

Toxicology Review of BLA/STN 125761 AV7909 Anthrax Vaccine Adsorbed, Adjuvanted

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Subject: Review of toxicology data

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4.2.3.1 Single-dose Toxicity
4.2.3.2 Repeat-dose Toxicity
4.2.3.5 Reproductive and Developmental Toxicology

Sponsor: Emergent Product Development Gaithersburg Inc.

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EXECUTIVE SUMMARY:

AV7909 Anthrax Vaccine Adsorbed, Adjuvanted (AV7909) was developed by Emergent Product Development Gaithersburg Inc. (Emergent) as a new vaccine indicated for postexposure prophylaxis (PEP) of disease following suspected or confirmed exposure to *Bacillus anthracis* (*B. anthracis*) when administered in conjunction with the recommended antibacterial regimen. Each 0.5 mL dose of AV7909 consists of (b) (4) of the immunostimulatory oligodeoxynucleotide, CPG 7909 and is administered as a two-dose regimen given two weeks apart.

Emergent has completed a single-dose acute toxicity study, a repeat-dose safety toxicity study, a pre- and postnatal developmental and reproductive toxicity (DART) study, as well as a juvenile repeat-dose toxicology study. In all studies, animals were dosed via intramuscular (IM) injection, the intended route for human use. All these GLP-compliant toxicology studies were performed in rats. The rat model was chosen for the safety evaluation of AV7909 as it is a widely used species for toxicology studies and rats were shown to respond appropriately to AV7909 and its component, CPG 7909, as confirmed by immunogenicity assessments conducted in each of the studies.

The treatment-related effects were very similar in the single dose toxicity study as well as in the repeat dose toxicity study in adult (full human dose given on Study Day [SD] 1, 15, 29) and juvenile rats (1/5th of the human dose given on SD 21, 28, 35). The test article related findings were transient and limited to the injection site, spleen and lymph nodes and were consistent with the immunostimulatory effects of CPG 7909 and the candidate vaccine. Test article-related findings were observed in the adjuvant alone (CPG 7909) group as well as the Anthrax Vaccine Adsorbed (AVA), adjuvanted group with similar frequency and similar severity, which suggests that the findings were mostly induced by the adjuvant. At the injection site, mild to marked necrosis occurred within the muscle, accompanied by mild to moderate chronic, chronic-active, subacute or granulomatous inflammation which often extended to the surrounding fascia and occasionally extended into the subcutis. After the treatment phase the subacute inflammation progressed to a chronic inflammation characterized by chronic or granulomatous inflammatory infiltrates observed at the injection site. The necrosis and granulomatous inflammation at the injection sites was considered adverse but were expected to resolve over time. Systemically, treated animals showed a transient increase in neutrophil, monocyte and basophil counts, as well as inflammatory markers. Additionally, increases in spleen weights, and hyperplasia in lymphoid tissues of the spleen and draining lymph nodes were observed. All findings were indicative of local and generalized immune system stimulation and reactogenicity.

In the submitted DART study AV7909 did not induce any reproductive or developmental toxicity. Administration of the AV7909 vaccine (full human dose, 0.5 mL) by IM injection to female (b) (4) rats 14 days prior to start of cohabitation, on the day of cohabitation and on Gestation Day (GD) 7 did not produce any effect on mating, fertility, pregnancy, embryo-fetal viability, growth, or morphologic development, parturition, maternal care of offspring or postnatal survival, growth or development.

PRODUCT: AV7909 Anthrax Vaccine Adsorbed, Adjuvanted

PROPOSED USE:

Post-exposure prophylaxis (PEP) of disease following suspected or confirmed exposure to *Bacillus anthracis* in persons 18 through 65 years of age when administered in conjunction with recommended antibacterial regimen.

INTRODUCTION:

AV7909 Anthrax Vaccine Adsorbed, Adjuvanted (AV7909) is a next-generation vaccine candidate comprised of (b) (4) Anthrax Vaccine Adsorbed (AVA) [DS; consisting of cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *Bacillus anthracis* (strain V-770-NP1-R) adsorbed to aluminum hydroxide], and (b) (4) of the immunostimulatory Toll-like receptor 9 (TLR9) agonist oligodeoxynucleotide (ODN), CPG 7909 adjuvant.

AV7909 Anthrax Vaccine Adsorbed, Adjuvanted is indicated for post-exposure prophylaxis of disease following suspected or confirmed exposure to *Bacillus anthracis* in persons 18 through 65 years of age when administered in conjunction with recommended antibacterial drugs. The proposed dosing regimen for AV7909 is to be administered via intramuscular injection in the deltoid muscle in two doses (0.5 mL each) given two weeks apart.

BioThrax® (Anthrax Vaccine Adsorbed; AVA), also licensed by Emergent BioSolutions, is an FDA-approved vaccine indicated for the active immunization for the prevention of disease caused by *Bacillus anthracis* in persons 18 through 65 years of age (US License number 1755, STN 103821). The vaccine is approved for both pre-exposure prophylaxis of disease in persons at high risk of exposure as well as post-exposure prophylaxis of disease following suspected or confirmed *B. anthracis* exposure, when administered in conjunction with recommended antibacterial drugs.

The AVA drug substance used to produce AV7909 is made with the same ingredients, (b) (4) and using (b) (4) manufacturing process as AVA contained in the licensed anthrax vaccine, BioThrax® (Anthrax Vaccine Adsorbed), License No. 1755; STN: BL 103821.

STUDIES SUBMITTED:

Single dose toxicity study:

1. Study no. 971-003 “Acute Intramuscular Toxicology Study in (b) (4) Rats with CPG 7909 and BioThrax Human Equivalent Dose”

Repeat dose toxicity study:

2. Study no. 1778-09072 “AV7909: Repeat-dose Safety Toxicity Study in Rats”

Reproductive and developmental toxicity study:

3. Study no. T05153 “AV7909 Vaccine: Combined Intramuscular Pre- and Postnatal Developmental and Reproductive Toxicity Study in Rats”

Juvenile toxicity study:

4. Study no. 98820 “AV7909: Repeat-dose Safety Toxicity Study in Juvenile Rats”

Proposed labeling text:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In clinically recognized pregnancies in the US general population, the estimated background risk of major birth defects is 2% to 4% and of miscarriage is 15% to 20%.

There are no adequate and well-controlled studies of CYFENDUS in pregnant individuals.

Available human data on CYFENDUS administered to pregnant individuals do not establish the presence or absence of vaccine-associated risks in pregnancy (see Human Data).

Data are available from a BioThrax observational study and pregnancy exposure registry. BioThrax is a licensed anthrax vaccine with the same active ingredient as CYFENDUS except that BioThrax does not contain CPG 7909 adjuvant. In the observational study there were more birth defects in infants born to individuals vaccinated with BioThrax in the first trimester compared to individuals vaccinated post pregnancy or individuals never vaccinated with BioThrax. Data from the BioThrax pregnancy exposure registry do not establish the presence or absence of vaccine-associated risks in pregnancy (see Human Data).

In a developmental study with an embryo-fetal development toxicity phase, female rats were administered a full human dose (0.5 mL) of CYFENDUS twice prior to mating and once during gestation. This study revealed no evidence of harm to the fetus, changes in reproductive performance or adverse effects on post-natal development due to the vaccine (see Animal Data).

Data

Human Data

In pre-licensure clinical studies of CYFENDUS, women underwent pregnancy testing immediately prior to administration of each dose of CYFENDUS. Despite this pregnancy screening regimen, some subjects were vaccinated with CYFENDUS very early in pregnancy before human chorionic gonadotropin was detectable (n=10) or in the 30 days prior to pregnancy onset (n=1). Of the 11 pregnancies (one twin pregnancy), 1 (9.1%) resulted in miscarriage and there were 2 infants (16.7 %) born with major birth defects.

An observational study examined the rate of birth defects among 37,140 infants born to US military service personnel who received BioThrax vaccine during pregnancy between 1998 and 2004. In this study, birth defects were slightly more common in first trimester-exposed infants (4.68%) when compared with infants of individuals vaccinated post pregnancy (3.85%) (odds ratio = 1.20; 95% confidence interval: 1.005, 1.43) or when compared to individuals never vaccinated with BioThrax (4.03%) (odds ratio = 1.20; 95% confidence interval: 1.02, 1.42)¹.

A pregnancy exposure registry was conducted in individuals who received BioThrax, a vaccine with the same active ingredient as CYFENDUS except that BioThrax does not contain CPG 7909 adjuvant. Of 91 individuals who reported pregnancy outcomes, the majority of exposures were in the first trimester (n=89), and there were two infants with major birth defects (2.2%) and X miscarriages (X%).

Animal Data

In a pre- and post-natal developmental study with an embryo-fetal development toxicity phase performed in female rats, a full human dose (0.5 mL) of CYFENDUS was administered by intramuscular injection on three occasions: 14 days prior to start of cohabitation, on the day of cohabitation, and on Gestation Day 7. No vaccine related adverse effects on fetal development, reproductive performance, or pre- and post-natal development up to post-natal day 21 in the offspring were reported.

8.2 Lactation

Risk Summary

It is not known whether CYFENDUS is excreted in human milk. Human data are not available to assess the impact of the vaccine on milk production, its presence in breast milk, or its effects on the breastfed child. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for CYFENDUS and any potential adverse effects on the breastfed child, or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of CYFENDUS in individuals less than 18 years of age has not been established.

8.5 Geriatric Use

Safety and effectiveness of CYFENDUS in individuals older than 65 years of age has not been established.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis and Impairment of Fertility

CYFENDUS has not been evaluated for carcinogenicity, mutagenic potential, or male infertility in animals. CYFENDUS administered to female rats had no effect on fertility [see *Use in Specific Populations (8.1)*].

13.2 Animal Toxicology and/or Pharmacology

Animal Pharmacology

Because it is not feasible or ethical to conduct controlled clinical trials with anthrax, the efficacy of CYFENDUS is based on animal studies. Pre-exposure prophylaxis studies conducted in animal models were used to derive protective antibody thresholds to bridge animal efficacy and human immunogenicity data and predict efficacy in humans. Efficacy studies were conducted in guinea pigs and cynomolgus macaques. Animals received two intramuscular vaccinations two weeks apart with various dilutions of CYFENDUS and were challenged with a lethal dose of aerosolized *B. anthracis* spores on Day 28 or 70. The studies demonstrated a strong correlation between pre-challenge serum TNA levels and survival.

The ability of CYFENDUS to increase survival after the cessation of the post-exposure antibacterial treatment, as compared with antibacterial treatment alone, was investigated in a guinea pig PEP study. In this study, guinea pigs were challenged with a lethal dose of aerosolized *B. anthracis* spores on Day 0 and treated with ciprofloxacin (7.5 mg/kg three times daily) for two weeks after the challenge. Various dilutions of the vaccine were administered on Days 1 and 8 after the challenge. Post-exposure vaccination increased animal survival in the 21-day period following cessation of antibacterial drugs, compared to antibacterial treatment alone, in a dose-dependent manner.

Single dose toxicology study:

Title: Acute Intramuscular Toxicology Study in (b) (4) Rats with CPG 7909 and BioThrax Human Equivalent Dose

Study number: 971-003

Final Report date: December 1, 2003

Animal species and strain: (b) (4) Rats; (b) (4)

Breeder/supplier: (b) (4)

Number of animals per group and sex: 5

Age: 6.5-9 weeks

Body weight range: Males: 236 to 287 g; females: 202 to 253 g

Route and site of administration: IM

Volume of injection: 0.5mL (split into 0.25mL and 0.25mL, injected into each thigh muscle)

Frequency of administration: Single dose

Means of administration: Needle and syringe

Report status: Final

Experimental design:

Group	Dose Level AVA (mL)	Dose Level CPG (mg)
1	0	0
2	0	0.5
3	0.5	0
4	0.5	0.1
5	0.5	0.5
6	0.5	1.25

Table 1: Design of acute intramuscular toxicity study with AVA (Biothrax) and CPG 7909 in rats; total dose volume: 0.55mL

There was no mortality, nor were there any treatment-related effects on clinical observations, body weights, or ocular findings, and no treatment-related macroscopic changes were noted at necropsy.

Treatment related findings were local inflammation at the injection site which were associated with muscle degeneration or necrosis and statistically significant increases in spleen weights of males at the terminal necropsy and females at both necropsies in group 6 (high CPG 7909 dose group) animals when compared to control animals (group 1). These findings are consistent with the immunostimulatory properties of CpG oligodeoxynucleotides. Treatment-related microscopic changes were confined to the injection sites, spleen, and bone marrow. The changes in the injection sites on SD 15 were consistent with local inflammation (chronic and/or active) and were characterized by lymphohistiocytic infiltrates, necrosis, fibrosis, and myofiber degeneration/necrosis and regeneration. The incidence and severity were greatest in groups 2, 5 and 6, suggesting that the local inflammation was largely attributable to the CPG 7909

immunostimulatory effect, although the occurrence of generally minimal to mild leukocytic infiltration in the injection sites of a few group 3 (BioThrax only) animals indicated that there may have been some minor contribution from BioThrax as well. Histopathologic changes in the spleen included an increased incidence in hematopoietic cell proliferation and lymphoid follicle-hyperplasia, which were seen in all groups treated with CpG 7909 (with or without BioThrax), but not in the BioThrax only group. The lymphoid follicle-hyperplasia in the spleen was slightly more pronounced in the groups receiving BioThrax and CpG 7909 suggesting a possible contribution of BioThrax. A very slight degree of hematopoietic cell hyperplasia was detected in the bone marrow from groups 5 and 6.

After the recovery phase (SD 29), treatment-related microscopic findings were limited to injection sites and bone marrow, and the incidence and severity were substantially reduced compared to SD 15.

Repeat dose toxicity study:

Title: AV7909: Repeat Dose Safety Toxicity Study in Rats

Study number: 1778-09072

Performing laboratory: (b) (4)

Study initiation date: March 9, 2009

Final Report date: October 20, 2009

Test article batch/lot:

AV7909 FDP Lot 1: E3075 (TC2807) (AV7909 finished drug product (FDP) Lot 1 – 0.5 mL AVA with 0.5 mg of CPG 7909)

AV7909 FDP Lot 2: E3076 (TC2808) (AV7909 FDP Lot 2– 0.5 mL AVA with 0.25 mg CPG 7909)

CPG 7909: GAI-07-121-S4-B1-5.23

Animal species and strain: (b) (4) Rats; (b) (4)

Breeder/supplier: (b) (4)

Number of animals per group and sex: 5

Age: 11 weeks

Body weight range: Males: 254.4 to 340.1 g; females: 178.5 to 228.4 g

Route and site of administration: IM (hind limb)

Volume of injection: 0.5 mL

Frequency of administration and study duration: Study Day (SD) 1, 15, and 29.

