findings were observed in the tissues examined histopathologically.

3.(iii).A.(2).4) Four-time repeat-dose toxicity study in rabbits (62369 study)

Since the vaccine group showed a significantly low platelet count in the 58678 study, reproducibility and dose-dependency of the change were assessed. A four-time repeated dose of the clinical dose (1HD group) or one-tenth of the clinical dose (1/10HD group) of the HPV-16/18 L1 VLP AS04 vaccine, or the clinical dose of AS04 adjuvant (AS04 group), or saline (control group) was given to rabbits in a similar way to that in the 58678 study.

One animal each in the control group (Day 133) and the AS04 group (Day 106) was sacrificed due to debility caused by body weight loss, but no other effects of administration were observed in clinical signs, body weight or body weight gain. Additionally, no changes were observed in platelet count and the reproducibility of the platelet count drop observed in the 58678 study was not confirmed. No systemic toxicity was observed as well, and though slight changes in the hematological parameters, high fibrinogen level (AS04 group, 1HD group) and high neutrophil level (1HD group) were observed, all changes were considered to be related to the predicted inflammation caused by the immune responses. The local reactions at the injection site were most prominent in the 1HD group.

3.(iii).A.(2).5) Four-time repeat-dose toxicity study in rats (62370 study)

Since platelet count decreased in a four-time repeat-dose toxicity study in rabbits (58678 study) but reproducibility was not observed in the retest 62369 study, a repeat-dose toxicity study in rats was conducted to evaluate species specificity. Based on the result of immunogenicity study in rats (PIMS20 0475), the dose was set at 1/5 HD that was more than about 25-50 times (varied depending on the body weight) of the planned clinical dose in terms of body weight (24- 56 times when the weight of human and that of rat were assumed as 30-70 kg and 0.25 kg, respectively).

In a four-time repeat-dose study in rats, intramuscular administration of 1/5 of the planned clinical dose of HPV-16/18 L1 VLP AS04 vaccine (vaccine group), AS04 adjuvant (AS04 group) or saline solution (control group) was given to Wistar rats (female, 20 per group) 4 times repeatedly (total 4 times, i.e., initial dosing, and after 2, 4 and 8 weeks following initial dosing), and local and systemic toxicities at dosing as well as recoveries after 13 weeks from the final dosing were evaluated. The animals were sacrificed after 3 days or 13 weeks from the final dosing.

There were no death cases and no changes were observed in the evaluation of clinical signs, skin reaction, ophthalmology, body weight, body weight gain or food consumption. No differences between the groups were found in the body temperature after four hours from the initial dosing. Even though the body temperature in the AS04 group and vaccine groups was higher with statistical significance than that of the control group after 24 hours from the initial dosing, there was no difference between the groups after 48 hours. In contrast, no differences between the groups were found in body temperature both four and 24 hours after the final dosing. In the hematological examination, an increased neutrophil count (absolute and relative numbers), an increased fibrinogen level and a decreased lymphocyte count (relative number) were observed in the AS04 group and the vaccine group, but no change in platelet count was observed. In the histopathological examination performed three days after the final dosing, changes associated with administration of the vaccine such as subacute inflammation, necrosis of muscle fiber and focal hemorrhage were observed at the injection site, and the degree of changes was most prominent in the vaccine group. However, the difference from the control group was not observed 13 weeks after the final dosing, showing recovery during the recovery period. In each necropsy, inflammatory reactions in the popliteal lymph nodes were observed in both AS04 and vaccine groups.

3.(iii).A.(3) Genotoxicity and carcinogenicity studies

In accordance with the EMEA guidance "Note for guidance on preclinical pharmacological and toxicological testing of vaccines" (CPMP/SWP/465/95) and "Guideline on adjuvants in vaccines for human use" (EMEA/CHMP/VEG/134716/2004), a genotoxicity study and a carcinogenicity study have not been conducted.

3.(iii).A.(4) Reproductive and developmental toxicity study (BVR 249/033160 study)

As a reproductive and developmental toxicity study, study of effects on female fertility, pre- and postnatal development was performed. The HPV-16/18 L1 VLP AS04 vaccine or AS04 adjuvant was administered intramuscularly to SD rats (56 females/group) [redacted] days prior to mating and on days [redacted], [redacted], [redacted] and [redacted] of gestation, five times in total, and the effects of the vaccine on female fertilitiy, prenatal development and postnatal survival were studied (Table 3). Since the EMEA guidance "Note for guidance on preclinical pharmacological and toxicological testing of vaccines" (CPMP/SWP/465/95) does not require determination of blood antigen concentration, toxicokinetics testing has not been conducted.

Table 3. Treatment groups in the BVR 249/033160 study

Group	[redacted] days prior to mating	Days [redacted], [redacted], [redacted] and [redacted] of gestation
1	Saline	Saline
2	Saline	HPV-16/18 L1 VLP AS04
3	HPV-16/18 L1 VLP AS04	HPV-16/18 L1 VLP AS04
4	AS04	AS04

The immune response induced by the HPV-16/18 L1 VLP AS04 vaccine (1/5HD) was evaluated, and the exposure to the vaccine and successful vaccination were confirmed in the pre-pregnant and pregnant female animals. In addition, it was also confirmed that the antibody transferred to fetuses and also to offspring via milk.

All animals given the HPV-16/18 L1 VLP AS04 vaccine or AS04 adjuvant before mating mated successfully and were pregnant, and no effect on female fertility (number of pregnant animals and implantations) was observed. In addition, since both the vaccine group and the AS04 group had no differences from the control group in estrous cycles, mating period, number of copulation plugs and sperm numbers in the vaginal washing, the applicant explains that the HPV-16/18 L1 VLP AS04 vaccine and AS04 do not affect estrous cycles and mating ability.

During the study, no changes in clinical signs, body weight and food consumption were observed and no affects on embryo-fetal development were observed. The birth rate of pregnant animals, the number of live fetuses, fetal weight, and the development, growth and viability of the offspring up to [redacted] days after birth were not also affected by the HPV-16/18 L1 VLP AS04 vaccine. The types of principal visceral and skeletal findings and the incidence in the vaccine group and the AS04 group were comparable with the control group. Small ventricular septal defect was observed in two animals (one each in the vaccine-vaccine group and AS04-AS04 group) but they disappeared soon after birth, and were within the range of natural occurrence of ventricular septal defect in SD rats. In addition, all of other findings observed only in the vaccine group were mild and were not considered to be meaningful variations when compared with the control group and the background data. Therefore, the applicant explains that the result of this study suggests that the administration of the HPV-16/18 L1 VLP AS04 vaccine before mating and during gestation do not affect the occurrence of fetal abnormalities.

3.(iii).A.(5) Local irritation study ([redacted] 1513 study, [redacted] 58678 study)

Local irritation at the injection site was evaluated in a repeat-dose toxicity studies and a local irritation study has not been conducted independently.

In the histopathological examination performed in [redacted] 1513 study, inflammation was observed in subcutaneous tissue and muscle at the injection site, but the incidence did not show an apparent dose-dependency. Inflammatory findings at the injection site were also observed in the aluminium hydroxide group, which was evaluated as a control group, but the vaccine group had a tendency of more extensive inflammation and longer recovery period than the aluminium hydroxide group.

Similarly, in the [redacted] 58678 study, the injection site in rabbits where AS04 adjuvant (AS04 group) or the HPV-16/18 L1 VLP AS04 vaccine (vaccine group) was given four times exhibited the changes associated with the administration. In a single-dose administration, the local reactions were milder than those observed in a repeat-dose administration, and the reactions in the vaccine group were considered to be comparable to those in the AS04 group.

Based on the studies mentioned above, the applicant explains that the results of the conducted studies have demonstrated the safety of HPV-16/18 L1 VLP AS04 vaccine.

3.(iii).B Outline of the review by PMDA

In a four-time repeat-dose toxicity study in rabbits, low platelet count was observed but was not reproducible, and as a result of additional testing using rats to examine the species specificity, a platelet count decrease in rabbits was not directly associated with the administration of

HPV-16/18 L1 VLP AS04 vaccine. Based on the above results, the applicant concludes that low platelet count is a change without toxicological significance.

PMDA considers that the applicant's view is acceptable. Since the animals sacrificed due to the debilitation caused by body weight decrease in [redacted] 62369 study were those in the control group and adjuvant group, and because no body weight decrease and no change in clinical signs were observed in the other toxicity studies, PMDA judged that the said event was incidental and had no influence on the evaluation.

PMDA asked the applicant to further explain about ventricular septal defect observed in one out of 166 animals in the vaccine (HPV-16/18 L1 VLP AS04)-vaccine group and in one out of 169 animals in AS04-AS04 group in the study of effects on female fertility, pre- and postnatal development in rats.

The applicant explained that the defects were not muscular but membranous and no other findings suggesting developmental delay were found.

Since there was no increase in other visceral and skeletal abnormalities nor any particular change in comparison with the control group, PMDA considers that the ventricular septal defect was an incidental finding.

At the injection site of HPV-16/18 L1 VLP AS04 vaccine and AS04 adjuvant, inflammation and related findings due to inflammation were observed with increasing number of administration, and local reactions were obviously more enhanced by administration of this vaccine than by administration of adjuvant alone. Therefore, PMDA asked the applicant to explain about the relevance of the number of administration to tissue injury.

The applicant explained that focal perivasculitis occurred at the injection site with increased number of administration but it was reversible.

PMDA considers as follows:
Since the administration was alternately performed in the right and left paraspinal muscle in the repeat dose toxicity study in rabbits, the irritation caused by plural number of injections to the same site was unknown. It is considered necessary to pay attention to the local irritation reaction in the clinical use such as by carefully observing the injection site and avoiding the injection to the previously injected site.

4. Clinical data

4.A Summary of the submitted data

In this application for approval, the results of the following clinical studies conducted in healthy female subjects have been submitted as evaluation data.

Phase	Study	Primary endpoint	Target age	Primary study	Follow-up	Extension	Ongoing/ Extension
I	HPV-002	Safety	18-30 y	56 days n = 49 (0)		54 months n = 7 (0)	
II a	HPV-003	Safety	18-30 y	12 months n = 61 (31)			
	HPV-004	Antibody response, safety	18-30 y	12 months n = 60 (20)		48 months n = 38 (12)	
	HPV-005	Dose range (immune response), safety	18-30 y	12 months n = 209 (63)		48 months n = 35 (25)	
II b	HPV-001 /007	Efficacy against incident infection	15-25 y	18 months n = 1113 (560)	Up to 27 months n = 1113 (560)	HPV-007 (M24) 60 months n = 776 (393)	HPV-007 (M36) 72 months n = 699 (359)
	HPV-032	Prevention of persistent infection (6 month definition)*	20-25 y (Japanese)	Up to 18.5 months n = 1040 (519)		Long term follow-up	
III	HPV-008	Efficacy against CIN2+ associated with HPV-16 and/or 18	15-25 y	Up to 15 months n = 18665 (9319)		48 months [20[redacted]]	
	HPV-012	Lot consistency of immunogenicity	10-25 y	7 months n = 770 (770)	12 months n = 733 (733)	48 months [20[redacted]]	
	HPV-013 / 013Ext	Safety (SAEs), immunogenicity (extension)	10-14 y	7 months n = 2067 (1035)	12 months n = 2023 (1014)	18 months n = 1245 (626)	48 months [20[redacted]]
	HPV-014 / 014Ext	Immune response (seroconversion rate), immunogenicity(ex-tension)	15-55 y	7 months n = 666 (666)	12 months n = 635 (635)	HPV-014 (M18) 18 months n = 524 (524)	48 months [20[redacted]]
	HPV-015	Efficacy against persistent infection (6 month definition), efficacy against CIN1+ associated with HPV-16 and/or 18 (combined endpoint)	Over 26 y	7 months n = 5751 (2880)		36 months [20[redacted]]	
	HPV-016	Immunogenicity (lot consistency)	18-25 y	7 months n = 798 (798)		12 months [20[redacted]]	
	HPV-046	Immunogenicity, safety	10-15 y (Japanese)	7 months n = 100 (100)			

Length of study period (days or months) represents the time after first vaccination.
n: number of subjects
( ): number of subjects who received this HPV 16/18 L1 VLP AS04 vaccine

*Persistent infection (6 month definition): at least two positive HPV DNA PCR assays for the same viral genotype with no negative DNA sample between the two positive DNA samples, over an approximate interval of 6 months (> 150 days)

The results of the major clinical studies are summarized below.

4.A.(1) Domestic phase IIb study (Study HPV-032, no publication, evaluation data, conducted from April 2006, data lock date: [redacted], 20[redacted] [Interim Analysis II])

A multi-center, randomized, double blind, parallel-group, comparative study was conducted at 13 centers in Japan in 20 to 25 year-old Japanese healthy female subjects (target number of subjects of 1,000; 500 per group) to evaluate the preventive effect of AS04-added HPV-16/18 (20 µg/20 µg) L1 VLP vaccine (the drug product of the same composition is referred to as the "HPV Vaccine" hereinafter in this section) against persistent infection with HPV-16 and/or HPV-18 (6-month definition: defined as at least two positive HPV DNA PCR assays for the same viral genotype with no negative DNA sample between the two positive DNA samples, over an approximate interval of 6 months [>150 days]), using a hepatitis A vaccine (Aimmugen [HAV, 0.5 µg]) as a control vaccine.

The major inclusion criteria includes "negative in the urine pregnancy test", "no possibility of pregnancy", "no previous vaccination with HPV vaccine or hepatitis A vaccine", or "no previous administration of 3-deacylated-4'- monophosphoryl lipid A (MPL)". The dosage regimen was that a total of three doses of the HPV Vaccine or HAV were to be administered intramuscularly at Months 0, 1 and 6.

Two interim analyses and final analysis were planned in this study. The Interim Analysis I were to be performed at the month 7 and the Interim Analysis II at the time point when at least 8 cases of persistent infection (6-month definition) with HPV-16 and/or HPV-18 has been accrued in the ATP (According To Protocol) cohort. In Interim Analysis I, the safety and immunogenicity were evaluated, and in Interim Analysis II, the efficacy, immunogenicity and safety were evaluated. The results of the interim analyses were not to be used to make decisions about early termination of the trial. For significance level adjustment accompanying interim analysis, the Wang-Tsiatis bounds (Δ=0.2, *Biometrics.* 1987;43: 193-199) were used, and the two-sided significance level was set at 0.01 for Interim Analysis II and 0.045 for the Final Analysis. Interim Analysis II was performed by external statisticians after the data were locked on [redacted], 20[redacted]. The final analyses of efficacy, immunogenicity and safety were to be performed at Month 24. As of [redacted], the result of Final Analysis has not been submitted.

In this study, 1,046 subjects were enrolled and 1,040 subjects (519 in HPV Vaccine group and 521 in HAV group) received at least one dose of either vaccine and were handled as the Total Vaccinated Cohort (TVC), which was regarded as the safety analysis cohort. After 38 subjects were excluded from the cohort because of reasons such as high-grade or missing cytology at baseline or failure to follow the protocol in vaccination, 1,002 subjects (HPV Vaccine group, 501 subjects; HAV group, 501 subjects) were handled as the ATP cohort for efficacy.

The primary endpoint was persistent cervical infection with HPV-16 and/or HPV-18 (defined as at least two positive HPV DNA PCR assays for the same viral genotype with no negative DNA sample between the two positive DNA samples, over an approximate interval of 6 months [> 150 days]), and was assessed in subjects who were, for the corresponding HPV type, seronegative at Month 0 and HPV DNA negative (by PCR) at Month 0 and Month 6.

Table 4 shows the efficacy results obtained from the Interim Analysis II on the persistent cervical infection with HPV-16 and/or HPV-18. The lower limit of 99% confidence interval of VE (Vaccine Efficacy; VE = (1-(n1/T1)/(n2/T2) x 100 (%), n1: number of subjects demonstrating persistent infection in the HPV Vaccine group, T1: total follow-up period for the HPV Vaccine group, n2: number of subjects demonstrating persistent infection in the HAV group, T2: total follow-up period for the HAV group) was higher than 0 in the HPV-16 and/or HPV-18 (hereinafter referred to as "HPV-16/HPV-18"), indicating a significant difference.

Table 4. Vaccine efficacy against persistent cervical infection (6-month definition) with HPV-16/HPV-18 (ATP cohort for efficacy, Interim Analysis II)

Event type	Group	n/N	T	VE (Vaccine Efficacy)	
				%	99% confidence interval (CI)
HPV-16/HPV-18	HPV	0/358	366.79	100	[20.5, 100]
	HAV	9/367	363.98		
HPV-16	HPV	0/305	311.59	100	[-44.5, 100]
	HAV	6/319	317.36		
HPV-18	HPV	0/321	330.17	100	[-373.6, 100]
	HAV	3/321	322.57		

N: number of subject included in analysis, n: number of subjects with persistent infection
T: total follow-up period (year)
VE = (1-(n1/T1)/ (n2/T2)) ×100 (%), n1: number of subject with persistent infection in the HPV Vaccine group, T1: total follow-up period for the HPV Vaccine group, n2: number of subject with persistent infection in the HAV group, T2: total follow-up period for the HAV group

Table 5 shows the primary results in regard to the secondary endpoints of efficacy.

Table 5. Primary results of secondary endpoint (ATP cohort for efficacy, Interim Analysis II)

Event type	Endpoint	Group	n/N	T	VE (Vaccine Efficacy)	
					%	99% CI
HPV-16/ HPV-18 (by PCR)	Incident infection	HPV	6/403	399.09	82.2	[46.2, 95.8]
		HAV	33/406	391.53		
	ASC-US+	HPV	1/402	349.36	83.4	[-116.2, 99.9]
		HAV	6/406	347.54		

N: number of subjects included in analysis, n: number of subjects in a given endpoint
T: total follow-up period (year),
ASC-US+ (abnormal cytology): ASC-US (atypical squamous cells of undetermined significance), LSIL (low-grade squamous intraepithelial lesions), HSIL (high-grade squamous intraepithelial lesions), ASC-H (atypical squamous cells), and AGC (atypical glandular cells)

For the safety results, there were no death cases at the Interim Analysis II. Serious adverse events were reported by 16 subjects (23 events) in the HPV Vaccine group and 15 subjects (17 events) in the HAV group. Of the serious adverse events in the HPV Vaccine group, a spontaneous abortion was reported 15 days after second vaccination, which was considered by the investigator as possibly related to vaccination. Table 6 shows the major adverse events (incidence of ≥5% in either group).

