

Toxicology Review of SmallPox Vaccine (Live, Attenuated)

BLA: 125678

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Sponsor: Bavarian Nordic A/S, Hejreskovvej 10A Kvistgaard, Denmark 3490

Product: Live Modified Vaccinia Virus Ankara

Proposed indication: Active immunization against smallpox in adults aged 18 years and older

Recommended dose: 0.5 x 10^{e8} infectious units per 0.5 mL dose

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Division name: OVRR/DVRPA

Proprietary Name: JYNEEOS

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Introduction:

Variola virus, an orthopox virus of the poxviridae family, is the cause of smallpox. In two variants variola virus occurred:

- 1- variola major, leading to the classical smallpox disease with a case fatality rate of 30–40%;
- 2- and, variola minor (alastrim) observed in the late phase of the smallpox era, leading to a less severe form with a case fatality rate of 1–2% (1) (Fenner, 1988).

Variola virus, which does not persist in humans and has no animal reservoir, infects only humans. The historically proven means of prevention is vaccination. Vaccinations dates back to 1798 when Edward Jenner demonstrated that inoculation with cowpox virus, a member of the same orthopox family as variola virus, provided protection against smallpox. Vaccinia virus (VV), another closely related virus within the orthopox family, replaced cowpox virus as immunity induced by one orthopox member cross protects against variola virus. Modified Vaccinia Ankara Bavarian Nordic (MVA-BN[®]) was developed by Bavarian Nordic A/S (BN), Kvistgaard, Denmark. This is a proprietary strain of the orthopox virus as a live, highly-attenuated, non-replicating viral vaccine for protection against smallpox disease.

Smallpox could be transmitted from person-to-person; mainly via the respiratory route. Early symptoms (following an incubation period of approximately 12 to 14 days) are characterized by sudden onset of fever, malaise, headache, backache and prostration. Two to 3 days later, the fever drops and a maculopapular rash with deeply embedded lesions appear on the mucosa of the mouth and on the face, hands and forearms, eventually progressing to the trunk, legs and feet in a centrifugal distribution pattern. The lesions appear in crops and progress to vesicular and pustular stages. Scabs form, which can eventually lead to depressed, depigmented, pitted scars after the onset of rash (after eight to 14 days). Typical complications of smallpox are bacterial co-infections,

keratitis with consecutive blindness, encephalitis, as well as spontaneous abortion and stillbirth in pregnant women. Thus, smallpox is considered a serious disease associated with morbidity that has substantial impact on day-to-day functioning and high likelihood to result in death or permanent disability.

A summary of the various forms of smallpox disease in humans is provided in the following table:

Table 1: Summary of the various forms of smallpox disease in man

	Form of smallpox disease		
	Hemorrhagic	Confluent	Discrete
Symptoms	Head/Backache, vomiting, rash, anorexia, fever	Rash, eruptions, papules, vesicles	Rash, eruptions, papules, vesicles
Time of symptoms to death (days)	4-7	>15	>15
Death rate (%)	90-100	40	<10
Time until diagnosis (days)	Up to 30 days after first case	>15	>15

In animal species (i.e. mice and monkeys) and humans, clinical and pre-clinical studies have shown that both humoral and cellular immune responses were induced by MVA-BN. These responses were comparable to immune responses induced by traditional replicating vaccines used to eradicate smallpox. Furthermore, challenge studies performed in mice and monkeys have shown that MVA-BN confers protection against pox virus infection and disease comparable to that conferred by traditional replicating vaccines used to eradicate smallpox.

Overview of the Clinical Development Program (CDP)

Two investigational new drug (IND) applications were used to develop the current vaccine in the US: IND 11596 for the liquid frozen (LF) formulation and IND (b) (4) for a (b) (4) formulation. A total of 7871 subjects have been vaccinated with MVA-BN in 22 completed clinical studies since the time that clinical development of MVA-BN was initiated in 1999. Out of the 22 studies, 16 trials were sponsored by BN (10 under IND 11596, one under IND (b) (4)) and 6 were sponsored by the NIH/DMID (under IND 11229). The purpose of these trials was to:

- 1- Identify an optimal dose and vaccination regimen;
- 2- Generate data indicating the protective efficacy of MVA-BN by comparison to replicating smallpox vaccines (Dryvax and ACAM2000);
- 3- Assess the safety and immunogenicity of MVA-BN in subjects 18-80 years of age, including healthy as well as at-risk populations with contraindications to receive traditional smallpox vaccines;
- 4- Compare the (b) (4) to the LF formulation of MVA-BN.