Means of administration: Needle and syringe

Report status: Final

Experimental design:

Group	Treatment
Group 1	Control Article
Group 2	CPG 7909 (0.5 mg)
Group 3	AV7909 FDP Lot 1 (0.5 mL AVA with 0.5 mg CPG 7909)
Group 4	AV7909 FDP Lot 2 (0.5 mL AVA with 0.25 mg CPG 7909)

Table 2: Experimental design

Randomization procedure: Animals were initially accepted into the randomization pool based upon pre study body weights, clinical observations, and ophthalmologic examinations. They were assigned to study groups using computer-generated random numbers such that the mean body weight for each group was not statistically different ($p < 0.05$) from the control mean. Males and females were randomized separately.

Statistical analysis plan:

Electronic data collection, including test material receipt and accountability, formulations, randomization, dosing, animal husbandry, environmental enrichment, clinical observations, body weights, body weight changes, food consumption, body temperatures, organ weights, gross pathology, and histopathology, was performed using (b) (4) Environmental monitoring is performed using (b) (4)

Quantitative data including body weights, body weight changes, food consumption, body temperatures, clinical pathology, CRP, and organ weight data were analyzed using the Kolmogorov-Smirnov test for normality, the Levene Median test for equal variance, and by one-way Analysis of Variance (ANOVA). If either the normality or equal variance test failed, then the analysis was continued using the non-parametric Kruskal-Wallis ANOVA on rank transformed data. For parametric data, if the ANOVA indicated statistical significance among experimental groups then the Dunnett's t-test was used to delineate which groups (if any) differed from the control. For non-parametric data, if the ANOVA indicated statistical significance among experimental groups then the Dunn's test was used to delineate which groups (if any) differed from the control. The probability value of less than 0.05 (two-tailed) was used as the critical level of significance for all tests.

Statistical analysis was conducted using (b) (4)

groups with sample sizes of one are excluded from statistical analysis; if the sample size of one occurred in the control group, no analysis was conducted. The term "significant" is used throughout the text of the report to indicate statistical significance at $p < 0.05$.

Parameters	Frequency of Testing
Cage side observation	≥ 2 Daily
Clinical observations	Weekly, one day prior to termination, and on the day of termination
Body weight	Weekly, one day prior to termination, and on the day of termination
Food consumption	Weekly, quantitative
Body temperature	Prior to dosing, 24 ± 2 hours post dose, and 48 ± 2 hours post dose
Ophthalmologic exam	Prior to necropsy (all surviving animals)
Clinical chemistry*	Prior to necropsy
Hematology*	Prior to necropsy
Coagulation*	Prior to necropsy
Immunological response*	Prior to necropsy
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Prior to dosing, 4 ± 0.5 hours post dose, and 24 ± 2 hours post dose
Necropsy	SD 31, SD 43
Tissues for histopathology	SD 31, SD 43

Table 3: Parameters evaluated; * abdominal aorta

Postmortem procedures: The following tissues were collected at necropsy.

Organ/Tissue	Collected	Not collected
Adrenal glands	*!	
Aorta	!	
Bone (sternum & femur)	!	
Bone marrow (sternum & femur)	!	
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	*!	
Cervix	!	
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)	!	
Gall bladder	!	
Gross lesions (if any)	!	
Harderian gland (if applicable)	!	
Heart	*!	
Ileum	*!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!	
Lacrimal glands		!
Larynx	!	
Liver	*!	
Lung (main stem; bronchi)	!	
Lymph nodes (cervical)	*!	
Lymph nodes (mandibular)	!	
Lymph nodes (iliac)	*!	
Lymph nodes (mesenteric)		
Lymph nodes (inguinal)	!	
Mammary glands	!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		!
Ovaries	*!	
Pancreas	!	
Peyer's patch (if applicable)		!
Pituitary gland	!	
Prostate	!	

Rectum	!	
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	*!	
Stomach (squamous and glandular)	!	
Testes	*!	
Thymus	*!	
Thyroid (w/ parathyroid glands)	!	
Tongue	!	
Trachea	!	
Ureters	!	
Uterus (w/ cervix)	*!	
Urinary bladder	!	
Vagina	!	

Table 4: Histology - Tissue examined. Tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'

Results:

Morbidity and mortality: One male animal in group 2 was euthanized early due to a non-treatment-related injury (injured spine). All remaining study rabbits survived to the scheduled necropsies

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Total bile acids

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
B) HEPATOBILIARY		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bilirubin
ACUTE PHASE REACTANTS	Fibrinogen: SD31: G2♂↑*1.4 SD31: G3♂↑*2.3 SD31: G4♂↑*2.1 SD31: G2♀↑*1.3 SD31: G3♀↑*2.6 SD31: G4♀↑*2.1	
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Creatine kinase Fasting triglycerides

Table 5: Clinical chemistry results: fold changes are listed if great than 1.5, Study Day (SD), Sex (M, F). Dose Group (G), direction and fold change

On SD 31, there was an adjuvant-related increase in plasma fibrinogen values in groups 2, 3, and 4. The adjuvant effect of increased FIB values was an expected finding consistent with immune stimulation associated with administration of a vaccine.

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	Neutrophil count SD31: G3♂↑*2.0 SD31: G4♂↑*2.1 SD31: G3♀↑*3.1 SD31: G4♀↑*2.8 Monocyte count: SD31: G3♂↑1.5 SD31: G4♂↑1.5 SD31: G3♀↑*2.5 SD31: G4♀↑*2.1 Eosinophils count: SD31: G3♀↑1.6 Basophils count: SD31: G2♂↑*2.8 SD31: G3♂↑*2.3 SD31: G4♂↑*2.6 SD31: G2♀↑*2.6 SD31: G3♀↑*2.6 SD31: G4♀↑*2.2	lymphocyte count Total leukocytes (WBC) Large unstained cells (LUC)
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

Table 6: Hematology results: fold changes are listed if great than 1.5, Study Day (SD), Sex (M, F), Dose Group (G), direction and fold change

On SD 31, there were significant increases in absolute neutrophil and absolute monocyte counts in groups 3 and 4. There was also an adjuvant-related increase in absolute basophil counts in groups 2, 3, and 4. The test article effects of increased mean neutrophil and monocyte counts values were mild in extent, and all mean values exceeded the historical reference range from (b) (4) rats. These test article effects were secondary effects caused by the microscopic inflammation and necrosis at test article injection sites.

Organ Weights:

<i>SEX</i>	<i>MALE</i>				<i>FEMALE</i>			
<i>GROUPS</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>
<i>NUMBER OF ANIMALS</i>	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
BODY WEIGHT (TERMINAL)	456.65/ 483.23	427.63/ 472.01	421.29/ 483.13	432.42/ 495.39	248.90/ 276.36	254.05/ 260.43	238.51/ 264.10	251.23/ 264.03
BRAIN	2.1394/ 2.1180	2.1729/ 2.0934	2.0752/ 2.1364	2.1198/ 2.1368	2.0052/ 1.9115	1.9925/ 1.9209	1.9522/ 1.9117	1.9737/ 2.0024
ADRENALS	0.07171/ 0.06748	0.07057/ 0.06440	0.06782/ 0.06751	0.06678/ 0.06414	0.07235/ 0.06777	0.07256/ 0.06692	0.06519/ 0.06463	0.07279/ 0.07167
HEART	1.6076/ 1.4663	1.4625/ 1.5632	1.4295/ 1.5405	1.5155/ 1.5867	0.9603/ 0.9859	0.9649/ 0.9513	0.9368/ 0.9412	1.0064/ 0.9747
KIDNEYS	3.2578/ 3.0657	3.2091/ 3.1801	3.0763/ 3.3215	3.2063/ 3.3666	1.9781/ 1.8739	2.0116/ 1.8567	1.9294/ 1.8894	2.0076/ 1.9171
LIVER	13.5148/ 12.8461	11.8857/ 12.4512	11.8054/ 13.2047	12.8065/ 13.6731	7.3358/ 7.5270	7.7226/ 7.2319	7.5011/ 7.1235	8.0558/ 7.4107
LUNGS	2.2367/ 2.2426	2.0271/ 2.3311	1.9972/ 2.2413	2.1469/ 2.3551	1.7590/ 1.5108	1.5850/ 1.4349	1.5589/ 1.6144	1.4960/ 1.5294
SPLEEN	0.9194/ 0.8967	0.9285/ 0.8664	1.0861/ 0.9939	0.9851/ 1.0992*	0.6002/ 0.5692	0.6228/ 0.5569	0.7463*/ 0.5957	0.7452*/ 0.6459
LYMPH Node, iliac (left)	0.02096/ 0.02012	0.02448/ 0.02496	0.02276/ 0.02881	0.01861/ 0.02825	0.01528/ 0.01520	0.07425/ 0.01573	0.02959/ 0.01525	0.01909/ 0.01846
LYMPH Node, iliac (left)	0.02303/ 0.02487	0.09748*/ 0.04301	0.17462*/ 0.06903*	0.07787/ 0.09141*	0.01296/ 0.01122	0.10237*/ 0.01977	0.15361*/ 0.06018*	0.06245*/ 0.06784*
THYMUS	0.4225/ 0.4141	0.3654/ 0.4189	0.3779/ 0.3747	0.3284*/ 0.4172	0.3651/ 0.3464	0.3698/ 0.3471	0.3299/ 0.3795	0.3759/ 0.3892
TESTES	4.5833/ 4.5750	4.6907/ 4.7945	4.5409/ 4.9155	4.7521/ 5.0454				
OVARIES					0.31310/ 0.12907	0.13298/ 0.12208	0.11445/ 0.13233	0.13461/ 0.13318
UTERUS					0.5512/ 0.7481	0.6057/ 0.5485	0.6331/ 0.6113	0.6345/ 0.6619

Table 7: Organ weight: absolute weights are expressed as mean (grams) [mean organ weight on SD 31 / mean organ weight on SD 43]; *different from controls at $P \leq 0.05$; **different from controls at $P \leq 0.01$.

Animals sacrificed at terminal necropsy had treatment-related weight increases for right iliac lymph nodes and spleen. Increased absolute right iliac lymph node weight occurred for animals in groups 2, 3, and 4 when compared to group 1. The increased weight correlated to minimal to moderate lymphoid hyperplasia. Absolute and relative spleen weights were increased in group 3 and 4 females. These findings are consistent with immune stimulation.

Animals sacrificed at recovery necropsy also had treatment-related weight increases for right iliac lymph nodes and spleen. Absolute and relative right iliac lymph node weights were increased for animals in groups 3 and 4, correlating to lymphoid hyperplasia. This finding was consistent with immune stimulation. Absolute spleen weight was increased for group 4 males, and spleen weight relative to body weight was increased in group 4 males and females. This change correlated microscopically to increased splenic hematopoiesis for group 4 males.

Gross Pathology:

<i>Treatment Group Findings – Treatment Phase</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>
Ln. iliac, right, enlargement	-	4	8	4	-	3	8	5
Ln. iliac, left, enlargement	-	-	-	-	-	1	-	-
Injection site, mass: white, intramuscular, right	-	-	-	-	-	-	1	-
Injection site, mass: yellow, intramuscular, right	-	-	-	-	-	-	-	1
Injection site, mass: right	-	-	3	2	-	-	2	1
Injection site, mass: right, intramuscular	-	-	5	3	-	-	6	2
Injection site, edema, intramuscular	-	-	1	-	-	-	-	-
Lymph nodes, inguinal, discoloration, yellow, right	-	-	-	-	-	-	-	1
Lymph nodes, inguinal, enlargement, right	-	-	-	-	-	-	1	1
Seminal vesicle, small, right	-	-	2	-	-	-	-	-

Table 8: Gross pathology - treatment phase

In animals sacrificed at terminal necropsy, test article-related changes occurred at the injection site of animals in groups 3 and 4, and in the inguinal lymph node of one group 4 female. Masses at the injection site were intramuscular, tan, white, or yellow, firm, semi-firm or soft (cut surface), often multi lobular, and contained caseous material. One group 4 female had an enlarged (4 x 4 x 2 mm) inguinal lymph node with yellow discoloration. These macroscopic changes correlated to microscopic necrosis and inflammation of the muscle and surrounding fascia, occasionally extending into the subcutis. Treatment related enlargement of the right iliac lymph node occurred in groups 2, 3, and 4; one group 3 female had enlargement of the right inguinal lymph node. These lymph node findings correlated microscopically to lymphoid hyperplasia.

<i>Treatment Group Findings – Recovery Phase</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>
Ln. iliac, right, enlargement	-	3	3	6	-	-	4	4
Ln. iliac, left, enlargement	-	-	-	1	-	-	-	-
Injection site, mass: intramuscular	-	-	-	-	-	-	1	1
Injection site, mass: right	-	-	-	-	-	-	-	1
Injection site, mass: right, intramuscular	1	1	5	7	-	-	5	3
Lymph node, mandibular, discoloration, red, bilateral	-	-	-	-	1	-	-	-
Kidney, dilation, fluid-filled, left	-	-	-	1	-	-	-	-
Skeletal muscle, mass, right, abdominal	-	-	1	-	-	-	1	-
Urinary bladder, calculus, white	-	1	-	1	-	-	-	-
Uterus: fluid-filled, clear, bilateral	-	-	-	-	-	-	-	2
Uterus: fluid-filled, bilateral	-	-	-	-	-	-	1	-

Table 9: Gross pathology - recovery group

In animals sacrificed at the recovery necropsy, test article-related injection site masses were observed in groups 3 and 4 and once in the adjuvant-treated group 2 and group 1. These masses were intramuscular, white or tan, firm, multi lobular, and contained caseous material. The masses ranged in size from 11 x 7 x 4 mm to 30 x 20 x 7 mm. The macroscopic masses observed in group 1 and group 2 animals were not present microscopically. These injection site masses in group 3 and 4 correlated microscopically

to necrosis and inflammation of the muscle and surrounding fascia, occasionally extending into the subcutis. Two group 3 animals had test article-related masses in abdominal skeletal muscle which were tan, firm, contained caseous material, and measured 8 x 8 x 8 mm and 20 x 15 x 5 mm. These masses correlated microscopically to necrosis and inflammation of the muscle and surrounding fascia. These lesions likely were an extension of changes at the adjacent injection site. Treatment-related enlargement of the right iliac lymph node also occurred in groups 2, 3, and 4; this finding correlated microscopically to minimal to mild lymphoid hyperplasia.

