SCIENTIFIC DISCUSSION

1. Introduction

Human Papillomaviruses and Disease Background

It is widely accepted that infection with human papillomavirus (HPV) is the central causal factor for the development of cervical cancer. Recent studies have shown that HPV can be identified in 98.7% of all cervical carcinomas. Furthermore HPV infections are among the most common sexually transmitted infections in most populations and estimates of exposure range from 25% in many European countries to 70% in the US or 95% in high risk populations in Africa.

HPV are non-enveloped, double-stranded DNA viruses that infect the epithelial cells of the skin or mucosa. Based on their genomic differences within the oncogenes E6 and E7 and the capsid protein L1 over 100 genotypes are described to date. Thereof approximately 40 different genotypes lead to infections of the anogenital tract and about 16 are highly oncogenic with HPV types 16 and 18, being the most frequent found in cervical cancer. HPV-16 is detected in about 54% of cervical cancer cases, and the second type is HPV-18, detected in about 17% of cases.

The time from occurrence of HPV infection to cancer development usually exceeds 20 years. However, persistent HPV infection is a necessary but not a sufficient factor for the development of cervical carcinoma. Other factors such as smoking, long-term use of oral contraceptives or high parity are suggested to play a role in the process that lead to cancer.
The majority of genital HPV infections (>90%) however are transient sub-clinical infections that will be cleared or suppressed below the limits of detection by host cell defences within one to two years. In addition, any cervical lesion may spontaneously regress to normal without treatment with a probability of about 57% for CIN1, 43% for CIN2 and 32% for CIN3. The determinants leading to regression are not well understood.

It is confirmed that persistent cervical infection by high risk HPV types is a precursor event to cervical cancer.

The primary screening tool for cytological abnormalities is still the PAP smear test. Due to limitations of the original PAP test new methods have been developed. Currently liquid based PAP tests are routinely used. Nowadays the Bethesda classification system is used. This classification system specifies squamous cell abnormalities into four categories: atypical squamous cells (ASC), atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC).

Although the immune response to HPV cervical infections is not fully understood, there is evidence that the host humoral and cell mediated immune responses are responsible for regression of HPV infection. With respect to the humoral immune responses anti-HPV antibodies are only detected in 50 to 70% of infected women. Whereas the antibodies occur usually after several months post infection, they are long-lasting with steady levels. Because of the high level of antigenic specificity of HPV capsid antigens, there is no cross protection among subtypes but a type specific immunity develops.

The interaction of cytokines, chemokines liberated during humoral and cell mediated immune reactions is assumed to be responsible for the regression associated with HPV. The severe outcome of HPV infection in immunosuppressed individuals as transplant recipients and HIV-positive women suggests that cell-mediated immunity is also important. Furthermore a polarisation of the immune response for a Th2 directed response was observed in woman with HPV infections that evolve into high grade lesions.

2. Quality aspects

Introduction

Cervarix, HPV-16/18 L1 AS04 vaccine contains recombinant C-terminally truncated major capsid L1 proteins of HPV types 16 and 18 as active ingredients. The L1 proteins of HPV-16 and HPV-18 are separately produced using a recombinant Baculovirus expression system and the insect cell line Hi-5 Rix4446 derived from *Trichoplusia ni*. After expression of the L1 proteins and further purification, the L1 proteins assembled separately as virus-like particles (VLP). The VLPs of each HPV type are formulated with the AS04 adjuvant system composed of aluminium hydroxide and 3-*O*-desacyl-4.-monophosphoryl lipid A (MPL). The MPL immunostimulant is a detoxified derivative of the lipopolysaccharide of the gram negative bacterium *Salmonella minnesota* R595 strain.

One dose of Cervarix, contains 20µg of HPV-16 L1 and 20µg of HPV-18 L1 proteins adjuvanted with AS04 and is presented as a sterile turbid liquid suspension for injection, filled as a 0.5ml monodose in either syringes or vials stored at 2-8°C. A shelf life of 3 years is proposed. The vaccination schedule includes 3 doses administered at 0, 1, and 6 months intramuscularly.

Active Substance
The drug substance consists of two monovalent antigen bulks each containing C-terminally truncated versions of the major capsid L1 proteins of either type 16 or 18 HPV. These proteins are produced in separate production runs using the Baculoviruses expression vector system (BEVS) using GSK´s insect cell line cloned from the Hi-5 Trichoplusia *ni* insect cell line and HPV-16 L1 or HPV-18 L1 encoding recombinant Baculoviruses. Following expression the L1 proteins are purified by a series of chromatographic and filtration procedures and are finally assembled into (VLP) closely resembling the configuration of native HPV virus particles.

- Manufacture

Cell and Seed Banking

The manufacturing of the HPV-16 L1 VLP and HPV-18 L1 VLP makes use of an established two-tiered cell bank system of the Hi-5 Rix4446 cell line (Master and Working Cell Banks (MCB and WCB)) and an established two-tiered baculovirus Seed Lot system set up for the HPV-16 L1 and HPV-18 L1 gene-encoding recombinant baculoviruses (HPV-16 or HPV-18 Master and Working Seeds (MS and WS)).

Satisfactory quality control testing are in place and satisfactory stability data and storage conditions have been described for these cell banks and seed lots. The MCB and WCB, as well as the End of Production Cells (EPC) were tested for identity, purity and safety (adventitious agents). The MS and WS, and their related control cells, were tested for identity, safety (adventitious agents) and Baculovirus content.

This application concerns the first vaccine for human use that has been produced with a Baculovirus Expression Vector System. Hence, an extensive characterization was performed to confirm the safety and applicability of the integral elements of this novel system. The biological properties and characteristics of the Hi-5 Rix4446 cell line were extensively investigated. In particular, the Hi-5 Rix4446 insect cell line has been examined for the presence of adventitious agents not only by applying the classical testing protocol but also by a variety of assays specifically designed for the detection of insect-specific viral contaminants. In addition, the tumorigenic potential of the cell line was investigated. The baculovirus seeds were also checked for classical and insect-specific contaminating viruses. Also, the construction and genetic stability of the recombinant baculovirus has been described in sufficient detail.

Manufacture and purification:

A two-step process has been established for the large scale expression of L1 proteins in the Hi-5 Rix4446 insect cells.

First, recombinant baculovirus inocula of each serotype are prepared by amplification of the respective working seeds in the Hi-5 insect cell cultures. Next, these inocula are used for the infection of Hi-5 production cell cultures. L1 protein is released from the cells by osmotic shock and subsequently purified by a series of chromatographic columns, a nanometric filtration, an ultrafiltration and a final sterile filtration to generate the L1 VLP purified bulks.

The capacity of the individual steps of the purification cascade to efficiently eliminate viral contaminants has been demonstrated in viral clearance studies.

The purified HPV-16 L1 VLP and HPV-18 L1 VLP protein bulks are stored in stainless steel vessels dedicated to the adsorption to aluminium for up to 3 days at 2°C-8°C or at room temperature.

Process control and validation:

The company has established a system of process control during the manufacturing of the active substance using in-process tests with assigned specifications and monitoring tests used to monitor the process consistency and performance.

Validation of the HPV-16 L1 VLP and HPV-18 L1 VLP antigen production process was performed by the demonstration of process consistency through compliance with the pre-established QC standards and by the identification and validation of the manufacturing process critical parameters.

Consistency was demonstrated for the three unit-steps of the drug substance production process: the recombinant HPV-16/18 baculovirus inoculum production process, the L1 single harvest production and L1 extraction and the L1 antigen purification process.

Critical process parameters have been satisfactorily identified and batch data provided.

Manufacturing process development:

The comparability of HPV-16/18 L1 VLP antigen purified bulks prepared during the phase IIb and phase III development, including the final process purified bulks, was assessed by subjecting each type of materials to a series of physico-chemical analyses and by evaluation of the immunogenic properties. The results demonstrated satisfactory comparability.

Elucidation of structure and other characteristics:

An extensive number of batches of HPV-16 and HPV-18 drug substance produced by the final process has to date been subjected to structural and functional characterization. The physico-chemical properties of the L1-VLP assemblies were assessed in a multidisciplinary approach by identifying the electrophoretic profiles and molecular weight of L1 proteins (SDS-PAGE, Western blotting, and capillary electrophoresis, mass spectrometry), the primary (amino acid analysis, peptide mapping, N- and C-terminal analysis), the secondary (Infra-Red and circular dichroism spectroscopy) and the VLP structure and size (size exclusion chromatography, electron microscopy, and disc centrifugation size analysis). In addition, drug substances were also characterized focussing on their immunological properties (antigenic activity, binding to polyclonal sera and immune response elicited in mice). The clear identity (match with the theoretical sequence) and consistent quality of drug substance material from different batches was confirmed in these studies.

Impurities:

The applicant has considered host cell proteins (HCP), DNA, and infectious recombinant baculoviruses as potential impurities to be expected in drug substance purified bulks. Impurities are removed by the process very efficiently. Residual amounts of HCP were quantified by western blot and ELISA procedures. Also, data have been gathered to adequately demonstrate that other impurities such as lipids or carbohydrates are present only in negligible trace amounts.

- Specification:

The release specifications for the drug substance include identity, purity by Coomassie-stained SDS-PAGE, endotoxin content, total protein content, and antigenic activity.

Analytical methods have been adequately described and validated.

Batch analysis data were presented for four (HPV-16 L1 VLP) and five (HPV-18 L1 VLP) batches produced according to the final manufacturing process scale. Additional information was provided for batches from earlier process developmental stages. The data were satisfactory.

The Reference Standards established for the testing of the antigenic activity of drug substance bulks are the routine purified bulk batches for HPV-16 and for HPV-18. These batches complied with the specifications for identity, purity, sterility, endotoxin content, and protein content as set for drug product release control.

Adjuvants:

Satisfactory specifications and controls have been applied as appropriate to the adjuvants: *O*-desacyl-4'- monophosphoryl lipid A (MPL) and aluminium hydroxide, hydrated ($Al(OH)_3$). Non-Pharmacopoeial methods have been satisfactorily validated.

A detailed description of the analytical methods and their validation has been provided for the MPL powder and it is considered that the methods used are adequate to control the MPL powder on a routine basis. Manufacturing of MPL has already been extensively assessed during the evaluation of Fendrix (EMEA/H/C/550, Hepatitis B (rDNA) Vaccine absorbed and adjuvanted): (http://www.emea.europa.eu/humandocs/Humans/EPAR/fendrix/fendrix.htm).
See also Section 3.3 of this Assessment report for non-clinical evaluation of MPL itself as well as Cervarix.

- Stability

The stability data obtained on purified bulks showed that the HPV-16 L1 VLP and HPV-18 L1 VLP antigen purified bulks are not affected by storage at +25 ± 2.5°C for 3 days or at + 2 to +8°C for 3 days. No loss of antigen integrity (both at the protein or the VLP structure levels) and L1 VLP antigenic activity were observed. Similarly, storage of the purified bulks has had no impact on the quality of the derived adsorbed monovalent bulks, as evidenced by the similar immunogenicity profiles in mice and similar quality properties for all adsorbed monovalent bulks irrespective whether they were made with stored or freshly produced purified bulk material.

Based on these data, the Company proposes a shelf life of 3 days at room temperature or at +2.0 to +8.0°C for the storage of HPV-16 L1 VLP and HPV-18 L1 VLP antigen purified bulks.

Medicinal Product
The HPV vaccine is prepared from HPV-16 L1 VLP and HPV-18 L1 VLP formulated with the AS04 adjuvant system composed of aluminium hydroxide and 3-*O*-desacyl-4.-monophosphoryl lipid A (MPL). The vaccine is presented as a sterile turbid liquid suspension for injection, filled as a monodose in either syringes or vials stored at 2-8°C.

- Pharmaceutical Development

Formulation development

The composition of Cervarix was determined based on the data obtained from non-clinical challenge animal models and from clinical epidemiological and natural history studies. These studies showed that in order to be efficacious against HPV infection and related clinical lesions an HPV vaccine would need to induce strong antibody responses against the L1 capsid protein (assembled as VLPs), B-cell memory and T-cell responses.

The Cervarix vaccine formulation was therefore selected to be a combination of L1 proteins assembled as VLPs, to insure the induction of protective anti-HPV L1 antibodies, and the proprietary adjuvant AS04 developed by GSK Biologicals, to insure the induction of persistently high levels of antibodies as well as the induction of a specific cell-mediated immunity.

Data from pre-clinical immunogenicity studies supported the formulation of the vaccine containing an antigen to aluminium to MPL weight ratio of 1:25:2.5, respectively. The clinical study HPV-005 evaluated three HPV-16/18 L1 AS04 candidate vaccine formulation with 6 µg, 20 µg or 60 µg of each antigen in comparison to the $Al(OH)_3$ adjuvanted vaccine. There was a trend for higher antibody titres with increasing antigen dose levels that suggested that the 12 µg L1 dose was less immunogenic than the other antigen doses. Based on clinical data it was concluded that the HPV-16/18 L1 AS04 vaccine formulated with 20µg HPV-16 L1 and 20µg HPV-18 L1 afforded an acceptable balance between vaccine tolerability and the immune response elicited.

The applicant quotes additional studies performed in the context of the approval of the Fendrix® anti hepatitis B vaccine (containing 20 µg of antigen in combination with 50 µg of MPL adsorbed to $AlPO_4$) in order to support the formulation development strategy chosen for Cervarix.

Manufacturing process development

To allow for the withdrawal of the nominal content (0.5 ml), each syringe or vial is filled with an overfill. No overage of the active ingredient is applied for the formulation of the HPV vaccine.

The Company made several optimisations during the development of the HPV-16/18 vaccine manufacturing process. The comparability of the drug product prepared during the phase IIb and phase III development, including the final process drug product, has been carried out by comparing properties of vaccine lots from different stages of the process development. Physicochemical characterization confirmed that the lots of the different process development stages were comparable. Preclinical comparison of the immunogenicity induced by the different process development stages vaccine lots showed that all lots were able to elicit humoral and cellular immune responses which were consistent and comparable.

Immunogenicity of different vaccine lots prepared during Phase IIb and Phase III development was further investigated in humans in clinical study HPV-012. The results showed that the Phase IIb and Phase III HPV vaccine formulations had comparable immunological properties (induction of antibodies, rate of seroconversion).

- Adventitious Agents

In general, robust measures and precautions have been taken to ensure the safety of the HPV vaccine against non viral as well as viral adventitious agents.

With respect to non-viral adventitious agents:

- Appropriate precautions have been taken during the preparation of the HPV vaccine to ensure the final product is free of bacteria, mycoplasma and fungi
- A cellular feature identified in the Hi-5 Rix4446 cells was not considered to be associated with replicative or infectious properties

- No materials of animal origin are used in the current routine production process of the HPV vaccine except casamino-acids that are used in the preparation of the MPL immunostimulant and for which the source is compliant with the current CPMP Note for Guidance (EMEA/CPMP/410/01).

With respect to viral adventitious agents:

The investigations performed demonstrated satisfactory viral clearance capacity of the HPV-16 L1 VLP and HPV-18 L1 VLP antigen production processes and the absence of infectivity during QC testing or specific infectivity experiments both in the starting materials as well as during routine production; thereby supporting the viral safety of the HPV vaccine.

- Manufacture of the Product

Drug product manufacture is a step-wise process in which the HPV-16 L1 VLP and HPV-18 L1 VLP as well as the liquid MPL are first individually adsorbed to $Al(OH)_3$ to generate adsorbed bulks. The final bulk vaccine is produced by mixing defined amounts of adsorbed bulks and adding buffer solutions and $Al(OH)_3$ to reach the intended final formulation. The MPL immunostimulant utilized here has previously been employed by GSK as a component of the Fendrix hepatitis B vaccine and the pertaining documentation has been reviewed and approved in the context of the licensing of the latter.

The final vaccine is filled as a monodose in either sterile syringes (type 1 glass) or sterilised type I glass vials. Both container types are closed with grey butyl rubber stoppers. The same container/closure material is used for other commercial vaccines manufactured by GSK Bio.

The qualitative and quantitative composition of Cervarix is shown below.

One dose of Cervarix (0.5 ml) contains:

Human Papillomavirus[1] type 16 L1 protein[2,3,4]	20 micrograms
Human Papillomavirus[1] type 18 L1 protein[2,3,4]	20 micrograms

[1]Human Papillomavirus = HPV
[2]adjuvanted by AS04 containing:

3-*O*-desacyl-4'- monophosphoryl lipid A (MPL)[3]	50 micrograms
[3]adsorbed on aluminium hydroxide, hydrated ($Al(OH)_3$)	0.5 milligrams Al^{3+} in total

[4]L1 protein in the form of non-infectious virus-like particles (VLPs) produced by recombinant DNA technology using a Baculovirus expression system which uses Hi-5 Rix4446 cells derived from *Trichoplusia ni*.

And the following excipients: Sodium chloride (NaCl), Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4.2\ H_2O$) and Water for injection.

A satisfactory description of the manufacturing process has been provided, including flow chart describing unit operations and controls.

Process Control and validation

The following intermediates are generated during the manufacture of the final drug product:

- HPV-16 and HPV-18 adsorbed monovalent bulks (AMBs)
- MPL liquid bulk (MLB)
- MPL adsorbed bulk (MAB)

GSK has established a system of process control during the manufacturing of the drug product using in-process tests with assigned specifications and monitoring tests used to monitor the process consistency and performance.

All critical tests ensuring the product quality are performed during the Quality Control testing of the HPV-16 L1 and HPV-18 L1 AMBs and MPL liquid bulk.

No process controls are applied for AMBs and MABs production. The process control of MLB production is tested by measuring the MPL particle size during the microfluidization procedure and before the final sterile filtration. In addition, the bioburden before sterile filtration is monitored.

The QC testing programme established for AMBs addresses identity of the L1 protein, sterility, protein content and completeness of adsorption. Specifications and results of analysis are provided for four HPV-16 L1 VLP and five HPV-18 L1 VLP AMB batches
Satisfactory validation of the methods used for control testing has been provided.

Validation of the HPV vaccine production process was performed by the demonstration of process consistency through compliance with the pre-established QC standards and by the identification and validation of the manufacturing process critical parameters.

These QC data met the specifications considered to be indicators of the consistency and robustness of the mentioned process steps.

For the MPL liquid bulk production process additional data have been collected for consistency batches by investigating the effect of different process parameters of the microfluidization step.

Critical process parameters have been satisfactorily identified and batch data provided.

The validation of final HPV vaccine production step was further confirmed by means of *in vivo* consistency studies addressing immunogenicity issues for the final product. For this, the ability of three final process HPV vaccine lots to induce the production of HPV-L1 specific antibodies and a cellular immune response (IFN-γ and IL-5) was studies in BALB/c mice. These experiments confirmed that all three batches were capable of inducing a consistent immune response.

In conclusion, validation of the manufacturing was satisfactory.

Control of excipients

Satisfactory specifications and controls (Pharmacopoeials and in-house) have been applied as appropriate to the excipients. Non-Pharmacopoeial methods have been satisfactorily validated.

- Product Specification

The final bulk vaccine is tested for sterility. Final containers are subjected to tests for identity, sterility, general safety, *in vitro* relative potency, pH, volume, protein content, aluminium content, MPL content and completeness of adsorption for MPL, HPV-16 L1 VLP and HPV-18 L1 VLP proteins.
Satisfactory data from the analysis of consistency batches and from stability studies have been provided.

Analytical methods used for release testing have been satisfactorily validated.

Satisfactory batch analysis data have been provided for 3 lots of each final bulk and final containers

Reference Standards or Materials

Two types of reference standard preparations are currently used for HPV vaccine testing:

- Purified bulk reference standard: Two routine HPV-16 L1 VLP and HPV-18 L1 VLP purified bulk preparations are used for testing completeness of adsorption to aluminium (unbound HPV-16 L1

VLP and HPV-18 L1 VLP, respectively). Batch analysis data for these two batches has been provided.
- Final container reference standard: A routine final container preparation of HPV vaccine is employed for potency testing.

Reference standards used for MPL testing:

- Three reference standard established for the testing of the MPL content of HPV final container vaccines is a routine MPL liquid bulk batch. This batch complied with the specifications set at the time of release.

- Stability of the Product

Final bulk:
Three final bulk lots have been followed in long-term stability studies. Results available at this time for the final process consistency lots stored in polyethylene containers at +2 to +8°C provide evidence for a stability period for at least 18 months.

