Toxicology Review of Human Papillomavirus 9-Valent Vaccine, Recombinant

BLA: 125508

Sponsor: Merck Sharp & Dohme Corp.

Product: GARDASIL®9 (Human Papillomavirus 9-Valent Vaccine, Recombinant)
Proposed indication: Prevention of Human Papillomavirus Types 6, 11, 16, 18, 31, 33, 45, 52, 58

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Introduction:

With approximately half of the HPV-infected people being adolescents and young adults, human papillomavirus (HPV) infection is one of the most common sexually transmitted diseases. The inability to grow HPVs in tissue culture or to infect species other than humans slowed the ability to develop vaccines for this virus. Kreider et.al.[1,2], were able to isolate of infectious HPV11 in a nude mouse xenograft system. It has been reported that immunization with the PV vaccinia vectors protected animals from viral challenge[3-7]. It has also been reported that expression of PV L1 or L1 + L2 genes in baculovirus expression system or by vaccinia vectors was shown to result in assembly of virus-like particles (VLPs)[8-12] which have been demonstrated to induce high-titered virus-neutralizing antibodies[9,12]. Jansen KU et.al., 1995[13], reported that rabbits were protected from cottontail rabbit papillomavirus CRPV-induced papilloma formation by vaccination with yeast-expressed CRPV virus-like particles. Suzich JA et.al., 1995[14], reported that immunization of dogs with canine oral papillomavirus (COPV) L1 VLPS, in the absence or presence of adjuvant, protected animals from experimental challenge with COPV.

Wise LD et.al., 2008[15], reported that there was no evidence of toxicity in the F0 Sprague-Dawley rats treated with either Merck aluminum adjuvant (MAA) or quadrivalent HPV vaccine. No effects on the fertility or reproductive performance of the F0 females and no evidence of developmental toxicity to the F1 generation, including fetal body weight and morphology, postnatal growth and development, behavior, and reproductive performance were reported in their study. In another study by Wise LD et.al., 2010[16], no evidence of toxicity and no effects on the Cesarean-section parameters of females or reproductive parameters of the cohabited males, including histomorphology of testes and epididymis, sperm count, and sperm motility were reported in the quadrivalent HPV vaccine-treated rats.

GARDASIL®9 is a recombinant vaccine prepared from the purified virus-like particles (VLPs) of the major capsid (L1) protein of HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58. The L1 proteins are produced by separate fermentations in recombinant *Saccharomyces cerevisiae* and self assembled into VLPs. GARDASIL®9 (9vHPV vaccine) is prepared by combining the adsorbed VLPs of each HPV type. Each 0.5- mL dose is formulated to contain 30/40/60/40/20/20/20/20/20 µg of HPV 6/11/16/18/31/33/45/52/58 L1 proteins, respectively.

9vHPV vaccine targets HPV types 6, 11, 16, and 18 also targeted by the licensed quadrivalent HPV vaccine (qHPV) as well as HPV types 31, 33, 45, 52, and 58. HPV 16 and 18 are responsible for ~70% of cases of cervical cancer. An additional ~20% of cases are due to HPV types 31, 33, 45, 52, and 58.

HPV types are classified into high-risk (HR) types, based on their potential to cause cancer, and low-risk (LR) types (causing generally benign lesions). The International Agency for Research on Cancer (IARC) has identified 12 HPV types as carcinogens that include the 7 HR HPV types represented in the 9vHPV vaccine (HPV 16, 18, 31, 33, 45, 52, and 58) and 5 HR HPV types not represented in the 9vHPV vaccine (HPV 35, 39, 51, 56, and 59). LR HPV types 6 and 11, responsible for ~90% genital warts and recurrent respiratory papillomatosis cases, are also included in the 9vHPV vaccine. Cervical dysplasia represents a substantial burden of disease in countries with organized cervical cancer screening programs and incidence rates of high-grade cervical dysplasia are consistent between such countries (e.g., the United States, Canada).

Clinical studies submitted to support this BLA:

Protocol V503-001: This is a double-blinded (with in-house blinding), controlled with qHPV vaccine, dose ranging, efficacy, immunogenicity, and safety study of the 9vHPV vaccine. The study used a seamless phase II/III adaptive design, which allowed prompt progression from phase II dose selection to phase III efficacy evaluation. The V503-001 part B was designed to demonstrate that compared with qHPV vaccine, the 9vHPV vaccine is highly efficacious in reducing the incidence of: (1) A composite endpoint of HPV 31/33/45/52/58-related high-grade cervical, vulvar, and vaginal disease (primary efficacy endpoint); (2) HPV 31/33/45/52/58-related persistent infection (secondary efficacy endpoint); and (3) HPV 31/33/45/52/58-related cervical, vulvar and vaginal disease (any grade) (secondary efficacy endpoint).

Protocol V503-002: No efficacy study was conducted in adolescents due to the ethical constraints in collecting samples and performing examinations with children and low exposure to HPV in this age group. The 9vHPV vaccine efficacy findings in females 16 to 26 years of age were bridged to females and males 9 to 15 years of age based on the demonstration of non-inferior immunogenicity. This study was also conducted to demonstrate clinical consistency of manufactured material through immunogenicity assessment of three different final manufacturing process lots of the 9vHPV vaccine.

Protocol V503-009/GDS01C: This supportive non-IND study, requested by the European Medicines Agency (EMA), was conducted to demonstrate that 9vHPV vaccine and qHPV vaccine have similar immunogenicity with respect to HPV 6, 11, 16, and 18 in females, 9 to 15 years of age. It strengthens the immunological bridging conclusions from protocol V503-002.

Protocol V503-006: It is possible that some girls and women previously vaccinated with qHPV vaccine may want to receive the 9vHPV vaccine in order to benefit from broader protection against HPV diseases after this vaccine is approved for use. In anticipation of this need, the 9vHPV vaccine was assessed for safety and immunogenicity in prior qHPV vaccine recipients in protocol V503-006.

Protocols V503-005 and V503-007: Both studies were conducted to document the immunogenicity and safety profile of the 9vHPV vaccine administered concomitantly with vaccines recommended for routine vaccination of adolescents. Protocol V503-005 addressed concomitant administration of 9vHPV vaccine with Menactra™ and Adacel™. Protocol V503-007 addressed concomitant administration of 9vHPV vaccine with Repevax™ in preadolescents and adolescents (11 to15 year olds). Repevax™ is available in the European Union (EU) and some other countries.

Stability Summary:

The proposed storage period is ---(b)(4)--- for ----(b)(4)---- HPV types 6, 11, 16, and 18. The proposed storage period is ---(b)(4)--- for ---(b)(4)--- types 31, 33, 45, 52, and 58. The proposed storage period is ---(b)(4)--- for HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 -(b)(4)-.

Toxicity studies submitted to support this BLA:

GENERAL TOXICOLOGY STUDIES:

V503 three-month intramuscular toxicity study in rats dosed once every 21 days with a 21-day recovery period (TT #07-1006)

REPRODUCTIVE STUDIES:

1- V503 intramuscular developmental toxicity and immunogenicity study in rats with postnatal evaluation (TT #09-7230)

2- V503 intramuscular developmental toxicity and immunogenicity study in rats with prenatal evaluation (TT #07-7400)

Summary of the toxicology studies:

STUDY # 07-1006: V503 three-month intramuscular toxicity study in rats dosed once every 21 days with a 21-day recovery period.

Précis:

In this multiple dose (study days 1, 22, 43, and 64) toxicology study, rats were treated with V503, phosphate buffered saline (PBS), or Merck aluminum adjuvant (MAA). Animals (20/group/sex) were treated with 0.5 mL/animal (0.25 mL/quadriceps) by intramuscular route. The control group (group 1) received phosphate buffer saline (PBS) and the placebo group (group 2) received Merck aluminum adjuvant (MAA). Groups 3, 4, and 5 were treated with low, medium, and high doses of V503 in MAA. Rats were treated every 21 days for 10 weeks

followed by 3 weeks recovery period. The proposed clinical dose was used in this study.

Title and study number: V503 three-month intramuscular toxicity study in rats dosed once every 21 days with a 21-day recovery period. Study number: TT #07-1006
Performing laboratory: Merck Research Laboratories, Merck & Co., Inc., West Point, Pennsylvania 19486 U.S.A.
Study initiation date: February 15, 2007
Final report date: August 16, 2007
Test article batch/lot:

Test article	Lot No.
V503 (also known as L-002001044)	L-002001044-003G001 (low-dose formulation)
V503 (also known as L-002001044)	L-002001044-001C001 (mid-dose formulation)
V503 (also known as L-002001044)	L-002001044-002E001 (high-dose formulation)
Negative control = Phosphate buffered saline (PBS)	----------(b)(4)----------
Placebo control = Merck aluminum adjuvant (MAA)	------(b)(4)---------

Animal species and strain: ----------(b)(4)---------- rats
Breeder/supplier: ----------(b)(4)----------
Number of animals per group and sex: 20 per sex per group (5 groups)
Age: 57 days
Body weight range: 211-290 g for males and 146-199 g for females
Route and site of administration: Intramuscular (into each quadriceps) administration.
Volume of injection: The dose volume was 0.5 mL/animal (0.25 mL/quadriceps)
Frequency of administration and study duration: Test article were administered once every 3 weeks (once on study days 1, 22, 43, and 64). Study duration was 13 weeks.

Dose: Variable, see the following table:

Ingredient[a]	L-002001047-000B001 (Placebo)	L-002001044-003G001 (V503 Low Dose)	L-002001044-001C001 (V503 Mid Dose)	L-002001044-002E001 (V503 High Dose)
	Concentration	Concentration	Concentration	Concentration
HPV Type 6 VLP	N/A	40 µg/mL	60 µg/mL	80 µg/mL
HPV Type 11 VLP	N/A	80 µg/mL	80 µg/mL	80 µg/mL
HPV Type 16 VLP	N/A	80 µg/mL	160 µg/mL	160 µg/mL
HPV Type 18 VLP	N/A	40 µg/mL	80 µg/mL	160 µg/mL
HPV Type 31 VLP	N/A	40 µg/mL	40 µg/mL	60 µg/mL
HPV Type 33 VLP	N/A	40 µg/mL	40 µg/mL	60 µg/mL
HPV Type 45 VLP	N/A	40 µg/mL	40 µg/mL	60 µg/mL

Ingredient[a]	L-002001047-000B001 (Placebo)	L-002001044-003G001 (V503 Low Dose)	L-002001044-001C001 (V503 Mid Dose)	L-002001044-002E001 (V503 High Dose)
	Concentration	Concentration	Concentration	Concentration
HPV Type 52 VLP	N/A	40 µg/mL	40 µg/mL	60 µg/mL
HPV Type 58 VLP	N/A	40 µg/mL	40 µg/mL	60 µg/mL
Aluminum[b]	1.097 mg/mL	0.788 mg/mL	1 mg/mL	1.097 mg/mL
Sodium borate	35 µg/dose	35 µg/dose	35 µg/dose	35 µg/dose
Sodium chloride	0.32M	0.32M	0.32M	0.32M
Histidine	10 mM	10 mM	10 mM	10 mM
Polysorbate 80	0.01%	0.01%	0.01%	0.01%
pH	6.2	6.2	6.2	6.2

[a] Prepared in water for injection.
[b] As aluminum hydroxyphosphate sulfate.
HPV = Human papillomavirus.
VLP = Virus-like particle.

