

BLA STN125259 Toxicology Review

Product: Cervarix HPV vaccine adjuvanted with Monophosphoryl Lipid A

Sponsor: Glaxo SmithKline

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FDA Reviewer's Overall Summary and Conclusions:

Nonclinical toxicology studies were conducted by Glaxo SmithKline to support the evaluation and licensing requirements for Cervarix

Glaxo SmithKline conducted six GLP-compliant nonclinical toxicology studies, which included a repeated-dose toxicity study in rats, four repeated-dose studies in rabbits, a local tolerance study in rabbits, and a developmental and reproductive toxicity study in rats. Two *in vitro* and one *in vivo* genotoxicity studies were conducted solely on the adjuvant MPL (AS04) as well.

Table 1 Toxicology program on HPV-16/18 L1 VLP AS04 vaccine^{1, 2}:

Study type and duration ³	Route of administration	Species	GLP	Study name
Repeat-dose				
71 days	im	Rabbits	Yes	(b)(4) 1513
59 days	im	Rabbits	Yes	(b)(4) 1753
147 days	im	Rabbits	Yes	58678
147 days	Im	Rabbits	Yes	(b)(4) 52369
147 days	Im	Rat	Yes	62570
Reproductive and development				
77 days	im	Rats	Yes	(b)(4) 249;C23160

1: All these studies were GLP compliant and were sponsored by GSK Biologicals or by (b)(4). 2: In addition to these studies, the profile of MPL was also analysed in specific toxicological studies, which are summarised in MPL part, Module 2.5 Section 2.5.6
Toxicology section summary: 3: duration -- 1 day; im: intramuscular

These toxicology studies were designed based on the composition, dosage and immunization schedule of the human vaccine (20 µg of each of HPV-16 VLP and HPV-18 L1 VLP, adjuvanted with AS04, 3 doses at 0, 1, 6 months). In addition, since the vaccine target population includes women of childbearing age, a reproductive and development toxicity study was carried out. AS04 treatment groups were included in those studies.

In summary, the toxicity of a single or four administrations in rabbits, of up to three times the human dosage of HPV-16/18 L1 VLP AS04 vaccine, or of once the human dosage of the adjuvant AS04 was analyzed in combined single-repeat-dose studies. Hematology, clinical chemistry, bone marrow smears assessments, organ weight data, macroscopic pathology, and clinical sign evaluations showed no consistent treatment-related or dose-dependent findings.

No deaths were recorded in those studies. A drop in platelet count was observed in the Scantox 58678 toxicity study, after four repeated administration of the vaccine in rabbits. No other findings related to the platelet count drop were observed in this study, neither in the bone marrow, nor in the reticulo-endothelial system. The hematology profile and clinical signs of the treated animals were otherwise normal, and the biological relevance of this observation is therefore unclear. This effect does not appear to be of toxicological

significance, even though a treatment-related effect at the investigated dosage (full human vaccine dose) and in the rabbit can not be fully excluded.

To determine if the reduction in platelets was species-specific, or related to the dose, additional studies were conducted to evaluate potential effects of a dose range of HPV-16/18 L1 VLP AS04 on hematology parameters in both rabbits (b)(4) Study No 62369) and rats (b)(4) Study No 62370).

In the local tolerance study, histopathology assessment of the vaccine injection site revealed evidence of inflammation, and focal degeneration or necrosis of myofibers at the injection site of the HPV-16/18 L1 VLP AS04 vaccine, as expected shortly after the injection of alum-containing vaccines, followed by clear signs of recovery afterwards.

The genotoxic and oncogenic potential of Cervarix were not evaluated. Such studies are not usually required for recombinant protein vaccines, consistent with WHO guidelines on vaccine nonclinical safety evaluation. The adjuvant MPL (AS04) was evaluated for genotoxicity in the following genetic toxicology studies: (b)(4). The adjuvant was not positive in each of the genotoxicity assays.

Recommendation:

The BLA submission for Cervarix HPV vaccine is acceptable with regards to nonclinical toxicology.

Introduction:

The Cervarix vaccine is composed of recombinant C-terminally truncated HPV-16 L1 and HPV-18 L1 proteins, assembled into virus-like particles (VLPs) and adjuvanted with GlaxoSmithKline Biologicals proprietary adjuvant system AS04.

The HPV-16 L1 and HPV-18 L1 proteins constitute the active ingredient of the vaccine and are produced with a recombinant Baculovirus expression system. The AS04 adjuvant is composed of an aluminum salt, $\text{Al}(\text{OH})_3$ 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 and is manufactured and supplied by Corixa Corporation (GSK Biologicals North America, Hamilton, Montana, USA).

One dose of Cervarix contains 20µg of HPV-16 L1 and 20µg of HPV-18 L1 proteins adjuvanted with AS04 which is composed of 500µg of aluminum hydroxide and 50µg of MPL.

In the Cervarix vaccine, the HPV-16 L1 protein, HPV-18 L1 protein and the MPL immunostimulant are separately adsorbed onto aluminum hydroxide following which the three adsorbed bulks are formulated in an -----(b)(4)-----.

The vaccine is a preservative-free product available as a 0.5 mL single-dose in 3 mL glass vials (fill volume = -(b)(4)-) and as a 0.5 mL single-dose in pre-filled, TIP-LOK® disposable 1.25 mL glass syringes (fill volume = -(b)(4)-).

The pharmaceutical form of the vaccine is a turbid liquid suspension for intramuscular injection. The vaccine is to be injected intramuscularly in the deltoid region and the proposed administration regimen for the candidate vaccine is a 3-dose injection schedule at 0, 1 and 6 months.

Cervarix is indicated for girls, adolescent and adult females ≥ 10 years of age for the prevention of cervical cancer (squamous-cell carcinoma and adenocarcinoma) by protecting against incident and persistent infections, cytological abnormalities including ASC-US, cervical intraepithelial neoplasia (CIN) CIN1 and precancerous lesions (CIN 2 and CIN 3) caused by oncogenic human papillomaviruses (HPV) types 16 and 18.

Product Summary

Cervarix vaccine is composed of recombinant C-terminally truncated HPV-16 L1 and HPV-18 L1 proteins, assembled into virus-like particles (VLPs) adjuvanted with GlaxoSmithKline Biologicals proprietary AS04 adjuvant.

The HPV-16 L1 VLP and HPV-18 L1 VLP proteins constitute the active ingredient of the vaccine and are produced with a recombinant Baculovirus expression system. The AS04 adjuvant is composed of an aluminum salt, $\text{Al}(\text{OH})_3$ 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 human dose of Cervarix contains 20 μg of HPV-16 L1 VLP and 20 μg of HPV-18 L1 VLP adjuvanted with AS04 consisting of 500 μg of aluminum (as aluminum hydroxide) and 50 μg of MPL.

Table 1 Composition of the HPV vaccine

Ingredients	Quantity (per 0.5ml dose)	Function	Reference
Active ingredients			
HPV-16 L1 VLP	20 μg	Antigen	GSKBio Mn 20090601
HPV-18 L1 VLP	20 μg	Antigen	GSKBio Mn 20091001
Excipients			
3- <i>O</i> -desacyl-4' monophosphoryl lipid A (MPL)	50 μg	Immunostimulant	GSKBio Mn 10026404
Aluminium (hydroxide salt)	500 μg	Adjuvant	GSKBio Mn 10026701
Sodium Chloride (NaCl)	4.4 mg (150 mM)	Buffer	(b)(4)
Sodium dihydrogen phosphate dihydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$)	0.624 mg (8 mM)	Buffer	
Water for injection	q.s. ad 0.5 ml	Solvent	

2.6.7.4 Toxicology: drug substance

Table 2 HPV-16 L1 VLP antigen and HPV-18 L1 VLP antigen components
Test Article: HPV-16/18 L1 VLP AS04 vaccine

	Drug Substance Antigen	Purity (% L1)	Impurities (in purified bulk)	Study Number	Type of Study
Impurity level in Clinical & consistency batches ⁽¹⁾	HPV-16 L1 VLP HPV-18 L1 VLP	> 95 % > 95 %	(b)(4)	-	-
Batch No.					
20JUL99 MJA 103629	HPV-16 L1 VLP HPV-18 L1 VLP	NA		(b)(4) 1513	Repeat-dose
99AH05AW	HPV-16 L1 VLP HPV-18 L1 VLP	> 95 % > 95 %		(b)(4) 1758	Repeat-dose
EHPV002Y	HPV-16 L1 VLP HPV-18 L1 VLP	> 95 % > 95 %		(b)(4) 249-033160	Reproductive and developmental
DHPV005A9	HPV-16 L1 VLP HPV-18 L1 VLP	> 95 % > 95 %		(b)(4) 59578	Repeat-dose
DHPVA017B	HPV-16 L1 VLP HPV-18 L1 VLP	> 95 % > 95 %		(b)(4) 62369 (b)(4) 62370	Repeat-doses

⁽¹⁾ Batches prepared during Phase IIb and Phase III development (clinical study HPV-001, HPV-006 and HPV-012 to HPV-016), including consistency batches proposed for marketing. ⁽²⁾ Note: HCP values for these batches were below the limit of quantification of the ELISA assay, which was initially reported as < 5%. Validation of the ELISA assay has later shown that the limit of quantification of assay is (b)(4) which is the value reported for these batches in the above table. Abbreviation: (b)(4) NA: not available.