Finished product
Stability data presented have been generated on the three consistency lots produced according to the final manufacturing process and on vaccine lots representative of the proposed consistency formulation and presentation but derived from earlier production process stages administered in Phases IIb/III of the clinical development.

A number of stability studies are ongoing and will be completed as post-approval commitments. During the procedure, stability data was updated and from the data provided, no signs of instability were evident.

The proposed shelf life for the vaccine finished product is 36 months at +2-8°C and is considered to be satisfactory.

Overall Conclusions on chemical, pharmaceutical and biological aspects

Cervarix is composed of recombinant C-terminally truncated HPV-16 L1 and HPV-18 L1 proteins, assembled into virus-like particles (VLPs) adjuvanted with AS04 adjuvant system, composed of an aluminium salt, Al(OH)3 and 3-O-desacyl-4'-monophosphoryl lipid A, (MPL). One dose of Cervarix contains 20µg of HPV-16 L1 VLP and 20µg of HPV-18 L1 VLP proteins adjuvanted with AS04 consisting of 500µg of aluminium and 50µg of MPL. Cervarix is a preservative-free vaccine presented as a 0.5 ml monodose in either glass syringes or glass vials and is presented as a turbid liquid suspension for injection.

The assessment focused on the novel Baculovirus expression vector system, which is used in a Marketing Authorisation Application for a medicinal product for human use for the first time. There were no major objections raised during the assessment.

The Applicant has provided sufficient detail for the manufacturing processes, including the monitoring, in process control and acceptance criteria. Extended characterisation studies were presented for the Trichoplusia ni Hi-5 Rix4446 insect cell bank system and the baculovirus seed system.

Satisfactory characterization of the HPV-16 L1 VLP and HPV-18 L1 VLP antigen Drug Substances, including impurities, and quality control of HPV-16 L1 VLP and HPV-18 L1 VLP adsorbed monovalents bulks and of HPV vaccines have been described.

The analytical control procedures are summarized and the validation parameters, acceptance criteria and validation results are reported for the drug substances, the intermediates and the bivalent vaccine as drug product.

Satisfactory stability data and data to demonstrate the removal of adventitious agents has been provided.

Following the assessment there were no outstanding quality concerns which would preclude the authorisation of Cervarix. A number of quality Follow Up Measures were raised to be addressed post-authorisation.

3. Non-clinical aspects

Introduction

Non-clinical pharmacological, pharmacokinetics and toxicology studies were undertaken on HPV-16/18 L1 VLP AS04 in parallel with studies on AS04 and MPL, based on

- the CPMP Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95)**,**
- the Guideline on adjuvants in vaccines for human use (EMEA/CHMP/VEG/134716/2004),
- the Note for Guidance on Reproductive Toxicology: Detection of Toxicity to Reproduction for Medicinal Products (CPMP/ICH/386/95).

Based on these same guidelines secondary pharmacodynamic, pharmacodynamic drug interaction, pharmacokinetics, genotoxicity and carcinogenicity studies were not performed on the HPV-16/18 L1 VLP AS04 vaccine and secondary pharmacodynamic, pharmacodynamic drug interaction and carcinogenicity studies were not performed on MPL.

GLP

Primary pharmacology studies were not performed under GLP conditions.
Safety pharmacology and toxicology studies were carried out under GLP conditions.

Pharmacology

- Primary pharmacodynamics

Given the species specificity of HPV precluding the evaluation of any human HPV-16/18 vaccine in protection animal models, primary pharmacodynamic studies aimed at the preclinical evaluation of the immunogenicity of the HPV-16/18 L1 VLP AS04 vaccine were carried out in mice and Rhesus monkeys.

- A first group of experiments in BALB/c mice compared the immunogenicity of different HPV-16/18 L1 VLP AS04 formulations containing various doses of aluminium salt (Al(OH)3), MPL or HPV-16/18 L1 VLP antigen.
- A second group of experiments in BALB/c mice targeted the characterisation of both humoral and cellular immune responses induced by HPV-16/18 L1 VLP AS04 by comparing the immune responses to the immunogenicity of plain HPV-16/18 L1 VLP and HPV-16/18 L1 VLP $Al(OH)_3$. These pre-clinical experiments demonstrated that the HPV-16/18 L1 VLP AS04 vaccine provides a superior immunogenicity profile for a protective HPV vaccine compared to the HPV-16/18 L1 VLP $Al(OH)_3$ and the plain HPV-16/18 L1 VLP formulations. The HPV-16/18 L1 VLP AS04 vaccine was indeed able to induce higher and more persistent anti-HPV-16/18 L1 VLP antibody levels, higher levels of IgG2a antibodies and superior levels of IFN-γ and TNF-α, cytokines with known antiviral properties. In addition, HPV-16/18 L1 VLP AS04 also induced elevated frequencies of memory B-cells which should have a positive impact on antibody persistence. The comparison of the immunogenicity of HPV-16/18 L1 VLP $Al(OH)_3$ and HPV-16/18 L1 VLP AS04 formulations in rhesus monkeys, more closely related to humans, confirmed the superior

immunogenicity profile associated to the use of the HPV-16/18 L1 VLP AS04 vaccine including the induction of higher levels of neutralizing antibodies.

Another series of experiments was performed to evaluate the immunostimulatory properties of the AS04 adjuvant containing 3-O-desacyl-4'-monophosphoryl lipid A (MPL), a derivative from *Salmonella minnesota* Lipopolyssacharides (LPS), and aluminium salt ($Al(OH)_3$).

- Two experiments, performed on human peripheral blood mononuclear cells (PBMCs) and on a human monocytic cell line (U937), demonstrated that AS04, like MPL, is a potent inducer of pro-inflammatory cytokine (IL-6 and TNF-α) secretion by monocytes *in vitro*.
- A third experiment performed in mice demonstrated that AS04 is functionally equivalent to MPL regarding the *in vivo* induction of antigen-presenting cell maturation as evaluated by the upregulation of CD40 expression.

- Secondary pharmacodynamics

No secondary pharmacodynamic studies were performed according to the Note for Guidance on Preclinical Pharmacological and Toxicological testing of vaccines (CPMP/465/95) and Guideline on Adjuvants in Vaccines for Human Use (EMEA/CHMP/VEG/134716/2004).

- Safety pharmacology programme

The possible side effects of the HPV-16/18 L1 VLP AS04 vaccine on cardiovascular and respiratory parameters in the anaesthetized SPF female Wistar rats were investigated. Intramuscular administration of 0.1ml/animal of the HPV-16/18 L1 VLP AS04 vaccine did not produce any treatment-related effects on blood pressure, heart rate, ECG (lead II) or respiration depth and rate.

- Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were performed according to the Note for Guidance on Preclinical Pharmacological and Toxicological testing of vaccines (CPMP/465/95) and Guideline on Adjuvants in Vaccines for Human Use (EMEA/CHMP/VEG/134716/2004).

Pharmacology / MPL

The activity of 3-*O*-desacyl-4'-monophosphoryl Lipid A (MPL), a derivative from *Salmonella minnesota* LPS, regarding the induction of co-stimulatory molecules (APC maturation) and the induction of pro-inflammatory cytokines and chemokines has been investigated. A first set of primary pharmacodynamic studies performed in mice or on murine cells demonstrated that MPL plays a key role in inducing antigen –presenting cell (APC) maturation by the up-regulation of CD80, CD86 and CD40 co-stimulatory molecules both *in vitro* and *in vivo*, and by inducing the secretion of pro-inflammatory cytokines (IL-6, TNF-α) by APC *in vitro*. The experiments emphasize that MPL plays a significant role in the activation of innate immunity, which is critical for the induction of antigen-specific adaptive immune response. The role played by MPL on the induction of co-stimulatory molecules, pro-inflammatory cytokines and chemokines on human cells was further confirmed by *in vitro* studies on peripheral blood mononuclear cells (PBMCs) or on whole blood. MPL stimulation of human whole blood was shown to induce a panel of cytokines (IL-12, IFN-γ, IFN-α, IL-10), pro-inflammatory cytokines (IL-6, TNF-α, IL-8, Il-1β) as well as chemokines (PCP-1, MIP-1α, MIP-1β). Finally, *in vitro* studies on human PBMCs were shown to be supportive of the hypothesis that MPL acts through interactions with the human TLR4 receptor, but not with the human TLR2 receptor, to activate peripheral blood monocytes.

A cardiovascular and respiratory safety pharmacology study was performed in Beagle dogs and did not show any statistically significant nor physiologically relevant effects on the blood pressure, heart rate, respiration depth or rate or any abnormalities in the electrocardiogram waveform.

The Pharmacology studies showed that MPL plays a significant role in the activation of innate immunity and does not induce undesirable pharmacological effects on cardio-respiratory functions in animal models.

Pharmacokinetics

Studies to demonstrate absorption, distribution, metabolism, and excretion of the active ingredients in Cervarix have not been performed for any of the component viruses. This is in line with Note for guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95).

Pharmacokinetics / MPL

The pharmacokinetics, absorption, distribution and elimination of ^{14}C-MPL have been investigated following a single intramuscular or a single intravenous administration to the male Han Wistar rat at nominal dose levels of 100 μg/kg bodyweight. For both routes of administration, the dose was formulated as an aqueous solution, and was administered as a bolus injection over ca. 30 seconds. The highest observed concentrations of radioactivity in blood were 14.9 (occurring at 4 hours post-dose) and 248 (occurring at 5 minutes post-dose) ng equivalents of MPL/g of sample, following intramuscular and intravenous administration, respectively. The concentration remained relatively constant thereafter following intramuscular administration, and at 168 hours post-dose was 11.4 ng equivalents of MPL/g. The elimination half-life following intravenous administration was 76.5 hours. Relatively low quantifiable levels of radioactivity were detected in all tissues investigated at 56 days post-dose in both studies, with the highest concentrations observed in the fat and spleen.

The routes and rates of elimination were similar following both intramuscular and intravenous administration. The major route of elimination of MPL-related material was via the faeces accounting for a mean of approximately 24 % of the dose for both routes, with urinary elimination accounting for a mean of approximately 4 % of the dose. Radioactivity in the expired air traps accounted for a mean of approximately 2 % of the dose for each set of air traps. The mean total of radioactivity in the tissues and residual carcass was approximately 6 % of the dose, with the injection site accounted for approximately 0.2 % of the dose. The mean total recovery of radioactivity (including cage washes and cage debris) was approximately 38 %. The low total recovery is considered to be due to the elimination of volatile organic molecules that had not been trapped efficiently by the chosen trapping solution used in these studies.
Based on the MPL-related material recovered in the faeces, urine, expired air traps, tissues (excluding the injection site) and residual carcass following intramuscular administration, approximately 35% of the dose appeared to be absorbed. However, the total absorption is likely to be much higher than this value since only 0.2% of the dose was recovered from the injection site.
In conclusion the pharmacokinetic studies conducted in rats have shown that MPL-related material is widely distributed throughout the body, notably to the fat and spleen, and is then likely eliminated mainly via the expired air, with only low levels of radioactivity remaining in the carcass.

Toxicology

- Single dose toxicity

Single-dose toxicity of the HPV-16/18 L1 VLP AS04 vaccine was assessed as part of the repeat-dose toxicity study in rabbits. The treatment was well tolerated and no treatment-related systemic effect was noticed on haematology, body-weight, clinical signs, mortality and clinical chemistry over a 14-day observation period.

- Repeat dose toxicity (with toxicokinetics)

Repeated-dose toxicity studies have been performed with Cervarix and AS04 (intramuscular administration in rabbits).

The HPV-16 /18 L1 VLP AS04 vaccine and the AS04 adjuvant were well tolerated. The organ-weight data, macroscopic pathology evaluation, and evaluation of systemic effects showed no consistent treatment-related findings. Signs of inflammation at the injection sites of the test vaccine were observed. Histological examination of the administration sites a few days after vaccination with HPV-16/18 L1 VLP AS04 revealed evidence of sub-acute inflammation with slight to moderate focal degeneration, necrosis, or regeneration of myofibres. Animals which solely received aluminium hydroxide or AS04 also showed evidence of inflammation at the injection site although the

inflammation was of shorter duration and less extensive. Examination after a treatment free period (4 or 13 weeks) revealed evidence of histological changes (i.e. myofibres regeneration) that were indicative of an ongoing process of recovery.

The injection site reaction was accompanied with a temporary increase in the number of neutrophils and in fibrinogen, which may be a consequence of the recruitment of inflammatory cells following injections of the formulations.

- Genotoxicity

According to the Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95) and the Guideline on adjuvants in vaccines for human use (EMEA/CHMP/VEG/134716/2004) genotoxicity studies are not required for this vaccine.

- Carcinogenicity

According to the Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95) and the Guideline on adjuvants in vaccines for human use (EMEA/CHMP/VEG/134716/2004) carcinogenicity studies are not required for this vaccine.

- Reproduction Toxicity

The reproductive and developmental toxicity of Cervarix and the AS04 adjuvant alone was assessed in a study in female rats of the CD strain. The influence of Cervarix on fertility, embryo-fetal, peri-natal and post-natal survival and development was examined.

The study did not show any effect of vaccination with Cervarix or administration of AS04 on any phases of reproduction and foetal development in rats.

An immune response to the vaccine was observed, and antibodies were transferred to the offspring during gestation and also during lactation.

- Local tolerance

Local tolerance was assessed as part of the toxicity studies by intramuscular administration of the vaccine in rabbits.

The in vitro mutagenicity (Ames) and clastogenicity (CHO cells) tests, and in vivo micronucleus assay demonstrated no genotoxic potential of the MPL adjuvant.

Toxicology / MPL

Pre-clinical toxicity studies have been carried out to detect potential toxic signs associated with MPL administration and are shortly summarized below:

Acute toxicity of MPL was tested in Sprague-Dawley rats by intraperitoneal injections of single dosages of 10, 40, 400, and 4000 μg of MPL per body weight kg. Dosage-related effects were restricted to a slight increase in the incidence and relative severity of interstitial infiltration of mononuclear inflammatory cells in the omentum most probably due to the route of injection (intraperitoneal). This slightly irritating effect of MPL was neither apparent at dosages below 400 μg/kg nor was it correlated with any other anatomical or clinical changes.

Repeat-dose toxicity of MPL administered intravenously was examined in two rat studies with dosage levels of 100, 1000, or 2500 μg/kg/day for 8 days, and of 40, 200, or 1000 μg/kg/day for 7 days, respectively, and in dogs with dosage levels of 6, 120, or 1200 μg/kg/day during 14 days. In the first study, a dosage of 5000 μg/kg/day was initially tested and decreased to 2500 μg/kg/day due to excessive mortality. In contrast, no mortality occurred in the second study. In rats, reversible decreased body weight gain and food consumption were observed at all dosages. The incidence of clinical signs was increased at MPL dosage of 2500 μg/kg/day while no remarkable clinical signs were

observed at lowest dosages. A decrease in platelets was observed in males at MPL dosage ≥ 1000 µg/kg/day level, but not in females. A dose-dependent decrease was noted in erythrocytes, haemoglobin and hematocrit. A dose–related increase in segmented neutrophils was also noted. Organ weight increase was noticed in all groups, with a wider organ distribution at higher MPL dosages. The histopathological examination performed in the first study revealed changes generally characterized by infiltration of mononuclear inflammatory cells in several organs. These effects were minor at the 40 µg/kg/day dose, which was therefore considered well-tolerated. In dogs, repeated intravenous administrations of MPL only produced mild toxicity at dosage levels of 120 and 1200 µg/kg/day. A dosage level of 6 µg/kg/day was considered a no-observed-effect-level for this study.

No **toxicokinetic** studies were performed during the repeated-dose toxicity study program, according to the Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95 guidance), and the Guideline on adjuvants in vaccines for human use (EMEA/CHMP/VEG/134716/2004).

Genotoxicity studies performed *in vitro* did no reveal any MPL-related mutagenic activity or genotoxic effect. *In vivo* studies did not show any evidence of increased frequency of micronucleated immature erythrocytes or bone marrow cell toxicity linked to MPL.

Reproductive and developmental studies carried out in rats and rabbits by subcutaneous administration of MPL at dosage levels of up to 100 µg/kg/day showed no indication of maternal or embryo toxicity or teratogenicity, and no adverse effects of the maternal treatment on the F_1 generation, their maturation and reproductive performance.

In conclusion, MPL showed effects expected from a strong stimulation of the immune system. Under the reported studies, MPL appears to have a high safety margin and is suitable as human vaccine immunostimulant at the proposed dosage of 50 µg/vaccine dose, which corresponds to a 1.7-0.7 µg/kg dose for a 30-70 kg-weighing person.

Ecotoxicity / environmental risk assessment

According the Guideline on the Environmental Risk Assessment (ERA) of medicinal products for human use (EMEA/CHMP/SWP/4447/00), an ERA is not required for vaccines.

Overall, it can be concluded that none of the ingredients of the HPV-16/18 L1 VLP AS04 vaccine will enter the environment in quantities that merit ecological concern following its prescribed use in humans. Therefore, no specific precautionary and safety measures need to be taken regarding the environmental release from use in patients, and disposal of unused products or waste materials derived from the vaccine

Discussion on the non-clinical aspects

Non-clinical data reveal no special hazard for humans based on conventional studies of safety pharmacology, acute and repeated dose toxicity, local tolerance, fertility, embryo-foetal and postnatal toxicity (up to the end of the lactation period).

4. Clinical aspects

Introduction

The clinical development programme to support licensure of the Cervarix consisted of 9 clinical studies. Approximately 24348 subjects were included.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Pharmacokinetics

No clinical pharmacology studies describing the pharmacokinetic properties of Cervarix were conducted in support of this application. This is in accordance with the Guideline on clinical evaluation of new vaccines (EMEA/VWP/164653/05), which states, that pharmacokinetic studies are generally not required for injectable vaccines because they do not provide information useful for establishing adequate dosing recommendations. Pharmacodynamic evaluations related to the humoral and cellular immune responses to HPV were assessed in Phase I through Phase III studies.

Pharmacodynamics / Immunogenicity

The pharmacodynamics of the vaccine relate to its interaction with the immune system. Therefore, this section will discuss the data on the systemic immune response to vaccination.

The antibody response and cell mediated immune response to the candidate HPV-16/18 L1 VLP AS04 vaccine was investigated in exploratory phase I/IIa studies HPV-003, HPV-004 and HPV-005 (see Table below) and pivotal studies HPV-012, HPV-013 and HPV-014 (see Table below). In addition, immunogenicity was also evaluated in the efficacy studies HPV-001, HPV-007 and HPV-008 (see section *Clinical Efficacy*).

Study No/ Phase / Countries	Study Design / Posology	Study population Gender / Total No /Age range	Key study endpoints (Initial Phase)	Duration
HPV-003 Phase I/II USA 1999-2001	Double-blind, randomised, controlled Arm 1: HPV-16/18 20µg/20µg, AS04 Arm 2: $Al(OH)_3$ 3 doses at 0, 1, 6 months	Healthy HPV DNA positive females N=61 18-30 years	**Primary endpoint:** Occurrence of SAEs throughout the entire study period (up to Month 7). **Secondary endpoints (selected):** Anti-HPV-16/18 antibody titres assessed at Months 0, 2, 7 and 12. Anti-HPV-16/18 antibody titres in HPV DNA positive women at study entry assessed at Months 2, 7 and 12.	3 years
HPV-004 Phase IIa USA 2000-2001	Double-blind, randomised Arm 1: HPV-16/18 20µg/20µg, AS04 Arm 2: HPV-16/18 20µg/20µg, $Al(OH)_3$ Arm 3: HPV-16/18 20µg/20µg 3 doses at 0, 1, 6 months	Healthy females N=60 18-30 years	**Primary endpoints:** Safety assessment Anti-HPV-16/18 antibody titres assessed 30 days after third injection **Secondary endpoints:** Anti-HPV-16/18 antibody titres at days 0, 7, 30, 60, 180, 210, 360 Neutralising anti-HPV-16/18 antibody titres at days 0, 60, 210, 360 Lymphoproliferation, IL-5 and IFN-y assays at days 0, 60, 210, 360	4 years Initial phase 12 months Extension to 4 years to evaluate long-term persistence of antibodies
HPV-005 Phase IIa USA 1999-2001	Double-blind, randomised Dose escalating Arm 1: HPV-16/18 6µg/6µg, AS04 Arm 2: HPV-16/18 20µg/20µg, AS04 Arm 3: HPV-16/18 60µg/60µg, AS04 Arm 4: HPV-16/18 20µg/20µg, $Al(OH)_3$ 3 doses at 0, 1, 6 months	Healthy females N=210 18-30 years	**Primary endpoints:** Safety assessment Evaluation of antibody response **Secondary endpoints:** Anti-HPV-16/18 antibody titres, neutralising HPV-16/18 antibody titres and CMI (lymphoproliferation, IL-5 and IFN-y) through 30 days after 3rd injection Anti-HPV-16/18 antibody titres, neutralising HPV-16/18 antibody titres and CMI through 360 days after 1st injection Antibody titres (ELISA, NT) in cervical and vaginal secretions at 30 and 180 days after 3rd injection	4 years Initial phase 12 months Extension to 4 years to evaluate long-term persistence of antibodies

Antibody titres and seroconversion rates following vaccination were investigated in main studies HPV-012, HPV-013 and HPV-14. In order to assess the long-term persistence of the antibody responses studies HPV-004, HPV-005, HPV-012, HPV-013 and HPV-014 were extended. NB study HPV-001/007 evaluated long-term antibody response for 5.5 years - should also be mentioned. The vaccination schedule was identical to those preferred for recombinant Hepatitis B vaccines, which is the 0, 1, 6 months interval schedule.