Table 1: Dose concentration

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the BLA. Stability summary was provided on page 5.

Means of administration: Intramuscular administration
Report status: Final report
Experimental design:
Animals were randomized and assigned to 5 different groups. Each group consisted of 20 males and 20 females. Animals were treated with 0.5 mL/animal (0.25 mL/quadriceps) by intramuscular route. The control group received PBS and the placebo group received MAA. A recovery group consisting of 10 males and 10 females rats per group were sacrificed 21 days after the interim necropsy (day 67). Study duration was 13 weeks. The details of the study design are listed in the following table:

Group	Test Material	Dose Volume mL/animal	Number of Animals (#/sex/group)*	
			Main	Recovery
1 (control)	PBS	0.5	10	10
2 (placebo)	MAA	0.5	10	10
3	Low-Dose in MAA	0.5	10	10
4	Mid-Dose in MAA	0.5	10	10
5	High-Dose in MAA	0.5	10	10

* The first 10 rats/sex/group were designated for interim necropsy on study day 67 (3 days after the last dose in study week 10); the remaining 10 rats/group were designated for final necropsy in study week 13 (after a 21-day recovery period).

Methods:
Randomization procedure: Yes.
Statistical analysis plan: Yes.

The following parameters were evaluated: Clinical observations (daily), detailed observations (on day of timed dosing, for 2 days after each dose, and during the recovery period [from study week 11]), body weights (pretest, once in study week 1, and twice per week thereafter), food consumption (2 days per week), body temperature (not recorded), ophthalmoscopy (study weeks 1, 10, and 12 [recovery period]), clinical chemistry, hematology, coagulation parameters, and urinalysis (study days 4, 67, and 81 [recovery period]). Immunogenicity (study days 21 and 85 [recovery period]). Organ weight, macroscopic examination, and tissue collection (terminal necropsy at weeks 10 and 13).

Parameters	Frequency of Testing
Clinical observation[1]	Daily
Clinical signs[2]	On day of timed dosing, for 2 days after each dose, and during the recovery period [from study week 11]
Body weight	Pretest, once in study week 1, and twice per week thereafter
Food consumption	Two days per week
Body temperature	Not recorded
Ophthalmologic exam	Study weeks 1, 10, and 12 [recovery period]
Clinical chemistry*	Study days 4, 67, and 81 [recovery period]
Hematology*	Study days 4, 67, and 81 [recovery period]
Coagulation parameters**	Study days 4, 67, and 81 [recovery period]
Urinalysis	Study days 4, 67, and 81 [recovery period]
Immunogenicity*	Study days 21 and 85 [recovery period]
Necropsy	Weeks 10 and 13
Organ weight, macroscopic examination, and tissues for histopathology	Weeks 10 and 13

* Blood was collected from orbital sinus.
** Blood was collected from jugular vein

[1] Cageside observations include mortality, morbidity, general health and signs of toxicity.
[2] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Results:

Morbidity and mortality: No treatment related morbidity or mortality was reported during the study. There were 8 deaths (PBS control female #07-1129, placebo treated female #07-1159, low-dose female #07-1199, mid-dose female #07 1225, and high-dose male #07-1272, female #07-1279, female #07-1277, and male #07-1274) observed during or shortly after jugular bleeding for hematology analysis on study days 4, 67, and 85. The cause of death was attributed to complications of the bleeding procedure

SYSTEMIC TOXICITY:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight, food consumption, ophthalmoscopy, or macropathology were reported.

HEMATOLOGY:

Compared to PBS control group:

Group's 3 females' leukocyte and males' lymphocyte levels were decreased at study day 4. Increases in neutrophil levels in groups 3, 4, and 5 males, at study day 67, were reported. Increases in neutrophil levels in groups 3 and 4 males, at study day 81, were reported. Increases in neutrophil levels in groups 3, 4, and 5 females, at study day 67, were reported. Decreases in neutrophil levels in groups 3 and 4 females, at study day 81, were reported.

At study day 67, increases in monocyte levels in groups 3, 4, and 5 males were reported. At study day 4, increase in monocyte levels in group 5 females was reported. At study day 67, increases in monocyte levels in groups 3, 4, and 5 females were reported. At study day 81, decrease in monocyte levels in group 3 females was reported.

At study day 67, increases in eosinophil levels in groups 3, 4, and 5 males were reported. At study days 4 and 81, decreases in eosinophil levels in groups 3 and 5 females were reported. At study day 67, increases in eosinophil levels in groups 3, 4, and 5 females were reported.

Group 3 males' large unstained cells (LUC) were decreased at study day 4. Group 5 males' LUC were increased at study day 67. Groups 3 and 5 females' LUC were increased at study day 67.

Compared to PBS control

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)	NOT OF NOTE
RED BLOOD CELLS		Reticulocytes: Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC)
WHITE BLOOD CELLS	Total leukocytes (WBC) SD 4 F ↓ ≤ 0.7 G3 Lymphocyte count SD 4 M ↓ ≤ 0.7 G3 Neutrophil count SD 67 M ↑ ≥2.0 G3 SD 67 M ↑ ≥2.2 G4 SD 67 M ↑ ≥2.2 G5 SD 81 M ↓ ≤ 0.7 G3 SD 81 M ↓ ≤ 0.7 G4 SD 67 F ↑ ≥2.2 G3 SD 67 F ↑ ≥2.5 G4 SD 67 F ↑ ≥2.4 G5 SD 81 F ↓ ≤ 0.6 G3 SD 81 F ↓ ≤ 0.7 G4 Monocyte count SD67 M ↑ ≥2.2 G3 SD67 M ↑ ≥2.7 G4 SD67 M ↑ ≥2.8 G5 SD4 F ↑ ≥1.6 G5 SD67 F ↑ ≥3.0 G3 SD67 F ↑ ≥3.0 G4 SD67 F ↑ ≥2.6 G5 SD81 F ↓ ≤0.7 G3 Eosinophils count SD67 M ↑ ≥2.0 G3 SD67 M ↑ ≥2.4 G4 SD67 M ↑ ≥1.9 G5 SD4 F ↓ ≤0.6 G3 SD67 F ↑ ≥2.1 G3 SD67 F ↑ ≥2.0 G4 SD67 F ↑ ≥1.7 G5 SD81 F ↓ ≤0.6 G5	Macrophage Basophils

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)	NOT OF NOTE
	Large Unstained Cells (LUC) SD 4 M ↓ ≤ 0.6 G3 SD67 M ↑ ≥2.0 G5 SD67 F ↑ ≥2.3 G3 SD67 F ↑ ≥2.0 G5	
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Prothrombin time Mean platelet volume Fibrinogen* Platelet count
OTHERS		Bone marrow cytology

* Not measured.

Table 2: Hematology results (compared to PBS group)

Compared to MAA control group:

Increases in neutrophil levels in groups 3, 4, and 5 males and females, at study day 67, were reported. Increases in neutrophil levels in group 5 males, at study day 81, were reported.

At study day 67, increases in monocyte levels in groups 4 and 5 males were reported. At study day 81, decreases in monocyte levels in group 5 males and group 3 females was reported. At study day 67, increases in monocyte levels in groups 3 and 4 females were reported.

At study day 67, increases in eosinophil levels in groups 3, 4, and 5 males were reported. At study day 4, decreases in eosinophil levels in groups 3 and 4 females were reported. At study days 67 and 81, increases in eosinophil levels in group 3 females were reported.

Group 3 males' large unstained cells (LUC) were decreased at study day 4. Group 5 males' LUC were increased at study day 67.

Compared to MAA placebo control

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)	NOT OF NOTE
RED BLOOD CELLS		Reticulocytes: Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC)
WHITE BLOOD CELLS	Neutrophil count SD 67 M ↑ ≥2.2 G3 SD 67 M ↑ ≥2.4 G4 SD 67 M ↑ ≥2.4 G5 SD 81 M ↑ ≥1.6 G5 SD 67 F ↑ ≥3.1 G3 SD 67 F ↑ ≥3.5 G4 SD 67 F ↑ ≥3.3 G5 Monocyte count SD67 M ↑ ≥1.7 G4 SD67 M ↑ ≥1.8 G5 SD81 M ↓ ≤0.7 G5 SD67 F ↑ ≥1.6 G3 SD67 F ↑ ≥1.6 G4 SD81 F ↓ ≤0.7 G3 Eosinophils count SD67 M ↑ ≥1.7 G3 SD67 M ↑ ≥2.0 G4 SD67 M ↑ ≥1.6 G5 SD4 F ↓ ≤0.6 G3 SD4 F ↓ ≤0.7 G4 SD67 F ↑ ≥1.6 G3 SD81 F ↑ ≥2.0 G3 Large Unstained Cells (LUC) SD 4 M ↓ ≤ 0.6 G3 SD67 M ↑ ≥1.7 G5	Macrophage Lymphocyte count Basophils Total leukocytes (WBC) Lymphocyte count
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Prothrombin time Mean platelet volume Fibrinogen* Platelet count
OTHERS		Bone marrow cytology

* Not measured.

Table 3: Hematology results (compared to MAA group)

CLINICAL CHEMISTRY:

Compared to PBS control

AST levels were decreased in groups 3, 4, and 5 males at study day 81. Bilirubin levels were decreased in groups 3, 4, and 5 females at study days 67 and 81. Triglyceride levels were decreased in groups 3 and 4 males and group 4 females at study day 67. A/G ratio were decreased in groups 3, 4, and 5 males and groups 4 and 5 females at study day 67.

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Aspartate aminotransferase (AST) SD 81 M ↓ ≤ 0.7 G3 SD 81 M ↓ ≤ 0.7 G4 SD 81 M ↓ ≤ 0.7 G5	Glutamate dehydrogenase Total bile acids Lactate dehydrogenase (LDH) Alanine aminotransferase (ALT)
B) HEPATOBILIARY	Total bilirubin SD 67 F ↓ ≤ 0.5 G3 SD 67 F ↓ ≤ 0.5 G4 SD 67 F ↓ ≤ 0.5 G5 SD 81 F ↓ ≤ 0.5 G3 SD 81 F ↓ ≤ 0.5 G4 SD 81 F ↓ ≤ 0.5 G5	Gamma-glutamyl transferase (GGT) Total bile acids Alkaline phosphatase (ALP)
ACUTE PHASE REACTANTS		C-reactive protein*, fibrinogen* (also under coagulation),
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Triglycerides: SD 67 M ↓ ≤ 0.7 G3 SD 67 M ↓ ≤ 0.7 G4 SD 67 F↓ ≤ 0.7 G4 A/G ratio SD 67 M ↓ ≤ 0.7 G3 SD 67 M ↓ ≤ 0.7 G4 SD 67 M ↓ ≤ 0.7 G5 SD 67 F ↓ ≤ 0.7 G4	Albumin (A) Cholinesterase* Total protein Creatine kinase* Total Cholesterol Globulin

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
	SD 67 F ↓ ≤ 0.7 G5	

* Not measured.