Table 2 Test Article: MPL¹

(b)(4)	
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Safety Pharmacology: Cervarix and MPL

Safety pharmacology studies were performed on HPV-16/18 L1 VLP AS04 in rats and on MPL in dogs. The intramuscular administration of 1/5 of human dose (0.1 ml/animal) of HPV-16/18 L1 VLP AS04 in the anaesthetized -(b)(4)- male -(b)(4)- rat, or the intravenous administration of ascending MPL doses up to 100 µg/kg bodyweight (50-100-fold the human dose of MPL in the AS04 adjuvant) showed no treatment-related effects on the examined cardiovascular or respiratory parameters.

Safety pharmacology studies on the MPL adjuvant showed no deleterious pharmacological effects on cardiovascular or-respiratory functions in animal models. Cervarix did not induce pharmacological effects on cardiovascular or respiratory functions in animal models.

MPL: Cardiovascular and respiratory effects in the anaesthetized dog following intravenous administration -(b)(4)- 1729/22)

The -(b)(4)- dog was used for the evaluation of possible side effects of MPL on cardiovascular and respiratory parameters in a GLP study performed by --(b)(4)-- -----, in 1999.

Two male and two female adult -(b)(4)- dogs were anesthetized using propofol anesthesia and received ascending doses of MPL at 1, 10 and 100 µg/kg at a dose volume of 1-2 ml/kg intravenously in the left jugular vein with at least 25 minutes between each dose. Two male and two female control animals received 3 volumes of vehicle (phosphate buffered saline), using the same regime as for test animals. Cardiovascular (blood pressure, heart rate, left ventricular pressure, mean femoral blood flow and ECG) and respiratory (peak inspiration and expiratory flow, respiration rate, tidal volume and minute volume) parameters were measured post-dose at 2, 10 and 20 minutes and were compared with pre-dose values. Cardiovascular and respiratory parameters were monitored continuously for a period of two hours post vaccination.

There was little effect of treatment at any dose level of MPL. A small and gradual increase in mean heart rate over the MPL study period (from 83 at baseline to 93 beats per minute by the end of the experiment) and a decrease in the mean height of the T wave (due to 2 out of 4 animals) were noted although not being statistically different from controls. Also, a small increase in mean respiratory rate (from 15 at baseline till 18 breaths per minute), after administration of the top dose of MPL, was not considered to be physiologically relevant.

HPV-16/18 AS04 Human Papillomavirus type 16 and type 18 candidate vaccine adjuvanted with AS04. Cardiovascular and respiratory evaluation in the anaesthetized rat (b)(4)- 371/033059)

In a GLP compliant study, the possible side effects of the HPV-16/18 L1 VLP AS04 vaccine on cardiovascular and respiratory parameters in the anaesthetized (b)(4)- male (b)(4)- rats were investigated by -----(b)(4)-----, in 2004.

The (b)(4)- rat is considered a suitable species for this test and (b)(4)- has experience in the use of this species for such studies. In two parallel groups of 4 (b)(4)- rats, either phosphate buffered saline or the HPV-16/18 L1 VLP AS04 were administered intramuscularly at 0.1 ml/animal (1/5 of a human dose), the route of human exposure to the vaccine. The dose exceed intended human dose relative to the bodyweight of the rat by about 24-56-fold. Cardiovascular (blood pressure, heart rate, electrocardiogram (ECG)) and respiratory (respiration rate, tidal volume and minute, volume) parameters were monitored continuously for a period of two hours post vaccination.

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

Toxicology Summary and Overview

Pharmacological and toxicological studies were performed on HPV-16/18 L1 VLP AS04, AS04 as well as on MPL.

Safety pharmacology studies were performed on HPV-16/18 L1 VLP AS04 and MPL. The intramuscular administration of 1/5 of human dose (0.1 ml/animal) of HPV-16/18 L1 VLP AS04 in the anaesthetized -(b)(4)- male -(b)(4)- rat, or the intravenous administration of ascending MPL doses up to 100 µg/kg bodyweight (50-100-fold the human dose of MPL in the AS04 adjuvant) showed no treatment-related effects on any recorded cardiovascular or respiratory parameters.

Toxicology studies were performed with HPV-16/18 L1 VLP AS04, AS04 adjuvant, and MPL (Cervarix).

Four repeated-dose toxicity studies on the HPV-16/18 L1 VLP AS04 vaccine were carried out in rabbits and one in rats. These studies showed that the vaccine and adjuvant were well tolerated, and no consistent signs of systemic toxicity were observed. Injection site inflammation remained local and showed signs of resolution. Up to three times the full human vaccine dosage was used in these toxicity studies, possibly increasing this local reaction.

HPV-16/18 L1 VLP AS04 or the AS04 adjuvant was not associated with other side effects, except for a platelet drop observed 13 weeks after the fourth administration of the HPV-16/18 L1 VLP AS04 vaccine in rabbits one of the repeat dose rabbit studies (-(b)(4)-- Study 58678). No other findings related to this platelet count decrease were observed in the nonclinical studies and no such platelet drop was observed during clinical trials. In order to assess if the reduction in platelets observed 13 weeks after the last dose in -(b)(4)-- Study 58678 was a rabbit-specific effect, or due to the strength of the investigated dose, two additional studies were performed to evaluate the potential effects of a dose range of HPV-16/18 L1 VLP AS04 on hematology parameters in rabbits (-(b)(4)-- Study 62369) and rats (-(b)(4)- Study 62370). The results of the repeated dose studies with rabbits (-(b)(4)- Study 62369) and rats (-(b)(4)- Study 62370) indicate that the decrease in platelet counts observed in the original repeated dose rabbit study (-(b)(4)- Study 58678) was neither reproducible in the rabbit at two vaccine dosages, nor was it observed in the rat. It appears that this effect is not toxicologically significant

Genetic Toxicology studies were conducted with MPL using both *in vitro* and *in vivo* assays. All genetic toxicology assay results were not positive.

No other vaccine- or adjuvant-associated adverse systemic effect was noted, even though the studied dosages exceeded the human dosage (up to more than 50-fold factor on a body weight basis), as well as the immunization schedule.

In conclusion, the pharmacology and toxicology studies performed in dogs, rabbits and rats showed that CERVARIX induces a strong and persistent

specific immune response and is safe in animal models. The HPV-16/18 L1 VLP AS04 vaccine did not demonstrate signs of systemic toxicity. The only conclusive effects seen were local and transient, showing signs of recovery, and are expected from formulations that induce recruitment of inflammatory cells. Induced injection site reactions were the sign of the immune response generated against the vaccine antigens and enhanced by the adjuvant. These studies support the utilization of CERVARIX for human vaccination against HPV infections.

The scope of this review is confined to safety pharmacology and toxicology studies of the HPV 16/18 vaccine and genetic toxicology studies of the adjuvant, AS04 (MPL). The toxicology of the adjuvant was reviewed by Elizabeth Sutkowski, Ph.D. and the reproductive toxicology of the vaccine was reviewed by Marion Gruber, Ph.D.

2.6.7.1 Toxicology: Overview

Table 1 Test Article: HPV-16/18 L1 VLP AS04 vaccine

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses	GLP Compliance	Testing Facility	Study Number	Location ^(a)
Single-Dose Toxicity	ND							
Repeat-Dose Toxicity	Rabbit	im	Day 1, 15, 29, 43	Aluminium hydroxide (1) or HD HPV-16/18 L1 VLP AS04 (2) or 3xHD HPV-16/18 L1 VLP AS04 (3)	Yes		1513	Module 4.2.2.1
	Rabbit	im	Day 1, 15, 29, 43	0.5 ml NaCl 0.9% or Aluminium hydroxide (1) or 3xHD HPV-16/18 L1 VLP AS04 (3)	Yes		1735	Module 4.2.2.2
	Rabbit	im	Day 1, 15, 29, 57	0.5 ml NaCl 0.9% or HD AS04 (4) or HD HPV-16/18 L1 VLP AS04 (2)	Yes		55575	Module 4.2.2.3
	Rabbit	im	Day 1, 15, 29, 57	0.5 ml NaCl 0.9% or HD AS04 (4) or 1/10 HD HPV-16/18 L1 VLP AS04 (2) or HD HPV-16/18 L1 VLP AS04 (2)	Yes		52539	Module 4.2.2.4
	MS	im	Day 1, 15, 29, 57	0.5 ml NaCl 0.9% or HD AS04 (4) or HD HPV-16/18 L1 VLP AS04 (2)	Yes		52570	Module 4.2.2.5
Genotoxicity	ND							
Carcinogenicity	ND							
Reproductive and Developmental Toxicity	MS	im	Day - 30, 6, 8, 11, 15 (6)	100 µl NaCl 0.9% or 1/5 HD HPV-16/18 L1 VLP AS04 or 1/5 HD AS04 (5)	Yes		333150	Module 4.2.3.5.2
Local Tolerance	In Repeat-dose toxicity							
Other Toxicity Studies	ND							

im = intramuscular; ND = Not done. Saline = NaCl 0.9%, (1) 500 µg Aluminium hydroxide; (2) HD HPV-16/18 L1 VLP AS04 refers to one human dose HPV-16/18 L1 VLP AS04 vaccine containing 20 µg of each of HPV 16 and HPV 18 L1 VLPs, 50 µg MPL, 500 µg Aluminium hydroxide; (3) 3xHD HPV-16/18 L1 VLP AS04 refers to three times the human dose of HPV-16/18 L1 VLP AS04 vaccine, containing 60 µg of each of HPV 16 and HPV 18 L1 VLPs, 50 µg MPL, 500 µg Aluminium hydroxide; (4) HD AS04 refers to one human dose of AS04 (50 µg MPL, 500 µg Aluminium hydroxide); (5) 1/5 HD HPV-16/18 L1 VLP AS04 refers to one fifth of human dose of HPV-16/18 L1 VLP AS04 vaccine, containing 4 µg of each of HPV 16 and HPV 18 L1 VLPs, 10 µg MPL, 100 µg Aluminium hydroxide; 1/5 HD AS04 refers to one fifth of human dose of AS04 (10 µg MPL, 100 µg Aluminium hydroxide); (6) Compared to pairing day; (7)