Microscopic finding:

<i>Treatment Group Findings – Treatment Phase</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>
Nerve sciatic: inflammation, subacute, perineural, diffuse, (mild, moderate)	1	6	3	2	-	-	4	-
Nerve sciatic: inflammation, subacute, perineural, focal, (minimal)	-	-	1	-	-	-	-	-
Nerve sciatic: inflammation, subacute, perineural, multifocal, (minimal, mild)	-	1	4	1	-	1	-	1
Iliac lymph node-left; hyperplasia; lymphoid (minimal to moderate)	-	1	1	-	-	1	-	1
Iliac lymph node-left, foamy macrophages; multifocal (minimal)	-	-	-	-	-	-	-	2
Iliac lymph node-right; hyperplasia; lymphoid (minimal to moderate)	-	7	7	6	-	4	8	7
Iliac lymph node-right, foamy macrophages; multifocal (minimal)	-	-	2	3	-	-	4	2
Harderian gland; inflammation; chronic; focal (minimal)	-	-	2	-	1	1	-	-
Harderian gland; inflammation; chronic; multifocal (minimal)	1	-	-	-	1	1	1	-
Thymus; hemorrhage; focal (minimal)	-	-	-	-	1	-	-	-
Thymus; hemorrhage; multifocal (minimal to mild)	2	-	4	5	-	-	1	1
Pancreas; inflammation; chronic; focal (minimal)	-	-	-	-	1	-	-	-
Pancreas; inflammation; chronic; multifocal (minimal)	-	-	-	-	-	1	-	-
Pancreas; inflammation; subacute; focal (minimal)	1	-	-	-	-	-	-	-
Pancreas; atrophy; lobular; focal (minimal)	1	-	-	-	-	-	-	-
Lymph node - mandibular; plasmacytosis; medullary (minimal to mild)	2	3	2	1	2	-	-	-
Lymph node - mandibular; hyperplasia; lymphoid (mild)	2	-	-	-	-	-	-	-
Lung; inflammation; acute; focal (minimal)	-	-	-	-	-	-	-	1
Lung; inflammation; acute; multifocal (mild)	1	-	-	-	-	-	-	-
Lung; inflammation; chronic; focal (minimal)	-	-	-	1	-	-	-	-
Lung; hemorrhage; focal (minimal)	-	-	-	-	-	-	-	1
Lung; hemorrhage; multifocal (minimal)	10	8	8	8	4	-	1	5
Lung; alveolar macrophages; increased; focal (minimal)	-	-	-	1	-	-	1	1
Lung; alveolar macrophages; increased; multifocal (minimal)	1	-	1	-	-	-	-	-
Lung; ectopia; osseous; focal (minimal)	-	-	-	1	-	-	-	-

Heart, degeneration; myofiber; focal (minimal)	1	-	-	1	-	-	-	-
Heart, infiltrate; mononuclear cell; myocardial; focal (minimal)	-	-	-	-	-	1	1	-
Heart, infiltrate; mononuclear cell; myocardial; multifocal (minimal)	1	-	-	1	-	-	-	-
Heart, hemorrhage; myocardial; focal (minimal)	-	-	-	-	-	-	1	-
Spleen, hematopoiesis; increased (minimal)	-	-	-	1	-	-	-	-
Spleen, macrophage; increased; diffuse (minimal)	-	-	-	-	-	-	-	4
Liver, infiltrate; mononuclear cell; multifocal (minimal)	10	8	8	7	10	8	8	8
Liver, necrosis; hepatocellular; multifocal (minimal)	1	-	-	-	-	-	-	-
Liver, vacuolation; hepatocellular; focal (minimal)	-	-	1	-	-	-	-	-
Liver, vacuolation; hepatocellular; multifocal (minimal, mild)	-	-	-	1	-	2	-	3
Kidney, fibrosis; interstitial; focal (minimal)	-	-	-	-	-	1	1	-
Kidney, infiltrate; mononuclear cell; interstitial; focal (minimal)	1	-	1	-	-	-	-	1
Kidney, infiltrate; mononuclear cell; interstitial; multi (minimal)	2	1	3	4	2	1	3	5
Kidney, inflammation; subacute; pelvic; unilateral; diffuse (minimal)	1	-	-	-	-	-	-	-
Kidney, cyst; focal (minimal)	-	-	-	-	-	-	1	-
Kidney, cyst; multifocal (minimal)	-	-	-	1	-	-	-	-
Kidney, basophilia; tubular; focal (minimal)	2	1	3	3	1	-	-	1
Kidney, basophilia; tubular; multifocal (minimal)	2	2	3	6	1	1	3	1
Kidney, tubular mineralization; focal (minimal)	1	-	-	-	-	1	-	-
Kidney, tubular mineralization; multifocal (minimal)	-	-	-	-	3	3	2	-
Colon, dilation, (mild)	-	-	1	-	-	-	-	-
Stomach; inflammation; acute; submucosal; fundic / glandular (minimal)	1	-	-	-	-	-	-	-
Lymph node - mesenteric; inflammation; subacute; mesenteric; adipose; (mild)	-	-	-	-	1	-	-	-
Testis; degeneration; tubular; multifocal (minimal)	-	1	1	-	-	-	-	-
Prostate; inflammation; acute; glandular; focal (minimal)	1	-	-	1	-	-	-	-
Prostate; inflammation; chronic; interstitial; multifocal (minimal, mild)	2	1	-	1	-	-	-	-
Seminal vesicle; secretion; decreased; unilateral (mild)	-	-	1	-	-	-	-	-
Epididymis; infiltrate; mononuclear cell; interstitial; focal. (minimal)	2	1	-	3	-	-	-	-
Epididymis; infiltrate; mononuclear cell; interstitial; multi. (minimal)	6	-	-	1	-	-	-	-
Skeletal muscle; degeneration / necrosis; myofiber; multifocal (minimal)	-	1	-	-	-	-	-	1
Skeletal muscle; inflammation; chronic active; multifocal (mild)	-	-	1	-	-	-	-	-
Skeletal muscle; inflammation; subacute; focal (minimal)	-	-	-	-	-	1	-	-
Skeletal muscle; inflammation; subacute; multifocal (minimal, mild)	-	10	2	4	-	2	2	2
Skeletal muscle; necrosis; multifocal (mild)	-	-	1	-	-	-	-	-
Skeletal muscle; hemorrhage; multifocal (minimal)	1	-	-	-	-	-	-	-
Skeletal muscle; macrophage; pigmented; multifocal (minimal)	-	-	-	-	-	1	-	-

Skin, inflammation; chronic; subcutaneous; focal (minimal)	-	1	-	-	-	-	-	-
Skin, inflammation; chronic; subcutaneous; multifocal (minimal)	-	-	-	-	-	-	-	1
Skin, inflammation; subacute; multifocal (minimal)	-	-	-	-	-	1	-	-
Skin, inflammation; subacute; subcutaneous; multifocal (minimal, mild)	-	-	-	-	-	1	1	-
Mammary gland; inflammation; chronic; interstitial; multifocal (minimal)	-	-	-	-	-	-	1	-
Mammary gland; inflammation; subacute; interstitial; multifocal (minimal)	-	-	-	-	-	1	-	-
Injection site; degeneration; myofiber; focal (minimal)	1	-	-	-	1	-	-	-
Injection site; degeneration; myofiber; multifocal (minimal)	1	-	-	-	-	1	-	-
Injection site; edema; focal (moderate)	-	-	1	-	-	-	-	-
Injection site; inflammation; chronic; diffuse (moderate)	-	-	-	-	-	-	-	1
Injection site; inflammation; chronic; multifocal (moderate)	-	-	3	2	-	-	2	1
Injection site; inflammation; chronic active; diffuse (moderate)	-	-	-	-	-	-	-	1
Injection site; inflammation; chronic active; multifocal (mild, moderate)	-	-	2	1	-	-	5	-
Injection site; inflammation; granulomatous; focal (moderate)	-	-	1	-	-	-	-	-
Injection site; inflammation; granulomatous; multifocal (mild, moderate)	-	-	2	-	-	-	1	1
Injection site; inflammation; subacute; diffuse (mild)	-	-	-	1	-	-	-	-
Injection site; inflammation; subacute; focal (minimal)	-	2	-	-	-	-	1	-
Injection site; inflammation; subacute; multifocal (minimal, mild)	1	5	1	4	-	3	1	1
Injection site; necrosis; diffuse (marked)	-	-	-	-	-	-	1	-
Injection site; necrosis; focal (mild, moderate)	-	-	2	-	-	-	2	-
Injection site; necrosis; multifocal (mild, moderate, marked)	-	-	6	3	-	-	5	4
Injection site; hemorrhage; focal (minimal, mild)	1	-	-	-	1	-	1	-
Injection site; hemorrhage; multifocal (minimal, mild)	1	-	2	-	-	-	-	-
Bone with marrow - femur; inflammation; chronic; synovial; focal (mild)	-	-	-	1	-	-	-	-
Bone with marrow - femur; inflammation; subacute; periosteal; multifocal (mild)	-	-	-	-	-	-	1	-
Bone with marrow - femur; inflammation; subacute; patella; diffuse (minimal)	-	-	-	-	-	1	-	-
Lymph node - inguinal; inflammation; chronic; multifocal (moderate)	-	-	-	-	-	-	-	1
Lymph node - inguinal; necrosis; diffuse (moderate)	-	-	-	-	-	-	-	1
Lymph node - inguinal; hyperplasia; lymphoid (minimal)	-	-	-	-	-	-	1	-
Uterus: dilation (minimal, mild)	-	-	-	-	-	2	1	-
Uterus: dilation; bilateral (moderate)	-	-	-	-	-	1	-	-
Uterus: dilation; unilateral (mild)	-	-	-	-	-	-	-	1

Table 10: Histopathology - treatment phase

At terminal necropsy, test article-related adverse changes occurred at the injection site in groups 3 and 4, with a higher frequency and severity in group 3. Mild to marked necrosis occurred within muscle at the injection site for groups 3 and 4, with a higher frequency and severity in group 3, accompanied by mild to moderate chronic, chronic-active, or granulomatous inflammation which often extended to the surrounding fascia and occasionally extended into the subcutis. Necrosis was characterized by single or multiple, sometimes coalescing, areas of amorphous, eosinophilic, cellular debris, often containing fragments or aggregates of neutrophils. Necrotic areas were surrounded by inflammation which varied in composition from chronic (predominantly mononuclear cells, with or without fibrosis), to chronic-active (chronic inflammation/fibrosis with areas of neutrophils), to granulomatous (numerous macrophages plus lymphocytes, with or without fibrosis). Necrosis and/or inflammation were occasionally observed in routine sections of skeletal muscle taken from areas adjacent to the injection site. These inflammatory changes correlated to alterations in peripheral leukocyte counts. Minimal to mild subacute inflammation at the injection site occurred in groups 2, 3, and 4 (primarily groups 2 and 4) without injection site necrosis. Subacute inflammation consisted of lymphocytes, plasma cells, and scattered neutrophils. Inflammatory infiltrates extended to surrounding tissues and were observed in routine sections of skeletal, sciatic nerve, and femur. These inflammatory changes correlated to alterations in peripheral leukocyte counts and were treatment related.

Test article-related changes were also observed in the lymph nodes. Scattered accumulations of foamy macrophages occurred in the iliac lymph nodes of animals in groups 3 and 4. One group 4 animal had test article-related necrosis and chronic inflammation of an inguinal lymph node, a change that was adverse. Treatment-related minimal to moderate lymphoid hyperplasia occurred in iliac lymph nodes (primarily in the right node, ipsilateral to the injection site) in groups 2, 3, and 4, and in the inguinal lymph node of one group 3 animal. This change was consistent with immune stimulation and correlated to increased serum globulin values for groups 3 and 4.

Four group 4 females had test article-related mild increases in macrophage numbers in the spleen, possibly correlating to increased spleen weight.

<i>Treatment Group Findings – Recovery Phase</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>
Nerve sciatic: inflammation, subacute, perineural, diffuse, (minimal, mild)	-	4	5	-	-	-	-	-
Nerve sciatic: inflammation, subacute, perineural, multifocal, (minimal, mild)	-	3	4	6	-	3	2	1
Pituitary; cyst; multiple (minimal)	-	-	-	-	-	-	1	-
Iliac lymph node-left; hyperplasia; lymphoid (minimal)	1	-	1	1	-	-	-	-
Iliac lymph node-left, foamy macrophages; multifocal (minimal)	-	-	2	2	-	-	2	-
Iliac lymph node-right; hyperplasia; lymphoid (minimal to mild)	-	4	8	7	-	-	4	5

Iliac lymph node-right, foamy macrophages; multifocal (minimal)	-	-	8	8	-	-	9	10
Adrenal gland, vacuolation; cortical; multifocal (minimal)	-	-	1	-	-	-	-	-
Thyroid gland; infiltrate; mononuclear cell; focal (minimal)	-	-	-	1	-	-	-	-
parathyroid gland; infiltrate; mononuclear cell; multifocal (minimal)	-	1	-	-	-	-	-	-
Harderian gland; inflammation; chronic; focal (minimal)	-	1	3	4	1	3	-	1
Harderian gland; inflammation; chronic; multifocal (minimal)	1	-	-	-	2	1	-	1
Thymus; hemorrhage; focal (mild)	-	-	1	-	-	-	-	-
Thymus; hemorrhage; multifocal (minimal to mild)	2	1	2	-	-	-	-	-
Pancreas; inflammation; chronic; focal (minimal)	-	-	1	-	1	1	-	-
Pancreas; inflammation; chronic; multifocal (minimal)	-	-	1	-	-	-	-	-
Pancreas; inflammation; subacute; focal (minimal)	-	1	-	-	-	-	-	-
Pancreas; inflammation; subacute; multifocal (minimal)	-	-	1	-	-	-	-	-
Pancreas; vacuolation; acinar cell; multifocal (minimal)	-	-	1	-	-	-	-	-
Pancreas; hemorrhage; multifocal (minimal)	-	-	-	1	-	-	-	-
Pancreas; atrophy; lobular; focal (minimal)	-	-	-	-	-	1	-	-
Mandibular salivary gland; inflammation; chronic; focal (minimal)	-	-	1	-	-	-	-	-
Lymph node - mandibular; plasmacytosis; medullary (minimal, mild)	1	2	2	2	3	-	-	1
Lung; inflammation; acute; focal (minimal)	-	1	-	-	1	-	1	-
Lung; hemorrhage; multifocal (minimal)	6	4	9	3	3	3	1	3
Lung; alveolar macrophages; increased; focal (minimal)	-	-	1	-	-	-	-	-
Lung; alveolar macrophages; increased; multifocal (minimal)	1	1	1	1	-	1	-	1
Heart, infiltrate; mononuclear cell; myocardial; focal (minimal)	-	1	2	-	1	1	1	-
Heart, infiltrate; mononuclear cell; myocardial; multifocal (minimal)	-	1	-	2	-	-	-	-
Spleen, erythrophagocytosis (minimal)	4	-	-	-	-	-	-	-
Spleen, hematopoiesis; increased (minimal, mild)	-	-	5	10	-	1	-	1
Spleen, macrophage; increased; diffuse (minimal, mild)	-	-	-	-	-	-	-	2
Liver, infiltrate; mononuclear cell; multifocal (minimal)	9	8	10	10	9	10	8	10
Liver, inflammation; acute; focal (minimal)	-	-	-	-	-	-	1	-
Liver, necrosis; hepatocellular; multifocal (minimal)	-	-	-	-	1	-	-	-
Liver, vacuolation; hepatocellular; multifocal (minimal)	-	-	-	-	1	-	-	-
Urinary bladder; infiltrate; mononuclear cell; submucosal; focal (minimal)	-	-	1	-	-	-	-	-
Urinary bladder; infiltrate; mononuclear cell; submucosal; multifocal (minimal)	-	-	1	-	-	-	-	-
Urinary bladder; inflammation; acute; submucosal; diffuse (mild)	1	-	-	-	-	-	-	-