MVA-BN has demonstrated a favorable safety profile and consistently demonstrated the ability to induce a rapid and strong vaccinia-specific immune response, i.e. neutralizing antibodies measured by plaque reduction neutralization test (PRNT) and total antibodies measured by enzyme-linked immunosorbent assay (ELISA). The purpose of the pivotal phase III clinical trial POX-MVA-006 was to demonstrate efficacy of MVA-BN. This was achieved by comparing neutralizing antibody titers to those induced by ACAM2000 and by assessing the attenuation of the take following ACAM2000

vaccination. This study met both of its co-primary endpoints, i.e. non-inferiority of MVA-BN compared to ACAM2000 in terms of vaccinia neutralizing antibody response and attenuation of the take when MVA-BN is administered prior to ACAM2000, demonstrating the efficacy of MVA-BN. The following table lists the completed clinical trials:

Table 2: Tabular overview of completed clinical trials

Table 2. Vaccination schedule of completed on-lead trials

Trial	Phase	Sponsor	Vaccine Lot(s)	Number of vaccinated subjects			
				Healthy	HIV	AD	Total
(b) (4) Formulation							
POX-MVA-002	1	NIH	0130303	75	0	0	75
POX-MVA-007	1	BN	0130303	29	0	31	60
POX-MVA-004	2	BN	0080902	164	0	0	164
POX-MVA-010	2	BN	0130303	60	91	0	151
POX-MVA-027	2	BN	F00105/C00007	324	0	0	324
POX-MVA-029	2	NIH	0130303	165	0	0	165
POX-MVA-036	2	NIH	F00105/C00009	435	0	0	435
HIV-NEF-004	2	BN	0130303	0	26	0	26
Subtotal				1252	117	31	1400
LF Formulation							
POX-MVA-001	1	BN	0021100	86	0	0	86
HIV-POL-002	1/2	BN	0120606	0	10	0	10
POX-MVA-005	2	BN	0170505	564	0	0	564
POX-MVA-006	3	BN	F00238	220	0	0	220
POX-MVA-008	2	BN	0170505 0040707	282	0	350	632
POX-MVA-009	1/2	NIH	0031105	199	0	0	199
POX-MVA-011	2	BN	0170505 0031105	97	482	0	579
POX-MVA-013	3	BN	F00100/C00001 F00101/C00002 F00102/C00003	999 1005 999	0	0	3003
POX-MVA-023	2	BN	0040707	152	0	0	152
POX-MVA-024	2	BN	0070808	120	0	0	120
POX-MVA-027	2	BN	F00103/C00004	327	0	0	327
POX-MVA-028	2	NIH	0050808/0111208	91	0	0	91
POX-MVA-029	2	NIH	0070808/0111208	359	0	0	359
POX-MVA-030	1	NIH	0050808/0111208	20	0	0	20
POX-MVA-037	2	BN	F00102	0	87	0	87
POX-MVA-03X	N/A	BN	F00052/0050808	22	0	0	22
Subtotal				5542	579	350	6471
Total (b) (4) + LF)				6794	696	381	7871

AD = Atopic Dermatitis; (b) (4) ; HIV = Human Immunodeficiency Virus; LF = Liquid Frozen; N/A = not applicable; POX-MVA-007: subjects with allergic rhinitis are included in the healthy subject population. POX-MVA-030: subjects with hematopoietic stem cell transplantation are included under "healthy" in this table as subjects underwent transplantation more than 2 years prior to enrollment and had to be in good general health without evidence of active malignancy and off immunosuppressant medications.

Stability Summary:

The stability of one batch of IMVAMUNE (MVA-BN® batch # 170505) was tested by determination of the virus titer at two different time points (0 and 2 hours incubation on wet ice) when either undiluted or 10-fold diluted. Protocol BN-PD-2005-021 was used to run stability analysis. The undiluted and the 10-fold diluted suspension of MVA-BN® batch 170505 were stable over 2 hours when stored on wet ice.

Toxicity studies submitted to support this BLA:

General toxicology studies:

- 1- Safety evaluation study of intramuscularly administered MVA-BN vaccine in male and female rabbits (study number M249-03).
- 2- Safety evaluation study of subcutaneously administered MV A-BN vaccine in male and female rabbits (study number M254-03).
- 3- Repeat dose toxicity study in (b) (4) rabbit following two subcutaneous administrations (study number HPA0006/074055).
- 4- (b) (4) and MVA-BN®: A 3 week repeat dose subcutaneous administration toxicity and local tolerance study in the adult rat followed by a 2 day and a 28-day treatment-free period (study number 2699/011).

Reproductive studies:

- 1- Developmental toxicity (teratology) study of subcutaneously administered MVA-BN® vaccine in pregnant rabbits (study number M350-06).
- 2- Peri- and postnatal developmental toxicity study of subcutaneously administered MVA-BN® vaccine in pregnant rats (study number M351-06).
- 3- Developmental toxicity (teratology) study of subcutaneously administered MVA-BN vaccine in pregnant (b) (4) rats (study number M349-05).
- 4- Study for effects on embryo-fetal development by subcutaneous route in rats (study number 40400 RSR).