(a) These sections are located in the CERVARIX™ part of module 4 Nonclinical study reports.

Materials used in the non-clinical safety pharmacology, pharmacokinetics and toxicological studies overall had comparable purity levels as the materials used for clinical studies and the materials proposed for marketing.

2.6.7.4 Toxicology: drug substance

Table 2 HPV-16 L1 VLP antigen and HPV-18 L1 VLP antigen components
Test Article: HPV-16/18 L1 VLP AS04 vaccine

	Drug Substance Antigen	Purity (% L1)	Impurities (In purified bulk)	Study Number	Type of Study
Impurity level in Clinical & consistency batches ⁽¹⁾	HPV-16 L1 VLP HPV-18 L1 VLP	> 95 % > 95 %		-	-
Batch No.					
20JUL99 MJA 1036/29	HPV-16 L1 VLP HPV-18 L1 VLP	NA		1513	Repeat-dose
99AH05AW	HPV-16 L1 VLP HPV-18 L1 VLP	> 95 % > 95 %		1758	Repeat-dose
EHPV002T	HPV-16 L1 VLP HPV-18 L1 VLP	> 95 % > 95 %		249/033160	Reproductive and developmental
DHPV005A9	HPV-16 L1 VLP HPV-18 L1 VLP	> 95 % > 95 %		58678	Repeat-dose
DHPVA017B	HPV-16 L1 VLP HPV-18 L1 VLP	> 95 % > 95 %		62369	Repeat-doses
				62370	

⁽¹⁾ Batches prepared during Phase Ib and Phase II development (clinical study HPV-001, HPV-008 and HPV-012 to HPV-016), including consistency batches proposed for marketing. ⁽²⁾ Note: HCP values for these batches were below the limit of quantification of the ELISA assay, which was initially reported as < 5%. Validation of the ELISA assay has later shown that the limit of quantification of assay is 0.5%, which is the value reported for these batches in the above table.
Abbreviation: NA: not available.

TOXICOLOGY

Toxicology Studies for Cervarix and MPL

Study name	Study type ⁽¹⁾	Test article	Location
1513	Repeat-Dose Toxicity	HPV-16/18 L1 VLP AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.7
1758	Repeat-Dose Toxicity	HPV-16/18 L1 VLP AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.7
58678	Repeat-Dose Toxicity	HPV-16/18 L1 VLP AS04 and AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.7
62339	Repeat-Dose Toxicity	HPV-16/18 L1 VLP AS04 and AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.7
62370	Repeat-Dose Toxicity	HPV-16/18 L1 VLP AS04 and AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.7
49/033160	Reproductive and Developmental Toxicity	HPV-16/18 L1 VLP AS04 and AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.13
DT127	Single-Dose Toxicity	MPL	MPL Module 2.6, Section 2.6.7, Item 2.6.7.5
3262.2	Repeat-Dose Toxicity	MPL	MPL Module 2.6, Section 2.6.7, Item 2.6.7.7
3262.4	Repeat-Dose Toxicity	MPL	MPL Module 2.6, Section 2.6.7, Item 2.6.7.7
3262.1	Repeat-Dose Toxicity	MPL	MPL Module 2.6, Section 2.6.7, Item 2.6.7.7
1729.3	Genotoxicity	MPL	MPL Module 2.6, Section 2.6.7, Item 2.6.7.8
1729.4	Genotoxicity	MPL	MPL Module 2.6, Section 2.6.7, Item 2.6.7.8
730/052195	Genotoxicity	MPL	MPL Module 2.6, Section 2.6.7, Item 2.6.7.9
1729.8	Reproductive & Developmental Toxicity	MPL	MPL Module 2.6, Section 2.6.7, Item 2.6.7.13
1729.7	Reproductive & Developmental Toxicity	MPL	MPL Module 2.6, Section 2.6.7, Item 2.6.7.13
1729.17	Reproductive & Developmental Toxicity	MPL	MPL Module 2.6, Section 2.6.7, Item 2.6.7.14

⁽¹⁾ All these studies were performed following GLP.

Table 1 Test Article: HPV-16/18 L1 VLP AS04 vaccine

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses	GLP Compliance	Testing Facility	Study Number	Location ⁽⁸⁾
Single-Dose Toxicity	ND							
Repeat-Dose Toxicity	Rabbit	Im	Day 1, 15, 29, 43	Aluminium hydroxide (1) or HD HPV-16/18 L1 VLP AS04 (2) or 3xHD HPV-16/18 L1 VLP AS04 (3)	Yes		1513	Module 4.2, Item 4.2.3.2.1
	Rabbit	Im	Day 1, 15, 29, 43	0.5 ml NaCl 0.9% or Aluminium hydroxide (1) or 3xHD HPV-16/18 L1 VLP AS04 (3)	Yes		1758	Module 4.2, Item 4.2.3.2.2
	Rabbit	Im	Day 1, 15, 29, 57	0.5 ml NaCl 0.9% or HD AS04 (4) or HD HPV-16/18 L1 VLP AS04 (2)	Yes		58678	Module 4.2, Item 4.2.3.2.3
	Rabbit	Im	Day 1, 15, 29, 57	0.5 ml NaCl 0.9% or HD AS04 (4) or 1/10 HD HPV-16/18 L1 VLP AS04 (2) or HD HPV-16/18 L1 VLP AS04 (2)	Yes		62339	Module 4.2, Item 4.2.3.2.4
	rats	Im	Day 1, 15, 29, 57	0.5 ml NaCl 0.9% or HD AS04 (4) or HD HPV-16/18 L1 VLP AS04 (2)	Yes		62370	Module 4.2, Item 4.2.3.2.5
Genotoxicity	ND							
Carcinogenicity	ND							
Reproductive and Developmental Toxicity	rats	Im	Day - 30, 6, 8, 11, 15(6)	100 µl NaCl 0.9% or 1/5 HD HPV-16/18 L1 VLP AS04 or 1/5 HD AS04 (5)	Yes		49/033160	Module 4.2, Item 4.2.3.5.2
Local Tolerance	In Repeat-dose toxicity							
Other Toxicity Studies	ND							

Im = intramuscular, ND = Not done; Saline = NaCl 0.9%, (1) 500 µg Aluminium hydroxide, (2) HD HPV-16/18 L1 VLP AS04 refers to one human dose HPV-16/18 L1 VLP AS04 vaccine containing 20 µg of each of HPV 16 and HPV 18 L1 VLPs, 50 µg MPL, 500 µg Aluminium hydroxide, (3) 3xHD HPV-16/18 L1 VLP AS04 refers to three times the human dose of HPV-16/18 L1 VLP AS04 vaccine, containing 60 µg of each of HPV 16 and HPV 18 L1 VLPs, 50 µg MPL, 500 µg Aluminium hydroxide, (4) HD AS04 refers to one human dose of AS04 (50 µg MPL, 500 µg Aluminium hydroxide), (5) 1/5 HD HPV-16/18 L1 VLP AS04 refers to one fifth of human dose of HPV-16/18 L1 VLP AS04 vaccine, containing 4 µg of each of HPV 16 and HPV 18 L1 VLPs, 10 µg MPL, 100 µg Aluminium hydroxide, 1/5 HD AS04 refers to one fifth of human dose of AS04 (10 µg MPL, 100 µg Aluminium hydroxide) (6) Compared to pairing day;

(8) These sections are located in the CERVARIX™ part of module 4: Nonclinical study reports.