Urinary bladder; inflammation; acute; submucosal; mucosal; diffuse (mild)	-	-	-	1	-	-	-	-
Urinary bladder; hyperplasia; urothelial-cell; diffuse (mild, moderate)	1	-	-	-	-	-	-	-
Kidney, infiltrate; mononuclear cell; interstitial; focal (minimal)	-	1	-	-	-	-	2	-
Kidney, infiltrate; mononuclear cell; interstitial; multi (minimal, mild)	1	1	6	6	1	3	1	2
Kidney, inflammation; subacute; pelvic; unilateral; diffuse (moderate)	-	-	-	1	-	-	-	-
Kidney, inflammation; subacute; pelvic; unilateral; diffuse (mild)	1	-	-	-	-	-	-	-
Kidney, hyperplasia; urothelial-cell; bilateral; diffuse (mild)	-	-	-	1	-	-	-	-
Kidney, cyst; focal (minimal)	-	1	-	-	-	1	1	-
Kidney, dilation; pelvic; bilateral (mild)	-	-	-	1	-	-	-	-
Kidney, dilation; pelvic; unilateral (mild)	-	-	1	-	-	-	-	-
Kidney, dilation; tubular; focal (minimal)	-	-	-	-	-	-	1	-
Kidney, dilation; tubular; multifocal (minimal, mild)	-	-	-	1	-	1	-	-
Kidney, basophilia; tubular; focal (minimal, mild)	3	4	3	1	1	-	1	1
Kidney, basophilia; tubular; multifocal (minimal, mild)	1	1	5	3	-	3	-	-
Kidney, tubular mineralization; multifocal (minimal)	-	-	-	-	5	2	6	2
Stomach; erosion; mucosal; non-glandular; focal (minimal)	-	-	1	-	-	-	-	-
Stomach; inflammation; acute; submucosal; fundic / non-glandular (minimal)	-	-	1	-	-	-	-	-
Stomach; hyperplasia; squamous; multifocal (mild)	-	-	1	-	-	-	-	-
Stomach; cyst; squamous; focal (minimal)	-	-	-	1	-	-	-	-
Prostate, fibrosis; interstitial; multifocal (moderate)	-	-	-	1	-	-	-	-
Prostate, inflammation; acute; glandular; focal (minimal)	1	-	1	-	-	-	-	-
Prostate, inflammation; acute; glandular; multifocal (minimal, mild)	1	-	1	1	-	-	-	-
Prostate, inflammation; chronic; interstitial; focal (minimal)	-	-	1	-	-	-	-	-
Prostate, inflammation; chronic; interstitial; multifocal (minimal to moderate)	6	5	2	6	-	-	-	-
Prostate, atrophy; lobular; multifocal (moderate)	-	-	-	1	-	-	-	-
Epididymis; infiltrate; mononuclear cell; interstitial; multifocal (minimal)	1	4	3	2	-	-	-	-
Uterus, dilation (minimal)	-	-	-	-	-	-	-	1
Uterus, dilation; bilateral (minimal, mild)	-	-	-	-	3	-	1	1
Skeletal muscle; inflammation; chronic; focal (minimal)	-	-	1	-	-	-	-	-
Skeletal muscle; inflammation; chronic; multifocal (minimal, mild)	-	5	5	1	-	2	2	1
Skeletal muscle; inflammation; granulomatous; focal (minimal)	-	-	-	1	-	-	-	-
Skeletal muscle; inflammation; granulomatous; multifocal (mild, moderate)	-	1	-	1	-	-	2	-
Skeletal muscle; necrosis; focal (mild, moderate)	-	-	1	-	-	-	1	-
mammary gland; inflammation; chronic; interstitial; multifocal (minimal)	-	-	-	-	-	1	-	1
Injection site; fibrosis; focal (minimal)	-	-	-	-	-	1	-	-

Injection site; fibrosis; multifocal (mild)	-	-	1	-	-	-	1	-
Injection site; inflammation; chronic; focal (minimal)	-	1	-	-	-	1	-	-
Injection site; inflammation; chronic; multifocal (minimal to moderate)	-	2	1	1	-	1	1	-
Injection site; inflammation; granulomatous; diffuse (mild)	-	-	-	-	-	-	-	1
Injection site; inflammation; granulomatous; multifocal (mild, moderate)	-	-	5	7	-	-	5	5
Injection site; necrosis; diffuse (marked)	-	-	1	-	-	-	-	-
Injection site; necrosis; focal (mild to marked)	-	-	-	-	-	-	3	1
Injection site; necrosis; multifocal (mild to marked)	-	-	4	7	-	-	3	5
Bone with marrow - femur; fibrosis; epiphyseal; focal (minimal)	-	1	-	-	-	-	-	-

Table 11: *Histopathology - recovery phase*

In animals sacrificed at the recovery necropsy, test article-related adverse changes occurred at the injection site of animals in groups 3 and 4. These changes correlated to masses observed macroscopically and consisted of mild to marked necrosis accompanied by minimal to moderate chronic, or mild to moderate granulomatous, inflammation. Necrosis was characterized by single or multiple, sometimes coalescing, areas of amorphous, eosinophilic, cellular debris. Necrotic areas were surrounded by inflammation which varied in composition from chronic (predominantly mononuclear cells, with or without fibrosis) to granulomatous (numerous macrophages plus lymphocytes, with or without fibrosis). Inflammation was occasionally observed in routine sections of skeletal muscle taken from areas adjacent to the injection site. In two group 3 animals, areas of necrosis and inflammation in the abdominal skeletal muscle were similar microscopically to changes at the injection site and correlated to macroscopic masses. These lesions likely were an extension of changes at the adjacent injection site. Treatment related minimal to mild chronic inflammation also occurred at injection sites in the absence of injection site necrosis in groups 2, 3, and 4. This represented a normal progression from the subacute inflammation present on Day 31 observed at injection sites that lacked necrotic changes. Chronic or granulomatous inflammatory infiltrates extended to surrounding tissues and were observed in routine sections of skeletal and sciatic nerve. These inflammatory changes were considered to be treatment-related and adverse.

Treatment-related minimal to mild lymphoid hyperplasia also occurred in the right iliac lymph nodes (ipsilateral to the injection site) in groups 2, 3, and 4. This change was consistent with immune stimulation, correlated to increased serum globulin values for groups 3 and 4, and was non-adverse. Test article-related changes were also observed in the iliac lymph nodes. Scattered accumulations of foamy macrophages occurred in the iliac lymph nodes of animals in groups 3 and 4. Two group 4 females had test article-related minimal to mild increases in macrophage numbers in the spleen. Minimal to mild increased splenic hematopoiesis was observed in group 3 and 4 males, likely a compensatory response to the decreased HGB and HCT levels noted on Day 31 and correlating to the increased spleen weight for group 4 males.

Body temperature

Group	Males	Females
1	0	5
2	0	9
3	0	0
4	0	0

Table 12: Occurrence of body temperature $\geq 40^{\circ}\text{C}$

No treatment related significant increases in body temperature were observed.

Local toxicity:

Macroscopic findings: Masses at the injection site were intramuscular, tan, white, or yellow, firm, semi-firm or soft (cut surface), often multi lobular, and contained caseous material.

One group 4 female had an enlarged (4 x 4 x 2 mm) inguinal lymph node with yellow discoloration. These macroscopic changes correlated to microscopic necrosis and inflammation of the muscle and surrounding fascia, occasionally extending into the subcutis.

Draize score: Treatment with AV7909 did not result in any dermal Draize test observations in either main study or recovery animals at any point throughout the study.

Serology: The samples were analyzed for seroconversion using an anti-recombinant protective antigen (rPA) (b) (4). High IgG anti-PA titers were observed at similar levels after administration of a human dose of A V7909 vaccine in group 3 and 4. After administration of A V7909, (b) (4) rats generated significant antibody levels with specificity for rPA which thereby served as a marker of administration of AV7909.

Test article related effects

- Injection site: (partially reversible)
 - Masses at the injection site (IM): tan, white, or yellow, firm, semi-firm or soft (cut surface), often multi lobular, and contained caseous material (group 3 and 4), correlated to microscopic necrosis (mild to marked) and inflammation (moderate chronic, chronic-active, or granulomatous) of the muscle and surrounding fascia, occasionally extending into the subcutis.
 - Enlargement of the iliac lymph node (minimal to moderate) correlated microscopically to lymphoid hyperplasia (group 2, 3 and 4), partially reversible.
 - Inflammation at the sciatic nerve (group 2, 3, and 4)
- Transient increase in neutrophil, monocyte counts and fibrinogen (group 3 and 4); as well as basophil counts (group 2, 3 and 4)
- Mild increases in macrophage numbers in the spleen, (partially reversible). Spleen hematopoiesis (minimal to mild, after recovery phase)

Assessment:

Treatment with adjuvant CPG 7909 or CPG 7909 combined with AVA (AV7909) had no effect on cage-side, clinical, or dermal Draize observations. There were no treatment-related effects on body weight or body weight changes, food consumption, body temperature and ocular observations. Following IM treatment with AV7909, rats elicited a strong, specific anti-protective antigen (PA) IgG antibody response.

Test article-related findings for Groups 3 (AV7909 FDP Lot 1) and 4 (AV7909 FDP Lot 2) were observed on SD 31 at injection sites, consisting of necrosis and inflammation which correlated to macroscopic observation of masses. Similar changes occurred in surrounding tissues and regional lymph nodes. Treatment (adjuvant)-related findings in Groups 2 (adjuvant control), 3, and 4 were also observed on SD 31 at the injection site, consisting of subacute inflammation without necrosis. Alterations in clinical pathology parameters and organ weights, and microscopic changes in lymphoid tissues were secondary effects related to injection site necrosis and inflammation, or were consistent with immune stimulation related to adjuvant or vaccine administration. Following the recovery period, there was no evidence of progression of the necrosis or inflammation noted on SD 31, and there was no evidence of delayed toxicity. Inflammation that was subacute on SD 31 had been replaced by chronic inflammation characterized by chronic or granulomatous inflammatory infiltrates observed at the injection site. This represented a progression from the subacute inflammation present on SD 31 observed at injection sites that lacked necrotic changes. Necrosis and granulomatous inflammation at the injection site was considered adverse, but was expected to resolve over time.

Reproductive and developmental toxicology study:

Title: AV7909 Vaccine: Combined Intramuscular Pre- and Postnatal Developmental and Reproductive Toxicity Study in Rats

Study no.: T05153

Conducting laboratory and location: (b) (4)

Date of study initiation: 12/14/2018

Final report: 10/09/2019

GLP compliance: Yes

QA reports: Yes

Drug, lot #: AV7909: lot number: 26000440; CPG 7909: lot number: 100001

Animal species: (b) (4) rats

Doses:

Group	Dose Material	Total Dose Volume (mL)
1	Control	0.5
2	Adjuvant (CPG 7909 + (b) (4))	0.5
3	AV7909	0.5

Table 13: Study design: Three intramuscular (IM, bolus) injections of the appropriate dose material was administered into the thigh muscle on SD 1, SD 15, and GD 7; 0.2 mL dose was injected into each thigh and the 0.1 mL was alternatively injected into the left and right thigh.

Species/strain: (b) (4) rats

Number/sex/group: 22

Route, formulation, volume, and infusion rate: Three intramuscular (IM, bolus) injections (0.2, 0.2, and 0.1 mL for a total injection volume of 0.5 mL) of the appropriate dose material was administered into the thigh muscle on SD 1 (14 days prior to start of cohabitation), SD 15 (day of cohabitation), and GD 7 (Day 1). The 0.2 mL dose was injected into each thigh and the 0.1 mL was alternatively injected into the left and right thigh

Study design:

Day	Dose location	Dose location
Volume of injection	0.2 mL	0.1 mL
SD1	Left and right thigh	Left thigh
SD15	Left and right thigh	Right thigh
GD7	Left and right thigh	Left thigh

Table 14: Injection site rotation (table provided by the sponsor)

Mating: Beginning on SD 15, each F0 female was continually housed on a 1:1 basis in the cage of a randomly selected male. Animals remained paired until evidence of mating was observed.

Parameters and endpoints evaluated:

Measurements and Observations – F0 Generation:

Clinical Observations: Observations for moribundity and mortality were performed twice daily during the quarantine and study periods, except on the day of receipt and the day of necropsy when animals scheduled for necropsy were observed once. During the lactation period, offspring were observed for moribundity and mortality at the time of observation of the dam. Detailed clinical observations were performed on all F₀ females at least once during pretest (once for group assignment) and at least once daily beginning SD 1. On days of dose administration, observations were performed twice: pre-dose and 1 to 2 hours post-dose.

Injection site observation: The injection site area of each F₀ female was observed for erythema (redness) and edema (swelling) prior to each dose administration and 1 to 2 hours following each dose administration. Subsequent observations were made daily (target 24-hour intervals after dose administration) for up to 1 week following each dose administration, until a score of 0 was obtained, or until termination

Body Weight: Individual body weights were recorded for F₀ females once on the day of arrival and once during quarantine, for group assignment. Body weights were recorded weekly during pre-mating (SD 1, 7, and 14), weekly during cohabitation, on GD 0, 3, 6, 9, 12, 15, 18, and 21, and on PND 1, 4, 7, 14, and 21.