Package insert:

The following has been submitted by the sponsor in the package insert in sections 8.1, 8.2, and 13.1:

8.1 Pregnancy

Animal Data

Four developmental toxicity studies were conducted in female rats and rabbits. Animals were administered the human dose (0.5 mL) of PROPRIETARY NAME in 2 or 3 repeated subcutaneous vaccinations. In the single study in rabbits, females were vaccinated 14 days prior to the day of sperm positivity (Day -14) and on gestation days 0 and 14. In three other studies done in female rat, animals were administered 0.5 mL of PROPRIETARY NAME on days -14, 0 and 14, or on days -14 and 0, or on days 0 and 6. No vaccine-related fetal malformations or variations and adverse effects on female fertility or pre-weaning development were reported in these studies.

8.2 Lactation

Risk Summary

It is not known whether JYNNEOS is excreted in human milk. Data are not available to assess the effects of JYNNEOS in the breastfed infant or on milk production/excretion.

The development and health benefits of breastfeeding should be considered along with the mother's clinical need for JYNNEOS and any potential adverse effects on the breastfed child from JYNNEOS or from the underlying maternal condition. For preventive vaccines, the underlying condition is susceptibility to disease prevented by the vaccine.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

JYNNEOS has not been evaluated for carcinogenic or mutagenic potential, or for impairment of male fertility in animals. Developmental toxicity studies conducted in rats and rabbits vaccinated with 2 to 3 doses of JYNNEOS revealed no evidence of impaired female fertility [see *Use in Specific Populations* (8.1)].

General toxicology studies review:

Study # 1: Safety evaluation study of intramuscularly administered MVA-BN vaccine in male and female rabbits (study number M249-03).

Performing laboratory: (b) (4)

Initiation date: August 18, 2003

Final report date: October 14, 2003

Batch/lot number of test article:

Test Article

MVA-BN vaccine (MV A-BN); 1 x 10⁸ TCID₅₀/vial

Sterile water

Dilution buffer [10 mM TRIS, 140 mM NaCl,

(b) (4)

Lot No.

130303

(b) (4)

(b) (4)

Animal species and strain: (b) (4) rabbits

Breeder/supplier: (b) (4)

Number of animal per sex per group: 10 per sex per group

Age: Specified as young adults

Body weight range: 3.2 to 4.5 kg

Route and site of administration: Intramuscular to the longissimus dorsi (muscle along the spine) and were moved to a different site on the left side of the spine for each injection.

Volume of administration: 0.5 (±10%, or ±0.05) mL/dose

Frequency of administration and study duration: Rabbits were treated at weeks 0, 3, and 6 and study duration was 56 days.

Dose/animal: See study design

Stability: The stability analysis provided by the applicant is presented in appendix B1-3. The stability of the test article, when reconstituted with water, was 1 hr when stored on wet ice (2-8°C).

Means of administration: Intramuscular (IM)

Report status: Final

Methods:**Study design**

Animals were randomized and assigned to 3 different groups. Each group consisted of 10 animals/sex/group. Animals were dosed by intramuscular route. The details of the study design are listed in the following table:

Table 3: Experimental design (study #1)

Group	Dose Route	MVA-BN Dose Levels (TCID ₅₀ /immunization) ^a	Number of Immunizations	Number of Animals	
				Main Study ^b	Recovery Group ^c
1	im	Vehicle Control	3	5M + 5F	5M + 5F
2	im	1×10^7	3	5M + 5F	5M + 5F
3	im	1×10^8	3	5M + 5F	5M + 5F

^aRabbits were immunized with the MVA-BN Vaccine or Control Article at Weeks 0, 3, and 6 (Days 0, 21, and 42).

^bMain Study: Five animals per sex per group were sacrificed 3 days after the last immunization (Day 45).

^cRecovery Group: Five animals per sex per group were sacrificed 2 weeks after the last immunization (Week 8; Day 56).

Randomization procedure: Yes.

Statistical analysis plan: Yes.

The following parameters were evaluated: Cage side observations (once daily), clinical observations (pre-injection and approximately 2-4 hr after injection on days of dosing, then once daily throughout the study), injection site evaluations (pre-injection and approximately 2-4, 24, 48, and 72 hr after each injection), body weights (before initiation of treatment on day 0 and weekly thereafter), food consumption (daily qualitatively and twice weekly quantitatively), body temperature (pre-injection and approximately 2-4, 24, 48, 72 and 96 hr after each injection), ophthalmoscopy (pretest and once during the final week), clinical chemistry, hematology, and coagulation (pretest, and then 3 days after each immunization [days 3, 24, and 45] and at necropsy), serology (pretest, 2 weeks after the first and second immunizations [days 14 and 35], and at necropsy 2 weeks after the third [final] immunization), urinalysis (once at scheduled necropsy). Gross anatomy at termination and organ weights and histopathology were evaluated/determined on selected tissues.