Several non-clinical toxicological studies were performed on the HPV-16/18 vaccine. Four repeated-dose toxicity studies were carried out in rabbits and one in rats. Studies -(b)(4)-1513 and -(b)(4)- 58678 evaluated the local and systemic toxicity after a single and repeated dose (up to four) administration of the vaccine, the latter study allowing longer term and more exhaustive post-treatment evaluations. In addition, the hematology portion of -(b)(4)- 1513 was repeated in study -(b)(4)-1758 as procedural problems in the former study precluded interpreting all hematology parameters. In order to assess if a reduction in platelets observed 13 weeks after the last dose in -(b)(4)- Study No.58678 was a rabbit-specific effect, or due to the strength of the investigated dose, GSK Biologicals initiated two additional studies to evaluate potential effects of a dose range of HPV-16/18 L1 VLP AS04 on hematology parameters in rabbits (-(b)(4)- Study No. 62369) and rats (-(b)(4)- Study No. 62370).

Because Cervarix is intended for administration to women of childbearing potential, the potential toxicity on reproductive functions was studied. For this purpose, a pre- and post-natal toxicity study in rats, including an assessment of the potential impact of vaccination on fertility was performed. The protocol for the investigation was submitted to IND -(b)(4)- (Serial No. 0065, December 9, 2002). The safety of AS04 was also examined, in parallel to HPV-16/18 L1 VLP AS04, in a combined single-repeat dose toxicity study and in a reproductive study.

The systemic and local toxicity of HPV-16/18 L1 VLP AS04 and AS04, after a single or up to four injections were assessed, including toxicological endpoints during 4- to 13-week recovery periods. The treatment regimen of up to 4 vaccine injections at 2 week intervals with up to three times the human dosage exceeds the total number of intended clinical injections.

The first 3 repeat-dose toxicity studies revealed no consistent treatment-related or dose-dependent findings following hematology and clinical chemistry assessment, organ weight data, macroscopic pathology, and clinical sign evaluation. Injection site examination revealed evidence of erythema and edema across all treatment groups (including controls), which were however more severe in several vaccine-treated rabbits. 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 myofibers. Animals which solely received aluminum 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. myofiber regeneration) that were indicative of an ongoing process of recovery. The additional 2 repeat-dose studies in rats and rabbits revealed findings consistent with the above.

The hematology analysis revealed a higher value of neutrophils and fibrinogen after AS04 or vaccine administration, which may be a consequence of the recruitment of inflammatory cells following injections of the formulations.

In -(b)(4)- study 58678, a decrease in platelet counts (mean 36% below control value) was observed in the HPV-16/18 L1 VLP AS04 group, 13 weeks after the last of four repeated administrations in rabbits. No other findings related to this platelet count decrease were observed, neither in the bone marrow (central mechanism), nor in the reticulo-endothelial system (peripheral mechanism). The hematology profile and clinical signs of the treated animals were otherwise normal, and the biological relevance of this observation was therefore unknown. This effect appears not to be of toxicological significance, even though a treatment-related effect at the dose studied (the human vaccine dose) and in the rabbit can not be excluded.

To assess if the reduction in platelets was species-specific, or due to the dose used, GSK Biologicals initiated additional studies to evaluate potential effects of a dose range of HPV-16/18 L1 VLP AS04 on hematology parameters in both rabbits (-(b)(4)- Study 62369) and rats (-(b)(4)- Study 62370).

In the repeated-dose study with rabbits (-(b)(4)- Study 62369), the full human dose (1 HD) as well as 1/10 of a human dose (1/10 HD) of the vaccine were tested in order to verify the reproducibility and dose-dependency of the decrease in platelet counts observed in -(b)(4)- Study 58678. The study was performed in 40 -(b)(4)- albino female -----(b)(4)----- rabbits and included a 13-week recovery period. Animals were allocated randomly to 4 groups of 10 animals each, and received intramuscular injections in the paravertebral muscles on Days 1, 15, 29 and 57. The rabbits in Group 1 were dosed with 0.9% NaCl, those in Group 2 received AS04 alone, and those in Groups 3 and 4 were injected with HPV-16/18 L1 VLP AS04 1 HD or 1/10 HD, respectively. Except for the higher value of fibrinogen on Day 4 (Groups 2 and 3) and the higher value in neutrophils on Day 2 (Group 3), both of which correspond with expected inflammation due to the immune response, no additional treatment-related effects on hematological parameters were seen. The platelet counts were unaffected by treatment. The bone marrow smears showed no treatment-related findings.

Individual Toxicology Studies: Cervarix

2.6.7.5 Single-Dose Toxicity

Single-dose toxicity was assessed as part of repeat-dose toxicity Section 2.6.7.7 CERVARIX, Repeat dose toxicity) and is summarized below.

Test article: HPV-16/18 L1 VLP AS04

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Dose (µg) ¹	Gender and #/ Group	Observed maximum non- lethal dose	Approximate lethal dose	Noteworthy findings (after dose 1)
-(b)(4)- Rabbit	im (Phosphate buffered saline/solution)	40, 120	6 females/ group2	No lethality observed	No lethality observed	-
-(b)(4)- Rabbit	im (Phosphate buffered saline/solution)	40	5 females/ group3	No lethality observed	No lethality observed	Injection site reactions (hemorrhage, discolorat inflammation, Degen./regen./ necrotic myofibres)

No noteworthy findings. 1: HPV-16 L1 VLP + HPV-18 L1 VLP content. 2: Three groups for each of the Aluminum hydroxide, 40 µg vaccine and 120 µg vaccine treatments. Per treatment, one group with 2 doses (necropsy 2 days post dose 2) and 2 groups with 4 doses (necropsy 2 or 28 days post dose 4). 3: Three groups for each of the saline, AS04, and vaccine treatments. Per treatment, one group with 1 dose (necropsy 3 days post dose 1) and 2 groups with 4 doses (necropsy 3 days or 13 weeks post dose 4).

2.6.7.7 Repeat-Dose Toxicity Pivotal studies

2.6.7.7.A. MEDI-517/SBAS4: An intramuscular Immunotoxicity Study in -(b)(4)- ----- Rabbit

Test Article: HPV-16 /18 L1 VLP AS04 Vaccine **Study No:** -(b)(4)- 1513

Species/Strain: -(b)(4)- Rabbits **Dosing:** Day 1, 15 or 1, 15, 29, 43 **Location in CTD:** CERVARIX

Initial Age: 15-19 weeks of age **Duration of Postdose:** 28 days Module 4.2. Item 4.2.3.2.1

Date of First Dose: July 27, 1999 **Method of Administration:** intramuscular

Vehicle/Formulation: Phosphate buffered saline/solution **GLP Compliance:** yes

Special Features: Each formulation was injected to three groups of female rabbits. Groups 1, 4, 7: 2 doses/2-day observation.

Group 2, 5, 8: 4 doses/2 day observation. Group 3, 6, 9: 4 doses/28 day observation. Left and right thighs were injected.

No Observed Adverse Effect Level: Not determined

Dosage	Aluminium hydroxide 500 µg (2 or 4 doses)	HPV-16/18 L1 VLP AS04 40 µg (20 µg of each antigen, 2 or 4 doses) ^e	HPV-16/18 L1 VLP AS04 120 µg (60 µg of each antigen, 2 or 4 doses) ^f
Number of Animals	Females: 18	Females: 18	Females: 18
Toxicokinetics: AUC ()	ND	ND	ND
<u>Noteworthy findings</u>			
Died or Sacrificed Moribund	0	0	0
Body Weight (% ^a)	3.9 kg (100)	(101)	(100)
Food Consumption Day 43-44 (% ^a)	183 g (100)	(101)	(101)
Water Consumption	ND	ND	ND
Ophthalmoscopy	ND	ND	ND
Electrocardiography	ND	ND	ND
Clinical Observations	-	-	-

- No noteworthy findings; + Mild ++ Moderate +++ Marked; ND= not done; * p<0.05 ** - p<0.01; NS = Not statistically significant; a: end of 4-dose period; b: L: left thigh R: right thigh; c: Groups 2, 5, 8. Six rabbits per group; d: Groups 3, 6, 9. Six rabbits per group; e: once the full human dosage; f: three-fold the human dosage. For controls, group means are shown; For treated groups, percent relative to controls are shown.

Dosage	Aluminium hydroxide 500 µg	HPV-16/18 L1 VLP AS04 vaccine 40 µg ^e (20 µg of each antigen, 2 or 4 doses)	HPV-16/18 L1 AS04 VLP vaccine 120 µg ^f (60 µg of each antigen, 2 or 4 doses)
	<u>Females:</u>	<u>Females:</u>	<u>Females:</u>
Number of Animals	18	18	18
Urinalysis	ND	ND	ND
Serum Chemistry	-	-	-
Organ Weights ^a (%)	-	-	-
Gross Pathology:			
- Injection site erythema	- to +	- to +++	- to +++
- Injection site edema	-	- to ++	- to ++
- Limb use impairment/mouse examined	0/18	1/18	0/18
Histopathology:			
- No. injection site granulomatous inflammation ^{a,c}	2L + 1R	0L + 1R	2L + 0R
- No. injection site perivasculitis ^{a,c}	0L + 1R	2L + 0R	4L + 2R
Postdose Evaluation:			
Number rabbit Evaluated	6	6	6
- No. injection site granulomatous inflammation ^{a,d}	0L + 0R	1L + 2R	2L + 0R
- No. Injection site perivasculitis ^{a,d}	0L + 0R	0L + 0R	0L + 0R

- No noteworthy findings; + Mild ++ Moderate +++ Marked; ND= not done; * p<0.05 ** p<0.01; NS = Not statistically significant; a: end of 4-dose period; b: L: left thigh R: right thigh; c: Groups 2, 5, 8. Six rabbits per group; d: Groups 3, 6, 9. Six rabbits per group; e: once the full human dosage; f: three-fold the human dosage.
For controls, group means are shown; For treated groups, percent relative to controls are shown.