Food Consumption: Quantitative food consumption parameters were recorded weekly during pre-mating (SD 1, 7, and 14), on GD 0, 3, 6, 9, 12, 15, 18, and 21, and on PND 1, 4, 7, and 14.

Litter Evaluation: A birth exam was conducted on PND 0, as soon as possible after parturition was deemed complete. Additional examination days were PND 1 through 4, 7, 10, 14, and 21. At each timepoint, each pup was examined for clinical observations (including any external developmental abnormalities), viability, and sex. The body weight of each live pup was recorded on PND 1, 4, 7, 10, 14, and 21.

Measures of pup physical development and reflex ontogeny were assessed pre-weaning, until achievement, as follows:

- Surface righting (from PND 1)
- Incisor eruption (from PND 7)
- Eye opening (from PND 10)
- Auditory startle reflex (from PND 10)
- Pupil constriction (on PND 21)

Acute Phase Protein Assessment: Alpha-2-macroglobulin and Alpha-1-acid glycoprotein: blood samples were collected from F₀ females in the laparohysterectomy cohort on GD 21

Immunogenicity: Blood samples for evaluation of antibody response were collected on SD1, GD21 and PND 21.

Results:

Mortality: There was no mortality during the study; all F0 females survived until scheduled termination.

Clinical Observations: Test article-related clinical observations in F0 females dosed with the test article were limited to a small mass (less than 5 mm) in the hindlimb in one female animal in the vaccine group during gestation and lactation (GD 6 through PND 21). The palpable mass correlated with the presence of a nodule at the injection site at necropsy on PND 21. One female in the adjuvant group had similar small palpable masses during gestation and lactation; however, there were no correlating nodules at necropsy. These masses are likely a local reaction due to injection of the adjuvant and test article into the hindlimb. Low incidences of abrasions/scabbing on the hindlimb(s) in the control, adjuvant and vaccine groups during premating, gestation and/or lactation are likely due to shaving of the injection site area. There were no abnormal maternal care observations during lactation.

Clinical observations in the test article group that were incidental included genital swelling, rough coat, eye discharge (red) and alopecia. These observations were not considered test article-related because they occurred at low incidences (1 or 2 animals) or at similar incidences as the control group.

Injection site observations (F0): Test article-related injection site edema occurred in F0 females but was not considered adverse because it was transient, and the severity was generally very slight to slight (scored as 1 or 2). The severity and duration of the edema were similar in the adjuvant and vaccine groups. The severity of the edema generally ranged from very slight or barely perceptible swelling to slight swelling, with moderate edema (score as 3) occurring sporadically after the second and third injections in a small number of animals in the adjuvant and test article groups. The highest incidence of edema was seen in the adjuvant and test article groups 24 hours after the SD 1, SD 15 and GD 7 injections. All 44 animals in the adjuvant and test article groups had edema 24 hours after the first injection compared to 12 animals in the control group. In contrast, the incidence of edema in the control, adjuvant and test article groups was similarly high 24 hours after the second injection (43, 44 and 44 animals, respectively). The edema severity was similar in the adjuvant and test article groups at the peak incidence 24 hours after the first and second injections (mean score) and 24 hours after the Erythema was not observed in the test article group during the study; one animal in the adjuvant group had transient very slight erythema after the second injection, 2 days post-administration. The edema was first seen approximately 1 hour or 24 hours after injection. The edema had an earlier onset in the test article group after the second injection; the number of animals with edema approximately 1 hour after the second injection was higher than after the first injection (23 vs. 12 animals). A similar earlier onset was seen in the adjuvant group. Most notably, the edema had an earlier onset in the control, adjuvant and test article groups after the third injection; the number of animals with edema approximately

1 hour after that injection was 25, 30 and 35 animals, respectively, compared to 4, 27 and 23 after the second injection.

In the control group, all edema had resolved within 5 days after injection. In the adjuvant and test article groups, the edema had resolved in most animals 7 days after injection.

Except for 2 animals that still had a score of 1 on SD 15, all animals had a score of 0 at the pre-dose injection site observation on SD 15 and GD 7, indicating that edema generally resolved within 2 to 3 weeks after the first and second injection.

Body Weight and Body Weight Changes: There were no treatment-related effects on mean body weights or mean body weight gains during the premating, gestation, or lactation periods.

Food Consumption: There were no treatment-related effects on mean food consumption during the premating, gestation, or lactation periods.

Reproductive Performance:

Parameter		F0 generation		
		Control	Adjuvant	AV7909
Female animals paired	N	44	44	44
Rats that mated	N (%)	44 (100)	44 (100)	44 (100)
Female Fertility Index (both groups)	N/N (%)	43 (97.7)	44 (100)	43 (97.7)

Table 15: Summary of mating and fertility: F0 generation female rabbits, N/N (number of pregnancies/number of rabbits that mated)

Reproductive Performance: Reproductive performance parameters were not affected by test article administration. All females on study had evidence of mating observed. Female fertility index in the control, adjuvant and test article groups was 98%, 100% and 98%, respectively. Gestation length among females that delivered a litter was comparable in the control, adjuvant and test article groups; the mean gestation length was 21.9, 21.8 and 22.0 days, respectively. Test article administration did not affect the number of viable litters, the gestation index, or litter survival; all litters were viable and survived to PND 21, nor did it affect the number of implantation sites (means of 15.0-15.2) or percentage post implantation loss (6.7% vs. 6.4% and 5.3% in control and adjuvant) in the natural delivery cohort.

Natural Delivery and Litter Observations:

Parameter		F0 generation		
		Control	Adjuvant	AV7909
Rats tested	N	22	22	22
Pregnant	N (%)	22 (100)	22 (100)	22 (100)
Delivered litters	N (%)	22 (100)	22 (100)	22 (100)
Viable litters	N (%)	22 (100)	22 (100)	22 (100)
Duration of gestation	MEAN±S.D.	21.9±0.3	21.8±0.4	22.0±0.4
Implantation sites per delivered litter	MEAN±S.D.	15±2	15±2	15±3
Post-Implantation loss	MEAN±S.D.	6.4±5.8	5.2±5.9	6.7±6.4
Gestation index	(%)	100.0	100.0	100.0
Litter survival	(%)	100.0	100.0	100.0

Table 16: Summary of natural delivery observation**Embryo-Fetal Survival, Fetal Weight, and Gravid Uterine Weight in the Cesarean section group**

Maternal performance – Cesarean section		F0 generation		
		Control	Adjuvant	AV7909
Number of females	N	22	22	22
Number of female pregnant	N	21	22	21
	%	95.5	100.0	95.5
Number of corpora lutea	Mean	15.8	15.2	15.5
	SD	2.9	1.8	2.2
Number of implantations	Mean	15.0	14.9	14.8
	SD	3.6	2.0	2.5
Late resorption (g)	Mean	0.0	0.0	0.0
	SD	0.0	0.0	0.0
% Pre-implantation loss	Mean	6.76	2.52	5.51
	SD	16.04	3.93	6.18
% Post-implantation loss	Mean	3.18	3.77	4.83
	SD	4.03	4.68	4.98
Total number of fetuses	Mean	14.5	14.3	14.1
	SD	3.6	2.2	2.4
Number of live fetuses	Mean	14.5	14.3	14.1
	SD	3.6	2.2	2.4
Live male fetus/litter	Mean	7.7	6.4	7.2
	SD	2.8	2.4	2.6
Live female fetus/litter	Mean	6.9	7.9	6.9
	SD	2.9	2.5	2.6
Number of dead fetuses	Mean	0.0	0.0	0.0
	SD	0.0	0.0	0.0
Early resorption (g)	Mean	0.5	0.5	0.7
	SD	0.6	0.7	0.7
Fetal Weight (g)	Mean	5.589	5.454	5.551
	SD	0.375	0.359	0.281

Table 17: Summary of maternal performance and mortality – Cesarean section; * $p < 0.05$; ** $p < 0.01$.

Test article administration had no effect on laparohysterectomy parameters, including the number of corpora lutea, implantations, live fetuses per litter, early resorptions, pre- and post implantation loss, fetal weight and sex ratio. No late resorptions or dead fetuses were observed in the study. Fetal weights (combined and separated by sex) were comparable across groups; combined (males and females) mean fetal weight per litter was 5.59, 5.45, and 5.55 grams in the control, adjuvant and test article groups, respectively. Fetal sex ratio was comparable in control, adjuvant and test article groups (55%, 45%, and 52% males).

Mating Index (%)	(Number with confirmed mating/Number cohabited) x 100
Fertility Index (%)	(Number pregnant/number with confirmed mating) x 100
Gestation index (%)	(Number of females with at least one live pup at birth/number pregnant) x 100
Pre-Implantation loss (%)	(Number of corpora lutea – number of implantation sites)/number of implantation sites x 100
Post-Implantation loss (%)	(Number of implantations – number of fetuses)/ number of implantation sites x 100
Live-Birth Index (%)	(Number of pups born alive/number of pups born) x 100
Survival Index Day 0-4 (%)	(Number of pups alive on PND 4/ number of pups born alive) x 100
Lactation Index (%)	(Number of pups alive on PND 21/ number of pups born alive) x 100
Litter Survival (%)	(Number of litters on PND 21/number of viable litters delivered) x 100

Table 18: Reproductive indexes calculations

Parameter: developmental landmarks F1		F0 generation		
		Control	Adjuvant	AV7909
Eye, Small - Malformation	Fetuses N(%)	0(0.00)	1(0.6)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Limb: Humerus/Femur, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(1.0)
	Litters N(%)	0(0.0)	0(0.0)	1(4.8)
Pelvic girdle: Pubis, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.6)	4(3.7)
	Litters N(%)	0(0.0)	1(4.5)	2(9.5)
Ribs: Costal cartilage, Not fused to sternum - Malformation	Fetuses N(%)	0(0.00)	1(0.6)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Ribs: Costal cartilage, Misshapen - Variation	Fetuses N(%)	0(0.00)	1(0.6)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Ribs: Incomplete ossification - Variation	Fetuses N(%)	2(1.3)	2(1.3)	2(1.7)
	Litters N(%)	2(9.5)	2(9.1)	2(9.5)
Ribs: Absent - Malformation	Fetuses N(%)	0(0.00)	1(0.6)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Ribs: Short - Variation	Fetuses N(%)	0(0.00)	1(0.6)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Ribs: Intercostal - Variation	Fetuses N(%)	0(0.00)	1(0.6)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Ribs: Fused - Malformation	Fetuses N(%)	0(0.00)	1(0.6)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Ribs: Wavy - Variation	Fetuses N(%)	1(0.6)	2(1.3)	3(2.4)
	Litters N(%)	1(4.8)	2(9.1)	1(4.8)
Skull: Hyoid, Unossified - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.7)
	Litters N(%)	0(0.0)	0(0.0)	1(4.8)
Skull: Incomplete ossification	Fetuses N(%)	0(0.00)	1(0.6)	2(1.7)

	Litters N(%)	0(0.0)	1(4.5)	2(9.5)
Skull: Isolated ossification site - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.7)
	Litters N(%)	0(0.0)	0(0.0)	1(4.8)
Skull: Supernumerary ossification sit - Variation	Fetuses N(%)	0(0.00)	1(0.6)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Sternebrae: Sternebra, Misaligned - Variation	Fetuses N(%)	0(0.00)	1(0.6)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Supernumerary Rib: Cervical, Rudimentary - Variation	Fetuses N(%)	1(0.6)	0(0.00)	0(0.00)
	Litters N(%)	1(4.8)	0(0.0)	0(0.0)
Supernumerary Rib: Thoracolumbar, Full - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.7)
	Litters N(%)	0(0.0)	0(0.0)	1(4.8)
Supernumerary Rib: Thoracolumbar, Rudimentary - Variation	Fetuses N(%)	12(8.6)	8(5.4)	6(3.9)
	Litters N(%)	10(47.6)	5(22.7)	4(19.0)
Vertebrae: Vertebral arch, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.6)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Vertebrae: Cervical centrum, Split - Malformation	Fetuses N(%)	0(0.00)	3(2.0)	0(0.00)
	Litters N(%)	0(0.0)	3(13.6)	0(0.0)
Vertebrae: Cervical centrum, Fused - Malformation	Fetuses N(%)	0(0.00)	1(0.8)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Vertebrae: Sacral arch, Misaligned	Fetuses N(%)	0(0.00)	0(0.00)	1(0.7)
	Litters N(%)	0(0.0)	0(0.0)	1(4.8)
Vertebrae: Thoracic arch, Misshapen - Malformation	Fetuses N(%)	0(0.00)	1(0.8)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Vertebrae: Vertebral centrum, Incomplete ossification - Variation	Fetuses N(%)	5(2.9)	9(5.9)	5(3.4)
	Litters N(%)	3(14.3)	7(31.8)	3(14.3)
Vertebrae: Thoracic centrum, Misaligned - Malformation	Fetuses N(%)	0(0.00)	1(0.8)	0(0.00)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)
Vertebrae: Presacral vertebrae, Greater than 26 - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.7)
	Litters N(%)	0(0.0)	0(0.0)	1(4.8)
Heart, Interventricular Septal Defect - Malformation	Fetuses N(%)	7(4.7)	4(2.1)	1(0.6)
	Litters N(%)	6(28.6)	2(9.1)	1(4.8)

Table 19: Summary of fetal abnormalities by finding – Cesarean section; * $p < 0.05$; ** $p < 0.01$

There were no fetal external, visceral, head or skeletal anomalies related to test article administration. Fetal external findings were not seen in any group. Soft tissue findings in the fetal head were limited to a small eye in the adjuvant group that was incidental due to the single observation. Visceral findings were limited to interventricular septal defects, which were incidental as they occurred at higher incidences in the control and adjuvant groups than in the test article groups (4.7%, 2.1%, and 0.6% fetuses per litter, respectively). There were no fetal skeletal malformations related to candidate vaccine administration.