Table 4: Parameters evaluated (study #1)

Parameters	Frequency of Testing
Cage-side observations	Once daily
Clinical observations	Pre-injection and approximately 2-4 hr after injection on days of dosing, then once daily throughout the study
Injection site evaluations	Pre-injection and approximately 2-4, 24, 48, and 72 hr after each injection
Body weight	Before initiation of treatment on day 0 and weekly thereafter

Parameters	Frequency of Testing
Food consumption	Daily qualitatively and twice weekly quantitatively
Body temperature	Pre-injection and approximately 2-4, 24, 48, 72, and 96 hr after each injection
Ophthalmoscopy	Pretest and once during the final week
Clinical chemistry*	Pretest, 3 days after each immunization [days 3, 24, and 45], and at necropsy
Hematology*	Pretest, 3 days after each immunization [days 3, 24, and 45], and at necropsy
Coagulation*	Pretest, 3 days after each immunization [days 3, 24, and 45], and at necropsy
Urinalysis	Once at scheduled necropsy
Serology*	Pretest, 2 weeks after the first and second immunizations [days 14 and 35], and at necropsy 2 weeks after the third [final] immunization
Necropsy	Days 45 and 56
Tissues for histopathology	Days 45 and 56

*Ear blood vessel

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.
 Table 5: Tissues collected at necropsy (study #1)

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	!	
Bone marrow smear		X
Bone marrow (histology and cytology, sternum)	!	
Bone (femur with distal metaphysis and epiphysis)	!	
Bone (sternum [examined along with the bone marrow section])	!	
Brain	!*	
Cecum	!	
Cervix	!	
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes	!	
Fallopian tubes		X

Organ/Tissue	Collected	Not collected
Gall bladder	!	
Gut Associated Lymphoid Tissue (GALT)		X
Gross lesions	!	
Harderian gland		X
Heart	!*	
Ileum	!	
Injection site	!	
Jejunum	!	
Kidneys	!*	
Liver	!*	
Lungs	!	
Lymph nodes (inguinal, iliac, mandibular, and mesenteric)	!	
Mammary gland	!	
Optic nerve	!	
Ovaries	!*	
Pancreas	!	
Parathyroid glands	!	
Pituitary gland	!	
Prostate	!	
Rectum	!	
Salivary glands	!	
Sciatic nerve	!	
Seminal vesicle	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (thoracolumbar junction) with spinal column	!	
Spleen	!*	
Stomach	!	
Testes	!*	
Thymus	!*	
Thyroid	!	
Tongue	!	
Trachea	!	
Ureters		X
Uterus	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland		X

Results:

Morbidity and mortality:

No test article-related morbidity or mortality was reported.

Clinical Chemistry, hematology, and coagulation:

Table 6: Clinical chemistry results (study #1)

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if Greater than 1.5 so Indicated Otherwise ≤ 1.5)	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, potassium, sodium, phosphorus
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Aspartate aminotransferase (AST or SGOT) SD24 F $\downarrow \leq 0.6$ G3	Alanine aminotransferase (ALT or SGPT)
B) HEPATOBILIARY	Alkaline phosphatase (ALP) SD56 F $\uparrow \geq 2.0$ G3	Total bilirubin
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation) C-reactive protein*
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Cholesterol SD24 M $\downarrow \leq 0.6$ G3 CPK SD56 M $\downarrow \leq 0.5$ G2 SD56 M $\downarrow \leq 0.6$ G3 SD3 F $\uparrow \geq 1.6$ G2 SD3 F $\uparrow \geq 2.2$ G3 SD24 F $\uparrow \geq 2.1$ G3 SD45 F $\uparrow \geq 2.0$ G3	Albumin (A) Total protein Carbon dioxide Globulin A/G ratio Fasting Triglycerides GGT Lactate dehydrogenase

* Not measured.

Clinical chemistry results show a decrease in AST levels in group 3 females at study day 24. ALP levels were increased in group 3 females at study day 56. Cholesterol levels were decreased in group 3 males at study day 24. Creatinine phosphokinase (CPK) levels were decreased in groups 2 and 3 males at study day 56. CPK levels were increased in groups 2 and 3 females at study day 3. CPK levels were increased in group 3 females at study days 24 and 45.