This study was carried out to assess the toxicity of a human papilloma virus vaccine, MEDI-5 17/SBAS4, when administered intramuscularly to female -----(b)(4)----- rabbits on up to 4 dose, at 2 week intervals, followed by an observation period after the last dose.

The test article or control/vehicle article (aluminum hydroxide; 1 mg/mL) was administered to 54 female rabbits (9 groups with 6 animals/group), as follows: Group 1 received 2 doses of Control/vehicle, followed by a 2 day observation period; Group 2 received 4 doses of control/vehicle, followed by a 2 day observation period; Group 3 received 4 doses of control/vehicle, followed by a 28 day observation period; Group 4 received 2 doses of 40 pg MEDI-517/SBAS4 (Low Dose), followed by a 2 day observation period; Group 5 received 4 doses of Low Dose test article, followed by a 2 day observation period; Group 6 received 4 doses of Low Dose test article, followed by a 28 day observation period; Groups 7, 8 and 9 underwent the same treatment as Groups 4, 5 and 6, respectively, except 120 pg MEDI-5 17/SBAS4 (High Dose) was administered at each injection. For all groups, the site of administration alternated between the right and left thigh for each injection.

Mortality was monitored twice daily during all phases of the study. During the treatment and observation periods, clinical signs (ill health, behavioral changes etc.) were evaluated at cage-side twice daily and a clinical examination of each animal was performed once weekly. In addition, the injection site(s) were examined daily for evidence of erythema, edema and limb use impairment, and the degree of erythema and edema was graded using the Draize scoring system.

Body weights were recorded for all animals once prior to initiation of treatment, and during the treatment and observation periods, were recorded weekly and prior to necropsy. Individual daily food consumption was recorded for all animals during the

pretreatment period and throughout the treatment/observation period. Hematology and clinical chemistry investigations were performed on each animal once during the pretreatment period and on all animals terminally, and each rabbit was then euthanized and subjected to a comprehensive necropsy, which included organ weight measurements.

Following necropsy, histopathological examination was performed on all injection sites (muscle tissue and its draining lymph nodes), gross lesions, liver, lungs, spleen, skin and subcutis, and thymus from all study animals.

There were no deaths on the study. Evaluation of the injection sites using the Draize scoring method showed erythema and edema in several rabbits, across all treatment groups, which in most cases was graded as very slight to slight. Moderate to severe erythema and moderate edema was recorded in a few MEDI-517/SBAS4-treated animals, and transient limb use impairment was noted in one test article-treated rabbit, however no dose-dependent trends were noted. For all animals, body weights and food consumption were considered normal for the duration of the study.

Intramuscular administration of the human papilloma virus vaccine, MEDI-517/SBAS4, to female -----(b)(4)----- rabbits resulted in erythema and edema at the injection sites in several rabbits, across all treatment groups, which in most cases was graded as very slight to slight. Moderate to severe erythema and moderate edema was recorded in a few MEDI-5 17/SBAS4-treated animals, and transient limb use impairment was noted in one test article-treated rabbit.

Hematology assessment showed reductions in terminal group means for white blood cells (WBC) relative to pretreatment group mean values in all treatment groups, including the control vehicle-treated groups. WBC differentials showed that mean neutrophil (NEUT), lymphocyte (LYMPH), monocyte (MONO), eosinophil (EOS) and basophil (BASO) counts were depressed in all groups at termination, relative to pretreatment. However, these findings were considered to be procedure-related (i.e. related to the site of blood sampling), and not due to effects of the control/vehicle or test articles. Histopathology examinations revealed evidence of inflammation in the subcutis and/or muscle at the sites of administration in MEDI-517/SBAS4 Low and High Dose groups following treatment, and after a 28-day observation period. There was no clear relationship between the incidence of the findings and the dose levels of MEDI-5 17/SBAS4. Animals which received control/vehicle article also showed evidence of inflammation at the injection sites, however the inflammation was less extensive and of shorter duration than that found in the test article-treated rabbits. Overall, intramuscular administration of MEDI-517/SBAS4, at dose levels of 40 and 120 pg/rabbit/dose, injected two or four times at 2 week intervals followed by an observation period after the last dose, was well tolerated in female -----(b)(4)----- rabbits.

**2.6.7.7.B MEDI-517/SBAS4: An intramuscular immunotoxicity study in -(b)(4)-
----- Rabbits**

Test Article: HPV-16 /18 L1 VLP AS04 Vaccine **Study No.** -(b)(4)- 1758

Species/Strain: -(b)(4)- Rabbits **Dosing:** Day 1, 15, 29, 43 **Location in CTD:** CERVARIX

Initial Age: ~ 16 weeks of age **Duration of Postdose:** 16 days Module 4.2. Item 4.2.3.2.2

Date of First Dose: March 7, 2000 **Method of Administration:** intramuscular

Vehicle/Formulation: Phosphate buffered saline/solution **GLP Compliance:** yes

Special Features: This study was conducted to evaluate hematology parameters that could not be evaluated in Study No -(b)(4)- 1513 due to procedure-related issue.

Laboratory investigation schedule: pre-treatment, days 3, 17, 31, 45, and terminally (day 59)

No Observed Adverse Effect Level: Not determined

Dosage	Saline control (4 doses)	Aluminium hydroxide 500 µg (4 doses)	HPV-16/18 L1 VLP AS04 vaccine 120 µg ^b (60 µg of each antigen 4 doses)
	<u>Females:</u>	<u>Females:</u>	<u>Females:</u>
Number of Animals	5	5	5
Toxicokinetics: AUC ()	ND	ND	ND
Noteworthy findings:			
Died or Sacrificed Moribund	0	0	0
Body Weight (% ^a)	4.1 kg (100)	(98)	(99)
Food Consumption (% ^a)	ND	ND	ND
Water Consumption	ND	ND	ND
Clinical Observations	-	-	-
Ophthalmoscopy	ND	ND	ND
Electrocardiography	ND	ND	ND

-: No noteworthy findings; + Mild ++ Moderate +++ Marked; ND= not done; * p<0.05 ** -p<0.01; NS = Not statistically significant; a: At end of dosing period; b: three-fold the human dosage; For controls, group means are shown; For treated groups, percent relative to controls are shown.

Dosage	Saline control (4 doses)	Aluminium hydroxide 500 µg (4 doses)	HPV-16/18 L1 VLP AS04 vaccine 120 µg ^b (60 µg of each antigen 4 doses)
	<u>Females:</u>	<u>Females:</u>	<u>Females:</u>
Number of Animals	5	5	5
Hematology	-	-	-
Serum Chemistry	ND	ND	ND
Urinalysis	ND	ND	ND
Organ Weights (%)	ND	ND	ND
Gross Pathology	ND	ND	ND
Postdose Evaluation:			
Number Evaluated	5	5	5
Bone Marrow smear	-	-	-

-: No noteworthy findings; + Mild ++ Moderate +++ Marked; ND= not done; * p<0.05 ** -p<0.01; a: At end of dosing period; b: three-fold the human dosage; For controls, group means are shown; For treated groups, percent relative to controls are shown.

This study was carried out to assess the potential toxic effects of MEDI-SI71SBAS4 on hematology parameters, when administered intramuscularly to -----(b)(4)----- rabbits on 4 doses at 2 week intervals, followed by a 16-day observation period. Due to a technical problem in a previous study (-(b)(4)- Study No. 1513) reliable hematology

parameters were not obtained, so the hematology section of the study was repeated and is presented here.

The test, vehicle (aluminum hydroxide; 1 mg/mL) or control (saline) article was administered to 15 female rabbits (3 groups with 5 animals/group), as follows: Group 1 received 4 injections of control article, Group 2 received 4 injections of vehicle article, and Group 3 received 4 doses of 120 pg MEDI-5 17/SBAS4. For all groups, the dose volume was 0.5 ml/injection/rabbit, and the site of administration alternated between the right and left thigh for each injection.

Mortality was monitored twice daily during all phases of the study. During the treatment and observation periods, clinical signs (ill health, behavioral changes etc.) were evaluated at cage-side twice daily, and a detailed clinical examination of each animal was performed once weekly. Body weights were recorded for all animals once prior to initiation of treatment, and during the treatment and observation periods, were recorded weekly and prior to necropsy. Hematology investigations were performed on each animal: once during the pretreatment period, 2 days following each treatment (i.e. Days 3, 17, 31, 45), and on completion of the observation period (terminally on Day 59). Terminal procedures included preparation and evaluation of bone marrow smears for each animal.

There were no deaths on the study. For all animals, clinical signs and body weights appeared normal for the duration of the study. Hematology assessment showed no treatment- or dose-dependent effects on any parameter. Evaluation of bone marrow smears showed no changes due to treatment.