Table 6. Solicited and unsolicited symptoms reported in ≥5% of subjects in either group (TVC, Interim Analysis II)

	Symptom	HPV Vaccine						HAV					
		AE			ADR			AE			ADR		
		N	n	%	N	n	%	N	n	%	N	n	%
Solicited local symptoms*	Injection site pain	512	508	99.2	-	-	-	510	214	42.0	-	-	-
	Injection site redness	512	455	88.9	-	-	-	510	287	56.3	-	-	-
	Injection site swelling	512	401	78.3	-	-	-	510	165	32.4	-	-	-
Solicited general symptoms	Arthralgia	512	123	24.0	512	113	22.1	511	61	11.9	511	48	9.4
	Fatigue	512	341	66.6	512	316	61.7	511	300	58.7	511	254	49.7
	Fever (Axillary) (≥37.5 °C)	512	41	8.0	512	28	5.5	511	28	5.5	511	19	3.7
	Gastrointestinal disorder	512	172	33.6	512	135	26.4	511	167	32.7	511	124	24.3
	Headache	512	250	48.8	512	201	39.3	511	222	43.4	511	181	35.4
	Myalgia	512	262	51.2	512	252	49.2	511	128	25.0	511	109	21.3
	Rash	512	33	6.4	512	30	5.9	511	24	4.7	511	22	4.3
Unsolicited symptoms	Injection site pruritus	519	83	16.0	519	83	16.0	521	15	2.9	521	15	2.9
	Injection site warmth	519	69	13.3	519	69	13.3	521	6	1.2	521	6	1.2
	Nasopharyngitis	519	110	21.2	519	11	2.1	521	91	17.5	521	7	1.3
	Headache	519	19	3.7	519	2	0.4	521	27	5.2	521	5	1.0

AE: adverse event, ADR: adverse drug reaction
Solicited (local and general) symptoms were reported during the 7 days (Days 0-6) post-vaccination.
Unsolicited symptoms were reported during the 30 days (Days 0-29) post-vaccination.
N: number of subjects with at least one vaccination, n: number of subjects reporting symptom(s), %: incidence rate
* For solicited local symptoms, causality (relationship to vaccination) were not assessed..

A serious adverse event leading to treatment discontinuation was reported in 1 subject (This female fell down stairways 40 days after the first vaccination and was admitted to hospital. The diagnosis was skull fracture and brain contusion). Five non-serious adverse events leading to treatment discontinuation occurred (4 cases in the HPV Vaccine group and 1 case in the HAV group), of which 3 cases were urticaria, 1 case was worsening dermographia, and 1 case was gastrointestinal symptom, which were considered by the investigator not related to vaccination.

New Onset of Chronic Disease (NOCD) was reported in 7 subjects (8 cases; 5 in the HPV Vaccine group and 3 in the HAV group) including 4 cases of urticaria (1 and 3 in respective groups). One case of rheumatoid arthritis and 1 case of allergic granulomatous angitis were classified as New Onset of Autoimmune Disease (NOAD).

Seventy-two2 pregnancies had been reported by 68 subjects. The outcome of these pregnancies was 27 elective terminations, 23 normal infants, 8 spontaneous abortion, 14 ongoing pregnancies [see “4.B.(4).6) Pregnancy”] .

4.A.(2) Domestic phase III study (Study HPV-046, no publication, evaluation data, study period: July 2007 - March 2008)

An open, uncontrolled study in Japanese healthy female subjects aged 10 to 15 years (target number of subjects of 100) was conducted at 8 centers to evaluate the immunogenicity and safety of 3 times vaccination of the HPV Vaccine. The HPV Vaccine was to be administered intramuscularly at Months 0, 1 and 6. In this study, 101 subjects were enrolled, of which, 100 subjects completed the vaccination of the HPV Vaccine and were handled as the TVC for safety. Of the 100 subjects, 99 subjects were included in the ATP cohort for immunogenicity excluding 1 subject, in whom the study vaccine was not administered in accordance with the protocol. The primary endpoints were antibody responses against HPV-16 and HPV-18 at Month 7 and assessment of the solicited adverse events reported within seven days (Days 0-6) after each vaccination.

Table 7 shows the immunogenicity results. One month after 3 vaccinations (Month 7), all initially seronegative subjects had seroconverted for both HPV-16 and HPV-18. Anti-HPV-16 antibody titers in initially seropositive subjects had also increased to a similar level to those achieved in the initially seronegative subjects.

Table 7. Seropositivity rates and GMTs for anti-HPV-16 and anti-HPV-18 antibodies by pre-vaccination status (ATP cohort for immunogenicity)

	Pre-vaccination status	Timing	N	Seropositivity rate (≥8 EU/mL)			GMT(EU/mL)			
				n	%	95%CI	GMT	95%CI	Min	Max
HPV-16	Seronegative	Pre vaccination	92	0	0	[0, 3.9]	4	[4, 4]	*	*
		Post dose 3	92	92	100	[96.1,100]	19513.8	[16837.7, 22615.3]	2930	120711
	Seropositive	Pre vaccination	7	7	100	[59, 100]	16.6	[10.1, 27.3]	9	39
		Post dose 3	7	7	100	[59, 100]	23101.1	[12334.5, 43265.7]	9804	58846
	Total	Pre vaccination	99	7	7.1	[2.9, 14]	4.4	[4.1, 4.8]	*	39
		Post dose 3	99	99	100	[96.3,100]	19748	[17147.7, 22742.7]	2930	120711
	Pre-vaccination status	Timing	N	Seropositivity rate (≥7 EU/mL)			GMT(EU/mL)			
				n	%	95%CI	GMT	95%CI	Min	Max
HPV-18	Seronegative	Pre vaccination	94	0	0	[0, 3.8]	3.5	[3.5, 3.5]	*	*
		Post dose 3	94	94	100	[96.2,100]	8998.4	[7746.7, 10452.2]	1710	61503
	Seropositive	Pre vaccination	4	4	100	[39.8,100]	12.2	[8.1, 18.3]	9	16
		Post dose 3	4	4	100	[39.8,100]	4730.3	[955.7, 23412]	2506	21186
	Total	Pre vaccination	98	4	4.1	[1.1, 10.1]	3.7	[3.5, 3.9]	*	16
		Post dose 3	98	98	100	[96.3,100]	8765.3	[7543.8, 10184.4]	1710	61503

*: Under cut-off value. Assay cut-off thresholds are 8 EL.U./mL for HPV-16 and 7 EL.U./mL for HPV-18. A subject is classified as seropositive if the titer is greater than or equal to the cut-off value of the assay (the cut-off thresholds were set based on the quantitation limit of the assay).
GMT: geometric mean antibody titer (Values under the cut-off thresholds were treated as half the cut-off value in calculating GMT).
N: number of subjects with baseline values available. n: number of seropositive subjects
(Data of HPV-18 titer was not available for 1 subject.)

The incidence of solicited local symptoms in 7 days after vaccination was 96.6% (288 of 298 doses). The local symptoms noted with a high incidence were injection site pain (95.0%, 283 of 298 doses), redness (72.5%, 216 of 298 doses) and swelling (66.4%, 198 of 298 doses). Solicited local symptoms of Grade 3 were noted with an incidence of 12.4% (37 of 298 doses). The incidence of solicited general symptoms in 7 days after vaccination was 40.6% (121 of 298 doses). The solicited general symptoms noted with a high incidence were fatigue (22.5%, 67 of 298 doses), headache (15.8%, 47 of 298 doses) and myalgia (13.1%, 39 of 298 doses). General symptoms of Grade 3 were noted as 3 events with an incidence of 0.7% (2 of 298 doses).

There were no death cases. No serious or non-serious adverse events leading to study discontinuation occurred. Major adverse events are shown in Table 8.

Table 8. Solicited and unsolicited symptoms reported in ≥5% of subjects (TVC)

	Symptom	AE			ADR		
		N	n	%	N	n	%
Solicited local symptoms*	Injection site pain	100	98	98.0	-	-	-
	Injection site redness	100	85	85.0	-	-	-
	Injection site swelling	100	81	81.0	-	-	-
Solicited general symptoms	Arthralgia	100	15	15.0	100	11	11.0
	Fatigue	100	40	40.0	100	37	37.0
	Fever (Axillary) (≥37.5°C)	100	10	10.0	100	6	6.0
	Gastrointestinal disorder	100	17	17.0	100	16	16.0
	Headache	100	33	33.0	100	31	31.0
	Myalgia	100	26	26.0	100	25	25.0
	Rash	100	5	5.0	100	5	5.0
Unsolicited symptoms	Abdominal pain	100	6	6.0	100	1	1.0
	Injection site pruritus	100	16	16.0	100	16	16.0
	Injection site warmth	100	9	9.0	100	9	9.0
	Nasopharyngitis	100	18	18.0	100	1	1.0
	Dysmenorrhoea	100	6	6.0	100	0	0.0

Solicited (local and general) symptoms were reported during the 7 days (Days 0-6) post-vaccination
Unsolicited symptoms were reported during the 30 days (Days 0-29) post-vaccination
N: number of subjects with at least one vaccination, n: number of subjects reporting symptom(s), %: incidence rate
* For solicited local symptoms, causality (relationship to vaccination) were not assessed.

4.A.(3) Overseas phase IIa study (Study HPV-005, no publication, study period: October 1999 - May 2001)

A randomized, double-blind, dose-comparison study was conducted at 25 centers in the US in healthy females aged 18 to 30 years (target number of subjects of 210) to evaluate the safety and immunogenicity of MEDI-517: HPV-16/18 VLP vaccine (containing virus-like particles [VLP] of HPV-16 and HPV-18 in an equal amount) added with SBAS4 (the same as AS04) or aluminum hydroxide as adjuvant. The immunogenicity was assessed by determining serum antibody titers to HPV-16 and HPV-18 by ELISA after 30 days from third vaccination.

The major inclusion criteria were seronegativity for HPV-16 and HPV-18 antibodies, negativity for high-risk HPV DNA, normal pap smear, etc. The following groups were set: groups of 12 µg, 40 µg and 120 µg of the study vaccine using SBA4 as adjuvant (hereinafter referred to as “SBA4 12 µg group” [target number of cases: 60, same below], “SBA4 40 µg group” [60] and “SBA4 120 µg group” [60], respectively), and a group of 40 µg study vaccine using aluminum hydroxide as adjuvant (“aluminum hydroxide 40 µg group” [30]). Each group was to be intramuscularly inoculated with 0.5 mL of study vaccine total 3 times (Days 0, 30 and 180).

This study included 210 subjects (60 subjects in the SBAS4 12-µg group, 64 subjects in the SBAS4 40-µg group, 59 subjects in the SBAS4 120-µg group, and 27 subjects in the aluminum hydroxide 40-µg group) and they received the study vaccine. Except for 1 subject who withdrew consent, 209 subjects were included in the safety analysis (60 subjects in the SBAS4 12-µg group, 63 subjects in the SBAS4 40-µg group, and 59 subjects in the SBAS4 120-µg group and 27

subjects in the 40-μg aluminum hydroxide group). Primary analysis of immunogenicity was performed with data from all subjects who received all three injections of the study vaccine (n=163; 52 subjects in the SBAS4 12-μg group, 48 subjects in the SBAS4 40-μg group, 42 subjects in the SBAS4 120-μg group and 21 subjects in the 40-μg aluminum hydroxide group).

In safety evaluation, no deaths occurred during the study and 5 subjects (2 in the SBAS4 12-μg group and 3 in the aluminum hydroxide group) had 6 serious adverse events (ovarian hyperstimulation syndrome due to egg donation [1 event], gastroenteritis [1 event], spontaneous abortion [2 events], and cholecystitis [2 events]) during the study. One serious adverse event (spontaneous abortion) was considered to be possibly related to study vaccine (in the SBAS4 12-μg group); however, the subject was taking other medications during her pregnancy that, in the opinion of the investigator, may have contributed to the event. Three subjects (1 subject in the SBAS4 40-μg group, 1 subject in the 120-μg group, and 1 subject in the 40-μg aluminum hydroxide group) had adverse events (injection site redness, tachycardia and throat tightness, and fatigue, respectively) that resulted in treatment discontinuation.

Major adverse events are shown in Table 9.

Table 9. Adverse events reported in ≥5% of subjects in either group

	Symptom	SBAS4 12μg			SBAS4 40μg			SBAS4 120μg			ALUM 40μg		
		AE			AE			AE			AE		
		N	n	%	N	n	%	N	n	%	N	n	%
Solicited local symptoms	Injection site pain	60	59	98.3	63	61	96.8	59	59	100.0	27	23	85.2
	Injection site redness	60	32	53.3	63	29	46.0	59	28	47.5	27	7	25.9
	Injection site swelling	60	22	36.7	63	25	39.7	59	31	52.5	27	5	18.5
Solicited general symptoms	Headache	60	42	70.0	63	39	61.9	59	36	61.0	27	19	70.4
	Gastrointestinal disorder	60	25	41.7	63	28	44.4	59	26	44.1	27	15	55.6
	Fatigue	60	38	63.3	63	38	60.3	59	35	59.3	27	15	55.6
	Rash	60	3	5.0	63	3	4.8	59	1	1.7	27	0	0.0
	Pruritus	60	12	20.0	63	11	17.5	59	12	20.3	27	8	29.6
	Fever (≥37.5°C)	60	29	48.3	63	28	44.4	59	29	49.2	27	11	40.7
Unsolicited symptoms	Injury	60	0	0.0	63	1	1.6	59	3	5.1	27	2	7.4
	Fever	60	1	1.7	63	2	3.2	59	0	0.0	27	2	7.4
	Headache	60	5	8.3	63	4	6.3	59	3	5.1	27	1	3.7
	Injection site reaction	60	8	13.3	63	7	11.1	59	10	16.9	27	1	3.7
	Nausea	60	0	0.0	63	2	3.2	59	3	5.1	27	0	0.0
	Dizziness	60	2	3.3	63	4	6.3	59	4	6.8	27	1	3.7
	Insomnia	60	4	6.7	63	0	0.0	59	0	0.0	27	0	0.0
	Pharyngitis	60	4	6.7	63	2	3.2	59	4	6.8	27	2	7.4
	Rhinitis	60	4	6.7	63	7	11.1	59	7	11.9	27	1	3.7
	Sinusitis	60	2	3.3	63	2	3.2	59	2	3.4	27	2	7.4
	Upper Respiratory Tract Infection	60	5	8.3	63	5	7.9	59	4	6.8	27	2	7.4
	Cervical smear abnormal	60	1	1.7	63	1	1.6	59	5	8.5	27	0	0.0
	Urinary tract infection	60	1	1.7	63	1	1.6	59	1	1.7	27	2	7.4
	Vaginal candidiasis	60	2	3.3	63	0	0.0	59	0	0.0	27	2	7.4

ALUM: aluminum hydroxide
Solicited (local and general) symptoms were reported during the 7 days (Days 0-6) post-vaccination
Unsolicited symptoms were reported during the 30 days (Days 0-29) post-vaccination
N: number of subjects with at least one vaccination, n: number of subjects reporting symptom(s), %: incidence rate

Table 10 shows the primary immunogenicity endpoints, serum anti-HPV-16 and anti-HPV-18 antibody titers (ELISA) at Month 7 in the AS04 adjuvant groups.

Table 10. Serum antibody titers against HPV-16 and HPV-18 at Month 7 (ATP cohort for immunogenicity)

	Group	N	GMT (EU/mL)	Titer (log10)	
				Average	95% CI
HPV-16	SBAS4 12 μg	51	3655.7	3.6	[3.4, 3.7]
	SBAS4 40 μg	47	5248.2	3.7	[3.6, 3.8]
	SBAS4 120 μg	42	5944.5	3.8	[3.6, 3.9]
HPV-18	SBAS4 12 μg	51	3402.6	3.5	[3.4, 3.7]
	SBAS4 40 μg	47	3443.4	3.5	[3.4, 3.7]
	SBAS4 120 μg	42	4228.5	3.6	[3.5, 3.7]

GMT: geometric mean antibody titer, N: number of subjects with baseline values available. (Data was not available for 1 subject each in the SBAS4 12 μg group and the SBAS4 40 μg group)

4.A.(4) Overseas phase IIb study (Study HPV-001, published [*Lancet*, 364: 1757-65, 2004], evaluation data, study period: Jan 2001 - April 2003)

A randomized, double-blind, placebo-controlled, comparative study was conducted at 32 centers in 3 overseas countries in healthy female subjects aged 15 to 25 years (target number of subjects of 1000; 500 in each group) to evaluate the preventive effect of AS04-added HPV-16/18 (20 μg/20 μg) L1 VLP vaccine (HPV Vaccine) against incident cervical infection with HPV-16 and HPV-18.

The major inclusion criteria were normal cervical cytologies (by liquid based methodology), seronegativity for HPV-16 and HPV-18 antibodies (by ELISA), and negativity for high-risk HPV DNA (by PCR) at screening. The dosage regimen was that a total of three doses of the HPV Vaccine or placebo (aluminum hydroxide) were to be administered intramuscularly at Months 0, 1 and 6.

In this study, three interim analyses (at the time of [redacted], 20[redacted], at the time when at least 28 subjects with incident infection for HPV-16/18 has been accrued or at the time of [redacted], 20[redacted], and at the time when the number of subjects with HPV PCR results obtained at Month 18 visits reaches at least equal to 50% of the number of subjects with results obtained at Month 12 visits) and the final analysis were planned. Only safety was evaluated in the first Interim Analysis, efficacy and immunogenicity in the second Interim Analysis and efficacy in the third Analysis. The significance level was adjusted for the interim analyses according to the O'Brien-Fleming adjustment. The significance level (two-sided) was 0.005 for the second Interim Analysis, 0.005 for the third Interim Analysis, and 0.046 for the Final Analysis. The results of the interim analyses were not used to make decisions about early termination of the trial. The second and

third Interim Analyses were performed by external statisticians in view of the use of study data in planning of subsequent development programs of the HPV Vaccine.