Table 4: Clinical chemistry results (compared to PBS group)

Compared to MAA placebo control

AST levels were decreased in group 4 females at study day 67. ALT levels were decreased in groups 3, 4, and 5 females at study day 67. ALT levels were decreased in group 4 females at study day 81. Triglyceride levels were decreased in groups 3 and 4 males at study day 67.

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Aspartate aminotransferase (AST): SD 67 F ↓ ≤ 0.7 G4 Alanine aminotransferase (ALT): SD 67 F ↓ ≤ 0.7 G3 SD 67 F ↓ ≤ 0.7 G4 SD 67 F ↓ ≤ 0.7 G5 SD 81 F ↓ ≤ 0.7 G4	Glutamate dehydrogenase Total bile acids Lactate dehydrogenase (LDH)
B) HEPATOBILIARY		Gamma-glutamyl transferase (GGT) Total bile acids Alkaline phosphatase (ALP) Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein*, fibrinogen* (also under coagulation),
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS,	Triglycerides: SD 67 M ↓ ≤ 0.7 G3 SD 67 M ↓ ≤ 0.7 G4	Albumin (A) A/G ratio Cholinesterase* Total protein

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
METHEMOGLOBIN, AND PROTEINS)		Creatine kinase* Total Cholesterol Globulin

* Not measured.

Table 5: Clinical chemistry results (compared to MAA group)

MEAN BODY AND ORGAN WEIGHTS:

SEX		MALES (WEEK 10/ WEEK 13)					FEMALES (WEEK 10/ WEEK 13)				
GROUPS		1 (CONTROL)	2 (PLACEBO)	3 (LOW)	4 (MID)	5 (HIGH)	1 (CONTROL)	2 (PLACEBO)	3 (LOW)	4 (MID)	5 (HIGH)
NUMBER OF ANIMALS		10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
BODY WEIGHT (terminal)		388/394	384/405	386/ 394	381/403	392/389	217/229	216/233	229/ 229	224/225	227/228
BRAIN		2.09/2.12	2.07/ 2.11	2.10/ 2.08	2.10/2.13	2.13/2.13	1.93/1.93	1.87/ 1.93	1.96/ 1.95	1.96/1.96	1.97/1.92
ADRENALS		0.05/0.05	0.05/0.05	0.05/0.04	0.05/0.05	0.05/0.05	0.05/0.06	0.06/0.06	0.06/0.05	0.06/0.06	0.06/0.05
EPIDIDYMIDES		NC	NC	NC	NC	NC					
HEART		1.37/1.28	1.36/ 1.29	1.31/ 1.27	1.35/1.33	1.41/1.34	0.92/0.87	0.87/0.87	0.95/ 0.86	0.92/0.88	0.98/0.89
KIDNEYS		3.04/2.65	2.94/ 2.57	2.95/ 2.58	3.00/2.68	3.14/2.70	1.78/1.78	1.73/ 1.68	1.83/ 1.61	1.84/1.74	1.89/1.64
LIVER		11.4/9.93	10.9/ 10.3	11.1/ 9.93	11.3/10.1	11.7/9.59	6.38/6.04	6.42/ 5.92	6.97/ 6.13	6.95/5.84	7.19/5.97
LUNGS AND BRONCHI		NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
ILLIAC LYMPH NODES	Right	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
	Left	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
SEMINAL VESICLES		NC	NC	NC	NC	NC					
SPLEEN		0.75/0.65	0.73/ 0.63	0.79/ 0.64	0.82/0.64	0.86/0.63	0.45/0.44	0.46/ 0.44	0.53/ 0.46	0.55/0.43	0.56/0.49
TESTES		3.40/3.47	3.41/ 3.39	3.47/ 3.26	3.32/3.55	3.46/3.53					
PITUITARY		0.011/0.011	0.011/0.011	0.011/0.010	0.012/0.01	0.012/0.011	0.013/0.013	0.012/0.013	0.014/0.014	0.013/0.014	0.014/0.013
PROSTATE		0.48/0.48	0.44/ 0.55	0.53/ 0.44	0.47/0.45	0.53/0.53					
THYROID and PARATHYROID		0.03/0.02	0.02/0.03	0.03/0.02	0.02/0.02	0.03/0.03	0.02/0.02	0.02/0.02	0.02/0.02	0.02/0.02	0.02/0.02
THYMUS		0.35/0.28	0.34/ 0.31	0.42/ 0.23	0.36/0.27	0.30/0.29	0.31/0.24	0.30/ 0.23	0.30/ 0.23	0.28/0.26	0.26/0.23
OVARIES							0.08/0.08	0.08/0.07	0.08/0.08	0.07/0.07	0.08/0.08
UTERUS AND CERVIX							NC	NC	NC	NC	NC

Absolute weights are expressed as mean (grams). NC = Not collected. Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and recovery phase of the study (main phase organ weight/recovery phase organ weight).

Table 6: Organs weight**.**

No treatment related-changes in organ weights were reported. Spleen weight was increased 15% in group 5 males at week 10. At week 10, females' spleen weight was increased 18%, 22%, and 24% in groups 3, 4, and 5, respectively. Males' thymus was decreased 18% in group 3 at week 10.

MACROPATHOLOGY:

Discoloration in group 1 males' thymus was reported. Increased in lymph node size was reported in 1, 6, 4, and 4 animals of groups 2, 3, 4, and 5 males, respectively. Focus skeletal muscle was reported in 4, 8, 6, and 5 animals of groups 2, 3, 4, and 5 males, respectively. Focus bone in one animal of group 2 males was reported.

Increased in lymph node size was reported in 1, 4, 5, and 6 animals of groups 2, 3, 4, and 5 females, respectively. Focus skeletal muscle was reported in 8, 7, 7, and 7 animals of groups 2, 3, 4, and 5 females, respectively. Focus bone in one

animal of group 2 females was reported. Focus stomach in 7 animals of group 2 females was reported. Decreased ovary size in one animal of group 2 females was reported. Focus at injection site and pallor lung in one animal of group 3 females was reported. Cyst in uterus in one animal of group 5 females was reported.

Group	Findings
1M	Discoloration in thymus (1/10)*
2M	Increased lymph node size (1/10); focus bone (1/10); focus skeletal muscle (4/10)
3M	Increased lymph node size (6/10); focus skeletal muscle (8/10)
4M	Increased lymph node size (4/10); focus skeletal muscle (6/10)
5M	Increased lymph node size (4/10); focus skeletal muscle (5/10)
1F	NF
2F	Increased lymph node size (1/10); focus bone (1/10); focus skeletal muscle (8/10); focus stomach (7/10); decreased ovary size (1/10)
3F	Focus at injection site (1/10); increased lymph node size (4/10); focus skeletal muscle (7/10); pallor lung (1/10)
4F	Increased lymph node size (5/10); focus skeletal muscle (7/10)
5F	Increased lymph node size (6/10); focus skeletal muscle (7/10); cyst in uterus (1/10)

NF = No findings. * (number of animals with the observation/total number of animals in the group).

Table 7: Macroscopic findings at terminal sacrifice

MICROSCOPIC FINDINGS:

Terminal sacrifice

Group	Findings
1M	Cellular infiltration in skeletal muscle (1/10)*; unilateral focal dilatation tubule in kidney (1/10) Injection site: Inflammation in muscle (10/10)
2M	Hyperplasia in iliac (10/10) and inguinal (2/10) lymph nodes Injection site: Inflammation in muscle (10/10); degeneration in muscle fiber (10/10); focal inflammation in skin (2/10); focal inflammation in periosteum (2/10); focal inflammation in femorotibial joint (2/10)
3M	NF
4M	NF
5M	Cyst in pituitary (1/10); mineralization in peyer's patches (1/10); depletion lymphoid tissue in spleen (1/10); hyperplasia in iliac (10/10),

Group	Findings
	inguinal (2/10), and renal (1/10) lymph nodes Injection site: Inflammation in muscle (10/10); degeneration in muscle fiber (10/10); focal inflammation in skin (3/10); focal inflammation in perineural tissue (1/10); focal inflammation in periosteum (1/10); focal inflammation in femorotibial joint (3/10)
1F	Injection site: Inflammation in muscle (3/10)
2F	Hyperplasia in iliac lymph node (9/10); hyperplasia in inguinal lymph node (1/10) Injection site: Inflammation in muscle (10/10); degeneration in muscle fiber (10/10); focal inflammation in skin (2/10); focal inflammation in perineural tissue (1/10); focal inflammation in periosteum (1/10); focal inflammation in femorotibial joint (1/10)
3F	Hyperplasia in iliac lymph node (1/10) Injection site: Inflammation in muscle (1/10); degeneration in muscle fiber (1/10)
4F	Hyperplasia in iliac lymph node (1/10) Injection site: Inflammation in muscle (1/10); focal inflammation in skin (1/10)
5F	Mineralization in peyer's patches (1/10); hyperplasia in iliac lymph node (9/10); hyperplasia in inguinal lymph node (2/10); cyst (1/10) and distention (1/10) in uterus Injection site: Inflammation in muscle (10/10); degeneration in muscle fiber (10/10); focal inflammation in skin (2/10); focal inflammation in perineural tissue (1/10); focal inflammation in periosteum (3/10); focal inflammation in femorotibial joint (3/10)

NF = No findings. * (number of animals with the observation/total number of animals in the group).

Table 8: Microscopic findings at terminal sacrifice

An extensive number of tissues were examined for histology. No increased incidences of histological findings indicative of potential adverse events were observed in the treated groups relative to the controls.

Serology:
Blood samples for antibody analysis were collected from --------(b)(4)------- rats on SD's 21 and 85. In order to detect antibodies to HPV type -6, -11, -16, -18, -33, and to HPV type -31, -45, -52 and -58 in rat serum, two immunoassays, using a Luminex competitive format, were used. The two HPV Luminex immunoassays are competitive immunoassays that allow the simultaneous quantification of antibodies to HPV type -6, -11, -16, -18, -33, and to HPV type -31, -45, -52, -58. The assays use ----------(b)(4)----------.