Table 7: Hematology results (study #1)

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if Great or Less than 1.51, ie, ≥ 1.6 or ≤ 1.6)	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	<p>Segmented neutrophil (ANS) SD3 M $\downarrow \leq 0.5$ G3 SD56 M $\downarrow \leq 0.6$ G2 SD56 M $\downarrow \leq 0.6$ G3 SD3 F $\downarrow \leq 0.6$ G2 SD24 F $\downarrow \leq 0.4$ G2</p> <p>White Blood Cells (WBC) SD24 F $\downarrow \leq 0.6$ G2</p> <p>Monocyte count: SD3 M $\uparrow \geq 2.3$ G3 SD24 M $\uparrow \geq 1.7$ G2 SD3 F $\downarrow \leq 0.6$ G2 SD24 F $\downarrow \leq 0.5$ G2 SD24 F $\uparrow \geq 2.5$ G3 SD56 F $\downarrow \leq 0.5$ G3</p> <p>Eosinophils count SD3 M $\uparrow \geq 2.5$ G2 SD3 M $\uparrow \geq 3.8$ G3 SD24 M $\downarrow \leq 0.6$ G2 SD24 M $\downarrow \leq 0.4$ G3 SD45 M $\downarrow \leq 0.5$ G3 SD3 F $\downarrow \leq 0.2$ G2 SD3 F $\downarrow \leq 0.6$ G3 SD24 F $\downarrow \leq 0.3$ G2 SD45 F $\downarrow \leq 0.5$ G2 SD45 F $\downarrow \leq 0.5$ G3 SD56 F $\downarrow \leq 0.3$ G3</p> <p>Basophils SD3 M $\downarrow \leq 0.2$ G2 SD45 M $\downarrow \leq 0.6$ G2 Pre-test F $\downarrow \leq 0.5$ G2 Pre-test F $\downarrow \leq 0.3$ G3 SD3 F $\downarrow \leq 0.4$ G3</p>	<p>Macrophage Leukocytes Large unstained cells (LUC) Lymphocyte count</p>

¹ With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if Great or Less than 1.51, ie, ≥ 1.6 or ≤ 1.6)	Not of NOTE
	SD24 F $\downarrow \leq 0.4$ G3 SD45 F $\downarrow \leq 0.4$ G3 SD56 F $\downarrow \leq 0.3$ G3	
Clotting potential	Platelet count (PLC) SD3 M $\downarrow \leq 0.6$ G2 SD56 M $\downarrow \leq 0.6$ G3 SD3 F $\downarrow \leq 0.5$ G2 SD24 F $\downarrow \leq 0.3$ G2 SD56 F $\uparrow \geq 1.6$ G2	Prothrombin time Activated partial-thromboplastin time clotting time Fibrinogen
Others		Bone marrow cytology

Hematology results show a decrease in ANS levels in group 3 males at study days 3 and 56 and in group 2 males at study day 56. ANS levels were decreased in group 2 females at study days 3 and 24. AMO levels were increased in group 3 males at study day 3. WBC levels were decreased in group 2 females at study day 24. Monocyte levels were increased in group 2 males at study day 24. Monocyte levels were decreased in group 2 females at study days 3 and 24. Monocyte levels were increased in group 3 females at study day 24. Monocyte levels were decreased in group 3 females at study day 56.

Eosinophil levels were increased in groups 2 and 3 males at study days 3 and 24. Eosinophil levels were decreased in group 3 males at study day 45. Eosinophil levels were decreased in groups 2 and 3 females at study days 3 and 45. Eosinophil levels were decreased in groups 2 and 3 females at study days 24 and 56, respectively.

Basophil levels were decreased in group 2 males at study days 3 and 45. Basophil levels were decreased in groups 2 and 3 females at pre-test. Basophil levels were decreased in group 3 females at study days 3, 24, 45, and 56.

PLC levels were decreased in groups 2 and 3 males at study days 3 and 56. PLC levels were decreased in group 2 females at study days 3, 24, and 56.

Systemic toxicity:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), food consumption, body temperature, urinalysis, ophthalmoscopic parameters, gross pathology, or microscopic anatomy were reported.

Organ Weight:

Table 8: Male's organ weight (study #1)

SEX		Males		
GROUPS		1 Day 45/56	2 Day 45/56	3 Day 45/56
NUMBER OF ANIMALS		5/5	5/5	5/5
BODY WEIGHT (terminal)		4000/3900	3800/4100	3900/3700
BRAIN		10.56/9.60	9.86/9.58	9.66*/9.79
ADRENALS		0.56/0.72	0.56/0.56	0.47/0.58
EPIDIDYMIDES		NC	NC	NC
HEART		9.47/8.49	9.21/9.13	10.25/9.04
KIDNEYS	Right	10.97/10.94	10.22/10.78	10.65/9.86
	Left	11.21/11.47	10.30/10.56	10.92/10.01
LIVER		110.9/123.0	118.7/125.9	130.8/107.0
LUNGS		NC	NC	NC
ILLIAC LYMPH NODES	Right	NC	NC	NC
	Left	NC	NC	NC
PROSTATE		NC	NC	NC
SPLEEN		1.48/1.39	1.30/1.72	1.81/1.47
TESTES		7.01/6.63	7.11/7.15	7.43/8.34
PITUITARY		NC	NC	NC
THYROID and PARATHYROID		NC	NC	NC
THYMUS		4.88/4.38	3.63/4.24	3.65/4.69
OVARIES				
UTERUS				

*Significant difference from control, $P \leq 0.05$.