Study No / Phase / Countries	**Study Design / Posology**	**Study Population / Total No / Age range**	**Key Study endpoints (Initial Phase)**	**Duration**
HPV-012 Phase III DK, FI, NL, Estonia, Greece, Russia 17 centres 2004-2005	Blinded, randomised, multicentric study with five parallel arms: - HPV-16/18 AS04, Lot 1 - HPV-16/18 AS04, Lot 2 - HPV-16/18 AS04, Lot 3 - HPV-16/18 AS04, previous formulation - HPV-16/18 AS04, Lot 1 3 doses, i.m., 0, 1, 6 months	HPV-16 / HPV-18 seronegative high risk HPV DNA negative healthy women N= 770 Age range: Arm 1-4: 15-25 years Arm 5: 10-14 years	**Primary endpoints** To demonstrate lot-to-lot consistency of HPV-16/18 vaccine (3 lots, 15-25 year olds) Clinical bridge to previous formulation **Secondary endpoints (selected)** Immunogenicity: immune non-inferiority in the 10-14 year old age group compared with 15-25 year old age group by ELISA (pooled lots, 15-25 years and single lot, 10-14 years) Safety assessment	1 year
HPV-013 Phase III CZ, FR, DE, NO, ES, SE, Australia, Columbia Honduras Korea Panama Taiwan 57 centres 2004-2005	Double-blind, randomised, controlled, multicentric study with two parallel groups: - HPV-16/18 L1 VLP AS04 - Havrix 3 doses, i.m., 0, 1, 6 months	HPV-16 / HPV-18 seronegative high risk HPV DNA negative healthy women N= 2067 Age range: 10-14 years	**Primary endpoint** Occurrence of SAEs throughout the entire study period (up to Month 7) by ELISA. **Secondary endpoints (selected)** Anti-HPV-16 and anti-HPV-18 antibody titres at month 0, 2 and 7 Anti-MPL antibody titres at month 0, 2 and 7 Safety assessment	1 year 3-year extension to evaluate long-term persistence of antibodies
HPV-014 Phase III DE, PL 6 centres	Open, multi-centre, age-stratified study with 3 arms 3 doses, i.m., 0, 1, 6 months - HPV-16/18 L1 VLP AS04 - HPV-16/18 L1 VLP AS04	Females N= 626 15-55 yrs 15-25 yrs 26-45 yrs	**Primary endpoint** Anti-HPV-16 and anti-HPV-18 seroconversion rates at Month 7 by ELISA in age groups 15-25 years and 26-45 years **Secondary endpoints (selected)** Anti-HPV-16 and anti-HPV-18 seroconversion rates at Month 7 (46–55 years). Anti-HPV-16 and anti-HPV-18 antibody titres (GMTs) at each time point in all	1 year

2004-2005	- HPV-16/18 L1 VLP AS04	46-55 yrs	subjects. Anti-HPV-16 and anti-HPV-18 seroconversion rates at Month 2 and 7 in all subjects. Safety assessment	

For all studies anti-HPV-16/18 antibody titres were summarized by geometric mean titres (GMTs) with their 95% confidence intervals prior to vaccination and at relevant time points following vaccination. Furthermore reverse cumulative distribution curves for these antibody titres were displayed. In addition seropositivity rates and their exact 95% confidence intervals were calculated.

Dose response study

In the dose escalating and selection study HPV-005 (for study outline see table in Pharmacodynamic section) the appropriate amount of the L1 derived VLPs for HPV-16 and HPV-18 were evaluated with respect to the safety profile and the immunogenicity (antibody responses). HPV-16/18 vaccine lots of different antigen content ranging from 6µg, 20µg and 60µg of each of the two HPV-16 and HPV-18 L1 VLP per dose were investigated. A total of 210 healthy women 18 to 30 years of age were enrolled in this study.

Study HPV-005: anti-HPV-16 and anti-HPV-18 antibody titres at Month 7 (expressed in Log_{10} ELISA titres) for volunteers who received all scheduled injections of study vaccine with AS04

Antibody	Group	N	GMT	Log_{10} mean	LL	UL
HPV-16	**AS04 12µg**	51	3655.7	3.6	3.4	3.7
	AS04 40µg	47	5248.2	3.7	3.6	3.8
	AS04 120µg	42	5944.5	3.8	3.6	3.9
HPV-18	**AS04 12µg**	51	3402.6	3.5	3.4	3.7
	AS04 40µg	47	3443.4	3.5	3.4	3.7
	AS04 120µg	42	4228.5	3.6	3.5	3.7

AS04 12µg = HPV-16/18 vaccine L1 VLP AS04 (6µg/6µg)
AS04 40µg = HPV-16/18 vaccine L1 VLP AS04 (20µg/20µg)
AS04 120µg = HPV-16/18 vaccine L1 VLP AS04 (60µg/60µg)
*In computation of means, titres <LOQ were set to 1.
N = number of subjects with available results
LL/UL = Lower/Upper limits of the 95% confidence interval on the Log_{10} mean titre

While differences in immunogenicity are marginal or not significant – depending on the type of recombinant L1 - choice of the 40 µg dose (20 µg HPV 16 L1 and 20 µg HPV 18 L1) appears to be appropriate and represents a conservative dose selection.

A candidate vaccine with an antigen content of 20µg HPV-16 L1 and 20µg HPV-18 L1 per dose was chosen for the further clinical development program.

AS04 Adjuvanting System

Consistently high immunogenicity of Cervarix is largely mediated by the use of AS04 as an adjuvanting system. The following table shows superiority of AS04 compared to conventional aluminium hydroxide or plain VLPs:

Study HPV-004: anti-HPV-16 and anti-HPV-18 ELISA titres at Month 7 and Month 12 for volunteers who received all scheduled injections (0, 1, 6 months schedule)

Antigen	Group	Timepoint	N	GMT	Log_{10} mean	LL	UL
HPV-16	**AS04 40µg**	**Month 7**	19	11199	4.0	3.9	4.2
		Month 12	18	4550.5	3.7	3.4	3.9
	ALU 40µg	**Month 7**	17	4076	3.6	3.4	3.8
		Month 12	15	2076.9	3.3	3.0	3.6
	No adjuvant	**Month 7**	20	2488	3.4	3.3	3.5
		Month 12	18	935.3	3.0	2.8	3.1
HPV-18	**AS04 40µg**	**Month 7**	19	4794	3.7	3.5	3.8
		Month 12	18	1536.1	3.2	2.9	3.4
	ALU 40µg	**Month 7**	17	1960	3.3	3.1	3.5
		Month 12	15	637.1	2.8	2.6	3.0
	No adjuvant	**Month 7**	20	1305	3.1	2.0	3.3
		Month 12	18	229.7	2.4	2.1	2.7

AS04 40µg = HPV-16/18 L1 VLP AS04 vaccine (20µg/20µg)
ALU 40µg = HPV-16/18 L1 VLP $Al(OH)_3$ vaccine (20µg/20µg)
*In computation of means, titres <LOQ were set to 1
N = number of subjects with available results
GMT = geometric mean titre
LL/UL = Lower/Upper limits of the 95% confidence interval on the Log_{10} mean titre

Results shown above (with regard to GMTs) are, in principle, representative and consistent for all clinical trials conducted throughout the pharmaceutical development program and support the use of AS04 as the preferred adjuvanting system for recombinant HPV 16/18 L1 particles.

Humoral immune response:

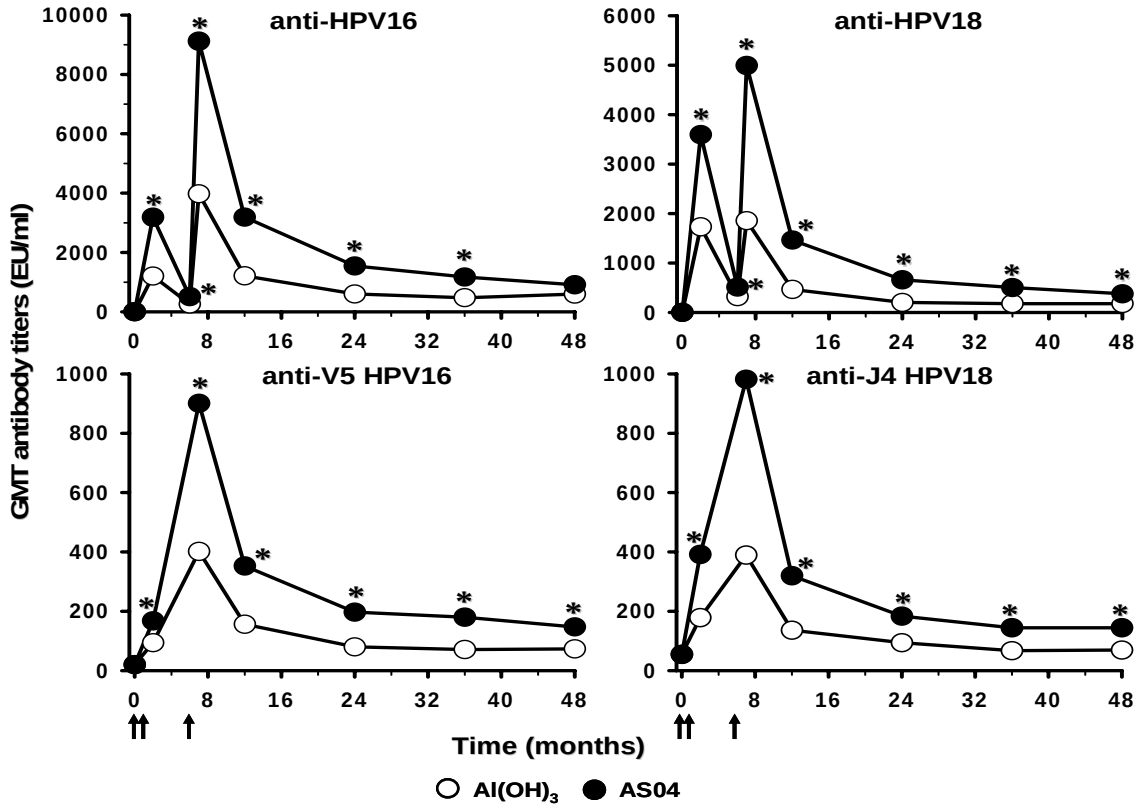
The immunogenicity induced by three doses of Cervarix has been evaluated in 5,303 female subjects from 10 to 55 years of age.
A number of different approaches were pursued, finally evolving into three different assay systems that were applied in all pivotal clinical studies for qualitative and quantitative analysis of the immune response induced. These assays include:

- An ELISA binding assay for antibody quantification (determination of GMTs)
- An inhibition assay to measure the amount of serum antibodies against neutralizing epitopes present on the surface of HPV 16 and 18
- A neutralization assay involving HPV pseudoparticles developed by the US National Cancer Institute

These assays were carefully cross validated against each other showing reasonable concordance of assay results. Thus, antibodies measured by ELISA in individual clinical trials are to a great extent functional. Consequently, the humoral part of the immunological mode of action of Cervarix may be explained by neutralization of wild-type HPV 16 and 18, respectively.

Measuring GMTs themselves does not provide sufficient information on the quality of the immune response. The following graphs provide some insight into the correlation between vaccine-induced serum antibody titres and their capacity to inhibit binding of neutralizing monoclonal antibodies (V5 and J4) to their corresponding epitopes on the viral surface. Moreover, the graphs illustrate again the superiority of the AS04 adjuvanting system over $Al(OH)_3$ with regard to GMTs, titres of functional antibodies and persistence of functional, i.e. virus neutralizing antibodies.

Figure: Studies HPV-004 and HPV-005 (pooled): Persistence of anti-HPV-16 and anti-HPV-18 antibodies (by binding ELISA and by inhibition ELISA) after vaccination



Significant differences ($p<0.05$) between the antibody titres of the AS04 and the aluminium group are indicated in the figure by asterisks (n=9-19 subjects for the aluminium salt group, n=21-37 subjects for the AS04 group).
Arrows indicate vaccination timepoints.
The two lower figures demonstrate the correlation between vaccine-induced serum antibody titres and their capacity to inhibit binding of neutralizing monoclonal antibodies (V5 and J4) to their corresponding epitopes on the viral surface.

Persistence of a quantitatively and qualitatively acceptable immune response is an important element to assure long-term protection from disease as well as to define the correct time point for booster vaccination.

Local immunity:

A key element to understand the immunological mode of action of Cervarix is to investigate the local activity of antibodies, such as IgGs transudated from the serum to the cervical mucosa.
The first issue, i.e. the local immune response, was addressed in the very early phase of the clinical development program and was aimed at measuring IgG activity in cervical samples and correlating these activities to serum antibody activities. These early results of **Study HPV-005** are presented in the table below.

Study HPV-005 : Summary of specific HPV-16 and HPV-18 activity* in serum and cervico-vaginal secretions at Month 7 and Month 12 after vaccination with the HPV-16/18 vaccine

Antibody	Study Group	Time point	N	CVS			Serum		
				n+	%+	Geometric Mean (units/mL)	n+	%+	Geometric Mean (units/mL)
HPV-16	HPV-16/18 L1 VLP AS04 12µg	PIII(M7)	7	4	57.1	0.040	6	85.7	0.036
		PIII(M12)	5	3	60.0	0.015	4	80.0	0.030
	HPV-16/18 L1 VLP AS04 40µg	PIII(M7)	10	9	90.0	0.076	9	90.0	0.090
		PIII(M12)	6	4	66.7	0.033	6	100	0.036
	HPV-16/18 L1 VLP AS04 120µg	PIII(M7)	8	8	100	0.130	8	100	0.166
		PIII(M12)	5	3	60.0	0.143	5	100	0.092
	HPV-16/18 L1 VLP $Al(OH)_3$ 40µg	PIII(M7)	4	4	100	0.108	4	100	0.116
		PIII(M12)	2	2	100	0.110	2	100	0.067
HPV-18	HPV-16/18 L1 VLP AS04 12µg	PIII(M7)	7	5	71.4	0.090	6	85.7	0.079
		PIII(M12)	5	2	40.0	0.067	4	80.0	0.034
	HPV-16/18 L1 VLP AS04 40µg	PIII(M7)	10	8	80.0	0.246	9	90.0	0.175
		PIII(M12)	6	4	66.7	0.061	6	100	0.055
	HPV-16/18 L1 VLP AS04 120µg	PIII(M7)	8	8	100	0.230	8	100	0.312
		PIII(M12)	5	4	80.0	0.147	5	100	0.185
	HPV-16/18 L1 VLP $Al(OH)_3$ 40 µg	PIII(M7)	4	4	100	0.159	4	100	0.175
		PIII(M12)	2	2	100	0.130	2	100	0.115

* Specific activity =reactivity per IgG
CVS = cervico-vaginal secretions
n+ = number of subjects positive
%+ = percentage of subjects positive
PIII(M7) = Post Dose III, Month 7
PIII(M12) = Post Dose III, Month 12

Careful interpretation of data allows the conclusion that there is a dose-effect-relationship, however, the clinical significance of local antibody activity and titres remains unclear. Nevertheless some evidence has been provided that i.m. injection of recombinant HPV 16 and 18 L1 particles do trigger an immune response at the target organ/tissue.

A subset of approximately 90 subjects in study HPV-014 provided blood samples and had cervico-vaginal secretion (CVS) samples collected one year after the third dose of the vaccine (Month 18). Cervico-vaginal HPV-16 VLP antibodies were detected in 85.7%, 80.8% and 77.3% of subjects in the [15-25], [26-45] and [46-55] age groups, respectively. Similarly, cervico-vaginal HPV-18 VLP antibodies were detected in CVS in 71.4%, 61.5% and 68.2% of subjects in the [15-25], [26-45] and [46-55] age group, respectively

Cell mediated immunity:

Vaccine efficacy and, in particular, long-term efficacy requires evaluation of the involvement of the cellular part of the immune system. A battery of methods has been developed and applied in a variety of studies to investigate activation of T-cell response and formation of memory B cells following

vaccination with Cervarix. A summary of these methodologies and clinical studies where these have been applied is outlined below.

Overview of analyses conducted to assess cell-mediated immunity to HPV-16/18 vaccine

Study No	T Cell Response			B Cell Response
	Lympho-proliferation	Cytokine production†	Intracellular Cytokine Staining‡	ELISPOT
HPV-002 (MI-CP-044)	Day 0 and 56 Day 112 and 140 * Months 18, 24, 30, 36, 42, 48 and 54 (planned)	Day 0 and 56 Day 112 and 140* Months 18, 24, 30, 36, 42, 48 and 54 (planned)	Day 0 and 56 Day 112 and 140*	ND
HPV-004 (MI-CP-055)	Days 0, 60, 210 and 360	Days 0, 60, 210 and 360:	Days 0, 60, 210 and 360	Days 0, 60, 210 and 360
	Months 18 and 24	Months 18 and 24:	Months 18 and 24	Months 18 and 24
HPV-005 (MI-CP-057)	Day 0, 60, 210 and 360	Day 0, 60, 210 and 360:	Days 0, 60, 210 and 360.	Days 0, 60, 210 and 360
HPV-TETRA-051	ND	Day 0, Month 2	Day 0, Month 2	Day 0, Month 2
		Month 7	Month 7	Month 7

ND = not done subjects in the HPV-16 group who received a third dose of vaccine
† = IFN-γ and IL-5
‡ = CD40L, IL-2 and TNF-α

In essence, vaccination with Cervarix apparently addresses a number of immunological compartments which is evidenced by specific expression of cytokine profiles. Expression levels and proliferative responses were strongly depended on antigen doses administered and reacted anamnestically on a multiple dose vaccination schedule indicating development of an immunological memory.
Also for the cell mediated immune response AS04 turned out to be superior with regard to its capacity to stimulate various elements of the cellular immune system compared to $Al(OH)_3$.
In conclusion, there is sufficient evidence that Cervarix induces a strong cell mediated immune response. For the time being none of the cell-mediated immunity (CMI) parameters measured can, however, be correlated with clinical efficacy.