In this study, 1113 subjects (560 subjects in the HPV Vaccine group, 553 subjects in the placebo group) were enrolled. Among those subjects, 1,081 subjects (540 subjects in the HPV Vaccine group, 541 subjects in the placebo group) other than 32 subjects excluded for reasons such as "administration of vaccine(s) forbidden in the protocol" or "randomisation failure" were handled as the ATP cohort for safety. Among these subjects, 721 subjects (366 subjects in the HPV Vaccine group, 355 subjects in the placebo group) other than 360 subjects excluded for reasons such as "seropositive for HPV-16 or 18, positive for oncogenic HPV DNA, or abnormal cytology results at study entry", "non compliance with vaccination schedule", "positive for HPV-16 or 18 DNA at Month 6" were handled as ATP cohort for efficacy.

The primary endpoint was that incident cervical infection with HPV-16 and/or HPV-18 (defined as at least one positive HPV-16 or HPV-18 DNA PCR assay). This was analysed using the HPV-16 and/or HPV-18 DNA results determined for all specimens (self-obtained cervicovaginal specimens and physician-obtained cervical specimens) at Months 6 to 18. Table 11 shows the efficacy against incident infection with HPV-16/HPV-18, HPV-16 alone, and HPV-18 alone after the third administration (VE = (1-(n1/N1)/(n2/N2) x 100 (%), N1: number of subjects in the HPV Vaccine group included in the analysis, n1: number of subjects demonstrating incident infection in the HPV Vaccine group, N2: number of subjects in the placebo group included in the analysis, n2: number of subjects demonstrating incident infection in the placebo group) from Month 6 to Month 18.

Table 11. Vaccine efficacy against incident infections with HPV-16 or HPV-18 (Months 6-18, ATP cohort for efficacy, Final Analysis)

Specimen Type	HPV type (s)	HPV			Placebo			Vaccine efficacy (VE)		
		N	n	Inci-dence	N	N	Inci-dence	%	95% CI	p-value *
All specimens (self-obtained cervico-vaginal specimens and physician-obtained cervical specimens combined)	HPV-16/ HPV-18	366	8	2.2	355	36	10.1	78.4	[54.3, 89.8]	< 0.001
	HPV-16	366	3	0.8	355	25	7.0	88.4	[61.8, 96.5]	< 0.001
	HPV-18	366	5	1.4	355	15	4.2	67.7	[12.0, 88.1]	0.023
Cervical specimens (physician-obtained)	HPV-16/ HPV-18	366	2	0.6	355	23	6.5	91.6	[64.5, 98.0]	< 0.001
	HPV-16	366	0	0	355	18	5.1	100	[79.4, 100]	< 0.001
	HPV-18	366	2	0.6	355	7	2.0	72.3	[-32.5, 94.2]	0.102

N: number of subjects included in the analysis, n: number of subjects with incident infection
VE = (1-(n1/N1)/(n2/N2))×100(%)
N1: number of subjects in the HPV Vaccine group included in the analysis, n1: number of subjects with incident infection in the HPV Vaccine group, N2: number of subjects in the placebo group included in the analysis, n2: number of subject with incident infection in the placebo group
*Fisher's exact test

For the secondary endpoint, Table 12 shows vaccine efficacy against persistent infection (defined as at least two positive HPV DNA PCR assays for the same viral genotype over a minimum interval of 6 months) from Month 6 to Month 18 and from Month 6 to Month 27.

Table 12. Vaccine efficacy against persistent infection with HPV-16 or HPV-18 (ATP cohort for efficacy, Final Analysis)

	Specimen Type	HPV type (s)	HPV Vaccine			Placebo			Vaccine efficacy (VE)	
			N	n	Inci-dence (%)	N	n	Inci-dence (%)	%	95% CI
Month 6-18	All specimens (self-obtained cervico-vaginal specimens and physician-obtained cervical specimens combined)	HPV-16/HPV-18	366	0	0	355	10	2.8	100	[63.0, 100]
		HPV-16	366	0	0	355	10	2.8	100	[63.0, 100]
		HPV-18	366	0	0	355	0	0	-	-
	Cervical specimens (physician-obtained)	HPV-16/HPV-18	366	0	0	355	7	2.0	100	[47.0, 100]
		HPV-16	366	0	0	355	7	2.0	100	[47.0, 100]
		HPV-18	366	0	0	355	0	0	-	-
Month 6-27	All specimens	HPV-16/HPV-18	366	0	0	355	16	4.51	100	[76.8, 100]
		HPV-16	366	0	0	355	13	3.66	100	[71.5, 100]
		HPV-18	366	0	0	355	4	1.13	100	[7.2, 100]

N: number of subjects included in the analysis, n: number of subjects with persistent infection

The Table 13 shows the vaccine efficacy against cytological abnormalities in the ATP cohort from Month 6 to 18 (note: the cervical samples for cytology were taken only until Month 18 according to the protocol). None of the subjects was diagnosed to have cervical adenocarcinoma or squamous cell carcinoma during the study period. During the period from Month 6 to 18, the histopathological diagnosis indicated cervical intraepithelial neoplasia (CIN) grade 1 or 2 in one subject in the HPV Vaccine group (CIN1, associated with HPV-18) and 3 subjects in the placebo group (CIN1 in 2 subjects and CIN2 in 1 subject, all three were associated with HPV-16).

Table 13. Vaccine efficacy against cytological abnormalities associated with HPV-16 and/or HPV-18 cervical infection (ATP cohort for efficacy, Final Analysis)

	Cytological abnormalities	HPV type(s)	HPV Vaccine			Placebo			Vaccine efficacy (VE)	
			N	n	Event rate	N	n	Event rate	Event rate	95% CI
Month 6-18	ASC-US	HPV-16 /HPV-18	366	0	0	355	8	2.25	100.0	[53.7, 100.0]
		HPV-16	366	0	0	355	5	1.41	100.0	[25.8, 100.0]
		HPV-18	366	0	0	355	5	1.41	100.0	[25.8, 100.0]
	LSIL	HPV-16 /HPV-18	366	1	0.27	355	8	2.25	87.9	[3.6, 98.5]
		HPV-16	366	0	0	355	8	2.25	100.0	[53.7, 100.0]
		HPV-18	366	1	0.27	355	0	0	-191	[-7019, 88.1]
	Any lesion (≥ ASCUS)	HPV-16 /HPV-18	366	1	0.27	355	15	4.23	93.5	[51.3, 99.1]
		HPV-16	366	0	0	355	12	3.38	100.0	[69.1, 100.0]
		HPV-18	366	1	0.27	355	5	1.41	80.6	[-65.2, 97.7]

N: number of subjects included in the analysis, n: number of subjects with cytological abnormalities with corresponding HPV type

If no case is observed in the control group, the estimation of the VE was obtained adding +0.5 to "N" and "n" in the table.

In the process of quality assurance of the data and monitoring related to GCP compliance by the sponsor for this study, the sponsor identified issues related to GCP compliance, etc. at two Brazilian study sites after completion of the Final Analysis, but before finalization of the study report. In response to the findings, the analysis was conducted again after correction and exclusion of the relevant data. However, the results obtained were the same as the initial efficacy analysis results.

Table 14 shows the major adverse events related to safety.

Table 14. Solicited symptoms reported in ≥5% of subjects in either group (ATP cohort, Final Analysis)

	Symptom	HPV Vaccine						Placebo					
		AE			ADR			AE			ADR		
		N	n	%	N	n	%	N	n	%	N	n	%
Solicited local symptoms*	Injection site pain	531	496	93.4	-	-	-	538	469	87.2	-	-	-
	Injection site redness	531	189	35.6	-	-	-	538	131	24.3	-	-	-
	Injection site swelling	531	182	34.3	-	-	-	538	113	21.0	-	-	-
Solicited general symptoms	Fatigue	531	308	58.0	531	214	40.3	538	289	53.7	538	189	35.1
	Gastrointestinal disorder	531	178	33.5	531	89	16.8	538	172	32.0	538	99	18.4
	Headache	531	331	62.3	531	180	33.9	538	329	61.2	538	194	36.1
	Itching	531	130	24.5	531	80	15.1	538	109	20.3	538	75	13.9
	Rash	531	60	11.3	531	31	5.8	538	54	10.0	538	32	5.9
	Fever (≥37.5°C)	531	88	16.6	531	37	7.0	538	73	13.6	538	28	5.2

Solicited (local and general) symptoms were reported during the 7 days (Days 0-6) post-vaccination
Unsolicited symptoms were reported during the 30 days (Days 0-29) post-vaccination
Placebo: Al(OH)3
N: number of subjects with at least one vaccination, n: number of subjects reporting the symptom, %: incidence rate
* For solicited local symptoms, causality (relation to vaccination) were not assessed.

There were no deaths of subjects. Serious adverse events were reported in 22 subjects in the HPV Vaccine group and 19 subjects in the placebo group. None of the serious adverse events were considered to be causally related to vaccination.

There were four treatment discontinuations related to adverse events, three due to non serious adverse events in the placebo group and one due to a serious adverse event in the HPV Vaccine group (spontaneous abortion 132 days after the second dose). None of these events were considered by the investigator to be causally related to vaccination. During the study, 88 pregnancies (44 in the HPV Vaccine group, 44 in the placebo group) were reported. The number of pregnancy outcomes reported as miscarriages/spontaneous abortions was low (5 subjects in the vaccine group and 3 subjects in the placebo group). There was one report of a neonatal death of

twins in the placebo group. All reported complications were considered as not related to vaccination.

4.A.(5) Overseas phase IIb study (Study HPV-007, published [*Lancet,* 367:1247-55, 2006], evaluation data, study period: November 2003 - August 2007)

A long-term follow-up study was conducted to evaluate the long-term vaccine efficacy in the prevention of cervical infection with HPV-16 and/or HPV-18 in women who received three doses of the study vaccine or placebo in Study HPV-001.

Of the 1113 women initially enrolled in Study HPV-001, all subjects who received three doses of HPV Vaccine/placebo and whose treatment allocation was not unblinded were eligible to participate. A total of 776 subjects (393 subjects who received the HPV Vaccine and 383 subjects who received the placebo) were enrolled in the long-term follow-up study HPV-007. During the study, blinding was to be maintained, and they were to be followed up for 36 months (7 visits at intervals of 6 months). In this study, two Interim Analyses (Month 12 and Month 24) and the Final Analysis (Month 36) were planned. For significance level adjustment accompanying interim analysis, the Haybitte-Peto method was used, and the two-sided significance level was set at 0.001 for the first Interim Analysis, 0.001 for the second Interim Analysis, and 0.049 for the Final Analysis. The purpose of these analyses was to provide an overview of long-term efficacy, immunogenicity and safety data to regulatory authorities. The Interim Analyses were performed by external statisticians.

Among the 776 subjects, after excluding 34 subjects for reasons such as “administration of vaccine(s) forbidden in the protocol” or “randomisation failure”, 742 subjects (373 subjects in the HPV Vaccine group, 369 subjects in the placebo group) were handled as the ATP cohort for safety. Among the 742 subjects, after excluding 53 subjects excluded for reasons such as “missing result (HPV DNA or Cytology or Serology) at Month 0 or at screening” or “seropositive (for HPV-16 or HPV-18) or abnormal cytology at screening”, 689 subjects (349 subjects in the HPV Vaccine group, 340 subjects in the placebo group) were handled as the ATP cohort for efficacy.

The primary endpoint was incident cervical infection with HPV-16 and/or HPV-18 (defined as positive reaction to HPV-16 or HPV-18 DNA detected at least once by PCR). The secondary endpoints include the efficacy against persistent infection (6-month and 12-month definition), and the preventive effect against CIN2+ (CIN2+ is defined as CIN2, CIN3, adenocarcinoma in situ [AIS] and invasive cervical cancer) associated with HPV-16 or HPV-18 infection (by PCR) detected within the lesional component of the cervical tissue. Table 15 shows the efficacy results

against incident infection, the primary endpoint (VE = (1-(n1/T1)/(n2/T2) x 100 (%), n1: number of subjects with incident infection in the HPV Vaccine group, T1: total follow-up period for the HPV Vaccine group, n2: number of subjects with incident infection in the placebo group, T2: total follow-up period for the placebo group).

Table 15. Vaccine efficacy against incident infection with HPV-16 and/or HPV-18 (ATP cohort for efficacy, Final Analysis)

Event type	Group	N	n	T	Vaccine efficacy (VE)		P-value*
					%	95% CI	
HPV-16/18	HPV	303	2	830.25	96.7	[87.4, 99.6]	<0.0001
	Placebo	267	47	644.66			
HPV-16	HPV	304	1	833.70	97.5	[85.3, 99.9]	<0.0001
	Placebo	270	33	676.11			
HPV-18	HPV	303	1	832.31	96.3	[77.5, 99.9]	<0.0001
	Placebo	281	24	731.50			

Cervical samples only,
N: number of subject included in the analysis (excluding subjects who were HPV DNA positive at pre-vaccination [Month 0 and 6 in Study HPV-001], n: number of subjects with incident infection
T: total follow-up period (year), VE= (1-(n1/T1)/ (n2/T2)) ×100 (%), n1: number of subject with incident infection in the HPV Vaccine group, T1: total follow-up period for the HPV Vaccine group, n2: number of subject with incident infection in the placebo group, T2: total follow-up period for the placebo group
*Fisher's exact test

Table 16 shows the efficacy against persistent infection (6-month and 12-month definition), the secondary endpoint.

Table 16. Vaccine efficacy against persistent infection with HPV-16 and/or HPV-18 (ATP cohort for efficacy, Final Analysis)

	Group	Persistent infection (6 months definition)				Persistent infection (12 months definition)			
		N	n	T	Vaccine efficacy (VE: %) [95% CI]	N	n	T	Vaccine efficacy (VE: %) [95% CI]
HPV-16/ 18	HPV	304	0	835.76	100 [85.9, 100]	304	0	835.76	100 [75.0, 100]
	Placebo	277	24	708.27		285	15	748.15	
HPV-16	HPV	304	0	835.76	100 [79.0, 100]	304	0	835.76	100 [67.5, 100]
	Placebo	277	17	722.75		285	12	755.03	
HPV-18	HPV	304	0	835.76	100 [59.4, 100]	304	0	835.76	100 [-39.7, 100]
	Placebo	285	10	760.84		285	4	770.74	

Cervical samples only
N: number of subject included in the analysis (excluding subjects who were HPV DNA positive at pre-vaccination [Month 0 and 6 in Study HPV-001] or subjects who reported persistent infection in Study HPV-001), n: number of subjects with persistent infection, T: total follow-up period (year)

The safety analysis was performed on the ATP cohort for safety (373 subjects in the HPV Vaccine group, 369 subjects in the placebo group), for whom data were collected from the end of Study HPV-001 throughout the entire HPV-007 study period. The number of subjects reporting an NOCD (assessed by the applicant) was 5 [1.3%] in the HPV Vaccine group and 6 [1.6%] in the placebo group. Among these subjects, only one case of NOCD (pneumonitis) was considered

as serious. The number of subjects reporting an NOCD based on investigator‘s assessment was similar in the HPV Vaccine group (18 subjects, 4.8%) and the placebo group (21 subjects, 5.7%).

NOCDs classified as NOADs were reported in 2 subjects in the HPV Vaccine group (0.5%) and 4 subjects (1.1%) in the placebo group. The NOADs were hypothyroidism, autoimmune thyroiditis and ulcerative colitis.

No deaths were reported. Serious adverse events reported were 36 cases in 31 subjects (7.9%) in the HPV Vaccine group and 46 cases in 39 subjects (10.2%) in the placebo group. None of the serious adverse events were considered as related to vaccination by the investigator.

During Study HPV-007, a total of 261 pregnancies (130 in the HPV Vaccine group and 131 in the placebo group) were reported in 217 subjects. The number of pregnancies resulting in abnormal outcomes (spontaneous abortion, abnormal infant, elective termination, missed abortion or still birth) was 19 in the HPV Vaccine group and 29 in placebo group.

4.A.(6) Overseas phase III study (Study HPV-008, published [*Lancet,* 369: 2161-70, 2007], evaluation data, conducted from May 2004, data cut-off date: [redacted], 20[redacted] [Interim Analysis])

A multi-center, double-blind, randomized, parallel-group, comparative study was conducted at 133 sites in 14 overseas countries in healthy female subjects aged 15 to 25 years (target number of subjects of 18,000; 9,000 in each group) to evaluate the efficacy of the HPV Vaccine in the prevention of cervical cancer associated with HPV-16 or HPV-18 persistent infection. Control was a hepatitis A vaccine (Havrix-based investigational formulation; 720 ELISA units of antigen and 500 μg aluminium hydroxide). The major inclusion criteria were healthy women, aged 15 to 25 years at the time of first vaccination, with a negative urine pregnancy test, no previous vaccination against HPV or hepatitis A or previous administration of MPL. A total of three doses of HPV Vaccine were to be administered intramuscularly at Months 0, 1 and 6 and the subjects were to be evaluated for about 48 months. In this study, one interim analysis (at the time point when 23 cases of CIN2+ associated with HPV-16 and/or HPV-18 has been accrued) and the final analysis (at the time point when 36 cases of CIN2+ associated with HPV-16 and/or HPV-18 has been accrued or at the time point when all subjects who did not withdraw from the study have completed the Month 48 visit). In the Interim Analysis, the efficacy, immunogenicity, and safety were evaluated. The results of the Interim Analysis were not used to make decisions about early termination of the trial. For significance level adjustment accompanying interim analysis, the O’Brien-Fleming method was used, and the two-sided significance level was set at 0.021 for the Interim Analysis and 0.039 for the Final Analysis. After the data were locked on [redacted],

20[redacted], the Interim Analysis were performed by external statisticians. Currently, the Final Analysis results have not been submitted.