Following intramuscular administration of the human papilloma virus vaccine, MEDI-5 17/SBAS4, to female -----(b)(4)----- rabbits at a dose level of 120 pg/rabbit/dose (injected four times at 2 week intervals), no mortalities occurred, and clinical signs and body weights were considered normal for -----(b)(4)----- rabbits. Hematology assessment, and evaluation of bone marrow smears, showed no treatment-related effects.

2.6.7.7.C. HPV pro/AS04D prophylactic Human Papilloma virus type 16 and type 18 candidate vaccine adjuvanted with AS04D (HPV pro 16/18 /AS04D). Repeated dose (4 times) toxicity in rabbits

Test Article: HPV-16 /18 L1 VLP AS04 or AS04 **Study No.** -(b)(4)- 58678

Species/Strain: -(b)(4)- Rabbits **Dosing:** Day 1, 15, 29, 57 **Location in CTD:** CERVARIX

Initial Age: 10-14 weeks of age **Duration of Postdose:** 91d post dose 4 Module 4.2 Item 4.2.3.2.3

Method of Administration: intramuscular

Date of First Dose: March 7, 2005 **Vehicle/Formulation:** Phosphate buffered saline/solution **GLP Compliance:** yes

Special Features: animal subsets were sacrificed 3d post dose 1, 3 day post dose 4, 13 weeks post dose 4

No Observed Adverse Effect Level: Not determined (no full dose range performed as not relevant to vaccine)

Dosage	Saline control (4 doses)	AS04 50 µg MPL, 500 µg Aluminium hydroxide (4 doses)	HPV-16/18 L1 VLP AS04 vaccine ^c 20 +20 µg L1 VLP antigens (4 doses)
	<u>Females:</u>	<u>Females:</u>	<u>Females:</u>
Number of Animals	15	15	15
Toxicokinetics: AUC ()	ND	ND	ND
Noteworthy findings			
Died or Sacrificed Moribund	0	0	0
Body Weight (%) ^a	3,992 kg (100)	(98) NS	(94) NS
Food Consumption – total (%) ^a	10,223 g (100)	(103) NS	(98) NS
Water Consumption	ND	ND	ND
Clinical Observations	-	-	-
Ophthalmoscopy	-	-	-
Electrocardiography	ND	ND	ND
Body Temperature	-	-	-

- : No noteworthy findings; + : Mild ++ : Moderate +++ : Marked; ND = not done; * p<0.05 ** - p<0.01; NS = Not statistically significant; a: At end of 4-dose period (Day 60); b: 91 days post dose 4. c: once the full human dosage; For controls, group means are shown; For treated groups, percent relative to controls are shown; Statistical significance is based on actual data (not on the percent differences). Statistical analysis: Bartlett's test, Dunnett's test, the Shapiro-Wilk method, Student's t-test, Kruskal-Wallis test, Wilcoxon Rank-Sum test

Study No. (b)(4) 58678 (continued)

Dosage	Saline control (4 doses)	AS04 - 50 µg MPL, 500 µg Aluminium hydroxide (4 doses)	HPV-16/18 L1 VLP AS04 vaccine 20 + 20 µg L1 VLP antigens (4 doses)
	Females:	Females:	Females:
Number of animals	15	15	15
Hematology: Platelet count x10 ⁹ /L			
End of the dosing period (%)	231 (102)	(92)	(86)
End of the post dose period (%)	215 (102)	(92)	(36) ^a
Serum Chemistry	-	-	-
Urinalysis	ND	ND	ND
Organ Weights ^a (%)	-	-	-
Gross Pathology (No. injection sites = 5)			
No. injection site hemorrhage ^a			
+	2	2	5
++	1	1	0
Total	3	3	5
No. injection site discoloration ^a	0	4	5
Histopathology (No. injection sites = 5)			
Inflammation, focal, subacute ^a			
+	0	4	1
++	0	0	4
Total	0	4	5
Degen./regen./necrotic myofibers, focal ^a			
+	0	3	1
++	0	1	3
+++	0	0	1
Total	0	4	5

- No relevancy findings; - Mild -- Moderate --- Marked ND= not done, * p<0.05 ** p<0.01; a: At end of 4-dose period (Day 60); b: 91 days post dose; c: once the full human dosage; d: For controls, group means are shown; For treated groups, percent relative to controls are shown; Statistical significance is based on actual data (not on the percent differences); Statistical analysis: Bartlett's test, Dunnett's test, the Shapiro-Wilk method, Student's t-test, Kruskal-Wallis test, Wilcoxon Rank-Sum test.

Study No. (b)(4) 58678 (Continued)

Dosage	Saline control (4 doses)	AS04 - 50 µg VP-, 500 µg Aluminium hydroxide (4 doses)	HPV-16/18 L1 VLP AS04 vaccine 120 µg antigen (4 doses)
Postdose Evaluation ^a :			
Gross Pathology (No. injection sites = 5)			
No. injection site discoloration	0	2	4
Histopathology (No. injection sites = 5)			
Inflammation, focal, subacute - Total	0	0	1 (+)
Regenerating myofibers, focal - Total	0	2	4

- No relevancy findings; - Mild -- Moderate --- Marked ND= not done, * p<0.05 ** p<0.01; a: At end of 4-dose period (Day 60); b: 91 days post dose; c: For controls, group means are shown; For treated groups, percent relative to controls are shown; Statistical significance is based on actual data (not on the percent differences); Statistical analysis: Bartlett's test, Dunnett's test, the Shapiro-Wilk method, Student's t-test, Kruskal-Wallis test, Wilcoxon Rank-Sum test.

Four intramuscular injections of 1 human dose (approximately 0.5 ml) of HPV pro16/18/AS04D prophylactic Human Papilloma virus type 16 and type 18 candidate vaccine adjuvanted with AS04D(HPV pro 16/18/AS04D) to rabbits resulted mainly in the change of a few hematological parameters and in a local reaction at the injection site. No systemic toxic effect was detected.

The changes in neutrophils and fibrinogen translate the inflammatory process due to the expected immune response induced by the treatment.

On Day 148, the females in Group 3 had a statistically significantly lower amount of platelets when compared to Group 1. The hematology profiles of the animals was normal and there was no evidence from other examinations (clinical signs, histopathology and bone marrow smears) It appears as a possibly treatment-related effect without other correlation as the last dose occurred on day 57.

Microscopically, no signs of systemic toxicity were observed in this study. The only

treatment-related change recorded was a local reaction at the intramuscular injection sites.

The type of the local reaction noted immediately after a single injection (Day 4), i.e. sub-acute inflammation with necrosis/degeneration/regeneration of myofibers, was similar for both vaccine and adjuvant. When the material is given on several occasions, the type of local reaction seen immediately after the last injection (Day 60) was similar than after the first injection but the severity and the incidence were slightly higher with the vaccine than with the adjuvant. Examination after 13 weeks post 4th intramuscular injection (Day 148) showed evidence of histological changes (i.e. myofiber regeneration) of partial recovery.

2.6.7.7.D. HPV-16/18 L1 AS04 Human Papilloma virus candidate vaccine. Repeated dose (4 times) toxicity in rabbits by intramuscular route

Test Article: HPV-16/18 L1 VLP AS04 Study No. LAB -(b)(4)- 62369

Species/Strain: -----(b)(4)----- **Dosing:** Day 1, 15, 29, 57

Initial age: 1.8-2.2 kg (at arrival) **Duration of Postdose:** 3 days or 13 weeks post IV

Location in CTD: CERVARIX Module 4.2 Section 4.2.3.2.4

Method of Administration: intramuscular

Date of First Dose: March 13 and 14, 2006 **Vehicle/Formulation:** Phosphate buffered saline/solution **GLP Compliance:** yes

Special Features: animal subsets were sacrificed 3 day post IV, 13 weeks post IV

No observed Adverse Effect Level: Not determined (no full dose range performed as not relevant to vaccine)

Dosage	Saline control (4 doses)	AS04 50 µg MPL, 500 µg aluminium hydroxide (4 doses)	HPV-16/18 L1 VLP AS04 vaccine - 1 HD 20 µg HPV-16 L1 VLP, 20 µg HPV-18 L1 VLP, 50 µg MPL, 500 µg aluminium hydroxide (4 doses)	HPV-16/18 L1 VLP AS04 vaccine - 1/10 HD 2 µg HPV-16 L1 VLP, 2 µg HPV-18 L1 VLP, 5 µg MPL, 50 µg aluminium hydroxide in 50 µl (4 doses)
	<u>Females:</u>	<u>Females:</u>	<u>Females:</u>	<u>Females:</u>
Number of Animals	10	10	10	10
Toxicokinetics: AUC (I)	ND	ND	ND	ND
Noteworthy findings				
Died or Sacrificed Moribund	1	1	0	0
Body Weight (%)	4325.6 g (100)	(97) NS	(97) NS	(96) NS
Food Consumption	ND	ND	ND	ND
Water Consumption	ND	ND	ND	ND
Clinical Observations	-	-	-	-

-: No noteworthy findings; - Minimal -- Slight --- Moderate ---- Marked; ND= not done; * p<0.05 ** p<0.01; NS = Not statistically significant; a: At end of dosing period. For controls, group means are shown; For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). Statistical analysis: Levene's test, Dunnett's test, the Sheppards/Wilk method, Student's t-test, Kruskal-Wallis test, Wilcoxon Rank-Sum test.