F1 Offspring data during lactation:

Viability, Survival, and Sex Ratio

Summary of Viability, Survival and Sex Ratio: F1 Litters		F1 generation		
		Control	Adjuvant	AV7909
Live birth index (%)	Mean	99.01	99.30	98.07
	SD	2.65	2.28	5.11
Survival index PND 0-4 (%)	Mean	99.21	98.08	97.52
	SD	2.66	4.15	4.02
Lactation Index	Mean	99.43	100.00	100.00
	SD	2.67	0.00	0.00
Sex Ratio	Mean	0.510	0.552	0.520
	SD	0.123	0.103	0.149
Total pups on PND 0	Mean	14.3	14.3	13.6
	SD	2.4	2.0	2.9
Live pups on PND 0	Mean	14.1	14.2	13.6
	SD	2.4	2.1	3.0
Live pups on PND 4	Mean	14.0	14.0	13.3
	SD	2.4	2.1	2.9
Live total – post cull d4	Mean	8	8	7.8
	SD	0.0	0.0	0.9
Live pups on day 7	Mean	8	8	7.8
	SD	0.2	0.0	0.9
Live pups on day 14	Mean	8	8	7.8
	SD	0.2	0.0	0.9
Live pups on day 21	Mean	8	8	7.8
	SD	0.2	0.0	0.9

Table 20: Summary of maternal performance and mortality – Cesarean section; * $p < 0.05$; ** $p < 0.01$.

The test article did not affect pup/litter viability or survival parameters during lactation or pup sex ratio within litters. The live-birth index and survival index for PND 0-4 ranged from 98% to 99%. The survival index for PND 4-21 (lactation index) ranged from 99% to 100%. The mean sex ratio within litters was similar across groups (0.51, 0.55 and 0.52). The mean total number of pups per litter on PND 0 ranged from 13.9 to 14.3 pups per litter, and the mean number of live pups per litter on PND 0 ranged from 13.6 to 14.2 pups per litter. The number of live pups per litter prior to and after culling on PND 4 and on PND 7, 14 and 21 were comparable between the groups. The number pups per litter were also comparable when sexes were analyzed separately.

Clinical Observations

There were no test article-related clinical observations in pups; observations were incidental and not attributed to the test article because they occurred at single or low incidence and/or at comparable incidence as in the control group. Similarly, clinical observations in pups in the adjuvant group occurred at single or low incidences that were comparable to the control group.

Body Weights and Growth Markers

No test article-related effects on body weight or body weight gain occurred in F1 offspring during lactation. Mean pup body weight per litter on PND 1 was 7.5, 7.4, and 7.6 grams in the control, adjuvant and test article groups, respectively, and continued to be comparable throughout the lactation period. Overall body weight gain in the control, adjuvant and test article groups for the lactation period (PND 1-21) was 57.9, 56.9, and 58.7 grams, respectively, and reflected comparable weight gain during all the lactation body weight gain intervals.

Pup Developmental Markers

The test article had no effect on pup developmental markers; the mean age of onset per litter for surface righting, incisor eruption, startle response and eye opening were comparable in the control, adjuvant and test article groups. There was no difference in age of onset for these parameters when sexes were analyzed separately or combined. All pups had a pupil reflex on PND 21. A statistically significantly earlier mean age of surface righting in female pups in the test article group was spurious and not related to test article administration because it was due to 2 outlier litters with an unusually early age of attainment and because of the lack of biological plausibility (the expected effect would be a delay rather than early onset).

Summary of Viability, Survival and Sex Ratio: F1 Litters		F1 generation		
		Control	Adjuvant	AV7909
Mean day of surface righting	Mean	5.2	5.1	4.7
	SD	0.9	0.7	0.8
Surface righting (M)	Mean	4.7	4.7	4.5
	SD	1.0	0.6	1.0
Surface righting (F)	Mean	5.8	5.6	5.0*
	SD	0.8	0.8	1.2
Mean day of incisor eruption	Mean	11.2	11.1	10.8
	SD	0.5	0.7	0.5
Incisor eruption (M)	Mean	11.3	11.2	11.0
	SD	0.6	0.7	0.5
Incisor eruption (F)	Mean	11.0	11.0	10.7
	SD	0.6	0.8	0.5
Mean day of startle	Mean	14.2	14.0	14.1
	SD	0.7	1.0	0.8
Startle (M)	Mean	14.6	14.1	14.2
	SD	0.9	1.1	1.1
Startle (F)	Mean	13.7	13.8	14.0
	SD	1.0	0.9	1.1
Mean day of eye opening	Mean	14.1	14.2	14.0
	SD	0.4	0.6	0.6
Eye opening (M)	Mean	14.2	14.2	14.1
	SD	0.4	0.7	0.6
Eye opening (F)	Mean	14.1	14.2	14.0
	SD	0.4	0.6	0.6
No with pupillary reflex	Mean	100.00	100.00	100.00
	SD	0.0	0.0	0.0

Table 21: Summary of litter mean development markers – F1 litter; * $p < 0.05$; ** $p < 0.01$

Pup Necropsy Observations

There were no test article-related post-mortem observations in pups; observations in the test article group were limited to interventricular septal defect in a pup found dead on PND 0 and dilated renal pelvis in several pups at scheduled necropsy on PND 21. These observations were incidental and not considered test article-related because they occurred at single incidences (interventricular septal defect) or were observed at higher incidence in the control group (dilated renal pelvis).

		Control	Adjuvant	AV7909
Number of litters examined:		22	22	22
Number of pups examined:		180	180	178
Heart: Intraventricular septal defect - malformation	Pups N(%)	0(0.0)	1(0.5)	1(0.5)
	Litters N(%)	0(0.0)	1(4.5)	1(4.5)
Limb: hyperflexion - malformation	Pups N(%)	1(0.5)	0(0.0)	0(0.0)
	Litters N(%)	1(4.5)	0(0.0)	0(0.0)
Renal pelvis: dilated -variation	Pups N(%)	7(3.9)	6(3.3)	3(1.7)
	Litters N(%)	4(18.2)	4(18.2)	1(4.5)

Table 22: Pup necropsy observation

Biomarker assessment:

	Control	Adjuvant	AV7909
AGP (µg/mL)	45	32	70
A2M (µg/mL)	1189	1121	884

Table 23: Mean serum AGP and A2M concentration in pregnant rats

Serum concentrations of the acute-phase inflammatory biomarkers AGP and A2M were evaluated in the serum of F0 females on GD 21 in control, adjuvant and test article groups by immunoassay using a commercially available multiplex assay kit qualified at (b) (4). AGP and A2M concentrations were comparable in pregnant rats in the control, adjuvant and AV7909 groups.

Immunogenicity:

	Control	Adjuvant	AV7909
F0 Females (SD 1)	<LOD [44]	<LOD [44]	<LOD [44]
F0 Females (GD 21)	<LOD [22]	<LOD [22]	14.390 (8.756) [22]
Fetuses (GD 21)	<LOD [21] ^a	<LOD [22]	1.306 (0.805) [21]
F1 Pups (PND 21)	<LOD [22]	<LOD [22]	9.990 (4.434) [22]

Table 24: Summary of htpTNA Response in F0 Females and F1 Fetuses and Pups: Standard deviation is presented in parentheses. Number of samples is shown in brackets. <LOD indicates all samples were below the limit of detection of the assay.

Antibodies against AV7909 were determined in serum samples from F0 females and their fetuses and pups in the control, adjuvant and test article groups using the high throughput toxin neutralization assay (htpTNA).

Toxin neutralization antibodies were detected in F₀ females and their fetuses at the end of gestation (GD 21) and in F₁ pups at the end of lactation (PND 21) in the group administered test article. The highest htpTNA response was observed in F₀ females on GD 21, and in F₁ pups on PND 21, with mean neutralizing factor-50 (NF₅₀) values of 14.390 and 9.990, respectively. High titers were observed in fetuses on GD 21, with a mean NF₅₀ of 1.306. Toxin neutralization antibodies were not detectable in the control, adjuvant or test article groups prior to the first dose (F₀ females), or in the control and adjuvant groups on GD 21 (F₀ females and their fetuses) or PND 21 (F₁ pups).

Assessment:

Administration of 0.5mL of the AV7909 vaccine by intramuscular (IM) injection to F₀ female (b) (4) rats 14 days prior to cohabitation, on the day of cohabitation (SD 15) and on GD 7 did not produce any developmental or reproductive toxicity. There was no effect on mating, fertility, pregnancy, embryo-fetal viability, growth, or morphologic development, parturition, maternal care of offspring or postnatal survival, growth or development. There was no adverse maternal toxicity in this study; test article-related findings in F₀ females were limited to non-adverse (transient, predominantly very slight to slight severity) injection site edema and non-adverse injection site nodules visible grossly at necropsy (small, transient). There was no systemic tissue injury or systemic inflammation in pregnant rats, based on evaluation of A2M and AGP two weeks after the last of three administrations of AV7909 vaccine. Administration of the AV7909 vaccine to female rats elicited high antibody response during gestation. As expected, based on the ability of maternally derived antibodies to cross the placenta and transfer via the mother's milk, AV7909 antibodies were detected in the serum of fetuses and pups. Therefore, exposure of the fetuses and pups to maternally-derived AV7909 antibodies did not result in any developmental toxicity.

Juvenile toxicity study:

Title: AV7909: Repeat Dose Safety Toxicity Study in Juvenile Rats

Study number: Study Number 98820

Performing laboratory: (b) (4)

Study initiation date: 10/30/2018

Final Report date: 08/30/2019

Test article batch/lot: AV7909: 26000440; CPG 7909: 100003; (b) (4)

Animal species and strain: (b) (4)

Breeder/supplier: (b) (4)

Number of animals per group and sex: 22

Age: 3 weeks. Twenty-six (26) dams with litter sizes of 2 male and 2 female pups were received on October 16, 2018 and Thirteen (13) dams with litter sizes of 4 males and 4 female pups were received on October 18, 2018. Upon receipt, dams were identified by a pre-test identification (ID) number on cage cards. Once pups were identified, they were individually observed and weighed, and then assigned to dose groups using a computer program (b) (4) ensuring no more than one rat/sex/litter was represented in a dose group. Pups remained with their dams until weaning on PND 21.

Body weight range: Males: 29.3 to 74.1 grams; females: 30.3 to 73.4 grams

Route and site of administration: 0.1 mL, between each dose administration, the injection site was rotated between the right and left quadriceps muscle

Frequency of administration and study duration: SD21, SD 28 and SD35

Dose: 0.1 mL

Means of administration: Needle and syringe

Stability: The sponsor submitted stability testing and a certificate of analyses; the test material was within the specifications.

Report status: Final

Experimental design:

Group	Dose Material	Dose Volume
1	AV7909	0.1 mL
2	Adjuvant	0.1 mL
3	Control	0.1 mL

Table 25: Study design

Randomization procedure: Pups were randomized based on body weight. Once pups were identified, they were individually observed and weighed, and then assigned to dose groups using a computer program (b) (4), ensuring no more than one rat/sex/litter was represented in a dose group.

Statistical analysis plan:

All appropriate quantitative in-life data collected at (b) (4) using the (b) (4) system were analyzed for test article effects by a parametric or nonparametric

analysis of variance (ANOVA). For all data, normality was determined by the Shapiro-Wilks test and homogeneity of variances was determined by Levene's test. Data was log-transformed to meet parametric assumptions. For parametric data determined to be normally distributed and homogeneous among groups, an ANOVA F-test was used to determine whether there were differences among the group means. If the ANOVA F-test was significant, then tests for differences between the control and each of the comparison groups were conducted using Dunnett's test, which adjusted for multiple comparisons. For nonparametric data that were not normally distributed and/or nonhomogeneous, a Kruskal-Wallis test was used to determine whether there were differences among the group means. If the Kruskal-Wallis test was significant, then tests for differences between the control and each of the comparison groups were conducted using Wilcoxon tests and the Bonferroni-Holm method to correct for multiple comparisons. All statistical tests were performed at the 0.05 level of significance ($p < 0.05$), after accounting for multiple comparisons where indicated.

Parameters	Frequency of Testing
Cage side observation for moribundity and mortality	Twice daily
Cage side observation	Dams and litters at least once daily
Detailed clinical observations	Pups on PND 20 and on dosing days (pre-dose, 1-2h post dose, 24h post-dose, 48h post-dose)
Body weight	On PND 10 and 14, 21, 23, 28, 30, 35, 36, 37, 42, 48, 49.
Food consumption	ND
Body temperature	ND
Ophthalmologic exam	ND
Clinical chemistry*	PND 37 for Core animals and on PND 49 for Recovery animals
Hematology*	PND 37 for Core animals and on PND 49 for Recovery animals
Coagulation**	PND 37 for Core animals and on PND 49 for Recovery animals
Immunological response	PND 37 for Core animals and on PND 49 for Recovery animals
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Prior to each dose administration and 1 to 2 hours following each dose administration. Subsequent observations were made daily (target 24-hour intervals after dose administration) for up to 1 week following each dose administration, until a score of 0 was obtained
Necropsy	PND 37, PND 49
Tissues for histopathology	PND 37, PND 49

Table 26: Parameters collected, *retro-orbital venous plexus, **vena cava or aorta

Postmortem procedures:

The following tissues were collected at necropsy.

Animal identification ^a	Mammary gland
Adrenal glands (paired)	Ovaries (paired)
Aorta	Pancreas
Bone (femur)	Parathyroid gland
Bone marrow (femur) ^b	Pituitary gland
Brain	Salivary gland (mandibular, sublingual)
Cecum	Sciatic nerve
Colon	Skeletal muscle
Duodenum	Skin (ventral abdomen)
Esophagus	Skin (injection site)
Eyes (with optic nerve, paired) ^c	Spinal cord (thoracolumbar)
Gross lesions	Spleen
Heart	Stomach
Ileum	Testes (paired with epididymides) ^d
Jejunum	Thymus
Kidneys (paired)	Thyroid glands
Liver	Trachea
Lungs with bronchi	Urinary bladder
Lymph node (mandibular, mesenteric, iliac)	Uterus (with cervix)

Table 27: Tissue collection: a) Collected but not processed, b) Bone marrow cytology smears were prepared for all animals at scheduled necropsy but were not evaluated due to lack of changes in peripheral blood, c) Preserved in Davidson's fixative, then transferred to 10% NBF, d. Preserved in Modified Davidson's fixative, then transferred to 10% NBF

Adrenal glands (paired)	Ovaries (with oviducts, paired)
Brain	Spleen
Heart	Testes (paired)
Kidney (paired)	Epididymides (paired)
Liver	Thymus
Lymph nodes, mesenteric	Uterus (with cervix)
Lung (with bronchi)	

Table 28: Organs weighed

Results:

Morbidity and mortality: All animals survived to their scheduled termination.

Clinical observations: Swelling of one or both hindlimbs was commonly observed in animals in the adjuvant (group 2) and AV7909 (group 1) groups. In contrast, swelling was noted for a single control female (group 3). Most adjuvant and AV7909-treated animals were noted with a firm nodule on the top of the anterior quadriceps of one or both legs. The nodules corresponded to nodules within the subcutis that were found during the macroscopic observation and also corresponded to inflammatory lesions seen during the histopathological examination. The nodules were considered attributable to adjuvant and AV7909 exposure.