In this study, 18,729 subjects were registered and 18,665 subjects were received study vaccine. Among the 18,665 subjects, after 21 subjects (described below) were excluded, 18,644 subjects (9,319 subjects in the HPV Vaccine group, 9,325 subjects in the control group) were handled as the Total Vaccinated Cohort, which was included in the safety analysis. Among those subjects, after 119 subjects were excluded for the reason of high-grade or missing cytology at baseline, 18,525 subjects (9,258 subjects in the HPV Vaccine group, 9,267 subjects in the control group) were handled as Total Vaccinated Cohort 1 for efficacy (TVC-1). In this study, the routine oversight activities by the sponsor for data quality assurance and GCP compliance identified potential problems on study conduct and data integrity at one site prior to the interim analysis. While the matter was still under investigation, all subjects from this site (N=21) were excluded from all the analyses.

The primary endpoint was that histopathologically-confirmed CIN2+ associated with HPV-16 or HPV-18 cervical infection detected within the lesional component of the cervical tissue specimen (by PCR). At the Final Analysis, objectives will be assessed post dose 3 in women who are DNA negative (by PCR) for HPV-16 or HPV-18 at Months 0 and 6.

In the Interim Analysis, efficacy have been assessed post dose 1 in women who were DNA negative (by PCR) for HPV-16/18 at Month 0 with normal or low-grade cytology at baseline. The analysis cohort included in the Interim Analysis (TVC-1) was all vaccinated subjects (at least one dose) for whom data of efficacy parameters were available and had a normal or low-grade cytology at Month 0. Table 17 shows the vaccine efficacy against CIN2+ (VE = (1-(n1/T1)/(n2/T2) x 100 (%), n1: number of subjects with CIN2+ in the HPV Vaccine group, T1: total follow-up period in the HPV Vaccine group, n2: number of subjects with CIN2+ in the control group, T1: total follow-up period in the control group).

Table 17. Vaccine efficacy against CIN2+ associated with HPV-16 and/or HPV-18 (TVC-1, Interim Analysis)

HPV Type	Group	N	n	T	Vaccine efficacy (VE)		P-value*
					%	97.9% CI	
HPV-16/18	HPV	7788	2	9613.75	90.4	[53.4, 99.3]	<0.0001
	Control	7838	21	9682.00			
HPV-16	HPV	6701	1	8279.75	93.3	[47.0, 99.9]	0.0005
	Control	6717	15	8284.32			
HPV-18	HPV	7221	1	8903.55	83.3	[-78.8, 99.9]	0.1249
	Control	7258	6	8947.82			

N: number of subjects included in the analysis, n: number of subjects with CIN2+
T: total follow-up period (year), VE = (1-(n1/T1)/ (n2/T2)) ×100 (%), n1: number of subjects with CIN2+ in the HPV Vaccine group, T1: total follow-up period for the HPV Vaccine group, n2: number of subjects with CIN2+ in the control group, T2: total follow-up period for the control group
*Fisher's exact test

When all subjects irrespective of their serostatus at baseline were included in the analysis, the vaccine efficacy was 91.6% (97.9% CI, [60.2, 99.4]).

Table 18 shows the efficacy against persistent infection (6-month and 12-month definition) in TVC-1.

Table 18. Vaccine efficacy against persistent infection (TVC-1, Interim Analysis)

	HPV type	HPV Vaccine			Control			VE	
		N	n	T	N	n	T	%	97.9% CI
Persistent infection (6 months definition)	HPV-16/18	6344	38	8149.58	6402	193	8129.85	80.4	[70.4, 87.4]
	HPV-16	5493	23	7076.92	5520	144	7035.66	84.1	[73.5, 91.1]
	HPV-18	5896	15	7590.88	5939	58	7635.60	74.0	[49.1, 87.8]
Persistent infection (12 months definition)	HPV-16/18	3386	11	5062.64	3437	46	5104.85	75.9	[47.7, 90.2]
	HPV-16	2945	7	4407.73	2972	35	4422.78	79.9	[48.3, 93.8]
	HPV-18	3143	4	4711.21	3190	12	4774.48	66.2	[-32.6, 94.0]

N: Number of subjects included in the analysis (subjects who were DNA negative and seronegative for the corresponding HPV type at baseline)
n: Number of subjects with persistent infection, T: Total follow-up period (year)

For safety, major adverse events at the time point of interim analysis are tabulated in Table 19. The incidence of solicited local symptoms (injection site pain, injection site redness, and injection site swelling) was higher in the HPV Vaccine group compared to the control group

Table 19. Solicited and unsolicited symptoms reported in ≥5% of subjects in either group (TVC, Interim Analysis)

	Symptom	HPV Vaccine						Control					
		AE			ADR			AE			ADR		
		N	n	%	N	n	%	N	n	%	N	n	%
Solicited local symptom*	Injection site pain	3077	2786	90.5	-	-	-	3080	2402	78.0	-	-	-
	Injection site redness	3077	1348	43.8	-	-	-	3080	851	27.6	-	-	-
	Injection site swelling	3077	1292	42.0	-	-	-	3080	609	19.8	-	-	-
Solicited general symptom	Arthralgia	3076	633	20.6	3076	390	12.7	3080	551	17.9	3080	317	10.3
	Fatigue	3076	1771	57.6	3076	1223	39.8	3080	1652	53.6	3080	1110	36.0
	Fever (Axillary) (≥37.5°C)	3076	381	12.4	3076	223	7.2	3080	337	10.9	3080	208	6.8
	Gastrointestinal disorder	3076	850	27.6	3076	473	15.4	3080	841	27.3	3080	458	14.9
	Headache	3076	1665	54.1	3076	943	30.7	3080	1579	51.3	3080	897	29.1
	Myalgia	3076	1606	52.2	3076	1209	39.3	3080	1382	44.9	3080	981	31.9
	Rash	3076	312	10.1	3076	183	5.9	3080	258	8.4	3080	131	4.3
	Urticaria	3076	298	9.7	3076	117	3.8	3080	244	7.9	3080	92	3.0
Unsolicited symptom	Influenza	3184	155	4.9	3184	6	0.2	3187	177	5.6	3187	3	0.1
	Headache	3184	218	6.8	3184	20	0.6	3187	241	7.6	3187	19	0.6

N; number of subjects with at least one vaccination, n: number of subjects reporting the symptom, %: incidence rate
Solicited (local and general) symptoms were reported during the 7 days (Days 0-6) post-vaccination.
Unsolicited symptoms were reported during the 30 days (Days 0-29) post-vaccination.

* For solicited local symptoms, causality (relation to vaccination) were not assessed.

Five deaths were reported (road traffic accident [2 subjects]; bone sarcoma, diabetic ketoacidosis, and victim of sexual assault and homicide [1 subject each]), which were all considered as not related to vaccination by the investigator. A total of 761 serious adverse events were reported in 653 subjects, of whom 330 were in the HPV Vaccine group and 323 were in the control group. The most frequently reported serious adverse events was spontaneous abortion (24 cases [0.3 %] in the HPV Vaccine group; 25 cases [0.3 %] in the control group). The number of subjects reporting serious adverse events for which a causal relationship to vaccination could not be denied was 9 in the HPV Vaccine group and 6 in the control group. Eight serious adverse events led to study discontinuation (3 events in the HPV Vaccine group; 5 events in the control group). Nine non-serious adverse events led to study discontinuation (6 events in the HPV Vaccine group; 3 events in the control group). Three subjects discontinued the study due to serious adverse events other than death (head injury, complete spontaneous abortion, and skin infection). None of these serious adverse events were assessed as possibly vaccine-related according to the investigator. The non-serious adverse events that lead to treatment discontinuation were urticaria, endometriosis, headache, hypoaesthesia, gastroenteritis, facial pain, rash, pain, and ovarian cyst. Among these events, urticaria, headache, facial pain and pain were assessed as possibly vaccine-related according to the investigator.

4.B Outline of the review by PMDA

4.B.(1) Clinical data package

About the clinical data package submitted for this application, the applicant explained as follows.

The core efficacy of the HPV Vaccine was evaluated in Studies HPV-001, HPV-007 and HPV-008 among the overseas studies. These studies were conducted in females between 15 and 25 years of age, in which population HPV infection occurs with the highest incidence and cervical tissues can be sampled. In Study HPV-001, females with no history of oncogenic HPV infection at screening prior to vaccination, i.e., the group with no previous exposure to HPV were included and evaluated. In contrast, in Study HPV-008, females irrespective of history of oncogenic HPV infection, i.e., the general population, were included and evaluated. These efficacy studies have not been performed in pre-adolescents but rather in adolescents and young adults because efficacy evaluation requires cervical sample (The evaluation of vaccine efficacy in terms of virological, cytological or histopathological endpoints in girls younger than 15 years is not feasible due to ethical and practical considerations), and the HPV infection rate is the highest in the adolescent and young adults population. For these reasons, the studies conducted in young adolescent females (aged 10-14 years) (Studies HPV-012, HPV-013, etc.) evaluated the immunogenicity, and the studies conducted in females aged ≥26 years (Study HPV-014, etc.) also evaluated the immunogenicity. Safety of Cervarix was evaluated not only by analysis of each

overseas study but also by pooled analysis of these studies. The clinical data package submitted for this application, based on the consultation with PMDA, has included the results of Study HPV-032 which was conducted in Japanese females aged 20 to 25 years to evaluate the efficacy against persistent infection, and the results of Study HPV-046 which was conducted in females aged 10 to 15 years to evaluate the immunogenicity.

PMDA decided to evaluate the results of Study HPV-008 conducted in foreign countries (target number of subjects of 18,000) as the core study for efficacy of the HPV Vaccine, and at the same time, for the efficacy and safety in the Japanese population, to evaluate the results of Studies HPV-032 and HPV-046 conducted in Japan together with overseas clinical studies. When preparing this Review Report (1), the results of Interim Analysis II (performed at the time point when 8 cases of persistent infection [6-month definition] associated with HPV-16 or HPV-18 were accrued in the ATP cohort for efficacy) have been submitted as the result of Study HPV-032. Prior to approval of the HPV Vaccine, PMDA intends to confirm that the Final Analysis results submitted in the future are not contradictory to the Interim Analysis II results.

4.B.(2) Pharmaceutical formulation

4.B.(2).1) Active ingredients

The applicant explained the background of development of an HPV vaccine containing the virus-like particles (VLP) of HPV-16 and HPV-18 as active ingredients for the purpose of prevention of cervical cancer as follows:

It was reported that protective immunity was induced against homologous papilloma virus infection and subsequent occurrence of lesions in 3 types of animal models (dog, cottontail rabbit and cow) by inoculation of species-specific L1 VLP vaccine (*Proc Natl Acad Sci*. 1995; 92:11553-7, *Virology*. 1992; 187:612-619, *Clinics in Dermatology*. 1997; 15:237-247, *Virology*. 1996; 219:37-44). Moreover, in a study conducted in humans, favorable correlation of HPV-16 L1 VLP IgG antibody concentration in the serum to that in the cervical mucous fluid was reported after administration of HPV-16 L1 VLP vaccine. (*J Natl Cancer Inst*. 2003;95 (15):1128-37), suggesting that the serum IgG antibody response induced by vaccine is important as a factor to provide protection against cervical infection with oncogenic HPV [see "3.(i).B.(1) Primary pharmacology"]. Meanwhile, a study performed by International Agency for Research on Cancer (IARC) has confirmed that the correlation between oncogenic HPV and cervical cancer is one of the strongest among those observed in human cancer so far and that persistent infection with the oncogenic HPV types is involved in almost all invasive cervical cancers. Particularly, it has been shown that HPV-16 and HPV-18 have higher risk to progress to persistent infection and cancer than other oncogenic HPV types (*Br J Cancer*. 2003;89:101-5, *Cancer Epidemio. Biomarkers*

Prev.2005;14(5):1157-64), and the two HPV types are reported to be involved in more than 70% of squamous cervical cancer and 85% of adenocarcinoma in the world. In Japan, results of a meta-analysis of 14 studies investigating the distribution of HPV types in the patients with invasive cervical cancer reported that HPV-16 and HPV-18 were the most frequently observed HPV types detected in more than 50% of 1,142 patients with invasive cervical cancer. Moreover, similar results were obtained in a meta-analysis of 7 reports on HPV types observed in Japanese females with high grade squamous intraepithelial lesion (HSIL) (total 338 subjects) (*Int J Cancer*. 2007;121 (3): 621-32).

PMDA considers as follows:

The involvement of HPV in the morbidity of cervical cancer has been known. Therefore, the development concept of this HPV Vaccine, prevention of cervical cancer and its precursor lesions by preventing persistent HPV infection, is understandable. Though there is a report suggesting that the ratio of infection with HPV-16 or HPV-18, known as oncogenic HPV, in patients with cervical cancer or HSIL in Japan are slightly different from those reported abroad (*Cancer Epidemiology Biomarkers & Prevention.* 2001;10:45-52), both types of infection are also detected at a high prevalence in Japan. Therefore, it is acceptable to target HPV-16 and HPV-18 and to set respective L1 VLP as the active ingredients in the vaccine development.

4.B.(2).2) Selection of adjuvant

The applicant explained the process of adjuvant selection as follows:

Based on the results of nonclinical studies, the adjuvant of this HPV Vaccine was selected, and the weight ratio of antigen, aluminum hydroxide and MPL was set at 1:25:2.5 [see "3.(i).A.(1).2) Examination of the contents of aluminum hydroxide, MPL and HPV antigen in this vaccine using mouse immune response as an index"]. In Study HPV-004, the serum antibody titer of 3 types of HPV-16/18 vaccines (containing aluminum hydroxide as an adjuvant, containing AS04 as an adjuvant and containing no adjuvant) that contained 20 µg of HPV-16 VLP and 20 µg of HPV-18 VLP was compared. As a result, the antibody titer (ELISA) was higher in the AS04 group than in the aluminum group or no-adjuvanted group in Months 7 and 12 (Table 20).

Table 20. Anti-HPV-16 and anti-HPV-18 ELISA titers (Study HPV-004, ATP cohort for immunogenicity)

Antigen	Group	Timepoint	N	GMT	Titer (log_{10})	
					Mean	95% CI
HPV-16	AS04 40µg	Month 0	20	1.2	0.1	[0.0, 0.2]
		Month 7	19	11199.0	4.0	[3.9, 4.2]
		Month 12	18	4550.5	3.7	[3.4, 3.9]
	Alum 40µg	Month 0	17	1.2	0.1	[0.0, 0.2]
		Month 7	17	4076.0	3.6	[3.4, 3.8]
		Month 12	15	2076.9	3.3	[3.0, 3.6]
	No adjuvant	Month 0	20	1.0	0.0	[0.0, 0.0]
		Month 7	20	2488.3	3.4	[3.3, 3.5]
		Month 12	18	935.3	3.0	[2.8, 3.1]
HPV-18	AS04 40µg	Month 0	20	1.2	0.1	[0.0, 0.2]
		Month 7	19	4794.2	3.7	[3.5, 3.8]
		Month 12	18	1536.1	3.2	[2.9, 3.4]
	Alum 40µg	Month 0	17	1.2	0.1	[0.0, 0.2]
		Month 7	17	1960.4	3.3	[3.1, 3.5]
		Month 12	15	637.1	2.8	[2.6, 3.0]
	No adjuvant	Month 0	20	1.2	0.1	[0.0, 0.2]
		Month 7	20	1305.2	3.1	[2.9, 3.3]
		Month 12	18	229.7	2.4	[2.1, 2.7]

N = number of subjects with available results
Titers below the quantitation limit were treated as 1 in calculation

When the neutralizing antibody activity was determined by RT-PCR (After reaction of diluted serum sample with HPV, the cells are infected, and the infection inhibition performance of serum is determined. For infection volume, mRNA of HPV in the cell is determined by RI-PCR), and the cellular immunity was determined through determination of lymphocyte growth response and IFN-γ and IL-5 production, the neutralizing antibody titer to HPV-16 tended to be the highest in the AS04 group while no difference was observed between the AS04 group and aluminum group in the response to HPV-18. The neutralizing antibody titers for both antigens were the lowest in the no-adjuvanted group. The changes in neutralizing antibody titer to HPV-16 and HPV-18 were similar to the changes in the immune response evaluated by ELISA. The antigen-specific humoral and cellular immune response to both HPV-16 and HPV-18 was observed after two inoculations of the investigational vaccine and the values was considerably higher than the baseline level even at Month 12 in all the groups. Since the above results indicated that the highest immune response was induced by AS04-added vaccine, it was decided to use the drug product adjuvanted with AS04 in the subsequent clinical trials. In the follow-up until 4 years after the first vaccination in the Studies HPV-005 and HPV-004, when the combined results of the 2 studies were analyzed for the AS04-adjuvanted 40 µg group and the aluminum-adjuvanted 40 µg group, the immunogenicity of the AS04-adjuvanted vaccine was higher than that of the aluminum-adjuvanted vaccine, and the anti-HPV-16/18 antibody titer were also higher in the AS04 group than the aluminum group until 4 years after vaccination. In the determination by competitive ELISA (the amount of neutralizing antibody was determined through competition

with monoclonal antibody to neutralizing epitope V5 or J4 in the serum) and pseudovirus particle method (evaluated in terms of inhibition of infection after co-culture of diluted serum sample, HPV L1, L2 and secretory type alkaliphosphatase gene-containing infectious pseudovirus particle with cells. The amount of infection was determined by alkaliphosphatase activity), the antibody titer of AS04-adjuvanted vaccine was also higher than that of aluminum-adjuvanted vaccine until 4 years after vaccination. Moreover, the AS04-adjuvanted vaccine showed high responses of T-cells and memory B-cells against HPV-16 and HPV-18.

PMDA considers as follows:
The selection of AS04 as adjuvant is considered appropriate from the efficacy viewpoint. Moreover, based on the evaluation of a new excipient MPL contained in AS04 [see "2.A.(2).5) Novel excipients," "2.B.(8) Novel excipients"] and the safety evaluation in clinical studies [see "4.B.(4) Safety"], it is judged that there is no particular problem at present from the safety viewpoint.

4.B.(3) Efficacy

4.B.(3).1) Study HPV-008

PMDA requested the applicant to provide a justification for establishing the CIN2+ (CIN2+ is defined as CIN2, CIN3, adenocarcinoma in situ [AIS] and invasive cervical cancer) associated with HPV-16 and/or HPV-18 infection at the lesion in the cervical tissues as a primary endpoint in the Study HPV-008 performed as a main study to show the efficacy of the HPV Vaccine.