No detectable anti-HPV type -6, -11, -16, -18, -33, -31, -45, -52, or -58 antibody response in the control groups (PBS or placebo [MAA]) at any time point was reported. In serum collected from groups 3, 4, and 5 (low-, mid-, and high-dose vaccine), an antibody response against all 9 HPV types was reported on study days 21 and 85. Following the fourth administration of the vaccine, antibody responses were generally more robust (in general, 2- to 6-fold increase in antibody response), when compared to that measured after a single vaccine administration. In conclusion, V503 vaccine induced antibody response to HPV type 6, -11, -16, -18, -33, -31, -45, -52, and -58 following one or four intramuscular injections to rats. No clear differences between males and females in their antibody responses were reported.

Antibody titer results at study day 21 (Males/females)

Group #	Serotype-Specific Antibody Titer (HPV U/mL)[a]								
	-6	-11	-16	-18	-33	-31	-45	-52	-59
1 (M/F)	20/20	50/50	4/4	18/18	25/25	14/15	31/31	91/91	35/35
2 (M/F)	20/20	50/50	4/4	18/18	25/25	14/14	31/31	91/91	35/35
3 (M/F)	1774/1918	20787/13282	7683/6686	14780/16336	4321/3392	22150/24666	4867/5520	78035/88404	17761/14113
4 (M/F)	1345/1383	9374/9371	4505/6880	7519/9161	3114/3382	14563/19803	4482/9501	40363/137335	18953/11911
5 (M/F)	3574/2197	11558/21561	15374/11360	10915/23608	6878/3946	16544/25387	21876/4723	54627/102908	29292/16171

[a] Cut-off values (HPV U/mL): HPV-6=40; HPV-11=100; HPV-16=8; HPV-18=36; HPV-31=28; HPV-33=50; HPV-45=62; HPV-52=182; HPV-58=70.

Table 9: Male's/female's geometric titer mean at day 21

Antibody titer results at study day 85 (Males/females)

Group #	Serotype-Specific Antibody Titer (HPV U/mL)[a]								
	-6	-11	-16	-18	-33	-31	-45	-52	-59
1 (M/F)	20/20	50/50	4/4	18/18	25/25	14/14	31/31	91/91	35/35
2 (M/F)	20/20	50/50	4/4	18/18	25/25	14/14	31/31	91/91	35/35

Group #	Serotype-Specific Antibody Titer (HPV U/mL)[a]								
	-6	-11	-16	-18	-33	-31	-45	-52	-59
3 (M/F)	1223/6077	12279/69695	1934/12279	50000/29823	1635/11048	130621/116489	113479/127544	197434/151232	157758/159012
4 (M/F)	4164/6253	28966/40919	8227/11983	27687/53658	9802/13351	49524/96328	24141/66252	55130/120976	60355/140494
5 (M/F)	12800/10696	89073/57621	46818/36058	26919/67006	24050/32651	142194/126949	59627/57439	129848/122591	115579/95557

[a] Cut-off values (HPV U/mL): HPV-6=40; HPV-11=100; HPV-16=8; HPV-18=36; HPV-31=28; HPV-33=50; HPV-45=62; HPV-52=182; HPV-58=70. M = Males. F = Females.

Table 10: Male's/female's geometric titer mean at day 85

Test article related effects are listed in the table below:

Test article related effects	Effects considered incidental
↑ Neutrophil levels ↑ Monocyte levels ↑ Eosinophil levels ↑ LUC ↓ Billirubin ↓ A/G ratio ↑ Lymph node size ↑ Focus skeletal muscle	↓ ALT

Assessment:

No treatment-related mortality or changes in clinical signs, body weight, food consumption, ophthalmic examination, urinalysis, organ weights, and macroscopic examination were reported.

Neutrophils are key components in the system of defense against infection. An individual with absence or scarcity of neutrophils (neutropenia) is vulnerable to infection. Monocyte, neutrophil, and eosinophil counts increases could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair.

LUC is a measurement of the large, peroxidase-negative cells which cannot be further characterized (i.e. as large lymphocytes, virocytes, or stem cells) present in a biological specimen. In LUC are found large lymphoid cells, more immature lymphocytes and other cells. If the value is higher than normal, blood counts should be checked under a microscope slide.

Bilirubin testing is used to check liver function and watch for signs of liver disease, such as hepatitis or cirrhosis, effect of medicines that can damage the liver, bile ducts blockage, or increased destruction of red blood cells.

The decrease in A/G ratio might be the result of an increase in immunoglobulin synthesis due to polyclonal activation of B lymphocytes by the adjuvant.

The decrease in ALT levels were considered incidental, because they would only be biologically significant if the levels had increased (and not decreased) by a corresponding amount.

The increase in lymph node size might be related to the immune response due to the vaccine treatment.

Focus skeletal muscle might be related to an inflammation due to the injection procedure. It has been reported by Knuf et. al., 2010 and Ajana et. al., 2008[17,18] that muscle is a site for immunization where vaccines appear to be better tolerated than when given by the subcutaneous route. Elicker S and Sipos W, 2009[19] reported that some local muscle damage occurs following animals vaccination, mainly as a result of the presence of adjuvant. Skeletal muscle damage might also develops secondary to other drug-induced effects such as electrolyte or metabolic disturbances, immunological reactions, ischemia, compression of muscle in states of altered consciousness or in response to excessive neuronal activation at the neuromuscular junction[20]. Whether of spontaneous nature or drug induced, muscle changes are also an important component of motor neuron damage.

GLP study deviations or amendments: No study deviations or amendments were included in this submission. It has been reported by the sponsor “On occasion, minor deviations occurred during the conduct of this study and are documented in the raw data. These deviations did not affect the quality and integrity of the study.”

Note: No body temperature was monitored during the study. No deviations or amendments were reported in this submission. Acute phase reactants (C-reactive protein or globulin fractions), fibrinogen, or creatine kinase were not measured in this study.

REPRODUCTION TOXICITY STUDIES:

Reproduction Toxicity Study # 1: V503 intramuscular developmental toxicity and immunogenicity study in rats with prenatal evaluation (TT #07-7400).

Key study findings: No significant findings were reported.
Study no.: TT #07-7400
Conducting laboratory and location: Merck Research Laboratories, Merck & Co., Inc., West Point, Pennsylvania l9486
Date of study initiation: 09/24/2007
Date of study completion: 02/20/2008
GLP compliance: Yes
QA reports: yes (X) no ( )

Drug, lot #, and % purity:

Test article	Batch/Lot #'s	Purity %
V503	L-002001044-004J001	*
PBS	---(b)(4)---	*
MAA	----------(b)(4)---------	*

* Not reported

V503 Formulation

Ingredients[a]	Concentration
HPV Type 6 L1 protein	60 µg/mL
HPV Type 11 L1 protein	80 µg/mL
HPV Type 16 L1 protein	160 µg/mL
HPV Type 18 L1 protein	110 µg/mL
HPV Type 31 L1 protein	60 µg/mL
HPV Type 33 L1 protein	60 µg/mL
HPV Type 45 L1 protein	60 µg/mL
HPV Type 52 L1 protein	60 µg/mL
HPV Type 58 L1 protein	60 µg/mL
Merck Aluminum Adjuvant (MAA)	1000 µg/mL
Sodium chloride	0.33 M
Sodium borate	35 µg/dose[b]
Histidine	10 mM
Polysorbate	80 0.01%

[a] Prepared in water for injection, pH 6.2.
[b] Dose = 0.5 mL.

MAA Formulation

Ingredients[a]	Concentration
MAA	1000 µg/mL
Sodium Chloride	0.33 M
Sodium Borate	35 µg/dose[b]
Histidine	10 mM
Polysorbate	80 0.01%

[a] Prepared in water for injection, pH 6.2.
[b] Dose = 0.5 mL.

Animal species and strain: ----------(b)(4)---------- rats
Breeder/supplier: ----------(b)(4)----------
Number of animals per group and sex: Ninety females. Animals were assigned to groups as follow:

	Number of Females Per Group
Control 1 (PBS)	25[a]
Control 2 (MAA)	25[a]
V503	40[b]

[a] Includes 5 extra females to obtain 20 females with identified mating for Cesarean section.
[b] Includes 12 females used only for blood sample collections and 8 extra females to obtain 20 identified mating for Cesarean section and at least 10 pregnant females for immunogenicity.

Age: 8 weeks.
Body weight range: 164-233 grams
Route and site of administration: Intramuscular injections into the quadriceps muscle of each hindlimb
Volume of injection: 0.5 mL per animal per dose (0.25 mL administered into each quadriceps muscle).
Frequency of administration and study duration: All female rats were dosed once at 5 weeks and 2 weeks prior to cohabitation, and all mated females were dosed on gestation day (GD) 6.
Dose Concentration: See V503 and MAA formulation tables above.

Stability:
Stability summary was provided on page 5.

Methods

Study design:
Rats were treated once at 5 weeks and 2 weeks prior to cohabitation, and all mated females were treated on gestation day (GD) 6. Animals were dosed with 0.5 mL per animal per dose. Animals were assigned to 3 different groups (groups 1 and 2 contained 25 animals and group 3 contained 40 animals). The details of the study design are listed in the following table:

Group	Treatment	Dosage Volume (mL/rat)	Treatment Days	Number of Animals
1	Control1 PBS	0.5	5 weeks and 2 weeks prior to cohabitation and GD 6	25
2	Control1 MAA	0.5	5 weeks and 2 weeks prior to cohabitation and GD 6	25
3	V503	0.5	5 weeks and 2 weeks prior to cohabitation and GD 6	40

Study design

PARAMETERS AND ENDPOINTS EVALUATED:

The following parameters were evaluated: mortality (daily), physical examination (once per week during the premating period, on GD 0, and then daily from GD 6 to termination), body weight (once per week during the premating period, and on GD's 0, 6, 10, 14, 18, and 21), food consumption (over 4-day intervals beginning on study days 1, 8, and 22 and over 2-day intervals beginning on GD's 6 and 18), serology (prior to the first dose, on the first day of cohabitation, and on GD 21), mating (daily), examination of F0 females and fetuses at Cesarean section for pregnancy status, early implantation and number of corpora lutea (GD 21). Placental morphology evaluated by gross examination, uterine implants, fetus weights, live fetus sex, viscera of the fetuses and externally malformed fetuses, and skeletal abnormalities (GD 21).

Randomization**:** Yes
Statistical methods**:** Yes

Results
Maternal findings:

Mortality/Clinical signs:
No test article-related effects on mortality or clinical observations were reported.

Food consumption and body weight gain:
No test article-related effects on body weight gain or food consumption were reported. Some inter-animal variability in food consumption was reported.

Mating Performance and Fertility:
No treatment-related changes in mating performance and fertility parameters were reported. The mating index was 100% in all groups, and the fecundity and fertility indexes were 96% to 100% across all groups.