Antibody response against MPL (AS04):

Study HPV-013: Seropositivity rates and GMTs for anti-MPL antibodies by pre-vaccination status (ATP cohort for immunogenicity)

Group	Pre-vacc status	Timing	N	n	%	LL	UL	GMT	LL	UL
HPV	**S-**	**PRE**	6	0	0.0	0.0	45.9	29.5	29.5	29.5
		PII(M2)	6	5	83.3	35.9	99.6	133.5	52.0	343.0
		PIII(M7)	6	6	100	54.1	100	274.1	152.3	493.5
	S+	**PRE**	132	132	100	97.2	100	156.3	141.8	172.3
		PII(M2)	132	132	100	97.2	100	423.5	373.9	479.8
		PIII(M7)	130	130	100	97.2	100	741.5	639.9	859.2
	Total	**PRE**	138	132	95.7	90.8	98.4	145.4	130.3	162.2
		PII(M2)	138	137	99.3	96.0	100	402.8	354.0	458.3
		PIII(M7)	136	136	100	97.3	100	709.7	613.2	821.3
HAV	**S-**	**PRE**	13	0	0.0	0.0	24.7	29.5	29.5	29.5
		PII(M2)	13	2	15.4	1.9	45.4	34.1	27.5	42.3
		PIII(M7)	13	1	7.7	0.2	36.0	32.4	26.4	39.7
	S+	**PRE**	128	128	100	97.2	100	149.5	137.5	162.5
		PII(M2)	127	120	94.5	89.0	97.8	138.9	124.4	155.1
		PIII(M7)	127	118	92.9	87.0	96.7	140.6	124.2	159.2
	Total	**PRE**	141	128	90.8	84.7	95.0	128.7	115.4	143.6
		PII(M2)	140	122	87.1	80.4	92.2	121.9	107.9	137.8
		PIII(M7)	140	119	85.0	78.0	90.5	122.7	107.2	140.3

HPV = HPV-16/18 L1 VLP AS04 (Hi-5 Rix4446) (10-14 years)
HAV = Havrix group
S- = seronegative subjects (antibody titre < 59 EU/ML) prior to vaccination
S+ = seropositive subjects (antibody titre >= 59 EU/ML) prior to vaccination
GMT = geometric mean antibody titre calculated on all subjects
N = number of subjects with pre-vaccination results available
n/% = number/percentage of subjects with titre within the specified range
LL/UL = Lower/Upper limit of the 95% Confidence Interval
PRE = Pre-vaccination; PII(M2) = Post Dose II (Month 2); PIII(M7) = Post Dose III (Month 7)

Only an MPL-containing vaccine such as Cervarix has a booster effect on a confirmed pre-existing anti-MPL immune response. A HAV (Hepatitis A) vaccine that does not contain MPL components has no such effect. However, even for Cervarix the booster effect on a pre-existing MPL “immunity” is weak to moderate and, most likely, will have no clinical effect. Such type of anti-MPL immune response is expected upon contact with any type of MPL-like substance which in most instances will be bacterial antigens containing MPL components in their outer cell wall structure.

Whether or not residual MPL “immunity” might impair immunogenicity of Cervarix has been addressed by grouping a subset of the study population from study HPV-013 into four different anti-MPL titer categories.

Study HPV-013: Seropositivity rates and GMTs for anti-HPV-16 and anti-HPV-18 antibodies according to anti-MPL level before vaccination in initially seronegative subjects (ATP cohort for immunogenicity):

Antibody	Group	Timing	N	nS+	%S+	LL	UL	GMT	LL	UL
Anti-HPV-16	**Level 1**	**PRE**	33	0	0.0	0.0	10.6	4.0	4.0	4.0
		PII(M2)	33	33	100	89.4	100	5413.9	4092.0	7162.9
		PIII(M7)	32	32	100	89.1	100	16765.5	11837.0	23746.2
	Level 2	**PRE**	32	0	0.0	0.0	10.9	4.0	4.0	4.0
		PII(M2)	32	32	100	89.1	100	6034.2	4443.0	8195.3
		PIII(M7)	32	32	100	89.1	100	26882.1	20997.9	34415.3
	Level 3	**PRE**	34	0	0.0	0.0	10.3	4.0	4.0	4.0
		PII(M2)	34	34	100	89.7	100	4728.1	3646.3	6130.8
		PIII(M7)	34	34	100	89.7	100	16998.1	12255.3	23576.4
	Level 4	**PRE**	34	0	0.0	0.0	10.3	4.0	4.0	4.0
		PII(M2)	34	34	100	89.7	100	6321.1	4685.2	8528.2
		PIII(M7)	34	34	100	89.7	100	22172.9	15684.1	31346.4
Anti-HPV-18	**Level 1**	**PRE**	32	0	0.0	0.0	10.9	3.5	3.5	3.5
		PII(M2)	32	32	100	89.1	100	4326.4	3312.9	5649.8
		PIII(M7)	32	32	100	89.1	100	6763.7	4838.2	9455.4
	Level 2	**PRE**	31	0	0.0	0.0	11.2	3.5	3.5	3.5
		PII(M2)	31	31	100	88.8	100	4296.7	3262.7	5658.5
		PIII(M7)	31	31	100	88.8	100	10529.5	7680.4	14435.6
	Level 3	**PRE**	34	0	0.0	0.0	10.3	3.5	3.5	3.5
		PII(M2)	34	34	100	89.7	100	4224.1	3312.1	5387.3
		PIII(M7)	34	34	100	89.7	100	6877.2	4946.5	9561.3
	Level 4	**PRE**	32	0	0.0	0.0	10.9	3.5	3.5	3.5
		PII(M2)	32	32	100	89.1	100	5595.1	4017.4	7792.4
		PIII(M7)	32	32	100	89.1	100	9079.2	6632.0	12429.4

Level 1 = HPV group with Anti-MPL<103 at PRE
Level 2 = HPV group with 103<=Anti-MPL<136 at PRE
Level 3 = HPV group with 136<=Anti-MPL<214 at PRE
Level 4 = HPV group with 214<=Anti-MPL at PRE
GMT = geometric mean antibody titre calculated on all subjects
N = number of subjects with available results
n S+/% S+ = number/percentage of subjects seropositive (antibody titre ≥ 8 EL.U/mL for HPV-16 or antibody titre ≥ 7 EL.U/ML for HPV-18)
LL/UL = Lower/Upper limit of the 95% Confidence Interval
PRE = Prevaccination
PII(M2) = Post Dose II (Month 2)
PIII(M7) = Post Dose III (Month 7)

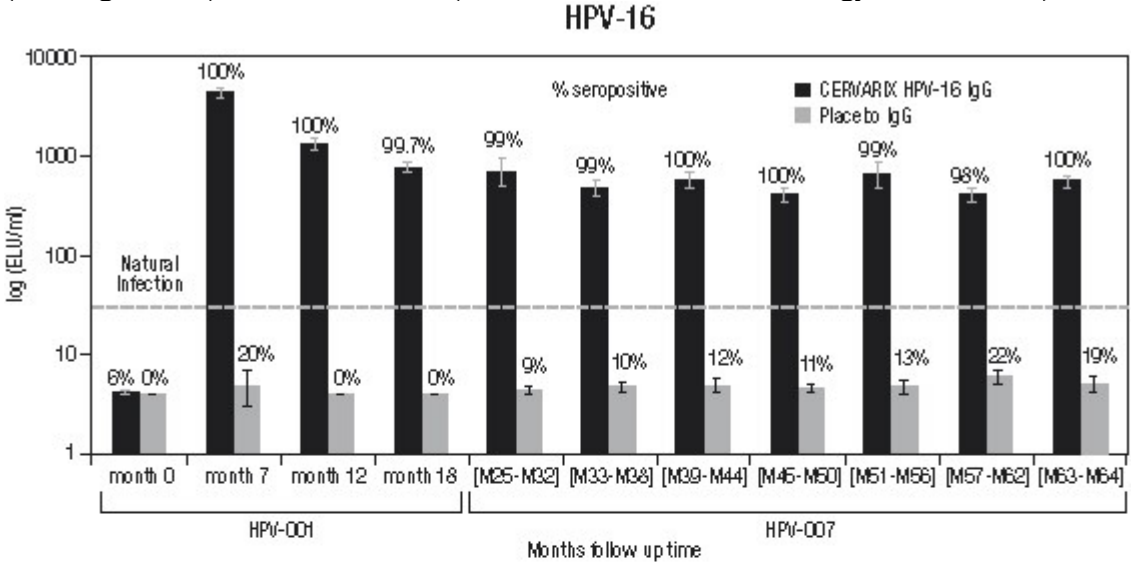
While no specific difference can be observed post dose II (PII(M2)) a titre-dependent stimulating effect of a pre-existing anti MPL "immunity" can be interpreted post-dose III (PIII(M7)). This effect is slightly more pronounced for HPV 16 L1 compared to HPV 18 L1. Overall, anti HPV L1 titres are higher in this specific subset of a study population. Apparently, pre-existing anti MPL "immunity" is an advantage rather than a risk with regard to the potential to trigger an optimal immune response following vaccination with Cervarix.

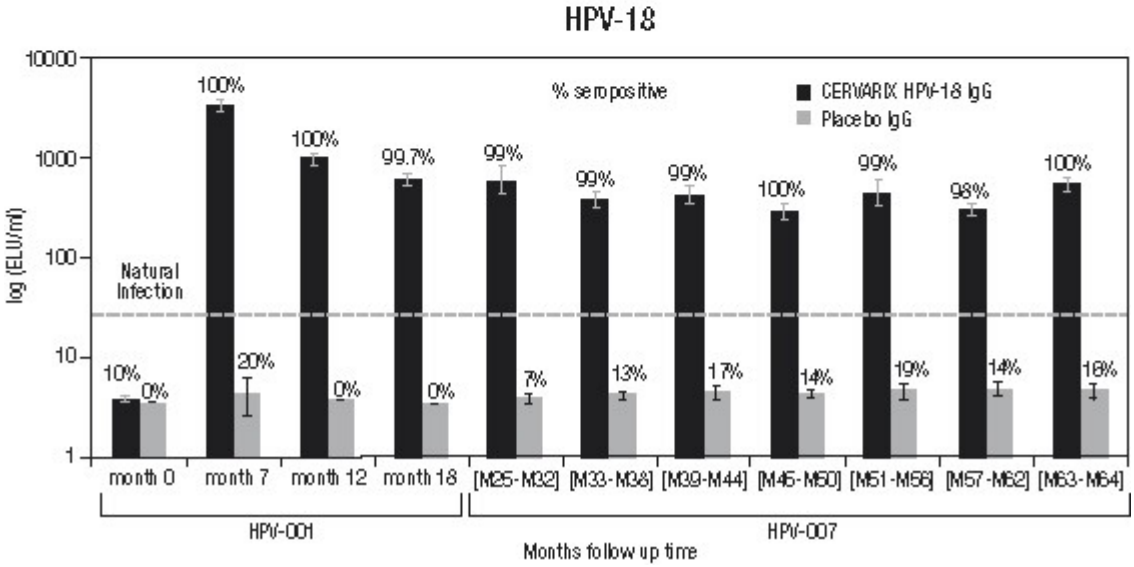
Other immunogenicity studies:

Persistence of Immunity

Phase IIb efficacy/long-term efficacy studies HPV 001 and HPV 007 provide the currently best available overview on the long-term persistence of immunity. The outline of these studies is described under *supportive studies* further below.

Studies HPV-001 and HPV-007: Persistence of Anti-HPV-16 and Anti-HPV-18 Antibodies (Binding ELISA) after Vaccination (0, 1, 6 Month Schedule, Serology Post-Dose III)





These data are a good indicator for long term persistence of vaccine induced immune response until at least month 64 post dose 1. Evaluation of the long-term persistence of the initial immune response is ongoing in a number of studies (see table below).

Timepoints for immunogenicity evaluations

	Day 0	Month 1	Month 2	Month 6	Month 7	Month 12	Month 18	Month 24	Month 36	Month 48
HPV-001	●	●		●	●	●	●			
HPV-007	●					●		(●)	(●)	
HPV-012	●		●		●	(●)		(●)	(●)	(●)
HPV-013	●				●	(●)	(●)	(●)	(●)	(●)
HPV-014	●		●		●	●	(●)	(●)	(●)	(●)

( ) = planned timepoint

The presented data does not allow conclusions about the need and timing of booster doses.

Rationale for bridging to 10-14 years old individuals

Study HPV-012

Information about the study outline can be found in the table provided in the beginning of the *Pharmacodynamic/Immunogenicity* section of this report.

A total of 770 subjects were vaccinated in this study. Of these, 458 subjects 15-25 years of age (mean: 20.2+/-3.0 years) received one of three lots of HPV-16/18 vaccine, 154 subjects 15-25 years of age (mean: 20.3+/-3.0 years) received a previous formulation and 158 subjects 10-14 years of age (mean: 12.4+/-1.4 years) also received one of the three vaccine lots.

At Month 7, all initially seronegative subjects, regardless of their age and regardless of the vaccine they received, had seroconverted to both HPV-16 and HPV-18.

Seropositivity rates and GMTs for anti-HPV-16 and anti-HPV-18 at Month 7 in initially seronegative subjects (binding ELISA) (ATP cohort for immunogenicity)

Antibody	Group	N	n S+	% S+	LL	UL	GMT	LL	UL
HPV-16	Lot 1	118	118	100	96.9	100	7438.9	6324.6	8749.6
	Lot 2	122	122	100	97.0	100	7150.3	6038.1	8467.3
	Lot 3	119	119	100	96.9	100	7297.2	6136.8	8677.0
	Pooled	359	359	100	99.0	100	7292.9	6623.7	8029.7
	Previous	111	111	100	96.7	100	9595.5	8027.4	11469.9
	10-14 years	143	143	100	97.5	100	17272.5	15117.9	19734.1
HPV-18	Lot 1	116	116	100	96.9	100	3070.1	2600.0	3625.4
	Lot 2	126	126	100	97.1	100	3173.4	2714.3	3710.2
	Lot 3	122	122	100	97.0	100	3743.3	3173.3	4415.7
	Pooled	364	364	100	99.0	100	3318.8	3023.1	3643.5
	Previous	117	117	100	96.9	100	4164.7	3552.0	4882.9
	10-14 years	141	141	100	97.4	100	6863.8	5976.3	7883.0

Lot 1 = 15-25 years
Lot 2 = 15-25 years
Lot 3 = 15-25 years
Pooled = Combined lots 1, 2 and 3 (15-25 years)
Previous = Previous formulation (15-25 years)
[10-14] = Lot 1 (10-14 years)
GMT = geometric mean antibody titres calculated on all subjects
N = number of subjects with results available
n S+/% S+ = number/percentage of subjects seropositive (antibody titre ≥ 8 EL.U/mL for HPV-16 or antibody titre ≥ 7 EL.U/ML for HPV-18)
LL/UL = Lower/Upper Limit of the 95% Confidence Interval

Both co-primary objectives (i.e., to demonstrate consistency of three lots in subjects 15-25 years and the clinical bridge) in subjects 15-25 years were met.

The secondary objective of demonstrating non-inferiority of the 10-14 year age group compared to the 15-25 year age group when receiving the same production lot was also met, since at Month 7, the upper limit of the 95% CI on the difference between the percentage of subjects who seroconverted in the older age group versus the younger age group was <10% and that the upper limit of the 95% CI on the GMT ratio between the older age group and younger age group was <2 . Overall, adolescents achieved significantly higher GMTs for both antigens (>2-fold) as compared to subjects 15-25 years of age.

Study HPV-013
Information about the study outline can be found in the table provided at the beginning of the Pharmacodynamic/Immunogenicity section of this report.

Secondary objectives included evaluation of antibody responses against HPV-16, HPV-18 and MPL (by ELISA) at Month 0, 2 and 7.

A total of 2067 subjects were vaccinated in this study: 1035 subjects in the HPV group (mean age: 12.1+/-1.4 years) and 1032 subjects in the control group (mean age: 12.1+/-1.4 years). Of these, 675 subjects were included in the immunogenicity subset.
After the third dose, all initially seronegative subjects had seroconverted for both antigens and had achieved GMTs of 19882.0 EU/mL for anti-HPV-16 antibodies and 8262.0 EU/mL for anti-HPV-18 antibodies.

Seropositivity rates and GMTs for anti-HPV-16 and anti-HPV-18 antibodies in initially seronegative subjects (binding ELISA) (ATP cohort for immunogenicity)

Antibody	Timing	N	n S+	% S+	LL	UL	GMT	LL	UL
HPV-16	PRE	630	0	0.0	0.0	0.6	4.0	4.0	4.0
	PII(M2)	625	622	99.5	98.6	99.9	4696.9	4388.6	5026.8
	PIII(M7)	619	619	100	99.4	100	19882.0	18626.7	21221.9
HPV-18	PRE	639	0	0.0	0.0	0.6	3.5	3.5	3.5
	PII(M2)	633	631	99.7	98.9	100	3741.7	3499.3	4000.9
	PIII(M7)	628	628	100	99.4	100	8262.0	7725.0	8836.2

GMT = geometric mean antibody titre calculated on all subjects (expressed in EU/ML)
N = number of subjects with pre-vaccination results available
n S+/% S+ = number/percentage of subjects seropositive by binding ELISA (antibody titre ≥ 8 EL.U/mL for HPV-16 or antibody titre ≥ 7 EL.U/ML for HPV-18)
LL/UL = Lower/Upper limit of the 95% Confidence Interval
PRE = Pre-vaccination
PII(M2) = Post Dose II (Month 2)
PIII(M7) = Post Dose III (Month 7)

Initially seropositive subjects also elicited immune responses to the vaccine and achieved GMTs levels for both antigens that were comparable to initially seronegative subjects.

In the above described two clinical trials (HPV-012 and HPV-013) performed in girls and adolescents aged 10 to 14 years, all subjects seroconverted to both HPV types 16 and 18 after the third dose (at month 7) with GMTs at least 2-fold higher as compared to women aged 15 to 25 years. On the basis of these immunogenicity data, the efficacy of Cervarix is inferred from 10 to 14 years of age.

Study HPV-014
In another clinical trial (study 014) performed in women aged 15 to 55 years, all subjects seroconverted to both HPV types 16 and 18 after the third dose (at month 7). The GMTs were, however, lower in women above 25 years. Nevertheless, all subjects remained seropositive for both types throughout the follow-up phase (up to month 18) maintaining antibody levels at an order of magnitude above those encountered after natural infection. Information about the study outline can be found in the table presented in the *Pharmacodynamics / Immunogenicity* section of this report.

Older age, smoking, and, even more pronounced a combination of both may significantly reduce the immunogenicity.

Factors such as hormone contraception, hormone replacement therapy, pre- or post menopausal status, do not significantly reduce immunogenicity of Cervarix.

Study HPV-014 further demonstrated, that seropositivity with regard to HPV 16 and 18 at the time of vaccination has some marginal influence on the kinetics of the immune response as well as on final GMTs and is more pronounced in older individuals compared to those who are younger:

Whether or not vaccination of a seropositive female population has any effect on clinical efficacy of Cervarix cannot be assessed for the time being.

Immunogenicity in Other Populations:

The effect of other medicinal products including vaccines on the immunogenicity of Cervarix has not been investigated.

Cervarix has not been investigated in male subjects.

Cervarix has not been investigated in pregnant and lactating women.

Clinical efficacy

The efficacy of Cervarix was assessed in 2 controlled, double-blind, randomised Phase II and III clinical studies that included a total of 19,778 women aged 15 to 25 years.

In the phase II HPV-001/007 study (described in this report under supportive studies) only HPV naïve women (negative for oncogenic HPV DNA [HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68], seronegative for HPV-16 and HPV-18 and normal cytology at study entry) were enrolled. In the pivotal phase III trial HPV-008 women were included without pre-screening for the presence of HPV infection, i.e. regardless of baseline cytology and HPV serological and DNA status.

Overview of studies to support the efficacy of Cervarix

Study No / Phase / Countries	Study Design / Posology	Study Population / Total No / Age range	Key Study endpoints (Initial Phase)	Duration
HPV-001 Phase IIb, Brazil, Canada, USA 32 centres 2001-2003	Multicentre, randomized, double-blind, placebo controlled study with two groups - HPV-16/18 AS04 (Hi-5/Sf-9) - $Al(OH)_3$ 3 doses, i.m., 0, 1, 6 months	HPV-16 / HPV-18 seronegative high risk HPV DNA negative healthy women N= 1113 Age range: 15-25 years	**Primary endpoints:** Incident cervical infection with HPV-16 and HPV-18 analysed using all HPV-16 and/or HPV-18 DNA results determined for all specimens (i.e. self-obtained cervicovaginal specimens and physician-obtained cervical specimens combined). In addition, a separate analysis of only physician-obtained cervical specimens was conducted. Defined as at least one positive HPV-16 or HPV-18 DNA PCR assay **Secondary endpoints (selected)** Persistent infection with HPV-16 and/or HPV-18 defined at least two positive HPV DNA PCR assays for the same viral genotype over a minimum interval of 6 months. Cytologically-confirmed or histopathologically-confirmed LSIL, HSIL, squamous cell cancer, or adenocarcinoma concurrently associated with HPV-16 and/or HPV-18 cervical infection	27 months
HPV-007 follow-up to study to HPV-001	Randomized, double-blind, placebo controlled study 3-year long-term extension of Study HPV-001 with interim analysis at Month 12 and 24* * Study vaccines were administered in study HPV-001	HPV-16 / HPV-18 seronegative high risk HPV DNA negative healthy women N= 776 Age range: 15-25 years	**Primary endpoint** Long-term efficacy against incident cervical infection with HPV-16 and/or HPV-18. **Secondary endpoints (Selected)** - Long-term Efficacy against persistent cervical infection (6-month definition) with HPV-16 and/or HPV-18. - Histopathologically-confirmed CIN 1+ or CIN 2+ associated with HPV-16 or HPV-18 detected within the lesional component of the cervical tissue specimen (by PCR). Long-term immunogenicity Long-term safety	3 years interim analysis after 24 months completed
HPV-008 **14 countries**	Randomized, double-blind, placebo controlled study	HPV-16 / HPV-18 seronegative high risk HPV DNA negative healthy women N=18665 Age range: 15-25 years	**Primary endpoint** Control in the prevention of histopathologically confirmed CIN2+ associated with HPV-16 or HPV-18 cervical infection detected within the lesional component of cervical tissue by PCR. **Secondary endpoints (selected)** - To evaluate the safety of the candidate vaccine in adolescent and young adult women	Final analysis when 36 cases CIN 2+ are found. Long-term follow-up to 48 months

			- Persistent cervical infection (6 and 12-month definitions) with HPV-16 and/or HPV-18. Histopathologically-confirmed CIN 1+ or CIN 2+ associated with HPV-16 or HPV-18 detected within the lesional component of the cervical tissue specimen (by PCR).	Interim analysis when 23 CIN 2 cases are found

- Main studies

The Applicant performed two sets of trials, the 001 – 007 set (of which the data were initially submitted) listed in this report under supportive studies and the 008 trial, of which the data of an interim analysis was submitted with the response to the CHMP LoQ at day 120 of the review. Both trials 007 and 008 are still ongoing and continue to generate data on both efficacy and safety. Data is available from studies HPV-001/007 (5.5 years) and HPV-008 (15 months).