Table of male's organ weight: Absolute weights are expressed as mean (grams). NC = Not collected. Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and at the end of the recovery phase (days 45/56).

At study day 56, adrenal weight was decreased 22% and 20% in groups 2 and 3 males, respectively. Left kidney weight was decreased 13% in group 3 at study day 56. Liver weight was increased 18% in group 3 males at study day 45. Liver weight was decreased 13% in group 3 males at study day 56. Spleen weight was decreased 12% in group 2 males at study day 45. Spleen weight was increased 22% in group 3 males at study day 45. Spleen weight was increased 24% in group 2 males at study day 56. Testes weight was increased 26% in group 3 at study day 56. At study day 45, thymus weight was decreased 26% and 25% in groups 2 and 3 males, respectively.

Table 9: Female's organ weight (study #1)

SEX		Females		
GROUPS		1 Day 45/56	2 Day 45/56	3 Day 45/56
NUMBER OF ANIMALS		5/5	5/5	5/5
BODY WEIGHT (terminal)		4100/4200	4100/4300	4000/4200
BRAIN		10.02/9.95	9.37/9.38	9.67/9.70
ADRENALS		0.48/0.50	0.60/0.46	0.49/0.50
EPIDIDYIMIDES				
HEART		8.96/8.69	9.52/9.24	9.37/7.96
KIDNEYS	Right	10.05/9.85	10.09/9.30	9.96/9.79
	Left	10.33/10.40	10.26/10.07	10.21/9.64
LIVER		102.5/101.7	97.82/102.4	85.84/89.23
LUNGS		NC	NC	NC
ILLIAC LYMPH NODES	Right	NC	NC	NC
	Left	NC	NC	NC
PROSTATE AND SEMINAL VESICLE				
SPLEEN		1.55/2.08	1.76/1.79	2.20/1.70
TESTES				
PITUITARY		NC	NC	NC
THYROID and PARATHYROID		NC	NC	NC
THYMUS		5.97/6.97	6.30/5.12	4.25/5.72
OVARIES		0.77/0.78	0.61/0.97	0.49/0.70
UTERUS		NC	NC	NC

Table of female's organ weight: Absolute weights are expressed as mean (grams). NC = Not collected. Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and at the end of the recovery phase (days 45/56).

Adrenal weight was increased 25% in group 2 females at study day 45. Liver weight was decreased 16% in group 3 females at study day 45. Liver weight was decreased 12% in group 3 females at study day 56. At study day 45, spleen weight was increased 14% and 42% in groups 2 and 3 females, respectively. At study day 56, spleen weight was decreased 14% and 18% in groups 2 and 3 females, respectively. Thymus weight was decreased 29% in group 3 females at study day 45. At study day 56, thymus weight was decreased 27% and 18% in groups 2 and 3 females, respectively. At study day 45, ovary weight was decreased 21% and 36% in groups 2 and 3, respectively. Ovary weight was increased 24% in group 2 at study day 56.

Injection site irritation

In all groups, some slight injection site irritation was reported following the second and/or third injections. The severity of irritation was slight in all cases and the injection sites of the animals that exhibited irritation usually returned to normal within 72 hr or less after injection. In 2 group 1 males, 3 group 2 males, and 2 group 3 males and females, slight erythema and/or edema was reported up to 48 hr or less after the second injection. In groups 2 and 3, the incidence of slight irritation after the third injection was higher than in the control group. In 2 group 2 females and 3 group 3 males, slight

erythema and/or edema was reported up to 72 hr or less after the third injection. None of these findings were reported in group 1.

Gross pathology:

Macroscopic findings are listed below:

Table 10: Male's and female's macroscopic findings (study #1)

Group	Males			Females		
	1	2	3	1	2	3
Animals examined	5	5	5	5	5	5
Iliac and inguinal lymph node						
Slightly enlarged	0	1	1	0	0	0
Inguinal lymph node						
Slightly enlarged	0	0	1	0	0	0
Slightly discolored red	0	0	1	0	0	0
Ovaries						
Multiple slightly black foci				1	0	0
Black foci 1-2mm				1	1	1
Black foci 2-4mm				0	1	0
Slightly black foci 1-2mm				1	0	0
Slightly red foci				0	0	1
Lungs with/bronchi						
Discolored dark red	0	0	0	0	1	0

No test article-related macroscopic findings were reported.