The applicant explained as follows:
Medical examination of the cervix is performed by multistep processes beginning with Pap smear, or collection of the cervical smear, used for cytological test. It has become possible to detect the precancerous lesions preceding cancer in situ by the introduction of the Pap smear in which cervical cells are collected and Papanicolaou stain are performed. For the subjects showing abnormalities in the cytological test, an examination by colposcopy and tissue diagnosis by biopsy of the cervix may be performed. At present, the Bethesda classification system has widely been used for assessment of the results in cytological test of the cervix, and its correspondence to tissue diagnosis is roughly shown in the figure below. Both cytological test and histological test diagnose low- and high-grade precancerous lesions, but it is necessary to pay attention to that there is no direct relationship between the grade in cytological diagnosis and that in histological diagnosis. Since it has been demonstrated that surgical treatment of CIN2+ diagnosed by histological test in the medical examination program reduces the occurrence of cervical cancer, the CIN2+ is considered an appropriate endpoint to show the efficacy in clinical studies of HPV

vaccines. Therefore, CIN2+ was decided to be used as an endpoint for evaluating the preventive effect of the HPV Vaccine against cervical cancer in the clinical trials for efficacy.

Bethesda System 1999	CIN System	Interpretation
Negative for intraepithelial lesions or malignancy	Normal	No abnormal cells
ASC ASC-US (atypical squamous cells of undetermined significance)		Squamous cells with abnormalities greater than those attributed to reactive changes but that do not meet the criteria for a squamous intraepithelial lesion
ASC-H (atypical squamous cells, cannot exclude HSIL)		
LSIL (low-grade squamous intraepithelial lesions	CIN 1	Mildly abnormal cells; changes are almost always due to HPV
HSIL (high-grade squamous intraepithelial lesions) with features suspicious for invasion (if invasion is suspected)	CIN2/3	Moderately to severely abnormal squamous cells
Carcinoma	Invasive squamous cell carcinoma Invasive glandular cell carcinoma (adenocarcinoma)	The possibility of cancer is high enough to warrant immediate evaluation but does not mean that the patient definitely has cancer

Figure. Bethesda and CIN Classification Systems

(*Clin Microbiol Rev.* 2003;1,6(1):1-17)

PMDA considers as follows:

It is appropriate to set CIN2+ as the primary endpoint in Study HPV-008 conducted for the purpose of investigating the preventive effect of the HPV Vaccine against cervical cancer. Although it is meaningful to investigate the efficacy of the HPV Vaccine during a certain observation period in a clinical study, in view of the duration from HPV infection to the onset of cervical cancer, it is also considered necessary to evaluate the preventive effect for a long time even after the clinical study. In this regard, it is understandable that the interim analysis was conducted using CIN2+ as an index and the efficacy thereafter has been continuously investigated. The results of interim analysis are considered to demonstrate the preventive effect of this HPV Vaccine in CIN2+ attributable to HPV-16 or HPV-18 [see "4.A.(6) Overseas phase III study (Study HPV-008)"]. In view of the progression process of cervical cancer and its precursor lesion, it is considered that the preventive effect, that is, the efficacy of this vaccine, has been demonstrated against cervical cancer caused by HPV-16 and/or HPV-18 and cervical atypical epithelium, that is, the precursor lesion. However, up to present, the efficacy of this vaccine has been evaluated for a limited period of time, for example, the mean follow-up period is about 4

years from the start of Study HPV-001 to Visit 3 in Study HPV-007. In view of the duration from HPV infection to the onset of cervical cancer and its precursor lesion, the applicant should continue the long-term collection of information, etc., bearing in mind whether or not the preventive effect of this HPV Vaccine is permanent is still unclear.

4.B.(3).2) Efficacy of the HPV Vaccine in Japan

In Japan, Study HPV-032 was performed to evaluate the preventive efficacy against persistent infection (6 months definition), immunogenicity and safety of the HPV Vaccine in women aged 20 to 25 years, and Study HPV-046 was performed to evaluate the immunogenicity and safety of the vaccine in women aged 10 to 15 years. An interim analysis was conducted in Study HPV-032 and the results were submitted. As is stated in "4.B.(3).1) Study HPV-008", PMDA considers it understandable that the efficacy of this HPV Vaccine was evaluated in the interim analysis after a certain period of observation and the efficacy thereafter has been continuously evaluated.

The applicant discussed the results of Studies HPV-032 and HPV-046 as follows:

The immunogenicity data obtained in Study HPV-032 was similar to the immunogenicity result obtained in the same age group in the overseas clinical studies, and the result of Study HPV-032 on "preventive efficacy against persistent infection (6-month definition)", that is, the primary endpoint, was also similar to that in Studies HPV-001 and HPV-008. Accordingly, it is considered that the efficacy against precursor lesions (CIN2+) identified in Studies HPV-007 and HPV-008 can be expected in Japanese women (15-25 years of age). Moreover, the immunogenicity result in the lower age group obtained in Study HPV-046 was similar to that obtained in the same age group in the Studies HPV-012 and HPV-013 performed in overseas countries. Additionally, the geometric mean antibody titer (GMT) after 7 months post dose in the overseas HPV-012 study was twice higher both for anti-HPV-16 antibody and anti-HPV-18 antibody in the age group of 10 to 14 years in comparison with that of 15 to 25 years. When the results of Study HPV-046 were compared with those of Study HPV-032, the GMT in the age group of 10 to 14 years was also twice as much or higher than that in the age group of 15 to 25 years old, suggesting the same trend as that in the overseas studies. Accordingly, it is considered that the efficacy results obtained from the Studies HPV-001, HPV-007, HPV-008 and HPV-032 can be used in evaluating the efficacy of the vaccine in the 10 to 15 years Japanese females.

PMDA considers as follows:

Since the preventive effect of this vaccine against CIN2+ associated with HPV-16 and/or HPV-18 was demonstrated in Study HPV-008 (Interim Analysis), and the preventive effect against persistent infection (6 months definition) was indicated in Study HPV-032 as with the case of overseas HPV-001 and HPV-008 studies, the preventive effect of this vaccine may also be

expected in Japanese females of 20 to 25 year of age against CIN2+ associated with HPV-16 and/or HPV-18, and against cervical cancer and its precursor lesion, i.e., atypical epithelium. However, as the applicant explained that a conclusion was not reached at present on whether the persistent infection precedes or correlates to the onset of lesion, the persistent infection is still a surrogate endpoint for the true endpoint, and its clinical significance has to be assessed with the findings obtained in the future including the results of post-marketing clinical study, etc. The immunogenicity in young females is evaluated as described below [see 4.B.(5).1) Age].

4.B.(3).3) Efficacy against HPVs other than HPV-16 or HPV-18

The applicant explained the efficacy of the HPV Vaccine against HPV types other than HPV-16 or HPV-18 as follows:

When the efficacy against persistent infection (6 months definition) of oncogenic HPVs (HPV-45, 31, 33, 52 and 58), which are detected with high frequency as a cause of cervical cancer following HPV-16/18, was evaluated in Study HPV-008, the VE was 36.1% (95% CI [0.5, 59.5]) for HPV-31, 59.9% [2.6, 85.2] for HPV-45 and 31.6% [3.5, 51.9] for HPV-52, showing significant efficacy. On the other hand, significant efficacy was not observed against HPV-33 or HPV-58, which are remotely related to HPV-16 and HPV-18. For the efficacy against persistent infection (12 months definition), since there were few subjects with 12-month persistent infection, no conclusion could be drawn. In the pooled analysis of Studies HPV-001 and 007, where a follow-up was undertaken for the incident infection with oncogenic HPVs for 6.4 years after the first vaccination in the Study HPV-001, the Kaplan-Meier curve for infection with each of oncogenic HPV types other than HPV-16 or HPV-18 (HPV-45, 31, 33, 52 and 58) was plotted. For HPV-31 and HPV-45, which are phylogenetically similar to HPV-16 and HPV-18, obvious dissociation was observed between the curve of the HPV Vaccine group and that of the control group, which suggests that the cross protection with HPV-31 and HPV-45 for incident infection may be kept for a long period of time. In the case of HPV-52, it was suggested that the persistence of cross protection was short. No result suggesting any efficacy was obtained for HPV-33 and HPV-58.

PMDA considers as follows:

The demonstrated preventive effect of the HPV Vaccine against HPV types other than HPV-16 or HPV-18 was not as high as the VE against HPV-16 and HPV-18 (HPV-16, 100% [79.0, 100]; HPV-18, 100% [59.4, 100]), and it is unknown whether or not the HPV Vaccine may have a preventive effect against cervical cancer and its precursor lesions associated with these HPV types.

4.B.(4) Safety

The adverse events observed with a relatively high frequency in all the clinical studies in Japan and overseas clinical studies were evaluated by classifying them into the following categories: solicited local symptoms (pain, redness and swelling at injection site that occurred within 7 days [Days 0-6] after vaccination), solicited general symptoms (fatigue, fever, gastrointestinal disorders [including nausea, vomiting, diarrhea and abdominal pain], headache and rash) and unsolicited symptoms.

PMDA evaluated the safety of this HPV Vaccine as described below mainly based on the results of clinical studies conducted in Japan (Studies HPV-032 and HPV-046) and Study HPV-008 conducted abroad in 18,000 subjects.

4.B.(4).1) Local reactions

The incidence of solicited local symptoms in the HPV Vaccine group was higher than that in the control group both in Studies HPV-032 and HPV-008. While the incidence of injection site pain was markedly higher than that of other 2 solicited local symptoms (redness and swelling at injection site) in overseas clinical studies, the incidences of all these 3 symptoms tended to be high in the studies in Japan. In Studies HPV-032 and HPV-046, no particular difference was observed in the incidences of solicited local symptoms (3 symptoms) [see 4.A.(1) Study HPV-032, 4.A.(2) Study HPV-046, 4.A.(6) Study HPV-008 for the number of cases and incidence].

PMDA considers as follows:

The incidence of solicited local symptoms tended to be higher in Japan in comparison with that observed abroad, and most of the symptoms were assessed as mild to moderate in severity. However, a tendency of higher incidence of severe local symptoms in comparison with the status abroad should be taken into consideration. Attention should also be paid to the tendency of increase in the incidence of redness and swelling at injection site with the increasing number of vaccination in Studies HPV-032 and HPV-008, though such tendency was not observed in Study HPV-046. On the other hand, the incidence of solicited local symptoms in Japan did not demonstrate any substantial difference by age. Furthermore, since most of these symptoms were reported as transient (mean duration 2.2-3.4 days), it is not considered necessary to discontinue the treatment with this vaccine simply because of concern about these events. Nor is it considered necessary to separately call for attention to these events on the basis of age difference. However, because the inflammatory findings in the injection site were also obtained in nonclinical studies [see “3.(iii).B Outline of the Review by PMDA”], and because the incidence of some symptoms tended to increase with the increase in the number of vaccination, it is considered necessary to

carefully observe the vaccinated site and pay attention to the local irritation reaction, such as by avoiding the injection to the previously injected site.

2) General reaction

Compared with the incidence of local symptoms, the incidence of solicited general symptoms was lower in all Studies HPV-032, HPV-046 and HPV-008. Major symptoms reported within 7 days (Days 0-6) after vaccination were fatigue, myalgia and headache. The incidence of all these symptoms tended to be higher in Study HPV-032 in comparison with Study HPV-046. In Study HPV-032, the incidences of fatigue, myalgia and arthralgia in the HPV Vaccine group were higher than those in the HAV group, and the incidence of severe symptoms also tended to be higher in the HPV Vaccine group than that in the HAV group. In Study HPV-032, the incidence of fatigue based on the number of administered doses and the number of vaccinated subjects (after a total of 3 doses has been administered to each of the subjects) were 1.8% (26 of 1,452 doses) and 4.5% (23 of 512 subjects) in HPV Vaccine group, and 0.8% (11 of 1,456 doses) and 2.0% (10 of 511 subjects) in the HAV group; The incidences of myalgia were 1.0% (14 of 1,452 dose) and 2.3% (12 of 512 subjects) in the HPV Vaccine group, and 0.1% (1 of 1,456 doses) and 0.2% (1 of 511 subjects) in the HAV group; The incidences of arthralgia were 0.6% (9 of 1,452 doses) and 1.6% (8 of 512 subjects) in the HPV Vaccine group, and 0.1% (1 of 1,456 doses) and 0.2% (1 of 511 subjects) in the HAV group. In addition, any increase in the incidence of clinically significant symptoms and that of grade 3 symptoms with the increasing number of vaccination were not observed. In Study HPV-008, the incidence of myalgia in the HPV Vaccine group was higher than that in the HAV group, and the incidence of severe myalgia was also higher in the HPV Vaccine group than the HAV group (The incidence of fatigue based on the number of administered doses and the number of vaccinated subjects [after a total of 3 doses has been administered to each of the subjects] were 1.8% [154 of 8,687 doses] and 4.6% [141 of 3,076 subjects] in the HPV Vaccine group, and 0.6% [52 of 8,751 doses] and 1.5% [47 of 3,080 subjetcs] in the HAV group).

As for the HPV Vaccine group, the adverse events for which a causal relationship to vaccination could not be denied (hereinafter referred to as “adverse reactions”) and which were observed in 10% or more subjects in both Studies HPV-032 and HPV-046 were arthralgia, fatigue, gastrointestinal disorders, headache and myalgia [see 4.A.(1) Study HPV-032 and 4.A.(2) Study HPV-046 for the number of cases and incidences], and the incidence of each symptom was higher in Study HPV-032 in comparison with that in Study HPV-046. Adverse reactions observed in 10% or more subjects in Study HPV-008 were similar to those that occurred in the clinical studies in Japan [see 4.A.(6) Study HPV-008 for the number of cases and incidences]. However, in comparison with Study HPV-008, no tendency of lower adverse reaction incidence was observed

in Study HPV-013 that was conducted abroad in the same age group (10-14 years old) as that in Study HPV-046.

PMDA considers as follows:
Unlike Studies HPV-008 and HPV-013, many solicited general symptoms for which a causal relationship to vaccination could not be denied were observed in both Studies HPV-032 and HPV-046. Attention should be paid to this point and the relevant trends should be investigated in the post-marketing survey and PSUR, etc. in future. Compared with the control group, the myalgia was severer in the HPV Vaccine group both in Studies HPV-032 and HPV-008. Since the incidence did not increase with increased number of vaccination and there was no substantial difference in the mean duration of myalgia between the HPV Vaccine group and control group (2.8 days and 2.3 days, respectively), the symptom is not considered to cause any particular problem even though attention should be paid to this symptom. The same applies to the fatigue and arthralgia also observed in addition to myalgia in the clinical studies in Japan. Compared with the age group of 15 to 25 years (20-25 years in the Japanese study), higher antibody titers were obtained in the age group of 10 to 15 years both in the studies in Japan and abroad [see 4.B.(3).2) Efficacy of the HPV Vaccine in Japan]. However, not much difference was observed in the incidence of fever between the age group of 15 to 25 years and that of 10 to 15 years. With reference to the above result, it is not considered necessary at present to pay particular attention to the difference in age groups in regard to the general reaction.

4.B.(4).3) Adverse events/serious adverse events leading to study discontinuation

a. Death

No adverse event resulting in death was reported in Japanese clinical studies (Studies HPV-032 and HPV-046).

In the Study HPV-008, 5 deaths were reported by the date of data lock, September 30, 2006, all of which were assessed not to be related to the vaccine by the investigator [see 4.A.(1) to 4.A.(6) for the outline of major clinical studies).

In the ongoing studies (Study HPV-007 [after interim analysis at Month 24], Study HPV-012Ext, Study HPV-013Ext [after interim analysis at Month 18], Study HPV-014Ext [after interim analysis at Month 18] and HPV-009), 7 deaths were observed by the date of data lock, September 30, 2006, all of which were reported in the Study HPV-009. Only one of 7 deaths was assessed as possibly related to vaccination by the investigator (Crohn’s disease: the key was opened only for this case). No event resulting in death was reported in other overseas clinical studies.

b. Serious adverse events

In Study HPV-032, a total of 37 serious adverse events were observed in 31 subjects among 1040 subjects (3.0%) until the time of Interim Analysis II. The primary system organ class (SOC) of serious adverse events reported most frequently was the "pregnancy, puerperium and perinatal conditions" (10 events) followed by "injury, poisoning and procedural complication" (7 events) and "infections and infestations" (7 events). Among them, 1 case of spontaneous abortion was assessed as related to vaccination by the investigator. In Study HPV-046, no serious adverse event was reported.

A pooled analysis was performed as to the serious adverse events reported in overseas clinical studies. The results are summarized in Table 21. The ratio of subjects who had at least one serious adverse event was 2.8% (459 of 16,142 subjects). The ratio was similar in the ALU group (2.2%, 75 of 3,454 subjects) and HAV360 group (2.4%, 25 of 1,032 subjects) but lower than that in the HAV720 group (3.5%, 323 of 9,325 subjects).

Table 21. Summary of serious adverse events (TVC)

	Group				Total
	HPV N = 16,142 D = 45,988	ALU N = 3,454 D = 9,217	HAV360 N = 1,032 D = 3,068	HAV720 N = 9,325 D = 26,956	N = 29,953 D = 85,229
Number of subjects with at least one SAE reported	459	75	25	323	882
Number of doses followed by at least one SAE	472	78	25	335	910
Number of SAEs classified by MedDRA Preferred Term*	533	84	28	372	1,017
Number of SAEs reported	542	86	28	372	1,028

HPV: Studies HPV-001/007, 003, 004, 005, 008, 012, 013, 013 Ext, 014, 014 Ext, 015 and 016
ALU: Studies HPV-001/007, 003 and 015
HAV360 (Hepatitis A control group containing 360 EL.U/mL hepatitis A antigen): Study HPV-013
HAV720 (Hepatitis A control group containing 720 EL.U/mL hepatitis A antigen): Study HPV-008
* Post-dose symptoms observed in a subject more than once and classified as the same Preferred Term are counted once.
N: Number of subjects
D: Number of doses

Among the serious adverse events observed in at least 0.1% of subjects in the HPV Vaccine group (Table 22), spontaneous abortion was the most frequently observed events, and the incidence was similar in the HPV Vaccine group and control groups (ALU group and HAV group). Moreover, the incidence of serious adverse events by age group was as follows: 2.3% (27 of 1,194 subjects) in the HPV Vaccine group and 2.4% (25 of 1,032 subjects) in the HAV360 group in the subjects aged 10 to 14 years; 3.4% (395 of 11,591 subjects) in the HPV Vaccine group, 8.4% (49 of 581 subjects) in the ALU group and 3.5% (322 of 9,315 subjects) in the HAV720 group in the subjects aged 15 to 25 years; and 1.1% (37 of 3,357 subjects) in the HPV

Vaccine group, 0.9% (26 of 2,873 subjects) in the ALU group and 10% (1 of 10 subjects) in the HAV720 group in the subjects aged 26 years or older. The incidences were similar between the HPV Vaccine group and HAV group but the incidence in the population of 15 to 25 year subjects was higher in the ALU group.