Study HPV-008

HPV-008 is a controlled, double blinded Phase III randomized clinical trial performed in Asia Pacific, Europe, Latin America and North America. HPV-008 has been conducted under the supervision of an Independent Data Monitoring Committee (IDMC) with duration of 48 months of follow-up.

The presented event-driven interim analysis was to be performed when at least 23 cases of CIN2+ with HPV-16 or HPV-18 DNA detected in the lesion.

METHODS

Study Participants and Sample sizes

A total of 18729 subjects were enrolled in the study. The study is conducted in 133 centres in 14 countries spread across four study regions: Asia Pacific, Europe, Latin America and North America. The target was to enrol approximately 18 000 unscreened women aged 15 to 25 years.

The inclusion and exclusion criteria were selected to allow enrolment of a broad population of women, including both those previously uninfected with HPV (HPV naïve) and those previously or currently infected with HPV (HPV non-naïve). No HPV serological testing, HPV DNA screening or cervical cytological screening was performed prior to enrolment.

Inclusion Criteria

- A woman between, and including, 15 and 25 years of age at the time of the first vaccination.
- Subject must be free of obvious health problems as established by medical history and clinical examination before entering into the study.
- Subject must have a negative urine pregnancy test.
- Subject must be of non-childbearing potential (e.g. surgically sterilized) or, if of childbearing potential, she must be abstinent or must be using adequate contraceptive precautions for 30 days prior to the first vaccination and must agree to continue such precautions for two months after completion of the vaccination series.
- Has had no more than 6 lifetime sexual partners prior to enrollment. This criterion may not be applicable in subjects less than 18 years of age, according to local regulatory/ethical requirements.
- Subject must have intact cervix (e.g. no history of cauterization or surgical treatment involving damage to the transformation zone of the cervix).

Main Exclusion Criteria

- Pregnant or breastfeeding. Women must be at least 3 months post-pregnancy and not breastfeeding to enter the study.
- A woman planning to become pregnant or planning to discontinue contraceptive precautions during approximately the first nine months of the study (Months 0-8).
- Previous administration of monophosphoryl lipid A (MPL) or AS04 adjuvant.
- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccine(s) within 30 days preceding the first dose of study vaccine, or planned use during the

study period.

- Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs within six months prior to the first vaccine dose. Inhaled and topical steroids were allowed.
- Planned administration of a vaccine not foreseen by the study protocol within 30 days before and 30 days after (i.e. days 0-29) each dose of vaccine.
- History of having had colposcopy or has planned a colposcopy to evaluate an abnormal cervical cytology (Pap smear) test.
- Received immunoglobulins and/or blood product within 90 days preceding enrollment. Enrollment will be deferred until the subject is outside of specified window.

Study cohorts/data sets analyzed

At the interim analysis analysis of efficacy is performed on the Total Vaccinated cohort for efficacy 1 (primary analysis) and on the Total Vaccinated cohort for efficacy 2.
Analysis of safety is performed on the Total Vaccinated cohort (primary analysis) and on the ATP cohort for safety.
Analysis of immunogenicity is performed on the ATP cohort for immunogenicity (primary analysis) and on the Total Vaccinated cohort.

Total Vaccinated cohort for efficacy 1 (TVC-1)
The Total Vaccinated cohort for efficacy 1 (TVC-1) includes all vaccinated subjects (at least one dose) for whom data concerning efficacy endpoint measures are available and who were HPV DNA negative and seronegative to the relevant HPV type (HPV-16 or HPV-18) at study entry. Women with high-grade or missing cytology (0.5%) were excluded from the efficacy analysis.
For this cohort, the follow-up time for a subject started at the day after Dose 1.

Total Vaccinated cohort for efficacy 2 (TVC-2)
A pre-specified analysis, Total Vaccinated cohort for efficacy 2 (TVC-2) was identical to the TVC-1 analysis except that it excluded women with abnormal cytology at study entry.
In addition, subjects must be negative for HPV DNA (by PCR) at Month 0 for the corresponding HPV type considered in the analysis (i.e. HPV type associated with the efficacy endpoint). For this cohort, the follow-up time for a subject started at the day after Dose 1. For all stratified efficacy endpoints, the principal analysis is performed on subjects who were seronegative (by ELISA) prior to vaccination for the corresponding HPV type considered in the analysis.
This analysis complements the analysis on TVC-1 and is performed at the interim analysis on primary and secondary endpoints that are associated with HPV-16 and/or HPV-18 only.

According-to-protocol (ATP) cohort for analysis of efficacy
The ATP cohort for analysis of efficacy includes all evaluable subjects (i.e. those meeting all eligibility criteria, complying with the procedures defined in the protocol, with no elimination violations) for whom data concerning efficacy endpoint measures are available and have a normal or low-grade cytology (i.e. negative or ASCUS or LSIL) at Month 0. In addition, subjects must be negative for HPV DNA (by PCR) at Months 0 and 6 for the corresponding HPV type considered in the analysis (i.e. HPV type associated with the efficacy endpoint). For this cohort, the follow-up time for a subject started at the day after Dose 3. For all stratified efficacy endpoints, the principal analysis is performed on subjects who were seronegative (by ELISA) prior to vaccination for the corresponding HPV type considered in the analysis. At the final analysis, the ATP cohort for efficacy will be the primary cohort for all endpoints, except for endpoints evaluated in HPV DNA positive woman at Month 0.

According-to-protocol (ATP) cohort for analysis of immunogenicity
The ATP cohort for analysis of immunogenicity includes all evaluable subjects (i.e. those meeting all eligibility criteria, complying with the procedures defined in the protocol, with no elimination criteria during the study) for whom data concerning immunogenicity endpoint measures are available. This includes subjects for whom assay results are available for antibodies against at least one study vaccine antigen component after vaccination. Subjects who acquire either HPV-16 or HPV-18 infection during the trial are excluded from the ATP cohort for immunogenicity.

Treatments

The candidate HPV vaccine and hepatitis A control vaccine were administered (0.5 ml dose) intramuscularly into the deltoid of the non-dominant arm according to a 0, 1, 6-month schedule. For the immunogenicity subset blood samples were drawn from all subjects at Months 0, 7 and 24 for HPV-16/18 serology testing by ELISA. Exit colposcopy was performed within 30 days after cytology results of the Month 48 visit depending on the cytological results.

Objectives

The primary objective of study HPV-008 was to demonstrate, that Cervarix protects individuals from histopathologically confirmed CIN2+ associated with HPV-16 or HPV-18 cervical infection detected within the lesional component of the cervical tissue specimen (by PCR), overall and stratified according to initial (Month 0) HPV-16 and/or 18 serostatus (by ELISA). CIN2+ is defined as CIN2, CIN3, adenocarcinoma in-situ (AIS) and invasive cervical cancer.

Secondary objectives included the prevention of CIN 1, protection against persistent infection with HPV-16 and/or HPV-18 and the immunological response to vaccination.

Outcomes/endpoints

The primary endpoint for the interim analysis was protection against CIN2+ lesions associated with HPV-16 and/or HPV-18 among subjects who are negative for HPV DNA (by PCR) at baseline for the corresponding HPV type.

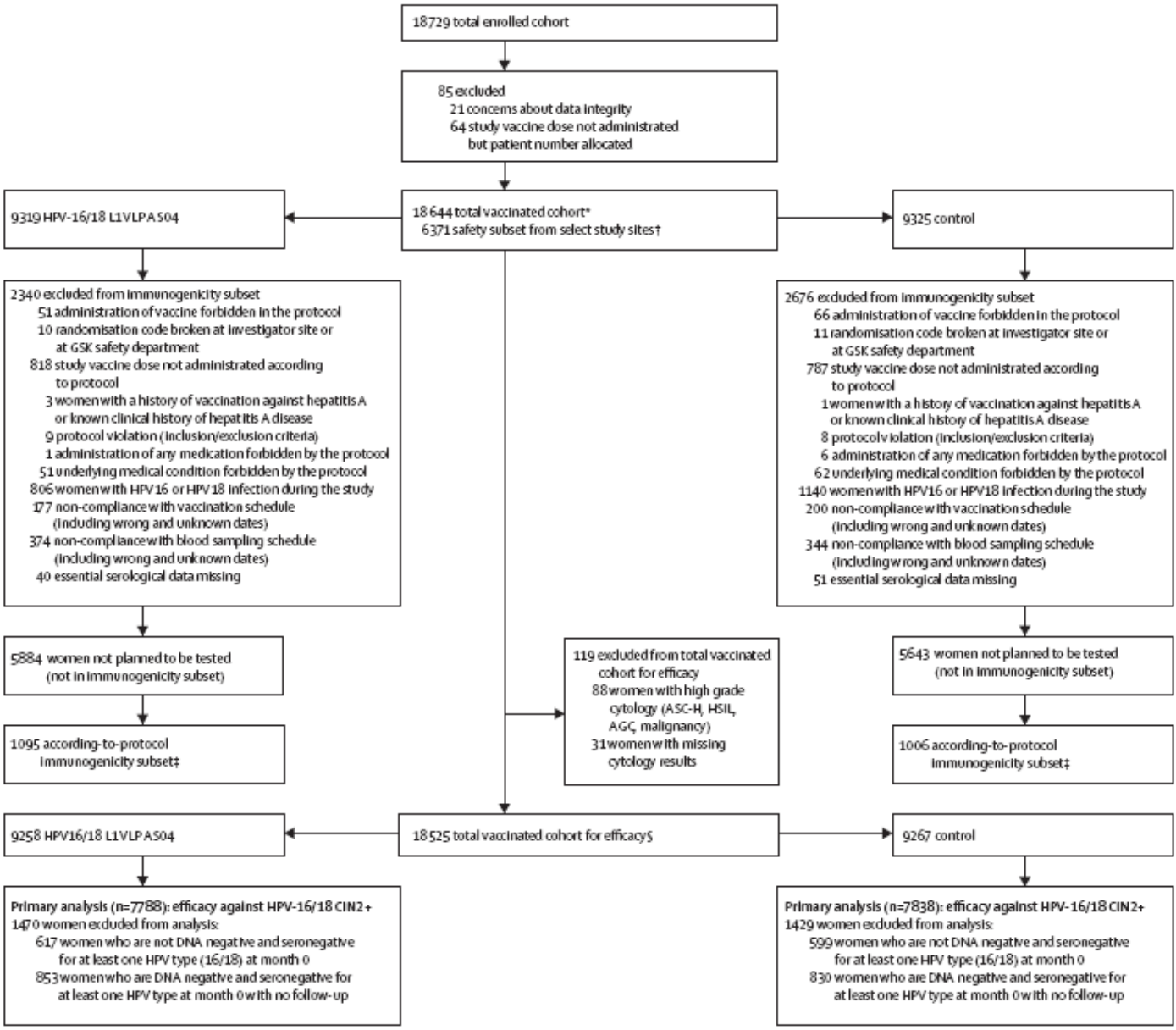
Secondary Endpoints included
- Protection against persistent infection (6-month and 12-month definition) with HPV-16 or HPV-18 among subjects who are negative for HPV DNA at baseline for the corresponding HPV type.
- Protection against persistent infection (6-month definition) with the following oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.
- Protection against CIN2+ and CIN1+ associated with HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.
- Immune response in a subset of subjects from selected study sites and immune correlates of protection against persistent infection (presented under PD/Immunogenicity).

Statistical methods

Incidence rates and vaccine efficacy against CIN2+ associated with HPV-16 and/or HPV-18 (by PCR) in HPV DNA negative and seronegative subjects at baseline have been calculated using the conditional exact method.

RESULTS

Participant flow/ Numbers analysed



18644 subjects were vaccinated and included in the study, of whom 9319 subjects received the HPV vaccine (vaccine group) and 9325 subjects received the hepatitis A vaccine (control group).
Of the 18644 vaccinated subjects in the study, 954 subjects withdrew from the study at the time of the interim analysis: 496 subjects in the vaccine group and 458 subjects in the control group (see table). The majority of these subjects (46.8%) were lost to follow-up (446 subjects), withdrew their consent not due to an adverse event (279 subjects), or migrated or moved from the study area (113 subjects). Eight subjects withdrew due to a SAE (3 subjects in the vaccine group and 5 subjects in the control group). Nine subjects withdrew due to a non-serious AE (6 subjects in the vaccine group and 3 subjects in the control group).

Recruitment

The study initiation date was 06 May 2004. An event-triggered interim analysis was planned when at least 23 cases of CIN2+ with HPV-16 or HPV-18 DNA were detected in TVC-1. At the time of the interim analysis, the majority of subjects (N=13046) had completed their Month 18 visit. The interim analysis has been performed on 03 November 2006.

Conduct of the study

Because potential study conduct and data integrity issues were identified at one study site, all 21 subjects from this site were excluded from the analyses. Additionally, 64 subjects were randomized but not vaccinated. There were four amendments to the study protocol dated 12 March 2004.

Baseline data

The demographic profiles of both groups of subjects were comparable with respect to mean age, regional distribution, racial distribution, mean height and weight. The mean age was 20 years and the population was predominantly of Caucasian or East & South East Asian origin (54.8% and 23.3%, respectively).
Overall, 29.8% of the subjects were identified as smokers, 92.2% of the subjects had less than three sexual partners during the past year, and 94.5% of the subjects were negative for Chlamydia. Regarding the HPV-16 status, 80.9% of the subjects were DNA negative and seronegative, 13.9% of the subjects were DNA negative and seropositive, 2.5% of the subjects were DNA positive and seronegative, and 2.8% of the subjects were DNA positive and seropositive. Regarding the HPV-18 status, 87.2% of the subjects were DNA negative and seronegative, 10.5% of the subjects were DNA negative and seropositive, 1.3% of the subjects were DNA positive and seronegative, and 1% of the subjects were DNA positive and seropositive.

Outcomes and estimation

In study 008 the primary analyses of efficacy included only women who were HPV DNA negative and seronegative to the relevant HPV type (HPV 16 or HPV 18) prior to study entry and had received at least one dose of Cervarix or the control. Women with high-grade or missing cytology (0.6%) were excluded from the efficacy analysis.

Overall, 74.0% of women enrolled were naïve to both HPV16 and HPV18 at study entry. A total of 22% of women enrolled into study 008 had abnormal low grade cytology and/or evidence of infection with an oncogenic HPV type at baseline.

Histopathology

Protection against CIN2+ associated with HPV-16/18 (primary endpoint)

In the protocol-specified primary endpoint interim analysis, the observed vaccine efficacy (VE) against CIN2+ associated with HPV-16 and/or HPV-18 was statistically significant (VE = 90.4% [53.4%, 99.3%], p<0.0001), i.e. 2 cases in the vaccine group vs. 21 cases in the control group. The observed vaccine efficacy against CIN2+ associated with HPV-16 alone was 93.3% [47%, 99.9%], i.e. 1 case in the vaccine group vs. 15 cases in the control group. The observed vaccine efficacy against CIN2+ associated with HPV-18 alone was not statistically significant 83.3% [-78.8%, 99.9%] due to a limited number of events at the time of the analysis, i.e. 1 case in the vaccine group vs. 6 cases in the control group.

Incidence rates and vaccine efficacy against CIN2+ associated with HPV-16 and/or HPV-18 (by PCR) in HPV DNA negative and seronegative subjects at baseline (primary endpoint)

					Person-year rate			VE			
Event Type	**Group**	**N**	**n**	**T(year)**	**n/T (Per 100)**	**LL**	**UL**	**%**	**LL**	**UL**	**P-value**
HPV-16/18*	HPV	7788	2	9613.75	0.02	0.00	0.09	90.4	53.4	99.3	<0.0001
	HAV	7838	21	9682.00	0.22	0.12	0.35	-	-	-	-
HPV-16	HPV	6701	1	8279.75	0.01	0.00	0.08	93.3	47.0	99.9	0.0005
	HAV	6717	15	8284.32	0.18	0.09	0.32	-	-	-	-

					Person-year rate			VE			
Event Type	**Group**	**N**	**n**	**T(year)**	**n/T (Per 100)**	**LL**	**UL**	**%**	**LL**	**UL**	**P-value**
HPV-18	HPV	7221	1	8903.55	0.01	0.00	0.07	83.3	-78.8	99.9	0.1249
	HAV	7258	6	8947.82	0.07	0.02	0.16	-	-	-	-

* Protocol-specified endpoint
HPV = HPV-16/18 L1 VLP/AS04 vaccine (three lots) = Vaccine group
HAV = Hepatitis A vaccine (three lots) = Control group
N=number of subjects included in each group
For single type: Subjects DNA negative and seronegative for the corresponding HPV type at Month 0
For combined types: Subjects DNA negative and seronegative for at least one HPV type at Month 0 (subjects are in the analysis of at least one single type)
n=number of subjects reporting at least one event in each group
Subjects with an event are DNA negative and seronegative for the corresponding HPV type at Month 0
T(years)=sum of follow-up period (censored at the first occurrence of an event) expressed in years in each group
Follow-up period starts at day after Dose 1
n/T=Incidence rate of subjects reporting at least one event
VE(%)=Vaccine Efficacy (conditional exact method)
LL,UL=97.9% Lower and Upper confidence limits
P-value=Two-sided Fisher Exact test

The regional distribution of the 23 cases of CIN2+ associated with HPV-16 and/or HPV-18 was as follows: 7 cases in Asia Pacific (N=5368), 10 cases in Europe (N=5934), 1 case in Latin America (N=2164) and 5 cases in North America (N=2160).

For this analysis, the association with HPV-16 and/or HPV-18 was based only on the detection of viral DNA by PCR in the biopsy sample and did not consider whether or not the HPV-16 and/or HPV-18 detected was likely to be responsible for the development of the lesion. Other oncogenic HPV types were frequently detected in lesions and in some cases subjects had longstanding infections with the other type(s) detected.

Therefore an additional *post-hoc analysis* was conducted to evaluate vaccine efficacy against lesions likely to be causally associated with HPV-16 and /or HPV-18. This post-hoc analysis (clinical case assignment) assigned causal association of an HPV type with the lesion based on the presence of the HPV type in cytology samples prior to detection of the lesion. Based on this case assignment, the analysis excluded 3 CIN2+ cases (2 in the vaccine group and 1 in the control group) which were not considered to be causally associated with HPV-16 or HPV-18 infections acquired during the trial.

The following table therefore presents results of the clinical case assignment analysis. The observed vaccine efficacy against CIN2+ associated with HPV-16 and/or HPV-18 was 100% [74.2%, 100%], i.e. 0 cases in the vaccine group vs. 20 cases in the control group. The observed efficacy against CIN2+ associated with HPV-16 alone was 100% [64.5%, 100%], i.e. 0 cases in the vaccine group vs. 15 cases in the control group. The observed efficacy against CIN2+ associated with HPV-18 alone was 100% [-49.5%, 100%], i.e. 0 cases in the vaccine group vs. 5 cases in the control group. Since the majority of CIN2+ cases in the control group resulted from infections, which were first detected prior to completion of the full three-dose schedule, the absence of cases in the vaccine group reflects the onset of a vaccine effect prior to completion of the full vaccination course.