Treatment Group	PBS	MAA	V503
Females Cohabited	25	25	40
Found Dead or Sacrificed Prior To Confirmed Mating	3	0	4
Mated Females	22	25	36
Scheduled Cesarean Section Females	20	20	20
Pregnant Females	20	19	20
Not Pregnant Females	0	1	0
Females Sacrificed Pregnant and Discarded [a]	2	5	4
Females for Immunogenicity	0	0	12
Pregnant Females	0	0	11
Not Pregnant Females	0	0	1
Matings Per 4-Day Periods of Cohabitation			
Days 1 to 4	22	25	33[b]
Days 5 to 8	0	0	0
Days 9 to 12	0	0	0
Days 13 to 16	0	0	0
Days 17 to 20	0	0	0
Time To Mating (4-Day Periods) ± SD	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00[b]
Mating Index, % [c]	100	100	100
Fecundity Index, %	100	96	97
Fertility Index, % [c]	100	96	97

Mating Index = Mated females/females cohabited (excluding females found dead or sacrificed during cohabitation).
Fecundity Index = Pregnant females/mated females (excluding females with an undetermined pregnancy status).
Fertility Index = Pregnant females/females cohabited (excluding females found dead or sacrificed during cohabitation or with an undetermined pregnancy status).
[a] Females discarded because quota for C-section obtained.
[b] Excludes 3 females with interrupted mating.
[c] Excludes females sacrificed prior to confirmed mating.

Table 11: Summary of reproductive performance

Gross examination:
No treatment-related changes in the gross examination of maternal thoracic and abdominal viscera.

F1 generation-fetuses from Cesarean sections

No test article-related effects on the embryonic/fetal survival as assessed by the numbers of corpora lutea, implantations, and live fetuses per female and the derived peri- and post-implantation loss calculations were reported. No test

article-related effects on implants numbers, resorption, or live fetal weight were reported.

There were no treatment-related changes in fetal sex ratios since the mean values were 47% to 53% across all groups.

Treatment Group	PBS	MAA	V503
Mated Females	20	20	20
Pregnant	20	19	20
Examined Live Litter	20	19	20
Resorbed or Dead Litter	0	0	0
Found Dead	0	0	0
Sacrificed	0	0	0
Not Pregnant	0	1	0
Live	0	1	0
Found Dead	0	0	0
Sacrificed	0	0	0
Corpora Lutea	300	305	322
Corpora Lutea/Pregnant Female ± SD	15.0 ± 1.6	16.1 ± 2.3	16.1 ± 2.4
% Peri-implantation Loss, Litter Mean ± SD	2.8 ± 5.1	4.9 ± 5.4	3.4 ± 5.0
Implants	291	290	311
Implants/Pregnant Female ± SD	14.6 ± 1.4	15.3 ± 2.3	15.6 ± 2.5
Resorptions	16	19	14
% Resorptions/Implants, Litter Mean ± SD	5.4 ± 4.7	6.5 ± 9.4	4.4 ± 5.0
Dead Fetuses	0	0	0
% Dead Fetuses/Implants, Litter Mean ± SD	0.0	0.0	0.0
% Post-implantation Loss, Litter Mean ± SD	5.4 ± 4.7	6.5 ± 9.4	4.4 ± 5.0
Live Fetuses	275	271	297
Females	142	145	137
Males	133	126	160
Sex Ratio, Litter Mean ± SD	0.51 ± 0.15	0.53 ± 0.12	0.47 ± 0.19
Live Fetuses/Pregnant Female ± SD	13.8 ± 1.4	14.3 ± 2.5	14.9 ± 2.4
Live Fetal Weight (g), Litter Mean ± SD			
Females	5.08 ± 0.24	4.99 ± 0.24	5.15 ± 0.35
Males	5.36 ± 0.24	5.27 ± 0.28	5.37 ± 0.40
% Peri-implantation Loss = ([No. Corpora Lutea - No. Implants] / No. Corpora Lutea) X 100. % Post-implantation Loss = ([No. Resorptions + No. Dead Fetuses] / No. Implants) X 100. Sex Ratio = Total No. Live Female Fetuses / (Total No. Live Female Fetuses + Total No. Live Male Fetuses).			

Table 12: Summary of Cesarean section data

No test article-related changes in the placental morphology were reported. The external abnormalities observed were singular occurrences.

Treatment Group		PBS	MAA	V503
Total Litters Examined		20	19	20
Live Fetuses/Litters Examined		275/ 20	271/ 19	297/ 20
Fetuses with Malformations (%L.M.± SD)		0	2(0.97± 3.1)	1(0.33± 1.5)
Litters with Malformations (%)		0	2(11)	1(5.0)
Fetuses with Variations (%L.M.± SD)		0	0	0
Placental Morphology No. Abnormal Placentas/Total Examined		0/275	2/271	3/297
Type and Number of Fetal Alterations (%L.M.± SD)	Class			
Omphalocele	M	0	1(0.66± 2.9)	1(0.33± 1.5)
Misdirected Tail	M	0	1(0.31± 1.3)	0
L.M. = Litter Mean. M = Malformation.				

Table 13: Summary of fetal external examinations

Treatment Group	PBS	MAA	V503
Total Litters Examined	20	19	20

Treatment Group		PBS	MAA	V503
Live Fetuses/Litters Examined		143/ 20	142/ 19	154/ 20
Fetuses with Malformations (%L.M.± SD)		0	1(0.53± 2.3)	1(0.63± 2.8)
Litters with Malformations (%)		0	1(5.3)	1(5.0)
Fetuses with Variations (%L.M.± SD)		0	1(0.75± 3.3)	0
Litters with Variations (%)		0	1(5.3)	0
Type and Number of Fetal Alterations (%L.M.± SD)	Class			
Ventricular Septal Defect	M	0	1(0.53± 2.3)[a]	0
Right-Sided Aortic Arch	M	0	1(0.53± 2.3)[a]	0
Renal Artery Malformation	M	0	0	1(0.63± 2.8)
Discolored Liver	V	0	1(0.75± 3.3)	0

L.M. = Litter Mean. M = Malformation. V = Variation.
[a] Multiple alterations observed for litter 07-3608, fetus 16.

Table 14: Summary of fetal visceral examinations

No test article-related coronal abnormalities were reported. The malformations in fetuses and litters were singular occurrences and were reported in the control groups.

Treatment Group		PBS	MAA	V503
Total Litters Examined		20	19	20
Live Fetuses/Litters Examined		132/20	131/19	143/20
Fetuses with Malformations (%L.M.± SD)		1(0.63± 2.8)	1(0.75± 3.3)	0
Litters with Malformations (%)		1(5.0)	1(5.3)	0
Fetuses with Variations (%L.M.± SD)		0	0	0
Litters with Variations (%)		0	0	0
Type and Number of Fetal Alterations (%L.M.± SD)	Class			
Retinal Malformation	M	1(0.63± 2.8)	0	0
Anophthalmia	M	0	1(0.75± 3.3)[a]	0
Microphthalmia	M	0	1(0.75± 3.3)[a]	0

L.M. = Litter Mean. M = Malformation.
[a] Multiple alterations observed for litter 07-3609, fetus 4.

Table 15: Summary of fetal coronal examinations

No test article-related skeletal abnormalities were reported. The variations in fetuses and litters reported in test article-treated group were not different from the control groups. Fetal alterations (cervical rib and supernumerary rib) reported in the test article-treated group were not different from the control groups.

Treatment Group	PBS	MAA	V503
Total Litters Examined	20	19	20
Live Fetuses/Litters Examined	143/20	142/19	154/20
Fetuses with Malformations (%L.M.± SD)	0	0	0
Litters with Malformations (%)	0	0	0
Fetuses with Variations (%L.M.± SD)	17(12 ±16.5)	18(13 ±15.5)	16(11 ±15.0)
Litters with Variations (%)	9(45)	10(53)	9(45)
Head Examination Total Litters Examined	20	19	20
Live Fetuses/Litters Examined	143/20	140/19	154/20
Fetuses with Malformations (%L.M.± SD)	0	0	0
Litters with Malformations (%)	0	0	0
Fetuses with Variations (%L.M.± SD)	0	0	0
Litters with Variations (%)	0	0	0

Treatment Group	PBS	MAA	V503
Type and Number of Fetal Alterations (%L.M.± SD) Class			
Cervical Rib V	2(1.3 ± 4.1)	3(2.3 ± 5.7)	2(1.1 ± 5.0)
Supernumerary Rib V	15(11 ±16.9)	15(11 ±15.2)	14(9.5 ±13.1)
L.M. = Litter Mean. V = Variation.			

Table 16: Summary of fetal skeletal examinations

The number of incomplete ossification in the fetuses and the litters of test article-treated group were higher than the PBS treated group but was not different from MAA treated group. The numbers of ossified sacrocaudal vertebrae were comparable across all treatment groups.

Treatment Group	PBS	MAA	V503
Torso and Limb Examination			
Total Litters Examined	20	19	20
Live Fetuses/Litters Examined	143/20	142/19	154/20
Fetuses with Incomplete Ossification (%L.M.± SD)	1(0.63± 2.8)	6(4.6 ± 7.2)	6(3.7 ± 7.1)
Litters with Incomplete Ossification (%)	1(5.0)	6(32)	5(25)
Number of Ossified Sacrocaudal Vertebrae ± SD	10.8 ± 0.7	10.7 ± 0.8	10.6 ± 0.6
Head Examination			
Total Litters Examined	20	19	20
Live Fetuses/Litters Examined	143/ 20	140/ 19	154/ 20
Fetuses with Incomplete Ossification (%L.M.± SD)	0	1(0.58±2.5)	1(0.63±2.8)
Litters with Incomplete Ossification (%)	0	1(5.3)	1(5.0)
Site and Number of Fetuses with Incomplete Ossification (%L.M.± SD) Class			
Incomp. Oss. Cervical Vertebra O	1(0.63± 2.8)	0	0
Incomp. Oss. Thoracic Vertebra O	0	1(0.75±3.3)	1(0.63±2.8)
Incomp. Oss. Sternebra O	0	5(3.9±6.9)	5(3.1±6.8)
Inc. Oss. Hyoid O	0	1(0.58±2.5)	1(0.63±2.8)
L.M. = Litter Mean. O = Ossification.			

Table 17: Summary of fetal ossification data

Immunogenicity:

Blood samples for antibody analysis were collected from -------(b)(4)------- rats on SD's 21 and 85. In order to detect antibodies to HPV type -6, -11, -16, -18, -33, and to HPV type -31, -45, -52 and -58 in rat serum, two immunoassays, using a Luminex competitive format, were used. The two HPV Luminex immunoassays are competitive immunoassays that allow the simultaneous quantification of antibodies to HPV type -6, -11, -16, -18, -33, and to HPV type -31, -45, -52, -58. The assays use ----------(b)(4)---------- (immunized with the 9-valent HPV vaccine) that was assigned arbitrary values expressed in HPV units per mL (HPV U/mL).