Table 22. Incidence of serious adverse events reported in ≥0.1% of subjects in the HPV Vaccine group (TVC)

System Organ Class	Preferred Term	HPV N = 16,142		ALU N = 3,454		HAV360 N = 1,032		HAV720 N = 9,325	
		n	%	n	%	n	%	n	%
Total		459	2.8	75	2.2	25	2.4	323	3.5
Infections and infestations	Appendicitis	25	0.2	4	0.1	5	0.5	23	0.2
	Pyelonephritis	11	0.1	2	0.1	0	0	5	0.1
	Pyelonephritis acute	12	0.1	0	0	0	0	5	0.1
Pregnancy, puerperium and perinatal conditions	Abortion spontaneous	34	0.2	14	0.4	0	0	25	0.3
	Abortion spontaneous complete	14	0.1	1	0	0	0	6	0.1
	Abortion spontaneous incomplete	23	0.1	2	0.1	0	0	12	0.1
Psychiatric disorders	Depression	16	0.1	2	0.1	0	0	12	0.1
Reproductive system and breast disorders	Ovarian cyst	10	0.1	0	0	1	0.1	4	0

HPV: HPV-16/18 vaccine group (Studies HPV-001, 003, 004, 005, 007, 008, 012, 013, 014, 015 and 016; from 10 years of age onwards)
ALU: Al(OH)3 control group (Studies HPV-001, 003, 007 and 015; from 15 years of age onwards)
HAV360 (Hepatitis A control group containing 360 EL.U./mL hepatitis A antigen): Study HPV-013
HAV720 (Hepatitis A control group containing 720 EL.U. /mLhepatitis A antigen): Study HPV-008
Total: number of subjects reporting at least one symptom (regardless of the MedDRA Preferred Term)
N: number of subjects with at least one administered dose
n: number of subjects reporting at least one symptom, %:incidence

Serious adverse events for which a causal relationship to vaccination was not denied by the investigator were reported by 103 subjects (121 events). The key codes were opened for the 114 events that were judged as serious and unpredictable with the breakdown of 60 events in the HPV Vaccine group and 54 events in the control groups (48 in the HAV group and 6 in the ALU group). In this regard, 85 events in 76 subjects were reported in Study HPV-009, about which the applicant explained that it was because the investigators decided to handle the causal relationship to vaccination as undeniable in the cases of abnormal pregnancy outcome in the subjects whose latest menstruation period (LMP) fell within 60 days after vaccination [see "4.B.(4).6) Pregnancy"].

c. Adverse events leading to study discontinuation

As for the ratio of subjects who discontinued the study due to adverse events in the Japanese clinical studies, in the Japanese Study HPV-032 (as of the Interim Analysis II), 460 of 519

subjects (88.6%) continued the study. There was no difference in the ratio of discontinuation cases between the HPV Vaccine group and control group. The study was discontinued due to serious adverse event in one subject (skull fracture and brain contusion due to the fall) but the causal relation to the investigational product was denied. The ratios of subjects who discontinued the study due to non-serious adverse events were 0.8% (4 of 519 subjects) in HPV Vaccine group and 0.2% (1 of 521 subjects) in the control group and the events were urticaria in 3 subjects, exacerbation of dermatographia in one subject and gastrointestinal symptom in one subject. The key codes have not been opened for these 5 subjects. In Study HPV-046, there was no subject who discontinued the study.

The ratio of subjects who discontinued the study due to adverse events in the overseas clinical studies was similar between the HPV Vaccine group (0.21%, 34 of 16,142 subjects) and control group (including ALU group, HAV360 group and HAV720 group; 0.15%, 21 of 13,811 subjects). Deaths occurred in 5 subjects (two in car accidents, one each due to homicide, osteosarcoma and diabetic ketoacidosis) (not including the number of deaths that occurred in the ongoing Study HPV-009). In addition, the study was discontinued due to serious adverse events in 4 subjects in the HPV Vaccine group (two by spontaneous abortion, and one each by multiple sclerosis and intervertebral disc protrusion), one subject in the HAV group (anorexia nervovosa), and 5 subjects whose key codes remained unopened (uterine prolapse, breast cancer, renal abscess, moderate skin infection and polytraumatism due to car accident). However, the causal relationship to the investigational product was denied in all these cases. The number of subjects who discontinued the study due to other non-serious adverse events was 40 of 29,953 subjects with the breakdown of 27 of 16,142 subjects (0.17%) in the HPV Vaccine group, 8 of 3,454 subjects (0.23%) in the ALU group, 2 of 1,032 subjects (0.19%) in the HAV group and 3 of 9,325 subjects (0.03%) in the HAV720 group. Except for the 29 events (injection site pain, arthralgia, fatigue, etc.), the investigators denied the causal relationship of these events to the vaccination.

PMDA considers as follows:
Since there was no difference between the HPV Vaccine group and control group in the incidences of death and serious adverse events in the studies in Japan and abroad, and no certain tendency was observed in the adverse event profile in the HPV Vaccine group, the risk leading to death and serious adverse events by vaccination of the HPV Vaccine is very low.

4.B.(4).4) New onset of chronic diseases and autoimmune disorder

In each of the studies excluding HPV-001, HPV-003, HPV-004 and HPV-005, the data on the New Onset of Chronic Disease (NOCD; autoimmune disorder, asthma, insulin-dependent diabetes mellitus and hypersensitivity as well as the signs and symptoms, etc. characteristic to

these diseases) and New Onset of Autoimmune Disorder (NOAD) were collected, and the results of a pooled analysis of 3 follow-up periods (0 to 7 months, 7 to 12 months and more than 12 months after vaccination) were investigated (Table 23).

Table 23. Incidence of new onset of chronic diseases by reporting period (TVC)

Reporting period	HPV N=13,591			ALU N=984			HAV360 N=1,032			HAV720 N=9,325		
	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI
Month 0-7	159	1.2	[1.0, 1.4]	10	1.0	[0.5, 1.9]	22	2.1	[1.3, 3.2]	87	0.9	[0.7, 1.1]
Month 7-12	46	0.4	[0.3, 0.5]	NA	NA	NA	6	0.6	[0.2, 1.3]	37	0.4	[0.3, 0.5]
> Month 12	44	0.4	[0.3, 0.5]	6	1.1	[0.4, 2.3]	3	0.3	[0.1, 0.8]	33	0.4	[0.2, 0.5]

N: number of subjects with at least one administered dose, n: number of subjects reporting the symptom, %: incidence
NA: There is no applicable clinical trial requiring reporting in the period

The incidences of NOCDs in the HPV Vaccine group (1.2%) during the period from 0 to 7 months after vaccination were lower than or the same as that in the control groups (1.0% in the ALU group, 2.1% in the HAV360 group and 0.9% in the HAV720 group), and no difference was observed between the vaccination groups thereafter. When assessing the incidences by age group, the 10 to 14 years age group tended to be higher in comparison with that in the age group of 15 to 25 years old, or in that of 26 years old or older, but the incidences were similar between the HPV Vaccine group and the control groups. Major NOCDs were asthma, urticaria and hypersensitivity, for which no difference in the incidence was observed between the vaccination groups. As for the clinical studies in Japan, the incidence of NOCDs in Study HPV-032 was 0.8% (4 of 519 subjects) in the HPV Vaccine group, which was similar to 0.6% (3 of 521 subjects) in the HAV group that served as the control group. Major NOCDs observed in Study HPV-032 were urticaria and asthma.

The NOADs were mainly related to thyroid gland disorder but the overall incidence was low, and there was no clear difference between age groups or vaccination groups. As for the clinical studies in Japan, the causal relationship of two NOADs (rheumatoid arthritis and allergic granulomatous angitis) that occurred in Study HPV-032 to the vaccination was denied. No event judged as NOCD or NOAD occurred in Study HPV-046.

Based on the above results, the applicant explained that even though no problem in safety was observed and the incidences of NOCD and autoimmune disorder were very low in the general population, it plans to continue evaluation in each of the ongoing clinical studies and further post-marketing investigation because there is a limitation in the assessment in the clinical development program.

PMDA accepted the applicant’s explanation.

4.B.(4).5) Occurrence of syncope, coma, etc.

As concerns Gardasil (Merck, unapproved in Japan) that is a drug comparable to this HPV Vaccine, the information on adverse events such as syncope, coma, Guillain-Barre syndrome, paralysis, etc. has been provided in the homepage of National Vaccine Information Center in the US (http://www.nvic.org/Diseases/HPV/HPVrpt.htm). PMDA requested the applicant to explain the occurrence status of similar adverse events of this HPV Vaccine in the clinical studies in Japan and abroad.

The applicant explained as follows:

Though the occurrence of coma, paralysis and Guillain-Barre Syndrome was not reported in the 15,469 subjects inoculated with this HPV Vaccine and 13,228 subjects with the control vaccine, in the ongoing or completed clinical studies (Studies HPV-008, HPV-012, HPV-012Ext, HPV-013, HPV-013Ext, HPV-014, HPV-014Ext, HPV-015 and HPV-016), 46 events of syncope were reported in 43 subjects, and 7 of these events were judged as serious. Of the 46 events, 5 events occurred on the day of vaccination with the breakdown of 3 (2 non-serious and 1 serious events) in the HPV Vaccine group and 2 (both of them non-serious) in the control group. The incidences per 1,000 vaccinated subjects were 0.16 (event) in the HPV Vaccine group and 0.15 (event) in the control group, indicating no difference between groups. In children and young adults, vasovagal syncope with a temporal relationship with vaccination by injection has been reported (*Arch Pediatr Adoless Med.* 1997;151:255-259). In this regard, syncope occurred within 5 minutes after vaccination in only one subject in the HPV Vaccine group but after 25 days or more from the vaccination in the remaining 42 subjects. In view of the onset time and clinical course, it was assumed that other underlying diseases induced syncope in most of the cases. Therefore, it is considered that the vaccination of this HPV Vaccine does not raise any particular safety concerns about increased risk of syncope, coma, Guillain-Barre Syndrome, or paralysis, etc. In addition, no report on syncope was included in the overseas post-marketing data.

PMDA considers as follows:

Based on the above explanation, the vaccination of this HPV Vaccine is not expected to increase the risk of inducing syncope, coma, Guillain-Barre Syndrome, or paralysis, etc. at present. However, it is necessary to continuously pay attention to these events and collect relevant information.

4.B.(4).6) Pregnancy

In the studies included in a pooled analysis of safety, a total of 1,737 pregnancies were reported by September 30, 2006, and their outcome is shown in Table 24. The incidence of spontaneous

abortion found in this analysis was lower than that in the general population reported in the epidemiological researches in the US (13%-16%) (*Epidemiology*. 1991;2(1): 33-39, *Vital Health Stat.* 2000;21(56)), and no inter-group difference was observed. Of the 6 cases of stillbirth, the key codes of 5 were not opened (HPV Vaccine group or HAV720 group).

Table 24. Number of pregnancies ** and outcome of pregnancy (TVC)

Outcomes	HPV N = 870		ALU N = 172		HAV360 N = 9		HAV720 N = 686		Total N = 1737	
	Value or n	%	Value or n	%	Value or n	%	Value or n	%	Value or n	%
Pregnancy ongoing	241	27.7	24	14.0	5	55.6	233	34.0	503	29.0
Normal Infant	399	45.9	104	60.5	2	22.2	264	38.5	769	44.3
Premature birth	16	1.8	3	1.7	1	11.1	17	2.5	37	2.1
Abnormal infant	6	0.7	4	2.3	0	0.0	8	1.2	18	1.0
Elective termination	103	11.8	13	7.6	1	11.1	93	13.6	210	12.1
Therapeutic abortion	1	0.1	0	0.0	0	0.0	2	0.3	3	0.2
Ectopic pregnancies	6	0.7	1	0.6	0	0.0	3	0.4	10	0.6
Spontaneous abortion	81	9.3	22	12.8	0	0.0	52	7.6	155	8.9
Still birth	*	*	1	0.6	0	0.0	*	*	6	0.3
Lost to follow-up	11	1.3	0	0.0	0	0.0	13	1.9	24	1.4
Not applicable	1	0.1	0	0.0	0	0.0	1	0.1	2	0.1

N : number of pregnancies, n ; number of pregnancies in a given category, Value : value of the considered parameter
% : n / Number of pregnancies with available results x 100
Spontaneous abortion includes missed abortion
Not applicable: e.g. mole, trophoblastic tumor
* : case which remains blinded to maintain the study investigator blinded to treatment allocation. Those cases remain blinded because Studies HPV-007, HPV-008 and HPV-015 are ongoing.
** : twin pregnancies counted as one pregnancy

When the outcome of pregnancy was compared among the groups, there was no substantial difference between the HPV Vaccine group and the control group. The incidence of fetal abnormalities (including congenital anomaly) was 0.7% (6 of 870 subjects) in the HPV Vaccine group, which was lower than that in the ALU group (2.3%, 4 of 172 subjects) and the HAV720 group (1.2%, 8 of 686 subjects). There were no specific events that occurred at a higher incidence among the 18 subjects.

It was reported that 11 neonatal deaths occurred in 9 subjects and 5 of these deaths were due to premature delivery. Other 3 deaths were due to neonatal respiratory distress syndrome (2 deaths) and intracranial hemorrhage (1 death). These were deaths due to typical conditions observed in premature infants. The remaining 3 deaths were due to pneumonia aspiration, congenital cystic kidney disease accompanied by oligohydramnios or gastroschiss.

In the clinical studies conducted in Japan, a total of 72 pregnancies (36 in the HPV Vaccine group and 36 in the control group) were reported by 68 subjects (Table 25). A follow-up is going on in regard to 14 cases (19.4%) of pregnancy. As of June 2009, no report is made on stillbirth and

outcome of fetal abnormalities (including congenital anomalies). No pregnancy was reported in Study HPV-046.

The applicant explains as follows:
Most of the subjects who became pregnant delivered normal babies, but even though the incidence of abnormal fetuses is very low, the relation to vaccination should be cautiously interpreted, and the applicant intends to continue information collection in the future.

Table 25. Reported number of pregnancies and their outcomes (Study HPV-032, TVC)

	HPV N = 36		HAV N = 36		Total N = 72	
Categories	n	%	n	%	n	%
Elective termination	12	33.3	15	41.7	27	37.5
Normal infant	10	27.8	13	36.1	23	31.9
Pregnancy ongoing	9	25.0	5	13.9	14	19.4
Spontaneous abortion*	5	13.9	3	8.3	8	11.1

N: number of pregnancies, n: number of pregnancies in a given category
%: n / Number of pregnancies with available results x 100
*Spontaneous abortion includes missed abortion

PMDA considers as follows:
Concerning the outcome of pregnancy related to vaccination of the HPV Vaccine, the data and responses submitted up to present indicated no tendency of substantial difference in the incidence of abnormal fetuses from the usual incidence. PMDA intends to discuss the matter in the Review Report (2), taking the latest information into consideration including the overseas post-marketing safety information that is being checked at present.

4.B.(5) Target population for vaccination

4.B.(5).1) Age

The clinical studies (HPV-001, HPV-007 and HPV-008) to investigate the preventive effect of the HPV Vaccine against cervical cancer and its precursor lesions were performed in women aged 15 to 25 years. Concerning the efficacy of the vaccine in other age groups, the applicant explained as follows:

In Studies HPV-013 and HPV-013Ext performed in women aged 10 to 14 years, immunogenicity and safety of the HPV Vaccine were confirmed. In Study HPV-012 conducted to evaluate the consistency of immunogenicity between 3 lots and the non-inferiority of seroconversion rate in women aged 10 to 14 years to that in women aged 15 to 25 years, seroconversion was identified in all the subjects, and the upper limit of two-sided 95% confidence interval of the difference in

the proportion of subjects showing seroconversion between the age groups 10 to 14 years and 15 to 25 years was lower than the pre-defined acceptance limit of 10% (Table 26).

Table 26. Seroconversion rates for anti-HPV-16 and anti-HPV-18 at Month 7 post-dose (Study HPV-012, ATP cohort for immunogenicity)

Antibody	Group	N	%	Group	N	%	Difference in seroconversion rate* (%)	95% CI
HPV-16	10-14 years	143	100	15-25 years	118	100	0.00	[-3.15, 2.62]
HPV-18	10-14 years	141	100	15-25 years	116	100	0.00	[-3.21, 2.65]

*: 15-25 year olds minus 10-14 year olds
N: number of subjects with available results
%: seroconversion rate (percentage of subjects with anti-HPV-16 IgG titer ≥ 8 EL.U./mL or anti-HPV-18 IgG titer ≥ 7 EL.U./mL)

Moreover, the GMTs for anti-HPV-16 and anti-HPV-18 at Month 7 were 7438.9 and 3070.1, respectively, in the group aged 15 to 25 years and 17272.5 and 6863.8, respectively, in the group aged 10 to 14 years.

The infection rate of HPV is the highest in young females who started sexual activity. Since biological changes of the uterine cervix occur in adolescent females, young females are considered particularly likely to be infected with HPV. The high incidence rate of oncogenic HPV in females aged 14 to 19 years in the US shows that HPV infection occurs immediately after the start of sexual activity in this age group. Thus, it is considered important to vaccinate this age group (10-14 years old) from the viewpoints of prevention of infection with oncogenic HPV and prevention of cervical cancer. Since the immune response of the HPV Vaccine has been confirmed to persist for at least 18 months (the longest follow-up period so far) after vaccination, vaccination of young females before starting sexual activity is expected to lead to long-term persistence of preventive effects.