Post-hoc analysis: Incidence rates and vaccine efficacy against CIN2+ associated with HPV-16 and/or HPV-18 (by PCR) in HPV DNA negative and seronegative subjects at baseline using conditional exact method (TVC-1/Clinical Case Assignment)

					Person-year rate			VE			
Event Type	**Group**	**N**	**n**	**T(year)**	**n/T (Per 100)**	**LL**	**UL**	**%**	**LL**	**UL**	**P-value**
HPV-16/18	HPV	7788	0	9614.95	0.00	0.00	0.05	100.0	74.2	100.0	<0.0001
	HAV	7838	20	9682.45	0.21	0.12	0.34	-	-	-	-
HPV-16	HPV	6701	0	8280.64	0.00	0.00	0.06	100.0	64.5	100.0	<0.0001
	HAV	6717	15	8284.32	0.18	0.09	0.32	-	-	-	-
HPV-18	HPV	7221	0	8903.86	0.00	0.00	0.05	100.0	-49.5	100.0	0.0625
	HAV	7258	5	8948.26	0.06	0.01	0.15	-	-	-	-

HPV = HPV-16/18 L1 VLP/AS04 vaccine (three lots) = Vaccine group
HAV = Hepatitis A vaccine (three lots) = Control group
N=number of subjects included in each group
For single type: Subjects DNA negative and seronegative for the corresponding HPV type at Month 0
For combined types: Subjects DNA negative and seronegative for at least one HPV type at Month 0 (subjects are in the analysis of at least one single type)
n=number of subjects reporting at least one event in each group
Subjects with an event are DNA negative and seronegative for the corresponding HPV type at Month 0
Additional analysis: The lesion is assigned to an HPV type found in the lesion if
a) the same HPV type is found in at least one of the two immediately preceding cytology samples, or
b) none of the HPV types found in the lesion are found in any of the two preceding cytology samples
T(years)=sum of follow-up period (censored at the first occurrence of an event) expressed in years in each group
Follow-up period starts at day after Dose 1
n/T=Incidence rate of subjects reporting at least one event
VE(%)=Vaccine Efficacy (conditional exact method)
LL,UL=97.9% Lower and Upper confidence limits
P-value=Two-sided Fisher Exact test

<u>Protection against cervical intraepithelial neoplasia (CIN) 1+ associated with HPV-16/18 (secondary endpoint)</u>

The following table shows the incidence rates and vaccine efficacy against CIN1+ associated with HPV-16 and/or HPV-18 in HPV DNA negative and seronegative subjects at baseline. It should be noted that for this analysis, the association with HPV-16 and/or HPV-18 was based on the detection of viral DNA by PCR in the biopsy sample and did not consider the presence of other oncogenic type(s) in the biopsy sample or the detection of HPV type(s) in the cervical samples prior to collection of the biopsy.

Incidence rates and vaccine efficacy against CIN1+ associated with HPV-16 and/or HPV-18 in HPV DNA negative and seronegative subjects at baseline

					Person-year rate			VE			
Event Type	**Group**	**N**	**n**	**T(year)**	**n/T (Per 100)**	**LL**	**UL**	**%**	**LL**	**UL**	**P-value**
HPV-16/18*	HPV	7788	3	9613.42	0.03	0.00	0.10	89.2	59.4	98.5	<0.0001
	HAV	7838	28	9681.19	0.29	0.18	0.44	-	-	-	-
HPV-16	HPV	6701	2	8279.42	0.02	0.00	0.10	88.9	44.6	99.2	0.0004
	HAV	6717	18	8283.71	0.22	0.12	0.37	-	-	-	-
HPV-18	HPV	7221	1	8903.55	0.01	0.00	0.07	90.9	22.1	99.9	0.0063
	HAV	7258	11	8947.48	0.12	0.05	0.24	-	-	-	-

* Protocol-specified endpoint
HPV = HPV-16/18 L1 VLP/AS04 vaccine (three lots) = Vaccine group
HAV = Hepatitis A vaccine (three lots) = Control group
N=number of subjects included in each group
For single type: Subjects DNA negative and seronegative for the corresponding HPV type at Month 0

For combined types: Subjects DNA negative and seronegative for at least one HPV type at Month 0 (subjects are in the analysis of at least one single type)
n=number of subjects reporting at least one event in each group
Subjects with an event are DNA negative and seronegative for the corresponding HPV type at Month 0
T(years)=sum of follow-up period (censored at the first occurrence of an event) expressed in years in each group
Follow-up period starts at day after Dose 1
n/T=Incidence rate of subjects reporting at least one event
VE(%)=Vaccine Efficacy (conditional exact method)
LL,UL=97.9% Lower and Upper confidence limits
P-value=Two-sided Fisher Exact test

Efficacy of Cervarix against CIN 1 associated with HPV16 and/or HPV 18 related CIN1 was 89.2% (97.9% CI: 59.4; 98.5). Statistically significance was reached for both HPV 16 and HPV 18 when analysed separately.

Virology

Protection against persistent infection (12-month definition) associated with HPV-16/18 (secondary endpoint) in HPV DNA negative and seronegative subjects at baseline.

The following table shows the incidence rates and vaccine efficacy against persistent infection (12-month definition) with HPV-16 and/or HPV-18 in HPV DNA negative and seronegative subjects at baseline.

					Person-year rate			**VE**			
Event Type	**Group**	**N**	**n**	**T(year)**	**n/T (Per 100)**	**LL**	**UL**	**%**	**LL**	**UL**	**P-value**
HPV-16/18	HPV	3386	11	5062.64	0.22	0.09	0.42	75.9	47.7	90.2	<0.0001
	HAV	3437	46	5104.85	0.90	0.62	1.26	-	-	-	-
HPV-16	HPV	2945	7	4407.73	0.16	0.05	0.36	79.9	48.3	93.8	<0.0001
	HAV	2972	35	4422.78	0.79	0.52	1.16	-	-	-	-
HPV-18	HPV	3143	4	4711.21	0.08	0.02	0.24	66.2	-32.6	94.0	0.0766
	HAV	3190	12	4774.48	0.25	0.11	0.48	-	-	-	-

HPV = HPV-16/18 L1 VLP/AS04 vaccine (three lots) = Vaccine group
HAV = Hepatitis A vaccine (three lots) = Control group
N=number of subjects included in each group
Subjects have at least 10 months of follow-up after Month 6
For single type: Subjects DNA negative and seronegative for the corresponding HPV type at Month 0
For combined types: Subjects DNA negative and seronegative for at least one HPV type at Month 0 (subjects are in the analysis of at least one single type)
n=number of subjects reporting at least one event in each group
Subjects with an event are DNA negative and seronegative for the corresponding HPV type at Month 0
T(years)=sum of follow-up period (censored at the first occurrence of an event) expressed in years in each group
Follow-up period starts at day after Dose 1
n/T=Incidence rate of subjects reporting at least one event
VE(%)=Vaccine Efficacy (conditional exact method)
LL,UL=97.9% Lower and Upper confidence limits
P-value=Two-sided Fisher Exact test

For HPV-16 protection against persistent infection (12-month definition) in HPV DNA negative and seronegative subjects at baseline reached statistical significance, whereas for HPV-18, the difference between the vaccine and control groups was not statistically significant

However, in a pre-specified analysis (TVC-2) who's cohort excluded women with abnormal cytology at study entry, the 12 month persistent infection endpoint for HPV 18 reached statistical significance with vaccine efficacy of 89.9% (97.9% CI: 11.3; 99.9). One case was observed in the vaccine group versus 10 cases in the control group.

Protection against persistent infection (6-month definition) associated with HPV-16/18 in HPV DNA negative and seronegative subjects at baseline.

The following table shows the incidence rates and vaccine efficacy against persistent infection (6-month definition) with HPV-16 and/or HPV-18 in HPV DNA negative and seronegative subjects at baseline.

					Person-year rate			VE			
Event Type	**Group**	**N**	**n**	**T(year)**	**n/T (Per 100)**	**LL**	**UL**	**%**	**LL**	**UL**	**P-value**
HPV-16/18	HPV	6344	38	8149.58	0.47	0.31	0.67	80.4	70.4	87.4	<0.0001
	HAV	6402	193	8129.85	2.37	2.00	2.80	-	-	-	-
HPV-16	HPV	5493	23	7076.92	0.33	0.19	0.52	84.1	73.5	91.1	<0.0001
	HAV	5520	144	7035.66	2.05	1.67	2.48	-	-	-	-
HPV-18	HPV	5896	15	7590.88	0.20	0.10	0.35	74.0	49.1	87.8	<0.0001
	HAV	5939	58	7635.60	0.76	0.55	1.02	-	-	-	-

HPV = HPV-16/18 L1 VLP/AS04 vaccine (three lots) = Vaccine group
HAV = Hepatitis A vaccine (three lots) = Control group
N=number of subjects included in each group
Subjects have at least 5 months of follow-up after Month 6
For single type: Subjects DNA negative and seronegative for the corresponding HPV type at Month 0
For combined types: Subjects DNA negative and seronegative for at least one HPV type at Month 0 (subjects are in the analysis of at least one single type)
n=number of subjects reporting at least one event in each group
Subjects with an event are DNA negative and seronegative for the corresponding HPV type at Month 0
T(years)=sum of follow-up period (censored at the first occurrence of an event) expressed in years in each group
Follow-up period starts at day after Dose 1
n/T=Incidence rate of subjects reporting at least one event
VE(%)=Vaccine Efficacy (conditional exact method)
LL,UL=97.9% Lower and Upper confidence limits
P-value=Two-sided Fisher Exact test

Study HPV-008 was designed to evaluate vaccine efficacy against 6 Month HPV-16/18 persistent infection as a secondary endpoint.
Despite the presented results in the table above 6-month protection against persistence HPV 16 / 18 infection is not accepted as a pre-defined endpoint and is therefore not considered in the assessment of efficacy.

Protection against persistent infection (6-month definition) associated with HPV-16/18 regardless of their initial serostatus at baseline.

The vaccine efficacy against persistent infection (6-month definition) with HPV-16 alone in HPV DNA negative subjects at baseline, regardless of their initial serostatus, was 81.1%; 95% CI; 0.5%, 88.4%]. The efficacy against persistent infection with HPV-18 alone was 72.6% (95% CI; 49.3%, 86.3%).

Furthermore statistically significant protection against persistent infection (6-month) associated with oncogenic HPV types in HPV DNA negative subjects at baseline was observed for HPV-31, HPV-45 and HPV-52 (VE = 36.1% (95% CI, 0.5%, 59.5%), 59.9% (95% CI, 2.6%, 85.2%) and 31.6% (95% CI, 3.5%, 51.9%), respectively). However, VE against 12-month persistent infection and CIN2+ did not reach statistical significance. There is currently no firm evidence for cross-protection against non-vaccine HPV types.

Ancillary analyses

- Clinical studies in special populations

Currently no data in the male population has been provided.

Supportive studies

HPV-001 and HPV-007

Study HPV-001 was a double-blind, randomised, controlled study conducted in 32 centres in the US, Canada and Brazil. The primary objective of Study HPV-001 was to evaluate vaccine efficacy against HPV-16 and/or HPV-18 incident infections which has not been an accepted endpoint for the demonstration of efficacy in the claimed indication. Main secondary objectives of the study were to evaluate vaccine efficacy against persistent infections, cytological abnormalities and histopathological lesions associated with HPV-16 and/or HPV-18 or associated with other high-risk HPV types. The study also evaluated the safety and immunogenicity of the vaccine. Study HPV-001 is completed.

Study HPV-007 is the extension of study HPV-001 and evaluates the long-term efficacy of the HPV-16/18 vaccine in a subset of subjects (70 %) vaccinated in Study HPV-001 for an additional 36 months. The maximum follow-up period in Study HPV-001 was 27 months. For all subjects, there was an interval between completion of Study HPV-001 and their first visit in Study HPV-007 (mean: 14 months). In total, from the time of first vaccination in Study HPV-001 until their Month 24 visit in Study HPV-007, subjects have been evaluated for up to 64 months (i.e., 58 months after the third dose), with a mean follow-up period of 60 months (i.e., 54 months after the third dose). Study HPV-007 is ongoing and interim data (a 24 months interim analysis, mean follow-up of 60 months after dose 1 administered in Study HPV-001) are presented.
The design of Study HPV-007 closely follows that of Study HPV-001 to ensure consistency in collection of information on efficacy of the vaccine.

Results

Prophylactic efficacy against HPV-16/18 in a population naïve to oncogenic HPV types

Women were vaccinated in study 001 and evaluated for efficacy up to month 27 and then **a subset (**N=776) **was** followed in study 007 up to 5.5 years after the first dose. The primary efficacy endpoint was incident infection with HPV-16 and/or HPV-18. Twelve-month persistent infection was evaluated as additional efficacy endpoint. There were five cases of 12-month persistent HPV-16/18 infection (4 HPV-16; 1 HPV-18) in the control group and one HPV-16 case in the vaccine group in study 001. In study 007 the efficacy of Cervarix against 12-month persistent HPV-16/18 infection was 100% (95% CI: 66.5; 100). There were ten cases of persistent HPV-16 infection, and four cases of persistent HPV-18 infection, all in the control group.

- Discussion on clinical efficacy

The efficacy of Cervarix was assessed in two controlled, double-blind, randomised Phase II and III clinical trials that included a total of 19,778 women aged 15 to 25 years.

The **phase III trial** (HPV-008) enrolled women regardless of baseline cytology and HPV serological and DNA status. The primary efficacy endpoint was CIN2+ associated with HPV-16 and/or HPV-18. Cervical Intraepithelial Neoplasia (CIN) grade 2 and 3 was used in the clinical trials as a surrogate marker for cervical cancer. The secondary endpoints included 12-month persistent infection.
The primary analyses of efficacy (TVC-1) included only women who were HPV DNA negative and seronegative to the relevant HPV type (HPV-16 or HPV-18) at study entry and had received at least one dose of Cervarix or the control (74.0% of women enrolled). An interim analysis with mean

follow-up 15 months was provided, however, the vaccine efficacy (ATP cohort post-dose 3) has not been evaluated in the primary efficacy population.

For HPV-16 all endpoints reached statistical significance. For HPV-18, the difference between the vaccine and control groups was not statistically significant for CIN2+ and 12 month persistent infection, due to a limited number of events at the time of the analysis. However, in a pre-specified analysis (TVC-2) that excluded women with abnormal cytology at study entry, the 12 month persistent infection endpoint for HPV-18 reached statistical significance with vaccine efficacy of 89.9% (97.9% CI: 11.3; 99.9).

An additional **post-hoc** analysis was conducted to determine vaccine efficacy against lesions likely to be causally associated with HPV-16 and/or HPV-18. This post-hoc analysis (clinical case assignment) assigned causal association of an HPV type with the lesion based on the presence of the HPV type in cytology samples prior to detection of the lesion. Based on this case assignment, the analysis excluded 3 CIN2+ cases (2 in the vaccine group and 1 in the control group) which were not considered to be causally associated with HPV-16 or HPV-18 infections acquired during the trial. Based on this analysis there were no cases in the vaccine group and 20 cases in the control group (Efficacy 100%; 97.9% CI: 74.2, 100).

The **phase II trial** (HPV-001/007) enrolled only women who were tested negative for 14 oncogenic HPV DNA types, seronegative for HPV-16 and HPV-18 and had normal cytology. The primary efficacy endpoint was incident infection with HPV-16 and/or HPV-18.
Women were vaccinated and evaluated for efficacy up to month 27 and then followed in study 007 up to 5.5 years. There were five cases of 12-month persistent HPV-16/18 infection (4 HPV-16; 1 HPV-18) in the control group and one HPV-16 case in the vaccine group. The efficacy of Cervarix against 12-month persistent HPV-16/18 infection was 100% (95% CI: 66.5; 100).

There was no evidence of protection from disease caused by the HPV types for which subjects were HPV DNA positive at study entry. However, individuals already infected with one of the vaccine-related HPV types prior to vaccination were protected from clinical disease caused by the remaining HPV type.

The **immunogenicity** induced by three doses of Cervarix has been evaluated in 5,303 female subjects from 10 to 55 years of age. No minimal antibody level associated with protection against CIN of grade 2 or 3 or against persistent infection associated with vaccine HPV types has been identified for HPV vaccines.
In clinical trials, 99.9% of initially seronegative subjects had seroconverted to both HPV types 16 and 18 one month after the third dose. Vaccine-induced IgG Geometric Mean Titres were well above titres observed in women previously infected but who cleared HPV infection. Initially seropositive and seronegative subjects reached similar titres after vaccination.
The **phase II study** (HPV-001/007) evaluated the immune response against HPV-16 and HPV-18 up to 64 months post dose 1. Vaccine-induced IgG Geometric Mean Titres for both HPV-16 and HPV-18 peaked at month 7 and then declined to reach a plateau from month 18 up to the end of the follow-up (month 64). At the end of the follow-up period, GMTs for both HPV-16 and HPV-18 were still at least 11-fold higher than titres observed in women previously infected but who cleared HPV infection and >98% of the women were still seropositive for both antigens.

In subjects above 25 years of age in **study 014** all subjects seroconverted to both HPV types 16 and 18 after the third dose (at month 7). The GMTs were, however, lower in women above 25 years. Nevertheless, all subjects remained seropositive for both types throughout the follow-up phase (up to month 18) with GMTs that were still at least 9 fold higher than in subjects from a control group who had cleared an HPV infection (seropositive and PCR negative).

In two clinical trials performed in girls and adolescents aged **10 to 14 years**, all subjects seroconverted to both HPV types 16 and 18 after the third dose with GMTs at least 2-fold higher as compared to women aged 15 to 25 years. On the basis of these immunogenicity data, the efficacy of Cervarix is inferred from 10 to 14 years of age.

Clinical safety

- Patient exposure

The safety information presented here is derived from the studies that were included in the pooled analysis of safety (HPV-003, 004, 005, 001, 007, 008, 012, 013, 014, 015 and 016 studies) as summarised in the following. The current pre-licensure safety database included females only.
Of the 29,953 healthy girls and women included in the overall clinical development program of the HPV-16/18 vaccine candidate, 16,142 subjects have received at least one dose of HPV 16-18 vaccine and 13,811 subjects have received one of the control vaccines (Havrix 720, Havrix 360 or Al(OH)3.

Pooled safety analysis: number and percentage of subjects who received study vaccine doses by group (Total vaccinated cohort)

	HPV N = 16142		ALU N = 3454		HAV360 N = 1032		HAV720 N = 9325	
Total number of doses received	n	%	n	%	n	%	n	%
1	436	2.7	81	2.3	9	0.9	266	2.9
2	1566	9.7	983	28.5	10	1.0	487	5.2
3	14140	87.6*	2390	69.2*	1013	98.2	8572	91.9
Any	16142	100	3454	100	1032	100	9325	100

HPV = HPV-16/18 vaccine group (Studies HPV-001, 003, 004, 005, 008, 012, 013, 014, 015 and 016)
ALU = Al(OH)3 control group (Studies HPV-001, 003 and 015)
HAV360 = Hepatitis A control group containing 360 EU hepatitis A antigen per dose (Study HPV-013)
HAV720 = Hepatitis A control group containing 720 EU hepatitis A antigen per dose (Study HPV-008)
N = number of subjects in each group included in the considered cohort
n/% = number/percentage of subjects receiving the specified total number of doses
Any = number and percentage of subjects receiving at least one dose
Data source = Pooled safety analysis
* Percentages are low, because vaccination in HPV-015 is ongoing.

Subjects included in various clinical trials covered a broad age range, starting from 10 years of age onwards. The studies were conducted in Europe, Asia Pacific, North and Latin America and consequently included a range of ethnicities, including subjects of Caucasian, Hispanic, Black and Asian origin.

All women and girls enrolled in the trials were to be free of obvious health problems based on medical history and clinical examination and had to have a negative urine pregnancy test prior to administration of each vaccine dose.