There were no detectable anti-HPV antibodies in the serum collected pretest from all F0 female rats. Very low antibody titer for HPV type 31 was reported. On cohabitation day 1 and on GD 21, antibodies to all 9 HPV serotypes in serum from V503-treated females were reported. In the serum of GD 21 fetuses from V503-treated dams, antibodies to all 9 HPV serotypes were reported.

Study Day	**Group/ Females**	**Serotype-Specific Antibody Titer (HPV U/mL)**								
		-6	**-11**	**-16**	**-18**	**-33**	**-31**	**-45**	**-52**	**-59**
Pretest	F_0[a]	20	50	4	18	25	15	31	91	35
Cohabitation (Day 1)	F_0[b]	11269	83276	36676	116270	25133	210801	93077	527216	165359
GD 21	F_0[b]	13251	58881	17174	56489	16451	91223	66415	148450	83186
	F_0 Fetuses[c]	2249	12788	2740	7052	2395	17385	12751	31810	12139
	Ratio Fetuses/ F_0	0.17	0.22	0.16	0.12	0.15	0.19	0.19	0.21	0.15

[a] Cut-off values (naïve rat serum) (HPV U/mL): HPV-6=40; HPV-11=100; HPV-16=8; HPV-18=36; HPV-31=28; HPV-33=50; HPV-45=62; HPV-52=182; HPV-58=70.
[b] Cut-off values (naïve female [pregnant] rat serum) (HPV U/mL): HPV-6=35; HPV-11=21; HPV 16=5; HPV-18=9; HPV-31=10; HPV-33=16; HPV-45=31; HPV-52=164; HPV-58=56.
[c] Cut-off values (naïve fetal rat serum) (HPV U/mL): HPV-6=57; HPV-11=54; HPV-16=5; HPV 18=23; HPV-31=148; HPV-33=23; HPV-45=143; HPV-52=246; HPV-58=42.

Table 18: Geometric titer mean for anti-HPV antibody response

Summary:
The objective of this study was to evaluate the effect of V503 vaccine, administered intramuscularly, on pregnant rats. Animals (25 or 40/group) were assigned to 3 different groups and treated by 500 µl/rat of PBS, MAA, or test article (V503).

Clinical symptoms, mortality, body weight, food consumption, serology, mating, examination of F0 females and fetuses at Cesarean section, placental morphology, uterine implants, fetus weights, live fetus sex, viscera of the fetuses and externally malformed fetuses, and skeletal abnormalities were evaluated.

F0 generation findings:
No test article-related effects on mortality, clinical observation, body weight gain, food consumption, mating performance, mating index, fecundity index, fertility index, and gross examination of maternal thoracic and abdominal viscera were reported.

F_1 generation-fetuses from Cesarean sections findings:
No test article-related effects on the embryonic/fetal survival (numbers of corpora lutea, implantations, and live fetuses per female and the derived peri- and post-implantation loss calculations), implants numbers, resorption, live fetal weight, or fetal sex ratios were reported.

No test article-related changes in the placental morphology, coronal abnormalities, or skeletal abnormalities were reported.

The number of incomplete ossification in the fetuses and the litters of test article-treated group were higher than the PBS treated group but was not different from MAA treated group. Thus, this effect might be related to the MAA (Merck aluminum adjuvant). No changes in the numbers of ossified sacrocaudal vertebrae due to test article-treatment were reported.

Conclusions

At 5 and 2 weeks prior to mating, and on GD 6, the anticipated human dose of V503 was administered by intramuscular injection. No treatment-related effects in the F0 females, including mating and fertility, were reported. No treatment-related developmental toxicity in the F1 generation was reported. Antibodies to all 9 HPV serotypes in serum of F0 and in serum of GD 21 fetuses from V503-treated females were reported.

Reproduction Toxicity Study # 2: V503 intramuscular developmental toxicity and immunogenicity study in rats with postnatal evaluation (TT #09-7230).

Key study findings: No significant findings were reported.
Study no.: TT #09-7230
Conducting laboratory and location: Merck Research Laboratories, Merck & Co., Inc., West Point, Pennsylvania l9486
Date of study initiation: 07/29/2009
Date of study completion: 05/18/2010
GLP compliance: Yes
QA reports: yes (X) no ( )

Drug, lot #, and % purity:

Test article	Batch/Lot #'s	Purity %
V503	L-002001044-005L001	*
MAA	----------(b)(4)---------	*

* Not reported

Formulation of Test Article

Ingredients[a]	Quantity
Virus-like particles of HPV types 6/11/16/18/31/33/45/52/58	60/80/120/80/40/40/40/40/40 µg/Ml
Merck Aluminum Adjuvant (MAA)	1000 µg/mL
Sodium chloride	0.33 M
Histidine	10 mM
Polysorbate-80	0.01%
Sodium borate	70 µg/mL
[a] Prepared in water for injection, pH 6.2.	

Formulation of Control Article

Ingredients[a]	Quantity
Merck Aluminum Adjuvant (MAA)	1000 µg/mL
Sodium chloride	0.33 M
Histidine	10 mM
Polysorbate-80	0.01%
Sodium borate	70 µg/mL
[a] Prepared in water for injection, pH 6.2.	

Animal species and strain: ----------(b)(4)---------- rats
Breeder/supplier: ----------(b)(4)----------
Number of animal per group and sex: Twenty F0 females per group. Twenty five animals were assigned to each of the two groups. Each group included 5 extra animals to obtain 20 mated females
Age: 8 weeks
Body weight range: 177-214 grams
Route and site of administration: Intramuscular injections into the quadriceps muscle of each hindlimb
Volume of injection: 0.5 mL per animal per dose (0.25 mL administered into each quadriceps muscle).
Frequency of administration and study duration: All female rats were dosed once at 5 weeks and 2 weeks prior to cohabitation, and all mated females were dosed on gestation day (GD) 6 and lactation day (LD) 7.
Dose: See the formulation tables for control and test article above.

Stability
Stability summary was provided on page 5.

Methods

Study design:
Rats were treated once at 5 weeks and 2 weeks prior to cohabitation, and the first 20 mated females were treated on gestation day (GD) 6 and lactation day

(LD) 7. Animals were dosed with 0.5 mL per animal per dose (0.25 mL injected into each hindlimb quadriceps muscle). Animals were assigned to 2 different groups (25 animals/group). Animals were euthanized on LD 21. The details of the study design are listed in the following table:

Group	Treatment	Dosage Volume (mL/rat)	Treatment Days	Number of Animals
1	Control MAA	0.5	5 weeks and 2 weeks prior to cohabitation and GD 6 and LD 7	25
2	V503	0.5	5 weeks and 2 weeks prior to cohabitation and GD 6 and LD 7	25

Study design

PARAMETERS AND ENDPOINTS EVALUATED:

The following parameters were evaluated: Physical signs observations (once per week during premating and daily beginning on GD 0 through sacrifice), mortality (daily), maternal body weight (premating once per week; on GD's 0, 6, 10, 14, 18, and 21; and on LD's 0, 3, 7, 10, 14, 17, and 21), food consumption (over 4-day intervals ending on premating days 5, 12, and 26; and on 2-day intervals ending on GD's 8 and 20).

Observation of parturition and length of gestation
Each female was observed on 4 occasions throughout the day (at approximately 7:30 AM, 10:00 AM, 12:30 PM, and 3:00 PM) from GD 21 until the completion of delivery.

Blood collection for immunogenicity assays
Blood samples were collected from the orbital sinus from the first 10 F0 female rats in the V503-treated group prior to the first dose and prior to the day of cohabitation. On LD 21, 10 F0 female rats in the V503-treated group and 1 pup/sex from these females were anesthetized with isoflurane gas and bled from the inferior vena cava. All females with pups were euthanized on LD 21.

F1 generation, preweaning (postnatal development [PND] 0 to 21)
Physical examinations (daily observation for mortality and physical signs), body weight (PND 0, 7, 14, and 21), serology (1 pup/sex from 10 litters in the treated group was bled on PND 21)

F1 generation, postweaning (PND 21 through sacrifice)
Physical examination (daily for mortality and twice weekly for physical signs), body weight (once a week from weaning to termination or cohabitation), body weight for mated females (GD's 0, 7, and 15), ophthalmologic examination (PND 45-49), passive avoidance (PND 35 ± 1 with previously untested animals and then repeated 1 week later with the same animals), auditory startle habituation (PND 63 ± 2), open field motor activity (PND 70 ± 2), blood for immunogenicity

(PND 70), reproductive assessment (PN week 12), scheduled termination and pregnancy status (GD's 15 and 17, PNW's 15 and 17)

Randomization**:** Yes
Statistical methods**:** Yes

Results

F0 generation results:

Mortality/clinical signs:

No test article-related effects on mortality or clinical observations were reported.

Food consumption and body weight gain:

No test article-related effects on body weight gain or food consumption were reported.

Mating performance and fertility:

No test article-related effects on mating performance and fertility parameters were reported. The mating index and fertility index in both groups were 100%. No test article-related effects on length of gestation or gross examination of maternal thoracic and abdominal viscera were reported.

Reproductive performance of F0 females

Treatment group	**MAA**	**V503**
Females Cohabited	25	25
Mated Females	20	20
Pregnant Females	20	20
Found dead during gestation	0	0
Found dead or sacrificed during parturition	0	0
Found dead or sacrificed during lactation	0	0
Females with live pups PND 0	20	20
Females with no live pups PND 0	0	0
Females with live pups PND 21	20	20
Pregnant Females for Immunogenicity	0	10
Females Sacrificed and Discarded[a]	5	5
Matings Per 4-Day Periods of Cohabitation		
Days 1 to 4	20	20
Days 5 to 20	0	0
Time To Mating (4-Day Periods) ± SD	1.00 ± 0.00	1.00 ± 0.00
Mating Index, %[b]	100	100
Fecundity Index, %	100	100
Fertility Index, %[b]	100	100
Females with Live Pups/Pregnant Females, %	100	100
Length of Gestation (Days ± SD)	22.2 ± 0.3	22.1 ± 0.3

Mating Index = Mated females/Females cohabited
Fecundity Index = Pregnant females/Mated females
Fertility Index = Pregnant females/Females cohabited
[a] Females discarded because delivery quota maintained.
[b] Excludes females sacrificed prior to confirmed mating.

Table 19: Summary of reproductive performance of F0 females

F1 generation (from birth until mating):

No test article-related effect on pup survival, % live pups delivered, live pups per litter, or pups death were reported.