In Study HPV-015 performed in women aged 26 years or older, the safety of the HPV Vaccine was confirmed, and the investigation of efficacy and immunogenicity is ongoing. In Study HPV-014 (performed in subjects aged 15-55 years) that was conducted to evaluate the non-inferiority of seroconversion rate in 26 to 45 years females and 46 to 55 years females to that in 15 to 25 years females, the GMT tended to decrease with an increase in age, but the GMT obtained in all the age groups (15 to 25, 26 to 45 and 46 to 55 years old) significantly exceeded the cut-off value of ELISA. Seroconversion to both antigens was observed 1 month after the third vaccination in all the age groups (Table 27).

Table 27. Seropositivity rates and GMTs against anti-HPV-16 and anti-HPV-18 antibodies (Study HPV-014, ATP cohort for immunogenicity)

Group	Age	Timing	N	S+			GMT	95% CI [lower/upper limits]
				n	%	95% CI		
HPV-16	[15-25]	PRE	224	29	12.9	[8.8,18.1]	5.1	[4.6, 5.6]
		PII(M2)	224	224	100	[98.4,100]	3162.2	[2875.7, 3477.4]
		PIII(M7)	219	219	100	[98.3,100]	7790.3	[6849.2, 8860.7]
	[26-45]	PRE	219	55	25.1	[19.5,31.4]	7.5	[6.4, 8.8]
		PII(M2)	219	219	100	[98.3,100]	2379.1	[2067.8, 2737.3]
		PIII(M7)	217	217	100	[98.3,100]	4060.0	[3510.8, 4695.0]
	[46-55]	PRE	199	60	30.2	[23.9,37.0]	7.1	[6.1, 8.2]
		PII(M2)	199	199	100	[98.2,100]	1768.7	[1522.3, 2055.0]
		PIII(M7)	196	196	100	[98.1,100]	2835.8	[2450.9, 3281.2]
HPV-18	[15-25]	PRE	223	16	7.2	[4.2,11.4]	3.9	[3.7, 4.2]
		PII(M2)	223	223	100	[98.4,100]	2234.4	[2009.6, 2484.2]
		PIII(M7)	218	218	100	[98.3,100]	3452.9	[3071.8, 3881.3]
	[26-45]	PRE	218	32	14.7	[10.3,20.1]	4.5	[4.1, 4.9]
		PII(M2)	218	218	100	[98.3,100]	1441.7	[1284.6, 1617.9]
		PIII(M7)	216	216	100	[98.3,100]	1880.9	[1660.7, 2130.3]
	[46-55]	PRE	201	26	12.9	[8.6,18.4]	4.4	[4.0, 4.9]
		PII(M2)	201	201	100	[98.2,100]	1015.8	[898.7, 1148.1]
		PIII(M7)	198	198	100	[98.2,100]	1377.5	[1212.8, 1564.6]

GMT: geometric mean antibody titer
N: number of subjects with pre-vaccination results available
n: number of seropositive subjects (i.e., with titer ≥ 8 EL.U./mL for anti-HPV-16 and ≥7 EL.U./mL for anti-HPV-18 by ELISA)
PRE: Prevaccination, PII(M2): Post Dose II (Month 2), PIII(M7): Post Dose III (Month 7)

The upper limit of two-sided 95% confidence interval of the difference in the proportion of subjects showing seroconversion for HPV-16 and HPV-18 between age groups of 15 to 25 years and 26 to 45 years or 46 to 55 years fell below the predetermined limit value of 10%.

Table 28. Seroconversion rate (Study HPV-014, ATP cohort for immunogenicity)

Antibody	Group 1	N	seroconversion rate(%)	Group 2	N	seroconversion rate (%)	Difference in seroconversion rate (Group 2 minus Group 1) (%)		95% CI
HPV-16	26-45 year olds	164	100	15-25 year olds	191	100	15-25 year olds minus 26-45 year olds	0	[-1.97, 2.29]
HPV-18	26-45 year olds	185	100	15-25 year olds	202	100	15-25 year olds minus 26-45 year olds	0	[-1.87, 2.03]
HPV-16	46-55 year olds	136	100	15-25 year olds	191	100	15-25 year olds minus 46-55 year olds	0	[-1.97, 2.75]
HPV-18	46-55 year olds	172	100	15-25 year olds	202	100	15-25 year olds minus 46-55 year olds	0	[-1.87, 2.18]

N: number of subjects with pre-vaccination results available and seronegative at baseline
Seroconversion rate: percentage of subjects seropositive at Month 7

The antibody titers to both antigens at Month 18 were markedly higher than those at natural infection, and the distribution of anti-HPV-16 antibody and anti-HPV-18 antibody in all the subjects was basically higher than the value obtained in Study HPV-007 in which persistence of

protection was confirmed. Based on the above, it is considered that the immune response observed in the subjects aged 26 years or older is sufficient to prevent infection with HPV-16 or HPV-18, a cause of cervical cancer. Since women having sexual activity are with the risk of new HPV infection and the risk of persistent infection with HPV increases with age (*J Infec Dis.* 2005;191:1808-16), the risk of onset of high-grade lesion or cervical cancer is also considered to increase in older-age groups. Thus, since women aged 26 years or older is always exposed to the risk of new infection and the infection-induced disease as well, the administration of the HPV Vaccine is considered necessary also in these age groups.

PMDA considers as follows:

From the ethical and feasibility viewpoints, it is understood to set immunogenicity as an endpoint in the clinical studies including females aged 10 to 14 years, but the correlation of the antibody titer with the long-term infection prevention effect, and consequently, the efficacy in the prevention of cervical cancer and its precursor lesion is not clear at present. However, on the basis of study results, the preventive effect of this vaccine against HPV infection can be also expected in this population and there were no event that raised any particular safety-related concern in the above mentioned age group in comparison with other age groups, and therefore, the said age group may be included as the subjects of vaccination. In this regard, because sufficient information has not been obtained at present on the duration of antibody titer persistence and the threshold antibody titer from which the preventive effect is expected, etc., information should be continuously collected including those to determine whether additional vaccination is needed. On the other hand, it is not appropriate to vaccinate females younger than 10 years because the efficacy and safety in this age group have not been established. As for females aged 26 years or older, in whom cervical samples can be collected, a clinical study to evaluate the efficacy should have been conducted in the first place. In view of the seroconversion rates, the efficacy of this HPV Vaccine is also suggested in these groups. However, since the number of those infected with HPV is assumed to increase along with increased age, it is necessary to bear in mind that the usefulness of this HPV Vaccine may be different from that in the lower age group. Comments on the vaccination to those already infected with HPV-16 or HPV-18 are given in the next section.

4.B.(5).2) Vaccination to those already infected with HPV-16 or HPV-18

PMDA asked the applicant to explain the appropriateness of vaccination of this HPV Vaccine to anti-HPV antibody positive or HPV-DNA-positive females including the necessity of screening before vaccination.

The applicant answered as follows:

It is said that approximately 50% of HPV-infected women do not seroconvert. Serologic studies have demonstrated that approximately 20% to 50% of women with history of infection with HPV and currently being DNA negative or with HPV-associated lesions do not have detectable type-specific anti-HPV antibodies (*J Infect Dis*. 2000;181: 1911-9). Moreover, it has been reported that naturally-acquired antibodies do not provide complete protection against re-infection with the same HPV type or with phylogenetically-related HPV types (*Cancer Epidemiology*. 2004;13: 324-327). To evaluate if antibodies acquired from natural infection is protective against re-infection, the applicant calculated the incidence of HPV-16- or HPV-18-associated efficacy endpoints (incident infection, persistent infection, CIN1+) in women 15 to 25 years of age included in the control arm of the Phase III efficacy Study HPV-008. As a result, the incidence of each endpoint caused by natural infection was similar between HPV DNA negative-seronegative females and HPV DNA negative-seropositive females. Therefore, it is thought that HPV-16 or HPV-18 seropositivity following natural infection does not provide protection against re-infection. When the efficacy of the vaccine in HPV DNA negative-seropositive women was investigated in Study HPV-008, the efficacy against persistent infection (6-month definition) with HPV-16 or HPV-18 was 56.1% (97.9% CI [0.4%, 82.3%]), indicating that the efficacy of this HPV Vaccine can be expected also in HPV seropositive women.

In the Studies HPV-008, HPV-015 and HPV-032, the females who were HPV-16 or HPV-18 DNA-positive at enrolment were also included as the subjects and received vaccination. In these studies, the females DNA-negative to both HPV types accounted for 95.9% to 100% of those aged 25 years or older while 90.1% to 92.8% in the 15 to 25 year-old females. The efficacy results obtained from the interim analysis of Study HPV-008 have shown that the HPV Vaccine has no statistically significant efficacy or any preventive effect in the females who were HPV DNA-positive and who had pathohistological lesion attributable to HPV-16/18 infection at the time of vaccination. However, even if the HPV DNA of either HPV-16 or HPV-18 is positive, the efficacy of this vaccine can be expected against the other DNA-negative virus type. Since the ratio of females who were already infected with both HPV types and who were not expected to benefit from administration of this vaccine was low (0.1% to 0.7%) regardless of age group, many females are considered to benefit from this vaccine. There is no evidence that administration of this vaccine aggravates existing cervical histopathological lesions attributable to HPV-16/18 infection in subjects who were HPV DNA-positive and had such lesions at the time of vaccination. Moreover, safety data in more than 6,000 women aged 26 years and older (Studies HPV-014 and HPV-015) do not show any increased risk of disease progression following vaccination of a woman who was DNA-positive for HPV-16/18 at baseline .

As described above, the HPV Vaccine has been shown to be safe and well-tolerated in women with past or current HPV infection. There should be little impact of pre-vaccination seropositivity on vaccine efficacy and no pre-vaccination screening (for antibodies and/or HPV DNA) should be necessary.

PMDA considers as follows:
Since there is little impact of pre-vaccination seropositivity on vaccine efficacy, there is no need to conduct anti-HPV antibody test before vaccination. On the other hand, in women who are DNA positive for either HPV type contained in this vaccine, the vaccine efficacy can not be expected against the corresponding HPV type. However, considering that the ratio of those who are DNA positive for both HPV-16 and HPV-18 is low, and that no particular problem is raised from the safety viewpoint as to vaccination to subjects who are HPV-DNA positive or anti-HPV antibody-positive, there is no need to mandate HPV DNA test before vaccination.

4.B.(6) Clinical positioning of this vaccine

PMDA considers as follows in regard to the clinical positioning of this HPV Vaccine:
As stated in "4.B.(2).1) Active ingredient", since the involvement of HPV in cervical cancer is well known, the significance of this vaccine, which prevents cervical cancer and its precursor lesion through prevention of persistent infection with HPV-16 and HPV-18 that are the virus types of high infection prevalence among the oncogenic HPVs, is recognized. This vaccine is considered useful for the primary prevention of cervical cancer since the efficacy against CIN2+ has been demonstrated in the efficacy clinical studies. However, the efficacy in cervical cancer and its precursor lesion attributable to oncogenic HPVs other than HPV-16 and HPV-18 has not been demonstrated. Therefore, it is necessary to especially bear in mind that cervical cancer is not completely prevented by this HPV Vaccine. Accordingly, the uterine cancer screening is necessary as the secondary prevention even after administration of this vaccine.

4.B.(7) Indications

The proposed indications are as shown below.
Prevention of cervical cancer (squamous-cell carcinoma and adenocarcinoma) by protecting against the following precursor lesions and infections caused by oncogenic human papillomavirus (HPV) types 16 and 18:
・Cervical intraepithelial neoplasia (CIN) grade 2 and grade 3
・Cervical intraepithelial neoplasia (CIN) grade 1
・Abnormal cytology (atypical squamous cells of undetermined significance [ASC-US], low-grade squamous intraepithelial lesions [LSIL] and high-grade squamous intraepithelial lesions [HSIL])

・Persistent infection

・Incident infection

Cervarix has also shown efficacy against persistent infection caused by oncogenic HPV types, in addition to HPV-16 and HPV-18, inclusive of phylogenetically-related types HPV-31and HPV-45.

PMDA considers as follows:

Since the preventive effect against CIN2+ associated with HPV-16 and 18 infection was demonstrated in Study HPV-008, the indication may include cervical cancer as well as CIN2 and 3 that are precursor lesions associated with these HPV types. On the other hand, the efficacy against cervical cancer and its precursor lesions associated with other HPV types other than HPV-16 and HPV-18 has not been clearly demonstrated. Moreover, it is not necessary to include abnormal cytology in the indications because cytological abnormality was not investigated as a primary endpoint in the clinical studies and this abnormality does not always correspond to histological abnormality on equal basis. As for the prevention of persistent infection, the applicant obtained the efficacy results against persistent infection that had been defined by the applicant itself (6-month and 12-month definitions) for the clinical studies and the applicant explained that no conclusion is reached at present on whether the persistent infection precedes, or correlated to, the onset of lesion. As for the incident infection, the relation to the true endpoint is not clear. Therefore, it is not particularly necessary to include the persistent infection and incident infection in the indications. Based on the above, the indication should be set as follows:

Prevention of cervical cancer (squamous-cell carcinoma and adenocarcinoma) and its precursor lesions (cervical intraepithelial neoplasia [CIN] grades 2 and 3) caused by infection with human papillomavirus (HPV) types 16 and 18.

A final conclusion on the appropriateness of the PMDA's judgment will be made later taking account of comments from the Expert Discussion.

4.B.(8) Dosage and administration

The proposed dosage and administration was "This vaccine should be administered in females 10 years or older as a single 0.5 mL injection by the intramuscular route into the deltoid region of the upper arm. The primary vaccination course consists of 3 doses according to the following schedule: 0, 1, and 6 months."

4.B.(8).1) Selection of dose

The applicant explained the reason for establishment of dose as follows:

In Study HPV-005, the immunogenicity after administration of 3 doses of AS04-adjuvanted HPV-16/18 vaccine (12, 40 and 120 µg of VLP) and 40 µg of aluminum hydroxide-adjuvanted HPV-16/18 vaccine were compared (serum antibody titer to HPV-16 and HPV-18 [determined by ELISA] at Month 7 in the AS04 adjuvanted group). As a result, the mean antibody titer (log10) was the highest in the AS04-adjuvanted vaccine 120 µg group for both HPV-16 and HPV-18. For HPV-16, the mean antibody titer was higher in the 40 µg group than in the 12 µg group, but for HPV-18, no difference was observed [see 4.A.(3) Study HPV-005]. Considering that the immunogenicity was lower at 12 µg in comparison with other doses and that the immune response was high at 120 µg, it was decided to use AS04-adjuvanted 40 µg in the clinical studies conducted after Study HPV-005.

PMDA considers as follows:
Since the efficacy of this HPV Vaccine has been shown in Study HPV-008 in which the dose of 40 µg (20 µg of HPV-16 VLP and 20 µg of HPV-18 VLP) added with AS04 was administered, and the clinical study results on the efficacy and safety of this vaccine were obtained in Japan and abroad using the drug product of said composition, the selection of HPV-16 VLP 20 µg and HPV-18 VLP 20 µg as the doses of active ingredient of this vaccine is appropriate.

4.B.(8).2) Selection of frequency of vaccination

In the phase I Study HPV-002, because enhancement of serum immune response was observed in all the subjects administered the third vaccination on Day 112 in comparison with the subjects administered twice (Days 0 and 28 of the study), the applicant decided to use the schedule of administration of 3 times at Months 0, 1 and 6 in the subsequent studies. In the Study HPV-002, it was also confirmed that there is no interference between HPV-16 and HPV-18, the active ingredients.

PMDA considers as follows:
Since the efficacy of the HPV Vaccine was demonstrated in the clinical studies conducted according to the schedule of total 3 vaccinations as described above, and no particular problem occurred from the viewpoint of safety and the rate of compliance with the 3 times vaccinations, it is appropriate to prescribe the said vaccination schedule as the dosage and administration regimen of the vaccine.

4.B.(8).3) Necessity of booster vaccination

The applicant explained the necessity of booster vaccination as follows:
In the Study HPV-007 (long-term follow-up study of the Study HPV-001), the subjects were followed up for 5.9 years (up to 6.4 years) after the first vaccination in the Study HPV-001, and

the long-term vaccine efficacy against incident infection, persistent infection (6 and 12 months definition), cytological abnormality and histological lesions was demonstrated. Moreover, the immune response to HPV-16 and HPV-18 has persisted up to 76 months, at least about 99% of subjects maintained seropositivity by ELISA, and the GMT value was kept at more than 10 times higher than the GMT obtained by natural infection with HPV-16 or HPV-18. In the pseudovirion neutralization method, the same functional immune response was observed, and the antibody titer did not decrease during the period. When these data was applied to 3 different mathematical models constructed, it was predicted that the antibody titer obtained by administration of the HPV Vaccine might exceed the antibody titer obtained by natural infection for at least 20 years after completion of 3 vaccination course (David *et al. Gynecologic Oncology*. 2009 in press). It is therefore considered not necessary to perform booster vaccination for 20 years after completion of 3 doses of the vaccine. As a follow-up study of HPV-001/007, Study HPV-023 is ongoing for the purpose of following up the vaccine efficacy and persistence of antibody titer in Brazilian cohort for further 3 years. The interim data shows the persistence of antibody titer for the maximum of 88 months after the first vaccination. The efficacy data of the study are not available at present.

PMDA considers as follows:

Since it has been shown that the antibody titer may persist for a long period of time, it is likely that the preventive effect of this vaccine against cervical cancer and its precursor lesion persists longer than the period observed up to present. However, it is still unclear at present whether or not the antibody titer exceeding the titer acquired from natural infection may demonstrate the preventive effect and the 20 year-persistence of antibody titer is still an estimated value. Therefore, it is necessary to continue information collection about the persistence of the preventive effect of this vaccine.