Study No. (b)(4) 62369 (continued)

Dosage	Saline control (4 doses)	AS04 50 µg MPL, 500 µg aluminium hydroxide (4 doses)	HPV-16/18 L1 VLP AS04 vaccine - 1 HD 20 µg HPV-16 L1 VLP, 20 µg HPV-18 L1 VLP, 50 µg MPL, 500 µg aluminium hydroxide (4 doses)	HPV-16/18 L1 VLP AS04 vaccine - 1/10 HD 2 µg HPV-16 L1 VLP, 2 µg HPV-18 L1 VLP, 5 µg MPL, 50 µg aluminium hydroxide in 50 µl (4 doses)
	<u>Females:</u>	<u>Females:</u>	<u>Females:</u>	<u>Females:</u>
Number of animals	10	10	10	10
Ophthalmoscopy	ND	ND	ND	ND
Electrocardiography	ND	ND	ND	ND
Body Temperature	ND	ND	ND	ND
Hematology:				
Neutrophils abs - Day 2	2.15 (100)	(105) NS	(138)*	(105) NS
Fibrinogen - Day 4	2.02 (100)	(129)*	(129)*	(124) NS
Serum Chemistry	ND	ND	ND	ND
Urinalysis	ND	ND	ND	ND
Bone Marrow smears	-	ND	-	ND

-: No noteworthy findings; - Minimal -- Slight --- Moderate ---- Marked; ND= not done; * p<0.05 ** p<0.01; NS = Not statistically significant; a: At end of dosing period. For controls, group means are shown; For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). Statistical analysis: Levene's test, Dunnett's test, the Sheppards/Wilk method, Student's t-test, Kruskal-Wallis test, Wilcoxon Rank-Sum test.

Study No. 61369 (continued)

Dosage	Saline control (2 doses)	AS04 50 µg MPL, 500 µg aluminium hydroxide (4 doses)	HPV-16/18 L1 VLP AS04 vaccine 20 µg HPV-16 L1 VLP, 20 µg HPV-18 L1 VLP, 50 µg MPL, 500 µg aluminium hydroxide (4 doses)	HPV-16/18 L1 VLP AS04 vaccine 2 µg HPV-16 L1 VLP, 2 µg HPV-18 L1 VLP, 5 µg MPL, 50 µg aluminium hydroxide in 50 µl (4 doses)
Number of animals	Females: 10	Females: 10	Females: 10	Females: 10
Organ Weights (%)	-	-	-	-
Gross Pathology				
Injection sites: slight yellow discoloration				
Day 1	0	4	2	0
Day 15	0	3	4	0
Day 29	0	4	3	0
Day 57	0	2	2	0
Histopathology 13 weeks after last injection				
Injection sites				
Interstitial inflammatory cell				
+	0	0	1	1
Total	0	0	1	1
Macrophages/vacuolated cells				
+	0	0	2	0
Total	0	0	2	0
Necrotic myofibers				
+	0	1	0	0
Total	0	1	0	0

0 = no noteworthy findings; + = Minimal; - = Slight; ++ = Moderate; +++ = Marked; N/D = not done; * p < 0.05; ** p < 0.01; *** p < 0.001; a = all end of dosing period; b = 13w post IV, 13 weeks (13 days weight); For controls group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on exact data (not on the percent differences). Statistical analysis: Levene's test, Dunnett's test, the Shapiro-Wilk method, Student's T-test, Kruskal-Wallis test, Wilcoxon Rank-Sum test.

In a previous repeated dose tox study in rabbit evaluating HPV-16/18 L1 AS04 (LAB (b)(4) Study No 58678), a drop in the platelet count was observed at the end of the recovery period. The purpose of the current study is to verify the reproducibility of this potential effect by assessing two different dose levels of the vaccine. The study included a 13-week recovery period, all the animals being killed on Day 148/149.

The study was performed in 40 (b)(4) albino female rabbits of the stock (b)(4) rabbits from (b)(4)

The animals were allocated randomly to 4 groups each of 10 animals. The animals received intramuscular injections in the paravertebral muscles on Days 1, 15, 29 and 57.

The animals in Group 1 were dosed with 0.9% NaCl and the animals in Group 2 with AS04. The animals in Groups 3 and 4 were treated with HPV-16/18 L1 AS04 receiving a full human dose (1 HD) or 1/10 of the human dose (1/10 HD) per injection respectively.

Clinical signs, body weight, haematology, organ weight, macroscopical and microscopical examinations were used as criteria to disclose any side adverse effect.

No treatment-related effect was seen on clinical signs, the body weight and body weight gain.

Except for the higher value of Fibrinogen on Day 4 (Groups 2 and 3) and the higher value in neutrophils on Day 2 (Group 3), which corresponded with the expected inflammatory process due to the immune response induced by the vaccine, no additional treatment-related effect was seen on the haematological parameters. The platelet counts were not affected by the treatment.

The bone marrow smear examination of the animals revealed no treatment-related findings and no adverse findings were seen on the organ weights.

Macroscopic treatment-related findings such as yellow discolouration were reported in all 4 injection sites in rabbits which had received AS04 or HPV-16/18 L1 AS04 1 HD.

Four intramuscular injections of 1 human dose or 1/10 of the human dose of the HPV-16/18 L1 AS04 candidate vaccine to rabbits had no effect on platelet counts. For this reason the original finding in LAB -(b)(4)- Study No 58678 appears to be not reproducible. Treatment resulted in a slight change of hematological parameters

Platelet counts were the main parameter investigated in this study. At none of the blood sampling time points was a statistically significant decrease, or a trend towards decrease observed in the platelet counts in any of the vaccine or adjuvant treated groups compared to the control group. When the platelet counts were below 150, a re-sample was performed, and it was confirmed that in two subsequent blood samples, collected from the same animal within a short time frame, the platelet counts showed variability

Local reaction at the injection site which was most pronounced in the group receiving the HPV-16/18 L1 AS04 candidate vaccine full HD per injection site. The observed changes were expected and considered to be related to the immune response induced by the vaccine. No signs of systemic toxicity were observed.

2.6.7.7.E. HPV-16/18 L1 AS04 Human Papilloma virus candidate vaccine. Repeated dose (4 times) toxicity in rats by intramuscular route

Test Article: : HPV-16/18 L1 AS04 Study No. LAB -(b)(4)- 62370

Species/Strain: -(b)(4)- rats **Dosing:** Day 1, 15, 29, 57

Initial weight: 211-255 g (at arrival) **Duration of Postdose:** 3 days or 13 weeks post IV

Location in CTD:

CERVARIX Module 4.2

Section 4.2.3.2.5

Method of Administration: intramuscular

Date of First Dose: February 28, 2006 **Vehicle/Formulation:** Phosphate buffered saline/solution

GLP Compliance: yes

Special Features: animal subsets were sacrificed 3 day post IV, 13 weeks post IV

No observed Adverse Effect Level: 0.1 ml (equal to 1/5 HD) dosed 4 times intramuscularly

Study No. (b)(4) 62370 (continued)

Dosage	Saline control (4 doses)	AS04 10 µg MPL, 100 µg aluminium hydroxide in 100 µl (4 doses)	HPV-16/18 L1 VLP AS04 vaccine 4 µg HPV-16 L1 VLP, 4 µg HPV-18 L1 VLP, 10 µg MPL, 100 µg aluminium hydroxide in 100 µl (4 doses)
	Females:	Females:	Females:
Number of Animals	20	20	20
Toxicokinetics: AUC ()	ND	ND	ND
Noteworthy findings			
Died or Sacrificed Moribund	0	0	0
Body Weight (%)	282.8 g (100)	(102) NS	(103) NS
Food Consumption (%)	2397.6 g (100)	(109) NS	(110) NS
Water Consumption	ND	ND	ND
Clinical Observations	-	-	-
Dermal reactions	-	-	-
Ophthalmoscopy	-	-	-
Electrocardiography	ND	ND	ND
Body Temperature	-	-	-

* No noteworthy findings. * Unremarkable. ** Slight. *** Moderate. **** Marked. ND = not done; † p<0.05; ** p<0.01; *** p<0.001; NS = not statistically significant; a, b, c and c' dosing period: b: 13w post IV. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). Statistical analysis: Levene's test, Dunnett's test, the Scheffé-Wilk method, Student's t-test, Kruskal-Wallis test, Wilcoxon Rank-Sum test.