Body weights: There were treatment related changes in body weights.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) Total bile acids
B) HEPATOBIILIARY		Alkaline phosphatase (ALP) Total bilirubin
ACUTE PHASE REACTANTS	Fibrinogen: SD37: G2♂↑*1.5 SD37: G1♂↑*1.8 SD37: G2♀↑* SD37: G1♀↑*1.6 AGP: SD37: G2♂↑*2.9 SD37: G1♂↑*6.3 SD37: G2♀↑*3.2 SD37: G1♀↑*4.7 A2M: SD37: G2♂↑*2.6 SD37: G1♂↑*3.2 SD37: G1♀↑*3.5	
KIDNEY FUNCTION		Creatinine, Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Creatine kinase SD37: G1♂↓ 0.4 SD37: G2♂↓ 0.4 LDH SD37: G1♂↓* 0.5 SD37: G2♀↓* 0.5 SD37: G1♀↓* 0.6 TRIG SD37: G1♂↓* 0.7 Globulin: SD37: G1♂↑* 1.4	Albumin (A) (G, calculated) or A/G Ratio Total cholesterol Total protein

Table 29: Clinical chemistry results, SD: study day, *different from controls at $P \leq 0.05$;
 **different from controls at $P \leq 0.01$.

On PND 37, LDH was decreased in animals receiving the adjuvant or AV7909. A reduction in creatine kinase was seen in male animals receiving the adjuvant or AV7909. Occasional animals across all groups had increased LDH and Creatine Kinase on Day 37. These findings were likely due, at least in part, to muscle damage/injury from procedural causes (e.g., IM injections, restraint, etc.). No changes were seen after the recovery phase.

On PND 37, AGP and A2M levels were systematically elevated in the adjuvant (group 2) and AV7909 (group 1) groups relative to the control group (group 3). Increased subject-to-subject variability was also observed in AGP response at PND 37. However, at the end of the recovery period (PND 49), A2M and AGP serum concentrations from animals in the AV7909 and adjuvant groups (groups 1 and 2, respectively) were comparable to those from the control group (group 3) for both sexes.

Transient increase in fibrinogen was observed on PND 37 in animals receiving the adjuvant or AV7909.

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
RED BLOOD CELLS	Reticulocytes: SD37: G2♂↓* SD37: G1♀↓	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): SD37: G1♂↑* SD37: G2♀↑*1.6 SD37: G1♀↑*1.5 Neutrophil count: SD37: G1♂↑*2.6 SD37: G2♀↑*2.2 SD37: G1♀↑*3.2 Monocyte count: SD37: G1♂↑* SD37: G2♂↑*2.1 SD49: G2♂↑*1.7 Basophils count: SD37: G2♂↑*1.7 SD37: G1♂↑*2.0 SD37: G2♀↑*2.3 SD37: G1♀↑*2.0 Large unstained cells (LUC) SD37: G2♂↑1.6 SD37: G1♂↑*1.9 Lymphocyte count: SD37: G2♂↑*1.5 SD49: G2♂↑* SD37: G2♀↑*3 SD37: G1♀↑*2.4	Eosinophil count
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen

Table 30: Hematology results. SD: Study Day, *different from controls at $P \leq 0.05$; **different from controls at $P \leq 0.01$

A transient increase in white blood cells neutrophils, neutrophils, monocytes, basophiles and lymphocytes was seen at PND 37, no increases were observed after the recovery phase.

Organ Weight:

<i>SEX</i>	<i>MALE</i>			<i>FEMALE</i>		
<i>GROUPS</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>1</i>	<i>2</i>	<i>3</i>
<i>NUMBER OF ANIMALS</i>	<i>10/10</i>	<i>10/10</i>	<i>10/10</i>	<i>10/10</i>	<i>10/10</i>	<i>10/10</i>
BODY WEIGHT (TERMINAL)	157.5/ 262.1	155.5/ 254.6	165.8/ 274.3	136.6/ 175.8	140.1/ 177.8	137.5/ 183.8
BRAIN	1.7966/ 1.9993	1.7890/ 1.9633	1.8340/ 2.0042	1.7323/ 1.8411	1.7304/ 1.8958	1.7484/ 1.8339
ADRENALS	0.0367/ 0.0492	0.0371/ 0.0468	0.0400/ 0.0491	0.0388/ 2.772	0.0380/ 0.0530	0.0370/ 0.0542
HEART	0.7579/ 1.1330	0.7573/ 1.1081	0.8283/ 1.1786	0.6877/ 0.7928	0.7088/ 0.8105	0.6760/ 0.8019
KIDNEYS	1.4965/ 2.2595	1.4743/ 2.1211	1.6205/ 2.3358	1.3623/ 1.5170	1.3835/ 1.5424	1.3301/ 1.6007
LIVER	5.1504/ 8.7347	4.9956/ 8.5536	5.5959/ 9.3580	4.6669/ 5.9801	4.7634/ 6.4068	4.6608/ 6.1309
LUNGS	1.7253/ 2.2970	1.6920/ 2.2488	1.8977/ 2.5389	1.3757/ 1.5458	1.5219/ 1.3898	1.5087/ 1.5509
SPLEEN	0.6607/ 0.7559	0.6022/ 0.7170	0.5264/ 0.7524	0.5415/ 0.5048	0.4977/ 0.4662	0.4497/ 0.4488
LYMPH Node, MESENTERIC	0.0815/ 0.1201	0.0883/ 0.1314	0.0883/ 0.1419	0.0770/ 0.1067	0.0851/ 0.1205	0.0957/ 0.1050
THYMUS	0.5595/ 0.8786	0.6519/ 0.8463	0.6169/ 0.8407	0.5180/ 0.6643	0.6397/ 0.6833	0.5533/ 0.6381
EPIDIDYMES	0.2041/ 0.3693	0.1887/ 0.3873	0.2143/ 0.4185			
TESTES	1.4053/ 2.3782	1.4033/ 2.4573	1.3912/ 2.5772			
OVARIES				0.0716/ 0.1089	0.0841/ 0.1023	0.0753/ 0.0991
UTERUS				0.3107/ 0.3958	0.2928/ 0.6113	0.3543/ 0.5253

Table 31: Organ weights absolute weights are expressed as mean (grams) [mean organ weight on SD 31 / mean organ weight on SD 59]; *different from controls at $P \leq 0.05$; **different from controls at $P \leq 0.01$.

Mean spleen weights were increased in male animals receiving AV7909 after the treatment phase. These increased spleen weights likely represent immune stimulation by the adjuvant with and without AV7909 vaccine material. Mean thymus weights were elevated in adjuvant (group 2) females. These findings were small in magnitude, only occurred in one sex and did not have a microscopic correlate.

After the recovery phase there were no statistically significant changes in mean terminal organ weights of the AV7909 (group 1) or adjuvant (group 2) animals.

Gross Pathology:

<i>Treatment Group Findings – Treatment Phase</i>	<i>3M</i>	<i>2M</i>	<i>1M</i>	<i>3F</i>	<i>2F</i>	<i>1F</i>
Bone: right femur thick white	-	1	-	-	-	-
Injection site: left mass, mottled	-	-	1	-	1	-
Injection site: left subcutaneous tissue, masses mottled	-	-	2	-	-	1
Injection site: left subcutaneous tissue, masses white	-	1	2	-	-	1
Injection site: right subcutaneous tissue, masses mottled	-	-	-	-	1	-
Injection site: right subcutaneous tissue, masses white	-	-	-	-	-	1
Injection site: bilateral, subcutaneous tissue, nodules white	-	-	-	-	1	-
Injection site: left, nodules, mottled	-	-	1	-	-	1
Injection site: left, nodules, white	-	1	-	-	1	-
Injection site: left subcutaneous tissue, nodules mottled	-	-	-	-	-	1
Injection site: left subcutaneous tissue, nodules white	-	1	2	-	4	2
Injection site: right subcutaneous tissue, nodules mottled	-	1	-	-	-	-
Injection site: right subcutaneous tissue, nodules white	-	-	1	-	-	1
Kidneys, bilateral, deformity	-	-	-	1	-	-
Kidneys, bilateral, dilatation	-	-	-	-	-	1
Kidney, left, medulla, dilatation, multiple	1	-	-	-	-	-
Kidney, left, pelvis, dilatation,	1	-	-	-	-	-
Kidney, left, cyst, clear, three	-	-	1	-	-	-
Kidney, right, cyst, clear, two	-	-	1	-	-	-
Lymph Node, iliac, enlarged, tan	-	-	1	-	-	1

Table 32: Gross pathology - treatment phase

After the treatment phase treatment-related gross lesions were confined to injection sites, iliac lymph nodes and the femur. Masses and nodules were noted in left and right injection sites within the subcutis. The masses and nodules were noted in AV7909 (group 1) and adjuvant (group 2) animals only and corresponded to inflammatory lesions seen microscopically.

Iliac lymph nodes were enlarged in Core AV7909 (group 1) animals. Enlargement corresponded to minimal lymphoid hyperplasia.

One animal in the Core adjuvant (group 2, Animal 209) had a thickened right femur which corresponded to periosteal inflammation.

Other lesions including kidney cysts were noted and not considered related to the test article or adjuvant.

<i>Treatment Group Findings – Recovery Phase</i>	<i>3M</i>	<i>2M</i>	<i>1M</i>	<i>3F</i>	<i>2F</i>	<i>1F</i>
Heart, atrium right dilatation	-	1	-	-	-	-
Heart, right ventricle, dilatation	1	1	-	-	-	-
Injection site: left, subcutaneous tissue, tan	-	-	1	-	1	1
Injection site: left, subcutaneous tissue, white	-	-	-	-	1	-
Injection site: right, subcutaneous tissue, mottled	-	1	-	-	-	-
Injection site: left, subcutaneous tissue, mottled	-	2	3	-	2	2
Injection site: left subcutaneous tissue, nodules pale	-	-	1	-	-	1
Injection site: left subcutaneous tissue, nodules tan	-	3	3	-	3	2
Injection site: left subcutaneous tissue, nodules tan two	-	-	-	-	-	1
Injection site: left subcutaneous tissue, nodules white	-	2	-	-	1	2

Injection site: right, subcutaneous tissue, mottled	-	-	1	-	1	-
Injection site: right subcutaneous tissue, nodules pale	-	-	-	-	-	1
Injection site: right subcutaneous tissue, nodules tan two	-	-	-	-	1	2
Injection site: right subcutaneous tissue, nodules white	-	1	1	-	-	-
Injection site: right subcutaneous tissue, nodules white two	-	1	-	-	-	-
Lymph Node, enlarged, tan	1	1	1	-	-	-
Lymph Node, iliac, enlarged, tan	-	-	1	-	2	1

Table 33: Gross pathology - recovery phase

After the recovery phase treatment-related gross lesions were confined to injection sites and iliac lymph nodes. Masses and nodules were noted in left and right injection sites within the subcutis. The masses and nodules were noted in AV7909 (group 1) and adjuvant (group 2) animals only and corresponded to inflammatory lesions seen microscopically.

Iliac lymph nodes were enlarged in recovery male AV7909 (group 1) and female adjuvant (group 2) animals. Enlargement corresponded to minimal lymphoid hyperplasia.

Other lesions including kidney cysts, enlarged mandibular lymph nodes, and nodules within the mesentery were noted and not considered related to the test article or adjuvant.

Microscopic findings:

<i>Treatment Group Finding – Treatment phase</i>	<i>3M</i>	<i>2M</i>	<i>1M</i>	<i>3F</i>	<i>2F</i>	<i>1F</i>
Femur, inflammation chronic active	-	1	-	-	-	-
Injection site, left skeletal muscle, inflammation, chronic-active (mild-moderate)	-	1	1	-	-	-
Injection site, left skeletal muscle, inflammation, granulomatous (mild-moderate)	-	-	1	-	1	-
Injection site, left subcutis, inflammation, chronic (moderate)	-	1	1	-	-	-
Injection site, left subcutis, inflammation, chronic-active (mild-moderate)	-	1	2	-	3	1
Injection site, left subcutis, inflammation, granulomatous (mild-moderate)	-	-	4	-	2	3
Injection site, right skeletal muscle, inflammation, chronic-active (mild-moderate)	-	5	5	-	5	4
Injection site, right skeletal muscle, inflammation, granulomatous (mild-moderate)	-	1	2	-	1	1
Injection site, left skeletal muscle, inflammation, histocyte (mild)	6	-	-	5	-	-
Injection site, left skeletal muscle, inflammation, histocyte (mild)	-	-	1	-	1	4
Injection site, right subcutis, inflammation, chronic-active (mild-moderate)	-	-	1	-	1	-
Injection site, right subcutis, inflammation, granulomatous (mild-moderate)	-	1	-	-	1	-
Injection site, right subcutis, inflammation, suppurative (mild)	-	-	-	-	-	-
Injection site, right skeletal muscle, necrosis, chronic-active (minimal-mild)	4	-	-	5	-	-
Kidneys, left, cyst	-	3	1	2	-	-
Kidneys, left, cyst, multiple	1	-	1	-	-	-
Kidneys, right, cyst,	1	-	1	-	-	-
Kidneys, right, cyst, multiple	-	-	1	-	-	-
Kidneys, nephropathy (minimal)	-	-	-	-	1	-
Kidneys, pelvis dilatation (mild)	-	-	-	-	-	1
Kidneys, left cortex, dysplasia (minimal)	-	1	-	-	-	-
Sciatic nerve, inflammation (chronic-active, mild)	-	-	-	-	-	1
Skeletal muscle, inflammation, neutrophilic (mild)	-	-	-	-	-	1
Skin, skeletal muscle inflammation, chronic (minimal)	-	-	-	-	1	-

Skin, skeletal muscle inflammation, chronic-active (minimal)	-	-	-	-	-	1
Skin, subcutis inflammation, chronic (mild)	-	-	-	-	-	1
Skin, subcutis inflammation, chronic-active (moderate)	-	-	1	-	-	1
Lymph node, iliac (minimal)	-	-	1	-	-	1

Table 34: Histopathology - treatment phase

Test article related microscopic lesions consisted of inflammation within the skeletal muscle or subcutis of injection sites. No remarkable differences between the AV7909 (group 1) and adjuvant (group 2) groups were noted, which suggests that the adjuvant was responsible for lesions in both groups. Inflammation in Core AV7909 (group 1) and adjuvant (group 2) animals sacrificed on PND 37 was primarily chronic active inflammation characterized by central deposit of amphophilic material (adjuvant +/- vaccine) with infiltrating foamy macrophages and cellular debris and surrounded by lymphocytes, plasma cells and variable numbers of degenerate neutrophils. Occasionally, only degenerate neutrophils were seen and coded as suppurative inflammation. Few animals had granulomatous lesions which were characterized by a central deposit of amphophilic material surrounded by foamy macrophages, lymphocytes, plasma cells and variable amount of fibrosis. These were identified as resolving chronic-active lesions. A few animals had chronic inflammation consisting of fibrosis, variable numbers of lymphocytes and plasma cells and neovascularization.