Pooled safety analysis: summary of demographic characteristics (Overall, Total Vaccinated Cohort)

		HPV N = 16142		ALU N = 3454		HAV360 N = 1032		HAV720 N = 9325		Total N = 29953	
Characteristics	Parameters or Categories	Value or n	%	Value or n	%	Value or n	%	Value or n	%	Value or n	%
Age [years]	N	16142	-	3454	-	1032	-	9325	-	29953	-
	Mean	23.2	-	34.2	-	12.1	-	20.0	-	23.1	-
	SD	8.8	-	9.1	-	1.4	-	3.1	-	8.7	-
	Median	21.0	-	34.0	-	12.0	-	20.0	-	21.0	-
	Minimum	10.0	-	15.0	-	10.0	-	15.0	-	10.0	-
	Maximum	72.0	-	68.0	-	14.0	-	33.0	-	72.0	-
Race	Black	488	3.0	117	3.4	10	1.0	358	3.8	973	3.2
	White/Caucasian	9594	59.4	1623	47.0	565	54.7	5117	54.9	16899	56.4
	Asian	3882	24.0	758	21.9	142	13.8	2957	31.7	7739	25.8
	Hispanic	1822	11.3	850	24.6	313	30.3	666	7.1	3651	12.2
	Other	356	2.2	106	3.1	2	0.2	227	2.4	691	2.3

HPV = HPV-16/18 vaccine group (Studies HPV-001, 003, 004, 005, 008, 012, 013, 014, 015 and 016)
ALU = Al(OH)3 control group (Studies HPV-001, 003 and 015)
HAV360 = Hepatitis A control group containing 360 EU hepatitis A antigen per dose (Study HPV-013)
HAV720 = Hepatitis A control group containing 720 EU hepatitis A antigen per dose (Study HPV-008)
N = number of subjects
n = number of subjects in a given category
Value = value of the considered parameter
% = n / Number of subjects with available results x 100
Data source = Pooled safety analysis

The current safety database allows for the detection of rare adverse events and vaccine-related rare events compared with the reporting of events in the control groups. Studies that were pooled had similar study designs with respect to vaccination schedule and methods of determination and capture of adverse events. Long-term follow-up data is available from studies HPV-001/007 (5.5 years), HPV-008 (approximately Month 24 for some subjects), HPV-013 (Month 18) and HPV-014 (Month 18).

- Adverse events

The following endpoints were evaluated in clinical studies:
Solicited symptoms: Occurrence of solicited local (injection site pain, redness and swelling) or general symptom (fatigue, fever, gastrointestinal symptoms, including nausea, vomiting, diarrhoea and/or abdominal pain), headache, rash, pruritus, myalgia, arthralgia and urticaria, (within 7 days (Day 0-6) after each vaccination)

  - Unsolicited symptoms: Occurrence of unsolicited symptoms within 30 days (Day 0-29) following each vaccination.
  - Serious Adverse Events (SAEs): Occurrence of SAEs following vaccination.
  - New Onset of Chronic Disease (NOCD)/ New Onset of Autoimmune Disease (NOAD)

- Medically Significant Conditions: Occurrence of medically significant conditions (MSCs) throughout the study period, regardless of causal relationship to vaccination and intensity.

- Pregnancies: Occurrence of pregnancies and pregnancy outcomes.

The following Adverse Reactions are reflected in the SPC (section 4.8)
Adverse reactions considered as being at least possibly related to vaccination have been categorised by frequency. Frequencies are reported as very common (≥1/10), common (≥1/100 to <1/10) and uncommon (≥1/1,000 to <1/100).

The most common adverse reaction observed after vaccine administration was injection site pain which occurred after 78% of all doses. The majority of these reactions were of mild to moderate severity and were not long lasting.

Nervous system disorders:
Very common: headache
Uncommon: dizziness

Gastrointestinal disorders:
Common: gastrointestinal symptoms including nausea, vomiting, diarrhoea and abdominal pain

Skin and subcutaneous tissue disorders:
Common: itching/pruritus, rash, urticaria

Musculoskeletal and connective tissue disorders:
Very common: myalgia
Common: arthralgia

Infections and infestations:
Uncommon: upper respiratory tract infection

General disorders and administration site conditions:
Very common: injection site reactions including pain, redness, swelling; fatigue
Common: fever (≥38°C)
Uncommon: other injection site reactions such as induration, local paraesthesia

A similar safety profile has been observed in subjects with prior or current HPV infection as compared to subjects negative for oncogenic HPV DNA or seronegative for HPV-16 and HPV-18 antibodies.

Assessment of the Safety data set:

The overall incidences for each of the three local symptoms were higher in the HPV group compared to the control groups. The most frequently reported solicited local symptoms reported was pain (reported following 78.0% of HPV, 52.5% of ALU, 41.3% of HAV360 and 58.9% of HAV720 doses). Most of the pain was mild to moderate in intensity, and grade 3 pain was reported following 6.3% of HPV doses. There was a slight increase in the incidence of redness and swelling with subsequent doses. Grade 3 redness and swelling occurred at low frequencies.

Reporting rates of fever and particularly urticaria within 30 minutes after vaccination were consistently low whereas reporting rates of rash or itching were common but similar between the HPV-16/18 vaccine group and the control groups.

Fatigue, headache and myalgia were the most frequently reported solicited general symptoms. There were no differences in the percentages of doses followed by any or grade 3 fatigue, gastrointestinal symptoms and headache between the HPV vaccine group and the HAV720 vaccine group. The incidence of grade 3 symptoms did not increase with subsequent doses.

There was a slightly higher incidence of arthralgia in the HPV group when compared to the control HAV720 vaccine (reported after 10.2% (95% CI [9.8; 10.6]) of HPV doses versus 8.6% (95% CI [8.0; 9.2]) of HAV doses). There were no differences in the percentages of doses followed by grade 3 arthralgia between the HPV vaccine group and the HAV720 vaccine group. The incidences of grade 3 symptoms did not increase with subsequent doses.

There was a slightly higher incidence of myalgia in the HPV group when compared to the HAV720 control vaccine (reported after 28.1% (95% CI [27.5; 28.7]) of HPV doses versus 26.5% (95% CI [25.6; 27.5]) of HAV doses). Grade 3 myalgia also occurred more frequently in the HPV group and was reported after 1.4% (95%CI [1.3; 1.6]) of HPV vaccine doses as compared to 0.2% (95% CI [0.1; 0.5]) of ALU, 0.5% (95% CI [0.3; 0.8]) of HAV360 and 0.6% (95% CI [0.4; 0.8]) of HAV720 doses (see following Table) . There was no significant increase of grade 3 myalgia with subsequent doses (1.3%, 1.1% and 1.7% following dose 1, dose 2 and dose 3 of HPV vaccine, respectively).

Pooled safety analysis: incidence of solicited local and general symptoms reported during the 7-day (Days 0-6) postvaccination period following all doses

		HPV					ALU					HAV360					HAV720				
					95 % CI					95 % CI					95 % CI					95 % CI	
Symptom	Type	N	n	%	LL	UL	N	n	%	LL	UL	N	n	%	LL	UL	N	n	%	LL	UL
Solicited local symptoms																					
Pain	All	22806	17785	78.0	77.4	78.5	4485	2353	52.5	51.0	53.9	3059	1264	41.3	39.6	43.1	8750	5150	58.9	57.8	59.9
	Grade 3	22806	1434	6.3	6.0	6.6	4485	154	3.4	2.9	4.0	3059	26	0.8	0.6	1.2	8750	156	1.8	1.5	2.1
Redness (mm)	All	22806	6753	29.6	29.0	30.2	4485	477	10.6	9.7	11.6	3059	418	13.7	12.5	14.9	8750	1401	16.0	15.2	16.8
	> 50	22806	126	0.6	0.5	0.7	4485	1	0.0	0.0	0.1	3059	4	0.1	0.0	0.3	8750	4	0.0	0.0	0.1
Swelling (mm)	All	22806	5876	25.8	25.2	26.3	4485	367	8.2	7.4	9.0	3059	262	8.6	7.6	9.6	8750	887	10.1	9.5	10.8
	> 50	22806	262	1.1	1.0	1.3	4485	2	0.0	0.0	0.2	3059	7	0.2	0.1	0.5	8750	16	0.2	0.1	0.3
Solicited general symptoms																					
Fatigue	All	22802	7545	33.1	32.5	33.7	4481	1021	22.8	21.6	24.0	3058	753	24.6	23.1	26.2	8751	3090	35.3	34.3	36.3
	Grade 3	22802	340	1.5	1.3	1.7	4481	54	1.2	0.9	1.6	3058	35	1.1	0.8	1.6	8751	113	1.3	1.1	1.6
Gastrointestinal symptoms	All	22802	2943	12.9	12.5	13.3	4481	522	11.6	10.7	12.6	3058	347	11.3	10.2	12.5	8751	1223	14.0	13.3	14.7
	Grade 3	22802	157	0.7	0.6	0.8	4481	33	0.7	0.5	1.0	3058	23	0.8	0.5	1.1	8751	62	0.7	0.5	0.9
Headache	All	22802	6730	29.5	28.9	30.1	4481	1161	25.9	24.6	27.2	3058	778	25.4	23.9	27.0	8751	2694	30.8	29.8	31.8
	Grade 3	22802	372	1.6	1.5	1.8	4481	53	1.2	0.9	1.5	3058	48	1.6	1.2	2.1	8751	119	1.4	1.1	1.6
Arthralgia	All	21222	2164	10.2	9.8	10.6	2916	222	7.6	6.7	8.6	3058	283	9.3	8.3	10.3	8751	751	8.6	8.0	9.2
	Grade 3	21222	82	0.4	0.3	0.5	2916	6	0.2	0.1	0.4	3058	5	0.2	0.1	0.4	8751	26	0.3	0.2	0.4
Myalgia	All	21222	5971	28.1	27.5	28.7	2916	289	9.9	8.8	11.1	3058	522	17.1	15.8	18.5	8751	2321	26.5	25.6	27.5
	Grade 3	21222	300	1.4	1.3	1.6	2916	7	0.2	0.1	0.5	3058	14	0.5	0.3	0.8	8751	52	0.6	0.4	0.8

HPV = HPV-16/18 vaccine group (Studies HPV-001, 008 subset, 012, 013, 014, 015 subset and 016; girls and women 10 years and above)
ALU = Al(OH)3 control group (Studies HPV-001 and 015 subset; adolescent girls and women 15 years and above)
HAV360 = Hepatitis A control group containing 360 EU hepatitis A antigen per dose (Study HPV-013; girls 10-14 years of age)

HAV720 = Hepatitis A control group containing 720 EU hepatitis A antigen per dose (Study HPV-008 subset; adolescent girls and women 15-25 years of age)
N= number of documented doses
n/%= number/percentage of doses followed by at least one type of symptom
95%CI= Exact 95% confidence interval; LL = lower limit, UL = upper limit
Grade 3 Pain: Spontaneously painful (HPV-001) or Pain that prevents normal activity (HPV-008, HPV-012, HPV-013, HPV-014, HPV-015 and HPV-016)
Data Source = Pooled safety analysis

For an additional evaluation of the incidence of myalgia and arthralgia, the safety data collected from the large controlled efficacy trial HPV-008 (conducted in subjects aged 15 to 25 years) were also reviewed. Although there was a slightly higher percentage of HPV doses followed by arthralgia, myalgia and grade 3 myalgia when compared to the HAV720 control, these symptoms were of short duration, not associated with other symptoms that may evoke a general disease associated with arthralgia/myalgia, and did not result in a high level of absenteeism from work or school.

- Serious adverse events

All reports of serious adverse events and case fatalities in studies HPV-003, HPV-004, HPV-005, HPV-001, HPV-007, HPV-008, HPV-012, HPV-013, HPV-014, HPV-015 and HPV-016 were included in the analysis. From a total of 29,953 subjects vaccinated in these studies, 882 subjects reported at least one SAE during the entire follow up period (see table below). There was no difference on the overall reporting rates of SAEs during the different follow-up periods.

Global Summary of Serious Adverse Events reported

	Group				
	HPV N=16142	ALU N=3454	HAV360 N=1032	HAV720 N=9325	Total N=29953
Number of subjects with at least one serious adverse event reported	459	75	25	323	882
Number of doses followed by at least one serious adverse event	472	78	25	335	910
Number of serious adverse event reported	542	86	28	372	1028

HPV = HPV-16/18 vaccine group (Studies HPV-001/007, 003, 004, 005, 008, 012, 013, 014, 015 and 016)
ALU = Al(OH)3 control group (Studies HPV-001/007, 003 and 015)
HAV360 = The control group who received Hepatitis A vaccine containing 360 ELU hepatitis A viruses and 250 µg Al(OH)3 (Study
HPV-013)
HAV720 = The control group who received Hepatitis A vaccine containing 720 ELU hepatitis A viruses and 500 µg Al(OH)3 (Study
HPV-008)
N = number of subjects with at least one administered dose
n/% = number/percentage of subjects reporting at least once the symptom
Data source = Pooled safety analysis

A total of 27 subjects reported SAEs assessed by the investigator as possibly related to vaccination. From these, 11 subjects received HPV-16/18 vaccine, 13 subjects received the control vaccine (either Aluminium or HAV) and 3 subjects received a blinded vaccine. Overall, the most frequently reported SAEs were pregnancy-related events: spontaneous abortions (2 HPV; 2 HAV; 3 ALU) and 2 foetal malformations (2 HAV). Five reported events were considered as potentially immune mediated (optic neuritis, thrombocytopenia, erythema nodosum, inflammatory bowel disease and arthritis reactive).

- Laboratory findings

Blood samples were collected to evaluate changes in biochemical and haematological parameters. For all studies where these parameters were evaluated, the haematology and biochemistry profiles were similar for all groups and there were no values out of range that were considered to be clinically significant.

- Safety in special populations

Regardless of serological HPV status, HPV DNA status or previous/current HPV disease status the incidence of solicited symptoms was closely aligned to that observed in HPV DNA negative and HPV seronegative subjects and to the one observed in the overall population (Pooled Total Vaccinated Cohort).

The pooled safety analysis for studies HPV-012, 013 and 014 was stratified according to age. A slight decrease in the reactogenicity profile of the HPV-16/18 vaccine with increasing age was observed.

Pregnancy

A total of 1,737 pregnancies have been reported over the entire follow-up period for completed or ongoing clinical studies (see following Table for details). A total of 503 pregnancies (29%) are still ongoing. The majority of pregnancies were reported in subjects between 15 to 25 years of age, i.e., the age group where the largest clinical study, HPV-008, is currently ongoing in more than 18,000 subjects. Of these 1,737 pregnancies, 769 (44.3%) resulted in the delivery of a normal infant, 210 (12.1%) pregnancies were terminated by an elective abortion and 155 (8.9%) resulted in a spontaneous abortion. Of note, the rates of spontaneous abortion (8.9%) from this analysis are below reported background rates of spontaneous abortion in the general population. Literature reviews show a range from 12-15% for clinically recognised pregnancies and from 17-22% for early pregnancy losses which may be defined as pregnancy losses within 14 days after conception, around the next menstruation, and which could be confused with menstrual bleeding .
In addition, a total of six stillbirths occurred, five of which were in women whose treatment status remains blinded (i.e., these subjects received either the HPV-16/18 vaccine or the HAV720 control vaccine).

When comparing the pregnancy outcomes per treatment group, there were no major differences in the rates of any specific pregnancy outcome between the HPV-16/18 vaccine group and the control groups. The rate of abnormal infants in the HPV-16/18 group was lower than the control group HAV720.
Concerning abnormal infants information available so far show that from the 18 reported pregnancy cases (6 in the HPV group, 4 in the ALU group and 8 in the HAV720 group), no cluster of events was identified. Nine pregnancies resulted in 11 neonatal deaths. Five of these deaths were related to prematurity. The remaining 6 neonatal deaths were related to respiratory distress syndromes (2 cases), aspiration pneumonia, intracranial haemorrhage, polycystic kidney disease with anhydramnios or gastrochisis.

Pooled safety analysis: number of subjects with pregnancies and pregnancy outcomes overall (Total vaccinated cohort)**

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

HPV = HPV-16/18 vaccine group (Studies HPV-001/007, 003, 004, 005, 008, 012, 013, 014, 015 and 016; adolescent girls and women 10 years of age onwards)
ALU = $Al(OH)_3$ control group (Studies HPV-001, 003 and 015; adolescent girls and women 15 years of age onwards)
HAV360 = Hepatitis A control group containing 360 EU hepatitis A antigen per dose (Study HPV-013; girls 10-14 years of age)
HAV720 = Hepatitis A control group containing 720 EU hepatitis A antigen per dose (Study HPV-008; adolescent girls and women 15-25 years of age)
N = number of pregnancies
n = number of pregnancies in a given category
Value = value of the considered parameter
% = n / Number of subjects with available results x 100
Spontaneous abortion includes missed abortion
Not applicable: e.g. mola, throfoblastic tumor
* = cases which remain blinded as studies HPV-001/007, HPV-008 and HPV-015 are ongoing
** = twin pregnancies counted as one pregnancy
Data source = Pooled safety analysis

A secondary analysis of pregnancies and pregnancy outcomes was conducted that focused on pregnancies in women for which their last menstrual period (LMP) occurred from -30 days to +45 days around vaccination. These pregnancies occurred despite the fact that subjects were strongly advised (through the informed consent process) to take precautions to avoid pregnancy until 2 months after the last vaccination, and that pregnancy testing was conducted prior to vaccination.

A total of 415 subjects reported pregnancies around vaccination (defined as LMP initiated from -30 days to +45 days around vaccination). 57.3% of subjects gave birth to normal infants, 16.1% of pregnancies resulted in elective abortion and 8.9% resulted in spontaneous abortion, whereas 9.4% of pregnancies are still ongoing. Except for a slight increase in the proportion of elective abortions, these rates are comparable to the outcome rates for all pregnancies. A total of 7 women delivered an abnormal infant. Outcomes in one of the control groups (HAV720), was observed when compared to the HPV group (5 in HAV720 group and 2 in HPV group). No reports of abnormal infants were received in the ALU and HAV360 control groups.

When comparing the outcomes between treatment groups, an imbalance with higher rates of spontaneous abortion was observed in the HPV group compared to the HAV720 group (11.0% versus 5.7%). This imbalance was not observed between the HPV group and the ALU group (11.0% versus 13.8%).

Pooled safety analysis: number of subjects with pregnancies around vaccinations and their outcomes (Total vaccinated cohort)

Outcome	HPV N = 210		ALU N = 29		HAV360 N = 1		HAV720 N = 175		Total N = 415	
	Value or n	%	Value or n	%	Value or n	%	Value or n	%	Value or n	%
Pregnancy ongoing	21	10.0	9	31.0	0	0.0	9	5.1	39	9.4
Normal Infant	115	54.8	10	34.5	0	0.0	113	64.6	238	57.3
Premature birth	7	3.3	1	3.4	1	100	4	2.3	13	3.1
Abnormal infant	2	1.0	0	0.0	0	0.0	5	2.9	7	1.7
Elective termination	37	17.6	5	17.2	0	0.0	25	14.3	67	16.1
Therapeutic abortion	*	*	0	0.0	0	0.0	*	*	1	0.2
Ectopic pregnancies	2	1.0	0	0.0	0	0.0	1	0.6	3	0.7
Spontaneous abortion	23	11.0	4	13.8	0	0.0	10	5.7	37	8.9
Still birth	*	*	0	0.0	0	0.0	*	*	1	0.2
Lost to follow-up	2	1.0	0	0.0	0	0.0	7	4.0	9	2.2
Not applicable	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

HPV = HPV-16/18 vaccine group (Studies HPV-001, 008, 012, 013, 014, 015 and 016; adolescent girls and women 10 years of age onwards)
ALU = $Al(OH)_3$ control group (Studies HPV-001 and 015; adolescent girls and women 15 years of age onwards)
HAV360 = Hepatitis A control group containing 360 EU hepatitis A antigen per dose (Study HPV-013; girls 10-14 years of age)
HAV720 = Hepatitis A control group containing 720 EU hepatitis A antigen per dose (Study HPV-008; adolescent girls and women 15-25 years of age)
N = number of pregnancies
n = number of pregnancies in a given category
Value = value of the considered parameter
% = n / Number of subjects with available results x 100
Pregnancies around-vaccinations: Pregnancy in subjects for which last menstrual period occurred between 30 days before and 45 days after vaccination (Subjects with missing date for last menstrual period are not included)
Spontaneous abortion includes missed abortion
Not applicable: e.g. mola, throphoblastic tumor
* = cases which remain blinded since HPV-001/007, HPV-008 and HPV-015 are ongoing
Data source = Pooled safety analysis

To further evaluate this observed imbalance, the ongoing study HPV-009 was also analysed in this respect.While cases of any abnormal pregnancy outcome experienced by a subject whose last menstrual period (LMP) occurred within 60 days of vaccination was unblinded the overall number of pregnancies per group are not available from study 009 since these pregnancies are still blinded and, contrary to study HPV-008, an interim analysis is not foreseen. Only the absolute numbers and not the rates of pregnancy outcomes can therefore be compared between the treatment groups. Therefore it is currently unknown whether the number of pregnancies would be comparable in the two treatment groups.