Study No. (b)(4) 62370 (continued)

Dosage	Saline control (4 doses)	AS04 10 µg MPL, 100 µg aluminium hydroxide in 100 µl (4 doses)	HPV-16/18 L1 VLP AS04 vaccine 4 µg HPV-16 L1 VLP, 4 µg HPV-18 L1 VLP, 10 µg MPL, 100 µg aluminium hydroxide in 100 µl (4 doses)
	<u>Females:</u> 20	<u>Females:</u> 20	<u>Females:</u> 20
Number of animals			
Hematology:			
Neutrophils abs - Day 4	1.03 (100)	(150) ^a	(150) ^a
Neutrophils % - Day 4	11.4 (100)	(144) ^{NS}	(145) ^{NS}
Neutrophils abs - Day 60	0.79 (100)	(156) ^{NS}	(176) ^{NS}
Neutrophils % - Day 60	13.0 (100)	(159) ^{NS}	(148) ^a
Fibrinogen - Day 4	2.56 (100)	(134) ^{NS}	(135) ^{NS}
Fibrinogen - Day 55	1.63 (100)	(112) ^{NS}	(114) ^a
Fibrinogen - Day 60	1.94 (100)	(202) ^{NS}	(171) ^{NS}
Fibrinogen - Day 146	1.80 (100)	(108) ^{NS}	(117) ^a
Lymphocytes % - Day 4	87.7 (100)	(94) ^a	(94) ^a
Lymphocytes abs - Day 60	5.11 (100)	(99) ^{NS}	(128) ^{NS}
Lymphocytes % - Day 60	85.9 (100)	(92) ^{NS}	(93) ^a
Serum Chemistry			
Globulin - Day 4	23.5 (100)	(114) ^a	(106) ^{NS}
Globulin - Day 60	23.5 (100)	(117) ^{NS}	(118) ^{NS}
Albumin - Day 4	50.9 (100)	(92) ^{NS}	(95) ^{NS}
Albumin - Day 60	52.3 (100)	(91) ^{NS}	(91) ^{NS}
Albumin/Globulin ratio Day 4	2.17 (100)	(81) ^{NS}	(90) ^a
Albumin/Globulin ratio Day 60	2.25 (100)	(78) ^{NS}	(76) ^{NS}
Urinalysis	ND	ND	ND

- : No noteworthy findings; - Minimal -- Slight --- Moderate ---- Marked; ND = not done; * p<0.05 ** p<0.01; NS = Not statistically significant; a: At end of dosing period; b: 13w post IV. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). Statistical analysis: Levene's test, Dunnett's test, the Shapiro-Wilk method, Student's t-test, Kruskal-Wallis test, Wilcoxon Rank-Sum test.

Study No. (b)(4) 62370 (continued)

Dosage	Saline control (4 doses)	AS04 10 µg MPL, 100 µg aluminium hydroxide in 100 µl (4 doses)	HPV-16/18 L1 VLP AS04 vaccine 4 µg HPV-16 L1 VLP, 4 µg HPV-18 L1 VLP, 10 µg MPL, 100 µg aluminium hydroxide in 100 µl (4 doses)
	<u>Females:</u> 20	<u>Females:</u> 20	<u>Females:</u> 20
Number of animals			
Organ Weights (% Day 60)			
Spleen	0.212	0.230	0.245 ^a
Organ Weights (% Day 148/149)			
Gross Pathology			
Histopathology 3 days after last inj.			
<u>Diagnoses</u>			
Inflammation, focal, subacute			
-	4	3	1
--	0	2	2
---	0	1	2
----	0	0	4
Total	4	5	9
Degeneration, necrosis, myofibers, focal			
-	0	0	1
--	0	3	1
---	0	0	2
----	0	0	3
Total	0	3	7
Hemorrhage, focal			
-	0	1	4
Total	0	1	4
<u>Rectal lymph nodes</u>			
Hyperplasia			
-	0	1	4
Total	0	1	4

- : No noteworthy findings; - Minimal -- Slight --- Moderate ---- Marked; ND = not done; * p<0.05 ** p<0.01; a: At end of dosing period; b: 13w post IV, c: Relative (% body weight); For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). Statistical analysis: Levene's test, Dunnett's test, the Shapiro-Wilk method, Student's t-test, Kruskal-Wallis test, Wilcoxon Rank-Sum test.

Dosage	Saline control (4 doses)	AS04 10 µg MPL, 100 µg aluminium hydroxide in 100 µl (4 doses)	HPV-16/18 L1 VLP AS04 vaccine 4 µg HPV-16 L1 VLP, 4 µg HPV-18 L1 VLP, 10 µg MPL, 100 µg aluminium hydroxide in 100 µl (4 doses)
Number of animals	Females: 20	Females: 20	Females: 20
<u>Pre/post lymph nodes</u> Hyperplasia			
+/+	0	2	8
Total	0	2	8
Histopathology 13 weeks after last injection			
Injection site			
Accumulation of macrophages			
+	0	0	3
Total	0	0	3
<u>Pre/post lymph nodes</u> Hyperplasia			
+	0	5	3
Total	0	5	3

+: No noteworthy finding; -: Minimal; ++: Slight; +++: Moderate; ++++: Marked; ND = not done; * p<0.05; ** p<0.01; a: At end of dosing period; b: 13w post IV; c: Relative (% body weight); For controls, group means are shown; For treated groups, percent differences from controls are shown; Statistical significance is based on actual data (not on the percent difference); Statistical analysis: Levene's test, Dunnett's test, the Shapiro-Wilk method, Student's t-test, Kruskal-Wallis test, Wilcoxon Rank-Sum test

The objective of this study was to evaluate the potential local and/or systemic toxic effects induced by repeated (4 times) intramuscular injections in rat of the HPV-16/18 L1 AS04 candidate vaccine and to evaluate the reversibility of potential toxic effects over a period of 13 weeks after the last injection.

In a previous repeated dose tox study in rabbit evaluating HPV-16/18 L1 AS04 (LAB -(b)(4)- Study No 58678), a drop in the platelet count was observed at the end of the recovery period. It was then decided to evaluate the vaccine in a second species (rat) in order to assess the species-specificity of the decrease in platelets.

Sixty (60) female ---(b)(4)--- rats were allocated to 3 groups of 20 animals per group. Each animal was dosed 4 times intramuscularly with a dose of 0.1 ml (equivalent to 1/15 of the human candidate vaccine dose) per injection on Days 1, 15, 29 and 57. Subgroups of 10 animals per group were sacrificed on either Day 60 (3 days after the last injection) or Day 148/149 (13 weeks after the last injection). Group 1 was treated with Saline, Group 2 with the adjuvant AS04 and Group 3 with the vaccine HPV- 16/18 L1 AS04 candidate vaccine.

Clinical signs, dermal observations, body weights, food consumption, body temperature, ophthalmoscopy, clinical pathology, organ weights as well as macroscopic and microscopic evaluation were used as criteria to detect any side adverse effect.

No treatment-related adverse clinical signs were observed and treatment did not result in any dermal reactions or adverse findings at ophthalmoscopy. Body weight and body weight gain as well as the food consumption were not affected by the treatment.

An increase in the body temperature was observed 24 hours after the first injection in both Groups 2 and 3. No differences between the control group (Group 1) and Groups 2 and 3 were observed at the 48 hour observation. No differences in body temperature were observed on Day 57.

The hematological examination revealed an increase in the value of neutrophils (absolute and/or relative) in both Groups 2 and 3 on Days 4 and 60, as well as a higher value of fibrinogen in the same groups on Days 4, 55 and 60 and in Group 3 on Day 146. Furthermore, a decrease in the relative value of lymphocytes in both Groups 2 and 3 was observed on Days 4 and 60. Platelet count was not affected by the treatment.

The clinical chemistry examination revealed an increase in the level of globulin and a decreased level of albumin as well as a lower albumin/globulin ratio in Groups 2 and 3 on Days 4 and 60.

The changes observed in the clinical pathology parameters were likely related to the expected inflammatory process caused by the immune response induced by the treatment. No test item related changes of toxicological importance were observed in the organ weights. The macroscopic examination revealed no test item related changes at any of the necropsy time points (Days 60 and 148/149).

The microscopic examination on Day 60 revealed test item related changes at the injection site such as subacute inflammation, myofiber necrosis and focal hemorrhage. The changes were most pronounced in the vaccine treated group (Group 3). However, as the examination performed on Day 148/149 revealed no differences from the control group, which showed that the changes resolved over time.

The histopathology examination showed an inflammatory response in the popliteal lymph nodes in Group 2 and 3 at both necropsy time points.

Four intramuscular injections of 0.1 ml (equivalent to 1/15 of the human dose) of the HPV-16/18 L1 AS04 candidate vaccine resulted in no signs of systemic toxicity. Treatment caused slight changes to a few hematological and clinical chemistry parameters as well as in the popliteal lymph node and transient local reactions at the injection site of both Group 2 and 3 animals. The local reaction at the injection site was most pronounced in the HPV-16/18 L1 AS04 candidate vaccine treated group. The observed changes were expected and considered to be related to the immune response induced by the vaccine. The platelet count was not affected by repeated intramuscular injections of HPV-16/18 L1 AS04 candidate vaccine in rat.

Genotoxicity Studies for MPL

2.6.7.8 Genotoxicity: In Vitro

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2.6.7.8.B. Monophosphoryl Lipid A: Induction of -----(b)(4)-----

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2.6.7.9 Genotoxicity: In Vivo

Report Title: MPL (--b(4)--Bulk) -----(b)(4)----- Test

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