Status of F1 generation prior to weaning

Treatment Group	MAA	V503
Parental Females §	20	20
Metrial Glands Per Female ± SD	16.8 ± 2.2	16.8 ± 2.5
% Post-Implantation Survival (L.M.) ± SD	94.1 ± 7.2	95.7 ± 5.4
Females with Live Pups Day 0 Lactation	20	20
Females with Live Pups Day 21 Lactation	20	20
Total Pups Delivered		
Live Pups (Sex Ratio, L.M.)	316(0.53)	320(0.55)
Dead Pups [N] (% L.M. ± SD)	7[6](2.0±3.5)	4[3](1.1±2.7)
% Live Pups Delivered (L.M. ± SD)	98.0 ± 3.5	98.9 ± 2.7
Live Pups Per Litter ± SD		
Postnatal Day 0	15.8 ± 2.3	16.0 ± 1.9
Postnatal Day 3	15.4 ± 2.2	15.9 ± 2.0
Postnatal Day 3	8.0 ± 0.0	8.0 ± 0.0
Postnatal Day 7	8.0 ± 0.0	8.0 ± 0.0
Postnatal Day 14	8.0 ± 0.0	8.0 ± 0.0
Postnatal Day 21	8.0 ± 0.0	8.0 ± 0.0
Pup Deaths [N](% L.M. ± SD)		
Postnatal Days 1 - 3	8[5](2.4±4.5)	3[3](0.9±2.3)
Postnatal Days 4 - 7	0	0
Postnatal Days 8 - 14	0	0
Postnatal Days 15 - 21	0	0
Postnatal Days 4 - 21	0	0

§ Excludes females dying or sacrificed prior to gestation day 21.
% Post-implantation survival = (Live pups delivered / Higher of [Metrial glands or total pups delivered]) x 100.
L.M. = Litter Mean.
Sex Ratio = (Total No. live female pups / Total No. live female and male pups).
[N] = Number of litters.

Table 20: Summary of status of F1 generation prior to weaning

Body weight at pre-weaning period:

No test article-related effects on body weight at pre-weaning period were reported.

Body weights of F1 pups prior to weaning

Treatment Group	MAA	V503
Live Female Pup Weight (g), Litter Mean ± SD		
Postnatal Day 0	6.11 ± 0.46	6.31 ± 0.49
Postnatal Day 7	16.4 ± 1.7	17.5 ± 1.5
Postnatal Day 14	35.5 ± 2.7	37.5 ± 2.5
Postnatal Day 21	57.6 ± 5.0	59.7 ± 3.9
Live Male Pup Weight (g), Litter Mean ± SD		
Postnatal Day 0	6.45 ± 0.51	6.68 ± 0.47
Postnatal Day 7	17.4 ± 1.8	18.2 ± 1.3
Postnatal Day 14	36.8 ± 2.8	38.4 ± 1.7

Treatment Group	MAA	V503
Postnatal Day 21	60.3 ± 5.4	62.2 ± 3.3

Table 21: Summary of body weights of F1 pups prior to weaning

External Examinations and Sex Ratio at Birth:
No test article-related effect on external malformations or variations or altered sex ratios at birth in F1 pups was reported.

No test article-related effect on physical signs in the F1 pups during the preweaning and postweaning periods was reported.

No test article-related effect on body weight at post-weaning period was reported. No unscheduled deaths during the postweaning period were reported.

External examinations postnatal day 0

Treatment Group	MAA	V503
Total Litters Examined	20	20
Delivered Pups (Live/Dead)/Litters Examined	(314/7)/20[a]	(320/4)/20
Intrauterine Pups (Live/Dead)/Litters Examined	(0/0)/0	(0/0)/0
Pups with Malformations (%,L.M.±S.D.)	0	0
Litters with Malformations (%)	0	0
Pups with Variations (%,L.M.±S.D.)	0	0
Litters with Variations (%)	0	0
(L.M.) = Litter Mean [a] See individual table for exclusion.		

Table 22: Summary of external examinations postnatal day 0

Developmental signs:
No test article-related effect on vaginal opening, preputial separation, ophthalmologic examination, and behavioral assessments (passive avoidance trials to criterion, auditory startle habituation, and open-field motor activity) was reported.

Mean developmental signs of F1 females

Treatment Group	MAA	V503
Vaginal opening (mean day of occurrence ± SD)	33.3 ± 2.0	32.2 ± 1.6
Preputial separation (mean day of occurrence ± SD)	45.7 ± 3.1	47.5 ± 4.5

Table 23: Summary of mean developmental signs of F1 females

Mean passive avoidance testing of F1 generation (females)

Treatment Group	MAA	V503
Days 34 to 36 postnatal - Session 1		
No. animals tested	20	20
Trials to criterion ± S.D.	5.9 ± 1.7	5.3 ± 1.6
No. not achieving criterion	2	1
Days 41 to 43 postnatal - Session 2		
No. animals tested	18	19

Treatment Group	MAA	V503
Trials to criterion ± S.D.	4.2 ± 2.3	4.4 ± 1.8
No. not achieving criterion	0	1

Table 24: Summary of passive avoidance testing of F1 generation (females)

Mean passive avoidance testing of F1 generation (Males)

Treatment Group	MAA	V503
Days 34 to 36 postnatal - Session 1		
No. animals tested	20	20
Trials to criterion ± S.D.	5.4 ± 1.5	5.3 ± 1.2
No. not achieving criterion	0	0
Days 41 to 43 postnatal - Session 2		
No. animals tested	20	20
Trials to criterion ± S.D.	3.5 ± 0.8	4.5 ± 1.2
No. not achieving criterion	0	0

Table 25: Summary of passive avoidance testing of F1 generation (males)

F1 generation from mating until sacrifice:

No test article-related effect on mortality, physical examinations, or maternal F1 body weight changes was reported.

No test article-related effects on reproductive performance as assessed by time to mating (in 4-day periods), mating index, fecundity index, and fertility index were reported.

Reproductive performance of F1 females

Treatment group	MAA	V503
Females Cohabited	25	25
Mated Females	20	19
Pregnant Females	20	18
Found dead during gestation	0	0
Sacrificed during gestation	0	0
Cesarean sectioned	20	18
Not pregnant females	0	1
Matings Per 4-Day Periods of Cohabitation		
Days 1 to 4	15	17
Days 5 to 8	3	2
Days 9 to 20	2	0
Time to Mating (4-Day Periods) ± SD	1.40 ± 0.82	1.11 ± 0.32
Mating Index, %[b]	100	95
Fecundity Index, %	100	95
Fertility Index, %b	100	90
Mating Index = Mated females/Females cohabited Fecundity Index = Pregnant females/Mated females Fertility Index = Pregnant females/Females cohabited		

Table 26: Summary of reproductive performance of F1 females

No test article-related effects on the variations in F2 embryonic/fetal survival parameters as assessed by the mean numbers of corpora lutea, implantations, and live fetuses per pregnant female and the derived peri- and post-implantation loss values were reported.

Cesarean section data of F1 females

Treatment group	**MAA**	**V503**
Mated Females	20	19
Pregnant Females	20	18
Examined Live Litter	20	18
Resorbed or Dead Litter	0	0
Found Dead or Sacrificed	0	0
Not Pregnant Females	0	1
Corpora Lutea	343	308
Corpora Lutea/Pregnant Female ± SD	17.2 ± 1.6	17.1 ± 3.1
% Peri-Implantation Loss, Litter Mean ± SD	5.2 ± 5.1	6.4 ± 7.5
Implants	325	290
Implants/Pregnant Female ± SD	16.3 ± 1.6	16.1 ± 3.4
Resorptions	27	11
% Resorptions/Implants, Litter Mean ± SD	8.4 ± 7.9	4.0 ± 5.1
Dead Fetuses	0	0
% Dead Fetuses/Implants, Litter Mean ± SD	0.0	0.0
% Post-implantation Loss, Litter Mean ± SD	8.4 ± 7.9	4.0 ± 5.1
Live Fetuses	298	279
Sex Not Examined	298	279
Live Fetuses/Pregnant Female ± SD	14.9 ± 2.1	15.5 ± 3.3
% Peri-Implantation Loss = ([No. Corpora Lutea - No. Implants] / No. Corpora Lutea) X 100.		
% Post-implantation Loss = ([No. Resorptions + No. Dead Fetuses] / No. Implants) X 100.		

Historical Control Data (2005-2009)

Parameter	Studies	Mean	SD	50.0 Percentile
Corpora Lutea/Pregnant Female	3	16.89	1.22	17.18
% Peri-implantation Loss	3	6.29	3.99	4.91
Implants/Pregnant Female	3	15.77	0.95	16.00
% Resorptions/Implants	3	5.03	0.97	5.26
% Dead Fetuses/Implants	3	0.13	0.23	0.00
% Post-implantation Loss	3	5.16	0.76	5.26
Live Fetuses/Pregnant Female	3	14.96	0.97	15.24

Table 27: Summary of Cesarean section data of F1 females

Immunogenicity:

Blood samples for antibody analysis were collected from -----(b)(4)----- rats at different time points (see table below). In order to detect antibodies to HPV type -6, -11, -16, -18, -33, and to HPV type -31, -45, -52 and -58 in rat serum, two immunoassays, using a Luminex competitive format, were used. The two HPV Luminex immunoassays are competitive immunoassays that allow the simultaneous quantification of antibodies to HPV type -6, -11, -16, -18, -33, and to HPV type -31, -45, -52, -58, respectively. The assays use ----------
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----------(b)(4)----------

----(b)(4)---- (immunized with the 9-valent HPV vaccine) that was assigned arbitrary values expressed in HPV units per mL (HPV U/mL).

There were no detectable anti-HPV antibodies in the serum collected pretest from all F0 female rats. Prior to the day of cohabitation, an antibody response against each of the nine HPV types was detected in ten of F0 females. HPV type -6 was the lowest in response where the cohabitation day/pretest antibody titer ratio was 856. In the mean time, HPV types -11 and -31 were the highest in response where the cohabitation day/pretest antibody titer ratios were 16,711 and 15,646, respectively.

On lactation day (LD) 21, similar or lower antibody responses against each of the nine HPV types were reported in the F0 females. On postnatal day (PND) 21, antibodies against all nine HPV types were reported in F1 generation. Similar antibody titers were reported in both males and females F1 generation. Therefore, the nine HPV types must have been transferred from the F0 females to the fetuses. HPV types -11 and -52 were the highest (0.86 and 0.84, respectively), and HPV type-6 was the lowest (0.24), in the antibody transferred. In addition, on PND 70, antibodies against the vaccine were reported in F1 generation.