The observed imbalance in study HPV-008 appears to be mostly concentrated in the group of women who had their LMP within 30 days before vaccination. In study HPV-009 a similar, albeit smaller, imbalance is seen. The reported frequencies of spontaneous abortions following HPV-16/18 vaccine administration among women whose pregnancy occurred around vaccination can be considered close to the lower limit (12-22%) of the range of background incidence rates of spontaneous abortion reported in the medical literature .

Several studies, including the large study HPV-008, are still ongoing and further follow-up will be collected on all pregnancies occurring among women in these studies. Close monitoring of pregnancy-related events is covered post-licensure (see RMP).

The effect on breast-fed infants of the administration of Cervarix to their mothers has not been evaluated in clinical studies.
Cervarix should only be used during breast-feeding when the possible advantages outweigh the possible risks.

- Safety related to drug-drug interactions and other interactions

In Studies HPV-001, 012, 013 and 014 the use of medication, including anti-pyretic medication, was recorded. Overall, no major differences concerning use of anti-pyretics was observed.

- Discontinuation due to adverse events/deaths

The number of subjects vaccinated, completed and withdrawn with the reasons for withdrawal are summarised in the table below. A total of 55 subjects were withdrawn from the study due to serious or non-serious adverse events (including 33 subjects in the HPV-16/18 vaccine group) as described below:

Five case fatalities were reported in the overall HPV clinical development. None of these were assessed by the investigator as possibly related to vaccination. Two case fatalities were associated with motor vehicle accidents. One case fatality was associated with homicide. The remaining 2 cases refer to dead due to osteosarcoma complications and ketoacidosis due to diabetes.

In addition to the 5 case fatalities described above, 13 foetal deaths were also reported to the Company as pregnancy outcomes. These cases were kept blinded as they were reported from studies that are currently ongoing. Although these case fatalities remain blinded, no safety signal has been identified based on the description of these events.

Number of subjects vaccinated and withdrawn due to serious or non-serious adverse events

	HPV-003		HPV-004	HPV-005	HPV-001		HPV-008		HPV-012	HPV-013		HPV-014	HPV-015		HPV-016	Total			
	HPV	ALU	HPV	HPV	HPV	ALU	HPV	HAV720	HPV	HPV	HAV360	HPV	HPV	ALU	HPV	HPV	ALU	HAV360	HAV720
Vaccinated	31	30	20	63	560	553	9319	9325	770	1035	1032	666	2880	2871	798	16142	3454	1032	9325
Withdrawal due to:																			
SAE	0	0	0	0	1	0	3	5	0	0	1	1	1	2	1	7	2	1	5
Non-SAE	0	0	0	0	0	3	6	3	5	0	2	3	8	5	4	26	8	2	3

HPV = HPV-16/18 vaccine group (Studies HPV-001, 003, 004, 005, 008, 012, 013, 014, 015 and 016)
ALU = Al(OH)3 control group (Studies HPV-001, 003 and 015)
HAV360 = The control group who received Hepatitis A vaccine containing 360 ELU hepatitis A viruses and 250 µg Al(OH)3 (Study HPV-013)
HAV720 = The control group who received Hepatitis A vaccine containing 720 ELU hepatitis A viruses and 500 µg Al(OH)3 (Study HPV-008)
Study HPV-007 is not included since subjects from this study were enrolled and vaccinated in Study HPV-001 and no vaccination was administered in Study HPV-007
Studies 008 and 015 are still ongoing and represent the information that was available at the time of the respective interim analyses in the different studies

New Onset of Chronic Disease (NOCD)
New onset chronic diseases (NOCD) were reported throughout the study periods regardless of causal relationship to vaccination and intensity. All adverse events reported during the trial were compared with a pre-defined list of symptoms/conditions that may evoke chronic diseases. The number of subjects experiencing an NOCD was similar between HPV vaccine and the control groups in any given reporting period (see following table).

The most commonly reported NOCD were asthma, urticaria and hypersensitivity, which were each reported at similar frequencies in the HPV vaccine and control groups.

Pooled safety analysis: percentage of subjects reporting the occurrence of New Onset of Chronic Diseases stratified by reporting period (Total vaccinated cohort)

Reporting period	HPV			ALU			HAV360			HAV720		
		95% CI			95% CI			95% CI			95% CI	
	%	LL	UL	%	LL	UL	%	LL	UL	%	LL	UL
Month 0-7[1]	1.2	1.0	1.4	1.0	0.5	1.9	2.1	1.3	3.2	0.9	0.7	1.1
Month 7-12[2]	0.4	0.3	0.5	NA	NA	NA	0.6	0.2	1.3	0.4	0.3	0.5
> Month 12[3]	0.4	0.3	0.5	1.1	0.4	2.3	0.3	0.1	0.8	0.4	0.2	0.5

1. HPV = HPV-16/18 vaccine group (Studies 008, 012, 013, 014, 015 subset and 016; girls and women from 10 years of age onwards)
ALU = Al(OH)3 control group (Study 015 subset; women 26+ years of age)
HAV360 = Hepatitis A control group containing 360 EU hepatitis A antigen per dose (Study HPV-013; girls 10-14 years of age)
HAV720 = Hepatitis A control group containing 720 EU hepatitis A antigen per dose (Study HPV-008; adolescent girls and women 15-25 years of age)
2. HPV = HPV-16/18 vaccine group (Studies 008, 012, 013 and 014; girls and women from 10-25 years of age)
ALU = Al(OH)3 control group (NA = not available, i.e., no data for this timepoint from Study 015)
HAV360 = Hepatitis A control group containing 360 EU hepatitis A antigen per dose (Study HPV-013; girls from 10-14 years of age)
HAV720 = Hepatitis A control group containing 720 EU hepatitis A antigen per dose (Study HPV-008; adolescent girls and women 15-25 years of age)
3. HPV = HPV-16/18 vaccine group (Studies 007, 008, 013 and 014; girls and women from 10-25 years of age)
ALU = Al(OH)3 control group (Study 007 in women vaccinated in Study 001; initially 15-25 years of age)
HAV360 = Hepatitis A control group containing 360 EU hepatitis A antigen per dose (Study HPV-013)
HAV720 = Hepatitis A control group containing 720 EU hepatitis A antigen per dose (Study HPV-008)
NA = not available % = percentage of subjects reporting at least once the symptom
95% CI= exact 95% confidence interval; LL = Lower Limit, UL = Upper Limit
Data Source = Pooled safety analysis

New Onset of Autoimmune Disease (NOAD)

Overall, the reporting rates of NOAD were low and no cluster of events has been detected in any treatment group. The most frequently reported events were related to thyroid disease as would be expected based on the background incidence rates of thyroid disease in young female population. No clear difference in the reported rates of thyroid disease between treatment groups in any of the follow-up periods has been observed to date. No cluster in terms of either time to onset of AEs and number of doses has been observed. The other events were kept blinded as most of these events have been reported only once. The low frequency of autoimmune diseases constitutes a limitation of their assessment in the clinical program. Therefore, they will be further evaluated in ongoing and the planned post-licensure activities.

Seven cases of neurological disorders (five cases in the HPV group and two in the control group) have been identified. These cases appear to be isolated events. Final diagnoses are unclear for 3 cases. The time to onset varies between 9 days to 7 months. Thus, no cluster in terms of either time to onset of AEs and number of doses has been observed. Considering the safety database and the age of the target population, the adverse events might be explained just by chance. The cases do not indicate an increased risk for demyelinating disease or nerve disorders. Autoimmune diseases including demyelinating neurological diseases as well as neurological diseases are addressed in the RMP.

- Discussion on clinical safety

The most frequently reported solicited symptoms after administration of the HPV-16/18 L1 VLP AS04 vaccine were injection site reactions including pain, redness and swelling, all reported more frequently in the HPV group as compared to the control groups. Solicited general symptoms were reported less frequently than local symptoms after vaccination. Among these, myalgia and arthralgia were reported more frequently in the HPV-16/18 vaccine group as compared to control groups. The majority of the reports were of low grade intensity and the symptoms were transient, not associated with any general disease and resolved without sequelae.
There was no clinically meaningful increase of symptoms with increasing number of doses. The majority of the solicited local and general symptoms reported were mild to moderate in intensity, and usually resolved within 3 or 4 days after vaccination.

Compliance with the full vaccination course was equally high in both HPV-16/18 vaccine and control groups, suggesting that the higher rates of local and general symptoms following HPV-16/18

vaccine did not negatively impact tolerability or acceptance of the vaccination treatment. There were no unusually high rates of occurrence of unsolicited adverse events, and there was no evidence that any unsolicited adverse event occurred more frequently in the HPV-16/18 vaccine group than in control groups. Separate analysis of NOAD revealed no clear trend or cluster, although as the incidence rates of autoimmune diseases are low, further post-marketing evaluation is necessary to fully assess the issue. Discontinuation rates due to adverse events were low and similar between groups.

The impact on pregnancy and pregnancy outcome could not be fully analysed at the time of assessment, since most of the pregnancies were ongoing and/or remained blinded concerning allocation to vaccine. Use of contraceptives was required per protocol for the period during the vaccination period and therefore, there is currently no robust data available on the use of Cervarix in pregnant women. A slight imbalance of spontaneous abortions in study HPV-008 was mostly observed in the group of women who had their LMP within 30 days before vaccination.

The reported frequencies of spontaneous abortions following HPV-16/18 vaccine administration among women whose pregnancy occurred around vaccination can be considered close to the lower limit (12-22%) of the range of background incidence rates of spontaneous abortion reported in the medical literature. However, vaccination should be postponed until after completion of pregnancy. Close monitoring of pregnancy-related events is covered postlicensure (see RMP).

The reporting rates of New Onset of Autoimmune Disease (NOAD) were low. No cluster in terms of either time to onset of AEs and number of doses has been observed. A review of the cases and analyses from the overall database do not indicate an increased risk for demyelinating disease or nerve disorders. Autoimmune diseases including demyelinating neurological diseases as well as neurological inflammatory disorders are addressed in the RMP.

Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan:

	Proposed pharmacovigilance activities	**Proposed risk minimisation activities**
Safety concern		
Auto-immune diseases (AIDs)	Ongoing Study HPV 008	Not applicable at this stage.
	Post-licensure prospective cohort study (AIDs study, Scotland)	Not applicable at this stage.

Missing information		
Use in HIV positive women	HPV-020 (South Africa)	SPC proposed wording "There are no data on the use of Cervarix in subjects with impaired immune responsiveness such as HIV infected patients or patients receiving immunosuppressive treatment. As with other vaccines, an adequate immune response may not be elicited in these individuals."
Use in co-administration with other vaccines	HPV-018 (US) HPV-029 (Europe) HPV-042 (Europe)	SPC proposed wording "Data have not been generated on the concomitant administration of Cervarix and other vaccines."
Pregnancy outcomes after vaccination	Ongoing Study HPV 008 Ongoing Study HPV 009 Ongoing Study HPV-007 Ongoing Study HPV-015	SPC proposed wording "These data are insufficient to recommend use of Cervarix during pregnancy. Vaccination should, therefore, be postponed until after completion of pregnancy."
	US-based Pregnancy Register	
	HPV-040 CRT (Finland)	
	Post-licensure prospective cohort study (Scotland)	
Other Plans and Considerations		
Long-term efficacy	HPV-008 LTFU (Finland cohort) via Finnish Cancer Registry	Not applicable at this stage.
HPV Type Replacement	HPV-040 CRT (Finland) Post-licensure prospective cohort study (Scotland)	Not applicable at this stage.
Males	HPV-011 (Phase I/II) HPV-040 CRT (Finland)	Not applicable at this stage.

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

Overall conclusions, risk/benefit assessment and recommendation

Quality

During the evaluation of Cervarix, no major objections were identified. A number of minor concerns were raised in regard to the novel insect cell production system and these have been adequately addressed, however several commitments have been made by the applicant, and several follow-up measures defined to provide further information post-approval. In conclusion, all quality issues can be considered resolved.

Non-clinical pharmacology and toxicology

No animal model exists for human papilloma virus infection. Consequently Cervarix has not been tested for protection. Pharmacology studies in animals show that MPL induces innate immunity and showed no induction of undesirable pharmacological effects on cardio-respiratory functions. Primary pharmacodynamic studies in mice and monkeys show that the activation of the innate immunity by AS04 is paralleled by the induction of a strong and persistent specific immune response by the Cervarix vaccine. Safety pharmacology studies on cardio-respiratory functions showed no undesirable pharmacological effects with Cervarix.

Studies on secondary pharmacology or pharmacodynamic drug interactions have not been performed, which is acceptable for this vaccine.

Two studies targeting the determination of the pharmacokinetics of MPL-related material following intravenous and intramuscular administration in rats were carried out. The MPL-related material is widely distributed throughout the body, notably in the fat and spleen, and is then likely eliminated via the expired air with only low levels remaining in the carcass.

Signs of inflammation at the injection sites of the test vaccine were observed. These injection site reactions were local and followed by a recovery process. This local effect is most likely the sign of the strong adjuvant effect induced by AS04. Furthermore, up to three times the full human dosage was used in the rabbit studies, probably amplifying this local reaction. The injection site reaction was accompanied with a temporary increase in the number of neutrophils and in fibrinogen, which may be a consequence of the recruitment of inflammatory cells following injections of the formulations.

In vitro mutagenicity and clastogenicity tests, and in vivo micronucleus assay demonstrated no genotoxic potential of the MPL adjuvant. The mutagenic and carcinogenic potential of the Cervarix vaccine was not further evaluated, which is accepted since the vaccine is based on L1 protein and HPV oncoproteins are absent.

The reproductive toxicity study in CD rats did not show any effects of vaccination or AS04 administration on fertility, embryofoetal development and pre- and post-natal development. Antibodies from vaccinated dams cross the placenta and are secreted in the milk.

Non-clinical data reveal no special hazard for humans based on conventional studies of safety pharmacology, acute and repeated dose toxicity, local tolerance, fertility, embryo-foetal and postnatal toxicity (up to the end of the lactation period).

Efficacy

The efficacy of Cervarix was assessed in two controlled, double-blind, randomised Phase II and III clinical trials that included a total of 19,778 women aged 15 to 25 years.

Endpoints included CIN2+ associated with HPV-16 and/or HPV-18 and 12-month persistent infection.
For HPV-16 all endpoints reached statistical significance.

For HPV-18, the difference between the vaccine and control groups was not statistically significant for CIN2+ and 12-month persistent infection due to a limited number of events at the time of the analysis. However, in a pre-specified analysis that excluded women with abnormal cytology at study entry, the 12-month persistent infection endpoint for HPV-18 reached statistical significance with vaccine efficacy of 89.9% (97.9% CI: 11.3; 99.9).

An additional **post-hoc** analysis based on the presence of the HPV type in cytology samples prior to detection of the lesion excluded 3 CIN2+ cases which were not considered to be causally associated with HPV-16 or HPV-18 infections acquired during the trial. Based on this analysis there were no cases in the vaccine group and 20 cases in the control group (Efficacy 100%; 97.9% CI: 74.2, 100).

There was no evidence of protection from disease caused by the HPV types for which subjects were HPV DNA positive at study entry. However, individuals already infected with one of the vaccine-

related HPV types prior to vaccination were protected from clinical disease caused by the remaining HPV type.

In clinical trials assessing immunogenicity, 99.9% of initially seronegative subjects had seroconverted to both HPV types 16 and 18 one month after the third dose. Vaccine-induced IgG Geometric Mean Titres were well above titres observed in women previously infected but who cleared HPV infection. Initially seropositive and seronegative subjects reached similar titres after vaccination.

Study HPV-014 demonstrated, that seropositivity with regard to HPV 16 and 18 at the time of vaccination has some marginal positive influence on the kinetics of the immune response as well as on final GMTs and is more pronounced in older individuals compared to those who are younger: Whether or not vaccination of a seropositive female population has any effect on clinical efficacy of Cervarix cannot be assessed for the time being.

In two further clinical trials performed in girls and adolescents aged **10 to 14 years**, all subjects seroconverted to both HPV types 16 and 18 after the third dose with GMTs at least 2-fold higher as compared to women aged 15 to 25 years. On the basis of these immunogenicity data, the efficacy of Cervarix is inferred from 10 to 14 years of age.

Safety

The most frequently reported solicited symptoms after administration of the HPV-16/18 L1 VLP AS04 vaccine were injection site reactions including pain, redness and swelling, headache, myalgia and arthralgia. Solicited general symptoms were reported less frequently than local symptoms after vaccination. The majority were of low grade intensity and the symptoms were transient and resolved without sequelae.

There was no clinically meaningful increase of symptoms with increasing number of doses. No unusually high rates of occurrence of unsolicited adverse events, and no evidence that any unsolicited adverse event occurred more frequently in the HPV-16/18 vaccine group than in control groups were observed. Discontinuation rates due to adverse events were low and similar between groups.

The impact on pregnancy and pregnancy outcome could not be fully analysed at the time of assessment, since most of the pregnancies were ongoing and/or remained blinded concerning allocation to vaccine. A slight imbalance of spontaneous abortions in study HPV-008 was mostly observed in the group of women who had their LMP within 30 days before vaccination.

The reported frequencies of spontaneous abortions following HPV-16/18 vaccine administration among women whose pregnancy occurred around vaccination can be considered close to the lower limit (12-22%) of the range of background incidence rates of spontaneous abortion reported in the medical literature. However, vaccination should be postponed until after completion of pregnancy. Close monitoring of pregnancy-related events is covered post authorisation (see RMP).

The effect on breast-fed infants of the administration of Cervarix to their mothers has not been evaluated in clinical studies. Cervarix should only be used during breast-feeding when the possible advantages outweigh the possible risks.

Administration of Cervarix should be postponed in subjects suffering from an acute severe febrile illness. However, the presence of a minor infection, such as a cold, is not a contraindication for immunisation.

The reporting rates of New Onset of Autoimmune Disease (NOAD) were low. Seven cases of neurological disorders were of particular interest (five cases in the HPV group and two in the control group). The cases appear to be isolated events and no cluster in terms of either time to onset of AEs and number of doses has been observed. The cases do not indicate an increased risk for

demyelinating disease or nerve disorders. Autoimmune diseases including demyelinating neurological diseases as well as neurological inflammatory disorders are addressed in the RMP.

Cervarix should not be used in case of hypersensitivity to the active substances or to any of the excipients.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

- User consultation

A user consultation has been performed and was considered to be satisfactory.

Risk-benefit assessment

The benefits of Cervarix are currently based on 2 controlled, double-blind, randomised Phase II and III clinical studies. Both trials are still ongoing and continue to generate data on both efficacy and safety. Data is available from studies HPV-001/007 (5.5 years) and HPV-008 (mean follow-up 15 months). The phase III interim analysis was event driven.

The primary efficacy composite endpoint, protection against CIN2+ associated with the HPV-16/18, was met.

Other composite HPV-16/18 endpoints (6-months and 12-months persistence, CIN1+ and ASCUS+) were also statistically significant but not considered for the demonstration of efficacy against cervical cancer.
For HPV-16 all endpoints reached statistical significance. For HPV 18 the difference between the vaccine and control groups was not statistically significant for CIN 2+ and 12-month persistent infection. However, in a pre-specified analysis that excluded women with abnormal cytology at study entry, the 12-month persistent infection endpoint for HPV-18 also reached statistical significance.

The safety profile of Cervarix was comparable to the control groups. No clear evidence for a serious safety risk has been identified.

Taking all of the data into consideration the benefits of Cervarix outweighs the risks and the risk/benefit ratio is positive.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns.

- no additional risk minimisation activities were required beyond those included in the product information.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Cervarix in

> The prevention of high-grade cervical intraepithelial neoplasia (CIN grades 2 and 3) and cervical cancer causally related to Human Papillomavirus (HPV) types 16 and 18.
>
> The indication is based on demonstration of efficacy in women aged 15-25 years following vaccination with Cervarix and on the immunogenicity of the vaccine in girls and women aged 10-25 years.
>
> See section 5.1 for information on the evidence that supports the efficacy of Cervarix in prevention of CIN grades 2 and 3 associated with HPV-16 and/or HPV-18.

was favourable and therefore recommended the granting of the marketing authorisation.