Microscopic findings are listed below:

Table 11: Male's and female's microscopic findings (study #1)

Group	Males			Females		
	1	2	3	1	2	3
Animals examined	5	5	5	5	5	5
Adrenal gland						
Extracapsular cortical tissue	2	0	3	0	0	0
Cortical tissue, Extracapsular				2	0	1
Aorta- Mineralization	0	0	1			
Brain						
Lipidosis, Choroid plexus				0	0	1
Heart						
Inflammation, Subacute				0	0	1
Injection site 1						
Acanthosis	2	1	1	2	1	4
Dermis, Chronic inflammation	3	4	2	2	4	3
Dermis, Fibrosis	1	2	1	5	4	3
Dermis, Granuloma				0	0	1
Dermis, Foreign body (IES)				0	0	1
Subcutis, Chronic inflammation	1	3	4	0	3	2
Subcutis, Necrosis	0	0	1			
Panniculus muscle, Chronic inflammation	0	0	1	0	2	0
Panniculus muscle, Fiber necrosis	0	0	1			
Deep skeletal muscle, Chronic inflammation	1	2	3	1	2	1
Deep skeletal muscle, Fiber necrosis	1	0	2			

Group	Males			Females		
	1	2	3	1	2	3
Animals examined	5	5	5	5	5	5
Injection site 2						
Surface exudate	1	0	0			
Acanthosis	2	3	1	1	2	1
Dermis, Chronic inflammation	3	2	5	4	4	5
Dermis, Fibrosis	2	4	4	5	4	3
Subcutis, Chronic inflammation	0	4	2	0	4	3
Subcutis, Necrosis				0	0	1
Subcutis, Foreign body (IES)	0	2	0	1	0	1
Subcutis, Hemorrhage				0	1	1
Panniculus muscle, Chronic inflammation	1	4	0	1	1	2
Panniculus muscle, Fiber necrosis	0	1	0			
Deep skeletal muscle, Chronic inflammation	0	1	1	1	1	1
Deep skeletal muscle, Fiber necrosis	0	0	1	1	0	0
Panniculus muscle, Foreign body (IES)	1	1	0			
Subcutis, Fibrosis	0	1	0	1	0	1
Subcutis, Granuloma (S)				0	0	1
Injection site 3						
Surface exudate	1	0	1	0	0	1
Acanthosis	4	4	5	0	2	3
Dermis, Chronic inflammation	4	5	5	4	4	5
Dermis, Fibrosis	3	3	4	3	3	5
Dermis, Necrosis				0	1	0
Dermis, Foreign body(IES)	0	1	0			
Subcutis, Chronic inflammation	2	3	4	1	3	3
Subcutis, Foreign body (IES)	0	1	1			
Subcutis, Hemorrhage	0	1	1	1	0	1
Panniculus muscle, Chronic inflammation	3	2	2	2	1	0
Panniculus muscle, Fiber necrosis	2	1	2			
Deep skeletal muscle, Chronic inflammation	1	0	0	1	1	3
Deep skeletal muscle, Fiber necrosis	1	0	0	0	1	0
Kidneys						
Mineralization	4	5	3	4	5	5
Pyelitis				0	0	1
Liver						
Cytoplasmic Vacuolization	0	3	4	3	1	0
Inflammation, Granulomatous	1	0	0			
Lungs with bronchi						
Granuloma	2	0	2			
Mineralization, Interstitium	1	0	0			
Alveolar edema	0	0	1	1	0	0
Proliferation, Alveolar macrophage				0	0	2
Lymph node, Inguinal						
Hyperplasia, Lymphoid	1	0	3	1	1	1
Hyperplasia, Reticuloendothelial	1	2	0	1	0	1
Plasmacytosis	0	0	1			
Lymph node, Iliac						
Hyperplasia, Lymphoid	0	2	1	0	0	2
Hyperplasia, Reticuloendothelial	0	0	1	0	0	1

Group	Males			Females		
	1	2	3	1	2	3
Animals examined	5	5	5	5	5	5
Hemorrhage	1	2	0	0	1	2
Plasmacytosis	0	2	1	0	2	3
Lymph node, Mandibular						
Hemorrhage, Fresh				0	1	0
Pancreas						
Apoptosis, Acinar cells, Increased				0	0	1
Parathyroid gland						
Branchial cyst/Devel. malform.				0	0	1
Pituitary gland						
Cyst	1	0	1			
Salivary gland						
Inflammation, Chronic	1	0	0			
Necrosis, Focal	1	0	0			
Skeletal muscle						
Inflammation, Chronic	1	0	0			
Skin, Abdominal						
Inflammation, subacute	3	0	2	4	0	5
Ulcer	1	0	0	0	0	1
Spleen						
Pigment., Hemosiderin. increase	0	0	1			
Testes						
Multinucleate giant cells, Seminiferous tubules	3	4	2			
Thymus						
Involution	0	1	2	2	3	1
Thyroid gland						
Cyst	4	0	2	3	0	5
Tongue						
Ulceration	0	0	1			
Trachea						
Inflammation, Acute	2	0	3	1	0	4

An extensive number of tissues were examined for histology. No test article-related microscopic findings were reported.