Study Day	**Group/ Females**	**Serotype-Specific Antibody Titer (HPV U/mL)**								
		-6	**-11**	**-16**	**-18**	**-33**	**-31**	**-45**	**-52**	**-58**
Pretest	F_0[a]	5.0	2.5	1.0	7.5	3.5	4.5	4.5	25	3.0
Pre-Cohabitation	F_0	4278	41779	11817	14291	14507	70407	16086	65374	38590
LD 21	F_0 (F)	4902	24154	7060	17681	14509	36179	16382	30913	24143
PND 21	F1 (M)	1083	20523	4889	9944	9929	25651	10646	25391	18645
	F1 (F)	1213	21045	5014	10520	10525	27759	11188	26204	18796
	F1 (F+M)	1146	20783	4952	10228	10312	26684	10914	25795	18720
PND 21/LD21	Ratio F1 (M+F)/F0 (F)	0.24	0.86	0.70	0.58	0.71	0.74	0.67	0.84	0.78
PND 70	F1 (M)	154	282	93	239	217	317	253	562	273
	F1 (F)	231	457	177	355	312	584	425	890	433
	F1 (M+F)	188	359	129	291	260	430	328	707	343

M = Male. F = Female

[a] All antibody titers with a value below the cut-off value for a given HPV type, were assigned a titer equal to the cut-off value x 0.5. Cut-off values were established during validation using naïve rat serum samples are as follows (HPV U/mL units): HPV-6=10; HPV-11=5, HPV-16=2; HPV-18=15; HPV-31=9; HPV-33=7; HPV-45=9; HPV-52=50; HPV-58=6.

Table 28: Geometric titer mean for anti-HPV antibody response

In conclusion, no detectable anti-HPV antibodies in the serum of F0 females collected prior to dosing were reported. In serum from F0 females collected prior to cohabitation and on LD 21, an antibody response against each of the 9 HPV types was reported. Antibodies against all 9 HPV types were transferred to the F1 generation during gestation and/or lactation. Up to PND 70, the antibodies persisted through the post-weaning period.

Summary:
The objective of this study was to evaluate the effect of V503 vaccine, administered intramuscularly, on pregnant rats. Animals (25 /group) were assigned to 2 different groups and treated by 500 µl/rat of MAA or test article (V503).

For F0 generation, clinical symptoms, mortality, body weight, food consumption, serology, parturition and length of gestation data were recorded. For F1 generation, physical examinations, body weights, external examination, serology data were recorded. For post-weaning F1 generation, physical examinations, body weights, developmental signs (vaginal opening and preputial separation), ophthalmologic examinations, behavioral assessments (passive avoidance, auditory startle habituation, and open-field motor activity), serology, reproductive assessment, and pregnancy status data were recorded.

Results
F0 generation:
No test article-related effects on mortality or clinical observations were reported. No test article-related effects on body weight gain or food consumption were reported. No test article-related effects on mating performance and fertility parameters were reported. The mating index and fertility index in both groups were 100%. No test article-related effects on length of gestation or gross examination of maternal thoracic and abdominal viscera were reported.

F1 generation (from birth until mating):
No test article-related effect on pup survival, % live pups delivered, live pups per litter, or pups death were reported. At pre-weaning period, no test article-related effect on body weight was reported. No test article-related effect on external malformations or variations or altered sex ratios at birth in F1 pups was reported. No test article-related effect on physical signs in the F1 pups during the pre-weaning and post-weaning periods was reported. No test article-related effect on body weight at post-weaning period was reported. No unscheduled deaths during the post-weaning period were reported. No test article-related effect on vaginal opening, preputial separation, ophthalmologic examination, and behavioral assessments (passive avoidance trials to criterion, auditory startle habituation, and open-field motor activity) was reported.

F1 generation from mating until sacrifice:
No test article-related effect on mortality, physical examinations, or maternal F1 body weight changes was reported. No test article-related effects on reproductive performance as assessed by time to mating (in 4-day periods), mating index, fecundity index, and fertility index were reported. No test article-related effects on the variations in F2 embryonic/fetal survival parameters as assessed by the mean numbers of corpora lutea, implantations, and live fetuses per pregnant female and the derived peri- and post-implantation loss values were reported.

No detectable anti-HPV antibodies in the serum of F0 females collected prior to dosing were reported. In serum from F0 females collected prior to cohabitation and on LD 21, an antibody response against each of the 9 HPV types was reported. Antibodies against all 9 HPV types were transferred to the F1 generation during gestation and/or lactation. Up to PND 70, the antibodies persisted through the post-weaning period.

Conclusions

At 5 weeks and 2 weeks prior to cohabitation, and at gestation day (GD) 6 and lactation day (LD) 7, the anticipated human dose of V503 was administered by intramuscular injection. No treatment-related effects in the F0 females, including mating and fertility, were reported. In the F1 generation, no treatment-related developmental toxicity, including behavioral assessments and F2 embryonic/fetal survival parameters, was reported. Antibody responses against each of the 9 HPV types were reported in F0 and F1 generations.

OVERALL SUMMARY:

General toxicology:

Animals were assigned to 5 different groups and each group consisted of 20 males and 20 females. Animals were treated with 0.5 mL/animal (0.25 mL/quadriceps) by intramuscular route. The control group received PBS and the placebo group received MAA. Groups 3, 4, and 5 were treated with low, mid, and high doses of V503 in MAA. A recovery group consisting of 10 males and 10 females rats per group were sacrificed 21 days after the interim necropsy (day 67). Study duration was 13 weeks.

No treatment-related mortality or changes in clinical signs, body weight, food consumption, ophthalmic examination, urinalysis, organ weights, and macroscopic examination were reported.

Monocyte, neutrophil, and eosinophil counts were increased in test article-treated groups. This could be an indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair. Total bilirubin levels were decreased in test article-treated groups. Testing of bilirubin levels is used to check liver function and watch for signs of liver disease, such as hepatitis or cirrhosis, effect of medicines that can damage the liver, bile ducts blockage, or increased destruction of red blood cells.

The decrease in A/G ratio might be the result of an increase in immunoglobulin synthesis due to polyclonal activation of B lymphocytes by the adjuvant. The increase in lymph node size might be related to the immune response due to the vaccine treatment. The decrease in ALT levels were considered incidental,

because they would only be biologically significant if the levels had increased (and not decreased) by a corresponding amount.

Focus skeletal muscle might be related to an inflammation due to the injection procedure.

Serology testing showed an immune response in all vaccine recipient animals.

Reproductive toxicology:

The objective of the reproductive studies was to evaluate the effect of V503 vaccine, administered intramuscularly, on pregnant rats at prenatal and postnatal. Animals (25 or 40/group) were assigned to 3 different groups in the prenatal developmental study. Animals (25 /group) were assigned to 2 different groups in the postnatal developmental study. Animals in prenatal and postnatal studies were treated with 500 µl/rat of PBS, MAA, or test article (V503) or with MAA or test article (V503), respectively.

In the prenatal study, clinical symptoms, mortality, body weight, food consumption, serology, mating, examination of F0 females and fetuses at Cesarean section, placental morphology, uterine implants, fetus weights, live fetus sex, viscera of the fetuses and externally malformed fetuses, and skeletal abnormalities were evaluated.

In the postnatal study, for F0 generation, clinical symptoms, mortality, body weight, food consumption, serology, parturition and length of gestation data were recorded. For F1 generation, physical examinations, body weights, external examination, serology data were recorded. For post-weaning F1 generation, physical examinations, body weights, developmental signs (vaginal opening and preputial separation), ophthalmologic examinations, behavioral assessments (passive avoidance, auditory startle habituation, and open-field motor activity), serology, reproductive assessment, and pregnancy status data were recorded.

At prenatal and postnatal studies and for F0 generation the results showed, no test article-related effects on mortality, clinical observation, body weight gain, food consumption, mating performance, mating index, fecundity index, fertility index, and gross examination of maternal thoracic and abodominal viscera were reported.

Prenatal F_1 generation-fetuses from Cesarean sections findings showed no test article-related effects on the embryonic/fetal survival (numbers of corpora lutea, implantations, and live fetuses per female and the derived peri- and post-implantation loss calculations), implants numbers, resorption, live fetal weight, or fetal sex ratios. No test article-related changes in the placental morphology, coronal abnormalities, and skeletal abnormalities were reported. The number of incomplete ossification in the fetuses and the litters of test article-treated group were higher than the PBS treated group but was not different from MAA treated

group. Thus, this effect might be related to the MAA (Merck Aluminum Adjuvant). No changes in the numbers of ossified sacrocaudal vertebrae due to test article-treatment were reported.

Postnatal F1 generation (from birth until mating) findings showed no test article-related effect on pup survival, % live pups delivered, live pups per litter, or pups death. At pre-weaning period, no test article-related effect on body weight was reported. No test article-related effect on external malformations or variations or altered sex ratios at birth in F1 pups was reported. No test article-related effect on physical signs in the F1 pups during the pre-weaning and post-weaning periods was reported. No test article-related effect on body weight at post-weaning period was reported. No unscheduled deaths during the post-weaning period were reported. No test article-related effect on vaginal opening, preputial separation, ophthalmologic examination, and behavioral assessments (passive avoidance trials to criterion, auditory startle habituation, and open-field motor activity) was reported.

Postnatal F1 generation from mating until sacrifice findings showed no test article-related effect on mortality, physical examinations, or maternal F1 body weight changes. No test article-related effects on reproductive performance as assessed by time to mating (in 4-day periods), mating index, fecundity index, and fertility index were reported. No test article-related effects on the variations in F2 embryonic/fetal survival parameters as assessed by the mean numbers of corpora lutea, implantations, and live fetuses per pregnant female and the derived peri- and post-implantation loss values were reported.

Serology testing showed no detectable anti-HPV antibodies in the serum of F0 females collected prior to dosing. An immune response in all vaccine recipient animals was reported. Antibodies against all 9 HPV types were transferred to the F1 generation during gestation and/or lactation. Up to PND 70, the antibodies persisted through the post-weaning period.

Pregnancy category: B

Justification: No data are available from adequate and well-controlled studies for V503 vaccine in pregnant women. Reproductive studies included in this BLA showed no adverse effects on mating, female fertility, pregnancy, parturition, lactation parameters, and embryo-fetal or pre- and post-weaning developments were observed. There were no vaccine-related fetal malformations or other evidence of teratogenesis. There were no treatment-related effects on developmental signs, behavior, reproductive performance, or fertility of the offspring.

Proposed label wording:

Pregnancy Category B

A reproductive and developmental toxicity study has been performed in female rats at a dose approximately 240 times the human dose **(on a mg/kg basis)** and has shown no evidence of impaired fertility or harm to the fetus due to V503 vaccine. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, V503 vaccine should only be continued during pregnancy if clearly needed. Treatment with V503 vaccine should not be initiated in pregnant women.

Carcinogenesis, mutagenesis, and impairment of fertility

No studies have been performed in animals to evaluate the carcinogenic potential and the genotoxic risk of V503 vaccine.
Reproduction studies have been performed in rats at doses up to 240 and 160 times the human dose on mg/kg basis and revealed no evidence of impaired fertility or harm to the fetus due to V503 vaccine.

OVERALL CONCLUSION:

Based on the nonclinical toxicity assessments of the V503 vaccine submitted in this BLA, there are no significant safety issues to preclude the BLA from being approved.

Concurrence: Martin D. Green

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