4.B.(9) Postmarketing

The applicant explained the postmarketing surveillance and postmarketing clinical study to be performed in Japan as follows:

After acquisition of marketing authorization of the HPV Vaccine, the applicant plans to perform a use-results survey to collect the information of a total of 1,500 doses (enrollment of 500 subjects) for the purpose of confirming the safety in actual use in Japan. In this surveillance, adverse events within 30 days after each vaccination (Months 0, 1 and 6) will mainly be evaluated. The target number of subjects has been set so that the total number of doses will be similar to that in Study HPV-032, considering that the data after a total of 1,777 doses of the HPV Vaccine (1,479 doses in 519 subjects in Study HPV-032 [20-25 years old]; 298 doses in 100 subjects in the Study HPV-046 [10-15 years old]) has already been accumulated in the Japanese Studies HPV-032 and

HPV-046. When combining the number of doses of vaccine in the conducted clinical studies and the use-results survey to be performed, the cumulative number of doses in Japanese women will become above 3,000, which will allow detection of adverse events with an incidence of 0.1% at ≥95% possibility. Moreover, it was decided to perform a long-term follow-up study (Study HPV-063) in the subjects who have participated in the Study HPV-032. It will be performed by following up with the subjects (HPV vaccine group or control HAV vaccine group) who completed their last visit of Study HPV-032 (Visit 7, at Month 24) for further 2 years (cumulative 4 years) to evaluate the efficacy, immunogenicity and safety.

PMDA considers as follows:
Since it is important to collect information related to the long-term efficacy and safety in the Japanese, the applicant's decision to conduct a long-term follow-up study in the subjects enrolled in Study HPV-032 is appropriate and it is important to properly provide information on the results obtained. In addition, the post-marketing survey is necessary to collect information under actual usage condition. Though the applicant proposes to set the target number of subjects for the post-marketing survey by taking into account the number of vaccinated subjects in Study HPV-032, PMDA considers it appropriate to set the number on the basis of the number of vaccine doses to be newly given in the post-marketing survey. As to the survey period, etc., there seems to be no particular problem in the proposed plan by the applicant.

A final conclusion on the post-marketing survey and study will be made later taking account of comments from the Expert Discussion.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based inspection and data integrity assessment were conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. Since the results indicated no particular problem, PMDA concluded that there should be no obstacle for performing a regulatory review based on the submitted product application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

GCP on-site inspection took place in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application (5.3.5.1.8, Study HPV-032 [Interim

Analysis II]; 5.3.5.2.8, Study HPV-046). As a result, it was found that there were cases where a study drug with a different number from the assigned one had been dispensed to subjects in some clinical trial sites, that the information on the said fact was not provided to the subjects, and that there were partial deviations from the protocol (some tests were not conducted, etc.) and inconsistencies between descriptions in case report forms and source documents (descriptions related to adverse events). As for the sponsor, there were cases where information on serious and unpredictable adverse reactions, etc. was not notified to the investigators or heads of clinical trial sites promptly after acquisition of such information. Moreover, regarding the above inconsistencies between descriptions in the case report forms and source documents, the situation did not suggest that the study conduct was appropriately monitored in accordance with the operational procedure. However, PMDA concluded that there should be no problem with performing a regulatory review based on the submitted product application documents.

IV. Overall Evaluation

It is concluded on the basis of submitted data that the preventive effect of this HPV Vaccine may be expected against cervical cancer attributable to human papilloma virus (HPV) type 16 and type 18 infections in Japan. As for safety, although it is still necessary to confirm that the response to PMDA's inquiry to be submitted includes no important problem, the data and responses submitted up to present do not seem to raise any particular problem. However, considering that this HPV Vaccine contains a new adjuvant ingredient and that it is the first recombinant drug product in Japan using insect cells as the protein-expressing cells, it is necessary to appropriately collect and assess safety-related information in the post-marketing survey, etc.

It has been concluded that, if there is no particular problem taking account of the comments from the Expert Discussion, this HPV Vaccine may be approved for marketing after confirming that the Final Analysis results of Study HPV-032 submitted in the future are not contradictory to the results of Interim Analysis II.

Review Report (2)

August 20, 2009

I. Product Submitted for Registration

[Brand name]	Cervarix
[Non-proprietary name]	Recombinant Adsorbed Bivalent Human Papillomavirus-like Particle Vaccine (derived from *Trichoplusia ni* cell)
[Applicant]	GlaxoSmithKline K.K.
[Date of application]	September 26, 2007

II. Content of the Review

Pharmaceuticals and Medical Devices Agency (PMDA) asked the expert advisors for comments on the Review Report (1). The outline of regulatory review based on the discussion with the expert advisors, the quality-related matters for which the review had not been completed by the time of preparing Review Report (1), and the matters on clinical study results submitted after the Expert Discussion are stated below.

The expert advisors for the Expert Discussion were appointed on the basis of requests, etc. from the expert members for the proposed product and in accordance with the provisions of "Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 8/2008 dated December 25, 2008)".

1. Quality

1.(1) Cytoplasmic cluster of particle-like structures observed in [redacted] cells

The applicant answered as follows:

Though the investigation of cytoplasmic cluster of particle-like structures [see Review Report (1) "II.2.B.(1) Cytoplasmic cluster of particle-like structures observed in [redacted] cells"] has been going on throughout the development period of the HPV Vaccine, there is no further information available at present than the already conducted characterization results. The applicant intends to continue the investigation and appropriately provide the information on characterization results if any effective approach for the structural analysis is identified in the future.

PMDA accepted the answer.

1.(2) Manufacturing process of MPL liquid bulk

In-process control test ( ) has been established for the manufacturing process of MPL liquid bulk [see Review Report (1) "II. 2.B.(5) Control of MPL liquid bulk manufacturing process"]. In this regard, the applicant explained the operational procedure of of MPL.

PMDA confirmed that the temperature, pressure, scale, etc. are controlled in the process.

1.(3) Specifications and test methods of drug product

Regarding the specifications and test methods of drug product (final container), PMDA asked the applicant to consider to set the insoluble particulate matter test for injections, content uniformity test and osmotic pressure test.

The applicant answered that the insoluble particulate matter test for injections will be included in the specifications and test methods by the end of December 2009 after validating the test method, and that the content uniformity test will also be included in the specifications and test methods. As to the osmotic pressure, the applicant presented the results of 145 lots (monitoring of final container products), and explained that, since consistent results were obtained, the osmotic pressure test will be handled as an in-process control test rather than included as the specifications and test methods.

PMDA accepted the answer.

1.(4) Shelf-life of this HPV Vaccine

The results obtained up to Month 12 of long-term testing of the drug product after the change in container closure system [see Review Report (1) "II. 2. B.(7) Shelf life of the drug product"] were additionally submitted after the Expert Discussion.

PMDA asked the applicant whether or not the product supply will be affected when the shelf-life of drug product is established on the basis of above mentioned study results obtained after the change in container closure system.

The applicant answered that it is considered difficult to supply the drug product in Japan with the shelf-life that can be established at present. In this regard, the applicant presented a future stability test plan.

PMDA judged that it is appropriate to have the drug product supplied in Japan with the former container closure system (in the condition before the change) in Japan for the time being, and to set the shelf-life for "36 months from the manufacturing date" as was proposed in the application because the long-term testing results of 36 months' storage have been obtained.

2. Study HPV-032

The final study report on Study HPV-032 dated [redacted], 20[redacted] was submitted. The primary endpoint of efficacy was persistent cervical infection (6-month definition) with HPV-16 and/or HPV-18. In this regard, the vaccine efficacy was 100% (95.5% CI, [71.3, 100]), in which the lower limit of 95.5% CI exceeded zero (0), indicating a significant difference. There were no subject reporting persistent infection in the HPV vaccine group versus 15 subjects in the HAV group. Furthermore, a total of 72 pregnancies had been reported up to the Interim Analysis II and 14 of them had been ongoing pregnancies. It was reported that the 14 pregnancies proceeded to delivery of normal infant without any problem in the mothers and babies. PMDA confirmed that the results of efficacy, safety and other issues of the final analysis stated in the said report were not contradictory to those of Interim Analysis II.

3. Indications

Since the expert advisors supported the conclusion of PMDA to set the indication as follows, PMDA asked the applicant to follow the judgment and the applicant accepted it.

[Indication]

Prevention of cervical cancer (squamous-cell carcinoma and adenocarcinoma) and its precursor lesions (cervical intraepithelial neoplasia [CIN] grades 2 and 3) caused by infection with human papillomavirus (HPV) types 16 and 18.

4. Dosage and administration

4.1) Target population for vaccination

The expert members supported the conclusion of PMDA to choose 10-year old or older females as the vaccination recipients, and to provide the information that the usefulness of this vaccine may be changed depending on the age stratum because the ratio of those already infected becomes higher with increasing age. Furthermore, the expert members also supported the conclusion of PMDA that the vaccination to the females younger than 10 years should not be recommended because of the absence of administration experience and the unclear efficacy and safety.

In this regard, an expert advisor commented that, even though the administration of the vaccine before the start of sexual activity is important, it is essential to check the long-term efficacy after vaccination to young females because the long-term efficacy is unknown. Issues on investigation of long-term efficacy are described below [see "7. Post-marketing investigation"].

4.2) Vaccination schedule

Considering that there is about 5 months of interval between the second and the third vaccination, an expert advisor commented that a measure to ensure completion of all three vaccinations needs to be considered. PMDA asked the applicant to explain how to ensure vaccination schedule compliance.

The applicant explained as follows:

It is planned to distribute a brochure with a card to record their vaccination history to vaccinated women in order to provide post-vaccination advice, e.g. recommendation of regular uterine cancer screening. This card contains spaces to be filled in with the dates of the three vaccinations and the clinic or hospital name, so that the necessity of receiving the second and third vaccinations in accordance with the schedule will be emphasized. It is also planned to develop a reminder mail system in which registered vaccine recipients will receive emails reminding them of the next vaccination dates (second and third vaccination) to encourage their next visits to the physician.

PMDA accepted the above answer. In this regard, PMDA considers that it is very important to provide appropriate information including post-vaccination advice to the vaccination recipients. A part of information materials (draft) for distribution to those giving and receiving vaccination was submitted during the preparation of this report [see "6. Information provision to vaccine recipients" for the information materials].

It was commented at the Expert Discussion that measures to be taken in the cases of pregnancy during the vaccination period need to be considered and that the efficacy of this vaccine might not be expected because of the immunosuppression during pregnancy.

Based on the information obtained up to present in regard to pregnancy during vaccination period and its outcome observed in the clinical studies, PMDA considers that there is no serious safety concern about the mother and baby even if pregnancy occurs after administration of this vaccine. Therefore, there is no need to terminate the pregnancy from the safety viewpoint. However, immune response during pregnancy may be different from that in the ordinary condition and the information on the efficacy of this vaccine used during pregnancy and on the necessity, or not, of

additional vaccination if pregnancy occurs after vaccination has not been accumulated and are still unknown. Based on the above, PMDA judged it necessary to provide the information in the package insert that the efficacy and safety of this vaccine have not been established in pregnant women and that the necessity, or not, of additional vaccination when pregnancy occurs after vaccination is still unknown.

5. Necessity/non-necessity of pre-vaccination screening

PMDA has judged that the HPV DNA test and anti-HPV antibody test do not need to be mandatory before administration of this vaccine. The expert advisors supported this judgment of PMDA.

It was commented by an expert advisor that a test may be considered before vaccination in some cases of already highly suspected of HPV infection (abnormal cytological result, etc.), and in such a case, the physician should consider the individual patient's condition for determining the necessity or not of HPV DNA test and should provide adequate explanation to the patient before the test and vaccination.

Though it is stated in the draft package insert that this HPV Vaccine is not expected to prevent progression of existing HPV-related lesions, PMDA considers that the said matter should be adequately explained to those receiving the vaccination and understood by them [see "6. Information provision to vaccine recipients"].

6. Information provision to vaccine recipients

At the Expert Discussion, an expert advisor commented that the onset of cancer in vaccinated women (due to causes other than HPV-16, 18) has been reported (*Obstet Gynecol*. 2009;113:550-552), and that an emphasis has been placed on the necessity of information provision to the vaccination recipients. In this regard, it was confirmed necessary to provide the information to vaccination recipients that the effect of this HPV Vaccine is limited to HPV-16 and -18 and is not expected to completely prevent the onset of cervical cancer, and that the duration of preventive effect is unknown because only information on a limited period is available at present. In addition, it was acknowledged that the lower rate of those receiving the uterine cancer screening in Japan in comparison with that abroad (the US, etc.) has been a problem and that an education campaign on uterine cancer screening is important as a secondary prevention next to the primary prevention of cervical cancer by the administration of this vaccine.

PMDA considers as follows:

It is stated in the draft package insert that this HPV Vaccine has no expected efficacy against other HPV types and it can not completely prevent cervical cancer, that the duration of protection is unknown, and that the uterine cancer screening is necessary even after vaccination with this drug (refer to the section on “Precautions for indications” in the package insert). Physicians should adequately explain these matters and other precautions as well as the usefulness of the vaccination to individual recipients, and confirm that the person has understood the explanation, before giving vaccination. Currently available information on the usefulness of this vaccine and the necessity of cancer screening also need to be accurately communicated to the vaccine recipients by using information materials, etc. Based on the above, PMDA asked the applicant to explain the measures to provide these information.

The applicant responded as follows:

Before giving vaccination, physicians will be required to explain the precautions stated in the prevaccination interview sheet to the vaccine recipients or their guardians in advance and ask them to sign the informed consent statement. The prevaccination interview sheet contains the information that should be provided (described above). In addition, a material to be used for explanation by physicians to the vaccine recipients or their guardians will be separately prepared and the contents similar to those mentioned above will be stated in the material. Furthermore, an information brochure to provide the post-vaccination advice will be distributed to the vaccine recipients. Other than that, the applicant plans to conduct a disease education campaign on the necessity of regularly receiving uterine cancer screening regardless of vaccination history with this vaccine.

As described in the above, a part of information materials (draft) for distribution to those giving and receiving vaccination was submitted during the preparation of this report. However, PMDA considers it necessary to further improve the description and is adjusting the contents with the applicant. It was also decided to check the contents of materials that have not been submitted yet by the market launch of this vaccine.

7. Post-marketing investigation

PMDA judged that the long-term follow-up of Study HPV-032 (further 2 years of follow-up of the subjects who have completed the final hospital visit [Month 24]) is meaningful in terms of collecting information on the efficacy, immunogenicity and safety of this HPV Vaccine in Japan. As to the post-marketing surveillance, PMDA judged it appropriate to set the target number of subjects without giving consideration to the number of doses administered in the clinical studies already conducted in Japan. Since the expert advisors supported the above judgment, PMDA

asked the applicant to reconsider the target number of subjects to collect in the post-marketing surveillance.

The applicant answered as follows:
The number of subjects to be investigated in the post-marketing surveillance is set as 1,000 (total 3,000 doses). The objective of this surveillance is to collect safety-related information under the actual use status of this vaccine. The registration period is 2 years and observation period is 30 days following each vaccination according to the 0, 1 and 6 month schedule.

In relation to the comment that the information should be collected for a longer period of time in Japan, PMDA asked the applicant to explain the status of overseas long-term follow-up studies .

The applicant responded as follows:
At present, a long-term follow-up study of Study HPV-008 is planned to be conducted. As to Studies HPV-012, HPV-013, HPV-014 and HPV-007, long-term follow-up clinical studies are ongoing. Meanwhile, although another long-term follow-up study was planned to evaluate the incidence of auto immune diseases following administration with this vaccine compared to a control group vaccinated with a hepatitis A vaccine and to evaluate the safety of this vaccine in women who are inadvertently exposed to the vaccine during pregnancy, it has been cancelled because the interest expressed by physicians to participate in this study was much lower than expected and it was not feasible.

PMDA considers as follows:
While it is important to collect information associated with long-term efficacy, it is currently difficult in view of the feasibility to further prolong the period of long-term follow-up study planned in Japan. However, it is considered important to conduct comprehensive assessment of primary and secondary prevention of cervical cancer in Japan performed as epidemiological research, etc. and to feed back the results to the medical practice.

At the Expert Discussion, an expert advisor mentioned the possibility that the adjuvant of this HPV Vaccine may be involved in autoimmune diseases, etc. and commented that the information related to autoimmune disease should be investigated in detail especially in the case of this vaccine that contains a new adjuvant.
In this regard, PMDA asked the applicant to describe the measures against the onset of autoimmune diseases after marketing of this vaccine.

The applicant responded as follows:
The potential risk of inducing and exacerbating an autoimmune disorder following vaccination has been evaluated in the ongoing overseas clinical trials. Also, a meta-analysis to evaluate MPL-containing vaccines as a whole has been performed, indicating no noteworthy safety

concerns up to present. The applicant intends to continue collecting information related to autoimmune diseases and other chronic diseases among safety information to be collected in the post-marketing surveillance and clinical studies (including the ongoing long-term extension clinical studies) in Japan and abroad. Moreover, the long-term safety of the HPV Vaccine, including potential occurrence of new onset autoimmune diseases, will be evaluated further in a large-scale study (Study HPV-040) to be conducted in Finland. In Japan, in addition to the long-term information collection in HPV-063, if any autoimmune disease is reported after the launch of the HPV Vaccine, the applicant intends to collect detailed information including the disease history and family history of the patient. The applicant will periodically evaluate the data thus accumulated in Japan and abroad for the occurrence status of autoimmune diseases and its tendency of occurrence, and consider whether any action needs to be taken, including changes in the description in the package insert.

In addition to the information related to the post-marketing clinical studies and surveillance, PMDA considers that any information related to long-term efficacy and safety of this HPV Vaccine that is obtained in Japan and abroad should also be provided promptly.

III. Overall Evaluation

Based on the above review, PMDA has concluded that this drug product may be approved for marketing after modifying the indication and dosage and administration as follows. PMDA has judged that the re-examination period of this drug product should be 8 years, that both the drug substance and drug product are classified as powerful drugs and that the drug product is regarded as a biological product.

[Indication]

Prevention of cervical cancer (squamous-cell carcinoma and adenocarcinoma) and its precursor lesions (cervical intraepithelial neoplasia [CIN] grades 2 and 3) caused by infection with human papillomavirus (HPV) types 16 and 18.

[Dosage and administration]

This vaccine should be administered in females 10 years or older as a single 0.5 mL injection by the intramuscular route into the deltoid region of the upper arm. The primary vaccination course consists of 3 doses according to the following schedule: 0, 1, and 6 months.