Iliac lymph nodes were enlarged by minimal lymphoid hyperplasia in single animals in the AV7909 (group 1). This may be due to induction of an inflammatory response.

In addition to inflammation at injection sites, single animals in the adjuvant (group 2) group showed a chronic-active inflammation of the right femur. Core AV7909 (group 1) Animal 157 had chronic-active inflammation of the axon nerve sheath. These lesions are likely due to an immune reaction to the test materials. Control animals (group 3) showed skeletal muscle necrosis at the injection site

A number of incidental findings were identified. Incidental kidney findings included cysts (all groups), nephropathy, mineralization, and/or cortical dysplasia.

<i>Treatment Group Finding – Recovery phase</i>	<i>3M</i>	<i>2M</i>	<i>1M</i>	<i>3F</i>	<i>2F</i>	<i>1F</i>
Cervix, inflammation, chronic-active (mild)	-	-	-	-	1	-
Heart, hemorrhage	-	1	-	-	-	-
Heart, pericardium, inflammation, lymphohistiocytic (mild)	-	1	-	-	-	-
Injection site, right, skeletal muscle, fibrosis (mild)	-	-	-	1	-	-
Injection site, left skeletal muscle, inflammation, granulomatous (mild)	-	1	2	-	-	-
Injection site, left skeletal muscle, inflammation, histocyte (minimal)	-	1	-	-	-	-
Injection site, left subcutis, inflammation, chronic (mild)	-	2	-	-	1	-
Injection site, left subcutis, inflammation, granulomatous (mild)	-	4	7	-	6	6
Injection site, left subcutis, inflammation, granulomatous multifocal (mild)	-	-	-	-	-	1
Injection site, right skeletal muscle, inflammation, chronic-active (mild)	-	1	-	-	-	-
Injection site, right skeletal muscle, inflammation, granulomatous (mild)	-	3	7	-	6	7
Injection site, right skeletal muscle, inflammation, lymphohistiocytic (minimal)	-	1	-	-	-	-
Injection site, right subcutis, inflammation, granulomatous (mild-moderate)	-	3	2	-	2	1

Injection site, right subcutis, inflammation, granulomatous multifocal (mild-moderate)	-	1	-	-	1	-
Injection site, right subcutis, inflammation, lymphohistiocytic (minimal)	-	1	-	-	-	-
Kidneys, left, cyst, bilateral	1	-	-	-	-	-
Kidneys, left, cyst	-	1	-	-	1	-
Kidneys, right, cyst	-	1	-	-	-	-
Kidneys, hemorrhage	-	-	1	-	-	-
Kidney, mineralization (minimal)	-	-	-	-	1	-
Kidneys, nephropathy (minimal)	-	-	-	-	-	1
Kidneys, right cortex, nephropathy (minimal)	-	-	1	-	-	-
Lymph nodes, mandibular, lymphoid hyperplasia (minimal)	1	1	1	-	-	-
Mesentery, inflammation, neutrophilic, (minimal)	-	1	-	-	-	-
Lymph node, iliac, lymphoid hyperplasia (minimal)	-	-	1	-	1	-

Table 35: *Histopathology - recovery phase*

Test article related microscopic lesions consisted of inflammation within the skeletal muscle or subcutis of injection sites. No remarkable differences between the AV7909 (group 1) and adjuvant (group 2) groups were noted, which suggests that the adjuvant was responsible for lesions in both groups. By PND 49, most lesions in the AV7909 (group 1) and adjuvant (group 2) consisted of granulomatous inflammation in skeletal muscle and subcutis of injection sites.

There were a few chronic-active lesions, as well as a few chronic lesions. Iliac lymph nodes of 1 recovery group 3 male and 1 recovery group 2 females were enlarged by minimal lymphoid hyperplasia suggesting continuation of the inflammatory response. Minimal lymphoid hyperplasia was found in mandibular lymph nodes of male animals in group 1, group 2 and group 3 and was considered an incidental finding.

Local toxicity:

Draize scoring:

Erythema

Very slight erythema was noted for a single male in the adjuvant group (group 2) and a single male and female in the AV7909 group (group 1). In all instances, erythema was noted on PND 29 only and no instances of erythema was seen for control (group 3) animals.

Edema

Two adjuvant-treated (group 2) females had very slight edema on PND 22 that resolved by the following day. Five males and 4 females had very slight, slight, or moderate edema starting on PND 29. All instances of edema were resolved by PND 33.

In the AV7909 group (group 1), 6 males and 2 females had very slight edema on PND 22. All resolved by the following day. On PND 29, 7 males and 8 females had very slight or slight edema. In all cases, no edema was seen by PND 34. No edema was noted for any control animal (group 3).

Serology: Serum samples for immunogenicity profile assessment using the htpTNA were collected on scheduled termination, PND 37 for core animals and PND 49 for recovery animals. The purpose of the TNA is to quantify the functional ability of serum to neutralize the lytic effects of anthrax lethal toxin on the (b) (4) cell line. In general, this assay is used to evaluate the neutralizing capacity of serum from subjects (human or animal) that have been vaccinated with anthrax vaccines, have been infused with anti-LT, or have mounted an endogenous immune response following exposure to *B. anthracis* organisms.

All subjects in the AV7909 (group 1) group demonstrated an immune response at PND 37 and PND 49. All subjects in the adjuvant group (group 2) were negative at PND 37 and PND 49 except for one animal. All subjects in the control group (group 3) were negative at PND 37 and PND 49.

<p><i>Test article related effects</i></p> <ul style="list-style-type: none"> • Injection site (seen in vaccine and adjuvant group): <ul style="list-style-type: none"> ○ Inflammation within the skeletal muscle or subcutis (mild-moderate chronic-active inflammation, mild-moderate granulomatous inflammation, mild suppurative inflammation); partial recovery. ○ Amorphous material surrounded by macrophages, lymphocytes, plasma cells and fibrosis, in some cases with neovascularization. ○ Lymphoid hyperplasia at iliac lymph nodes ○ Single animals showed chronic-active inflammation of right femur and axon -nerve sheath ○ Slight erythema and edema in single animals ○ Swelling and nodules • Transient increase in mean spleen weights • Transient increase (mild) in white blood cells neutrophils, neutrophils, monocytes, basophiles and lymphocytes • Transient increase in fibrinogen, AGP, A2M,
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Assessment:

The purpose of this study was to investigate the local tolerance and potential local and systemic toxic effects induced by multiple intramuscular (IM) injections of the AV7909 vaccine or adjuvant (b) (4) + CPG 7909) and to evaluate the reversibility of any observed toxic effects over a period of 2 weeks following the last injection in juvenile rats compared to control animals. Juvenile (3-week-old) rats were dosed three times at weaning (PND 21), PND 28, and PND 35, at a dose volume of 0.1 mL per injection with either AV7909, adjuvant or the control article.

Findings were indicative of immune system stimulation and were seen in both the adjuvant as well as the AV7909 groups at similar severity but not in the control animals. At the injection site erythema was noted for a limited number of animals in the adjuvant and AV7909 groups. Edema was noted for a few adjuvant animals and was observed at a

greater frequency for AV7909 animals. Edema was primarily observed starting at PND 22, with all instances resolved by PND 34. Swelling of the hindlimbs and subcutaneous nodules near the injection sites were seen for most animals in the adjuvant and AV7909 groups, which corresponded to inflammatory lesions seen during the histopathological examination as a result of immune stimulation.

Systemically, changes in clinical pathology parameters indicative of inflammation and were seen in animals receiving the adjuvant or AV7909; increased neutrophil, monocyte, and large unstained cell count, fibrinogen, and globulin, and decreased albumin and albumin/globulin (A/G) ratio were observed on PND 37. Additionally, AGP and A2M levels were systematically elevated for both adjuvant and AV7909 groups at the end of treatment phase; reversibility was observed after the recovery phase.

Histopathologic lesions in the AV7909 group were indistinguishable from lesions produced by the adjuvant alone and were likely due to the adjuvant. Findings were limited to local inflammatory lesions of the injection site subcutis or skeletal muscle or tissues adjacent to the injection site. Lesions at PND 37 (end of treatment phase) were mainly active chronic inflammation characterized by a central pool of amphophilic material with infiltrating foamy macrophages and surrounding lymphocytes, plasma cells and variable number of degenerate neutrophils. At PND 49, there was partial resolution and lesions were more granulomatous with loss of neutrophils and replacement by fibrous connective tissue. Further, an enlargement of iliac lymph nodes due to minimal lymphoid hyperplasia in was observe in animals receiving AV7909 or adjuvant. The only noted systemic effects were increases in spleen weights after treatment phase in animals receiving adjuvant or AV7909. The spleen and lymph node changes are likely due to nonspecific immune stimulation.

During immunogenicity testing, performed using the high throughput toxin neutralization assay (htpTNA), all animals in the AV7909 group demonstrated a robust immune response at PND 37 and PND 49. All subjects in the adjuvant and control groups were negative at PND 37 and PND 49 except for an individual animal in each group which was below the limit of detection (LOD) of the assay.

OVERALL ASSESSMENT:

AV7909 Anthrax Vaccine Adsorbed, Adjuvanted (AV7909) was developed by Emergent Product Development Gaithersburg Inc. (Emergent) as a new vaccine indicated for postexposure prophylaxis (PEP) of disease following suspected or confirmed exposure to *Bacillus anthracis* (*B. anthracis*) when administered in conjunction with the recommended antibacterial regimen. Each 0.5 mL dose of AV7909 consists of (b) (4) AVA and (b) (4) of the immunostimulatory oligodeoxynucleotide, CPG 7909 and is administered as a two-dose regimen given two weeks apart.

Emergent has completed a single-dose acute toxicity study, a repeat-dose safety toxicity study in adult rats, a pre- and postnatal developmental and reproductive toxicity (DART) study in rats, as well as a juvenile repeat-dose toxicology study in rats. In all studies, animals were dosed via intramuscular (IM) injection, the intended route for human use. The rat model was chosen for safety evaluation of AV7909 as it is a widely used species for toxicology studies and rats were shown to respond well to AV7909 and its component, CPG 7909, as confirmed by immunogenicity assessments conducted in each of the studies.

In the single-dose acute toxicity study (Study 971-003), male and female rats were dosed IM with vehicle only, 0.5 mg of CPG 7909 alone, human dose of AVA (0.5 mL, full human dose) alone, or human dose of AVA in combination with 0.1, 0.5, or 1.25 mg of CPG 7909. There weren't any treatment-related effects on clinical observations, body weights, or ocular condition, and no treatment-related macroscopic changes noted at necropsy. The only effects observed were consistent with effect of AVA and immunostimulatory properties of CpG oligonucleotides, such as local inflammation at the injection sites, increases in spleen weights, and hyperplasia in lymphoid tissues of the spleen and draining lymph nodes. Similar findings were noted in the subsequent repeat-dose toxicity study in adult rats (Study 1778-09072); animals were administered three IM injections, two weeks apart, of vehicle only, adjuvant only, or one of two AV7909 0.5 mL doses formulations (human dose) containing 0.5 or 0.25 mg of CPG 7909. In this study, test article-related findings were observed at injection sites, consisting of necrosis and inflammation which correlated to macroscopic observation of masses; as well as increases in spleen weights, and hyperplasia in lymphoid tissues of the spleen and draining lymph nodes. Changes in clinical pathology parameters and organ weights, and microscopic changes in lymphoid tissues were consistent with immune stimulation. At the injection site, mild to marked necrosis occurred within muscle, accompanied by mild to moderate chronic, chronic-active, subacute or granulomatous inflammation which often extended to the surrounding fascia and occasionally extended into the subcutis. The subacute inflammation noted after the treatment phase had been replaced by chronic inflammation characterized by chronic or granulomatous inflammatory infiltrates observed at the injection site. The necrosis and granulomatous inflammation at the injection sites was considered adverse but would be expected to resolve over time. Additionally, the severity of the inflammatory effects at the injection site was likely exacerbated by the repeat administration of the test articles in the same, rather than, alternating, site and the fact that in the rat the muscle mass at the injection site is much

less than in a human. The observed local and systemic inflammatory responses and findings are primarily due to the immunostimulatory effect of CPG 7909.

In the DART study (Study T05153), female rats were dosed IM with water for injection, adjuvant (CPG 7909 plus (b) (4) , or AV7909 (full human dose, 0.5 mL). The animals received three vaccinations: 14 days prior to start of cohabitation (SD 1), on the day of cohabitation (SD 15) and on GD 7. The DART study demonstrated that AV7909 administration did not induce any reproductive or developmental toxicity. There was no effect on mating, fertility, pregnancy, embryo-fetal viability, growth, or morphologic development, parturition, maternal care of offspring or postnatal survival, growth or development. There was also no adverse maternal toxicity, with findings limited to non-adverse, transient injection site edema and injection site nodules.

Subsequently a repeat-dose study was conducted in juvenile rats (Study 98820) to support the potential pediatric use of the vaccine in the event of an anthrax emergency; animals received three vaccine administrations one week apart, starting at weaning, of vehicle only, adjuvant (CPG 7909 plus (b) (4) , or AV7909 (0.1mL, 1/5th of human dose). Findings in the juvenile toxicity study were transient and indicative of local and generalized immune system stimulation. At the injection site mild moderate inflammation with microscopic necroses (mild to marked) was observed as well as lymphoid hyperplasia at the draining lymph nodes and spleen; partial recovery was observed for most observations. However, after the recovery phase chronic or granulomatous inflammation were observed at the injection site, these inflammatory changes were considered to be treatment-related and adverse but were expected to resolve over time.

All studies demonstrated a robust antibody response induced by AV7909 vaccination.

CONCLUSIONS:

In the BLA (125761) adequate nonclinical toxicology data regarding CPG 7909 combined with AVA (AV7909) have been presented for the safety. No issues regarding non-clinical toxicology have been identified that preclude approval of the BLA in healthy adults.