Body temperature:

No test article-effects on body temperature were reported. Body temperatures were fell within the normal range for laboratory rabbits (101.5 - 104.2°F; average 103.1°F). Individual variations from this range did not exhibit a dose-related trend in occurrence.

Serology:

The ANTI-MV A and ANTI-CEF antibody response in rabbits were quantified using ELISA assay.

A strong antibody (Ab) response was reported in group 3 two weeks post immunization. For the high-dose group, the mean anti-MVA response (\log_{10} titer \pm SEM) was 3.535 ± 0.083 . In group 2, only 5 of the 10 rabbits mounted an anti-MVA Ab response 2 weeks post immunization, as reflected by the low group mean, 1.681 ± 0.241 . In groups 2 and 3, two weeks after the second IM injection and two weeks after the third IM injection, strong Ab responses to MVA were reported.

For both treatment groups (2 and 3), the response to the CEF component of the vaccine mirrored that of the MVA response. However, the responses were significantly lower at all time points ($p < 0.01$). While none of the rabbits in group 2 exhibited a primary response to CEF, five of the 10 rabbits in group 3 generated Ab to CEF following the first IM injection, with a group mean, 1.620 ± 0.209 . In both treatment groups, the Ab response to CEF increased with time following the second and third IM injections. In group 2, the mean anti-CEF response was 2.091 ± 0.260 at the 5-week time point and 3.355 ± 0.081 at the 8-week time point. In group 3, the mean anti-CEF response was 3.746 ± 0.098 at the 5-week time point and 4.258 ± 0.103 at the 8-week time point. The secondary and tertiary Ab responses in group 3 were significantly greater ($p < 0.01$) than those in group 2.

No statistically significant difference in the response of males when compared to females at each time point.

Table 12: Anti-MVA antibody response in rabbits by sex^a (study #1)

Treatment	Sex	ELISA titer at week ^b			
		Pretest	2	5	8
Vehicle	M	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
	F	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
10^7 MVA-BNTCID ₅₀	M	1.000 ± 0.000	1.901 ± 0.396	4.288 ± 0.204	4.950 ± 0.147
	F	1.000 ± 0.000	1.460 ± 0.286	3.746 ± 0.321	5.010 ± 0.213
10^8 MVA-BN TCID ₅₀	M	1.000 ± 0.000	3.686 ± 0.074	5.130 ± 0.153	5.191 ± 0.153
	F	1.000 ± 0.000	3.385 ± 0.120	5.070 ± 0.113	5.191 ± 0.074

^aserum samples were analyzed for Ab reactivity to MVA by ELISA. Data presented as mean \pm SEM of the \log_{10} Ab titer. Antibody titers are reported as the \log_{10} of the reciprocal of the largest serum dilution in which the O.D. values at A₄₅₀₋₅₄₀ were ≥ 0.200 . O.D. values ≤ 0.200 were arbitrarily given a value of 1.000.

^bRabbits were immunized at weeks 0, 3, and 6. Sera were collected pretest and 2 weeks post immunization. The \log_{10} Ab titer mean \pm SEM was 4.370 ± 0.068 (8 determinations) for the positive control, rabbit anti-MVA-BN antiserum. Pooled normal rabbit serum, used as the negative control, had a \log_{10} Ab titer mean \pm SEM 1.000 ± 0.000 (8 determinations). Assays were performed in triplicate.

Table 13: Anti-CEF antibody response in rabbits by sex^a (study #1)

Treatment	Sex	ELISA Titer at Week ^b			
		Pretest	2	5	8
Vehicle	M	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
	F	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
10 ⁷ MVA-BN TCID ₅₀	M	1.000 ± 0.000	1.000 ± 0.000	2.282 ± 0.334	3.264 ± 0.113
	F	1.000 ± 0.000	1.000 ± 0.000	1.901 ± 0.418	3.445 ± 0.113
10 ⁸ MVA-BN TCID ₅₀	M	1.000 ± 0.000	1.981 ± 0.252	3.806 ± 0.135	4.288 ± 0.181
	F	1.000 ± 0.000	1.260 ± 0.260	3.686 ± 0.153	4.227 ± 0.120

^aSerum samples were analyzed for Ab reactivity to CEF by ELISA. Data presented as mean ± SEM of the log₁₀ Ab titer. Antibody titers are reported as the log₁₀ of the reciprocal of the largest serum dilution in which the O.D. values at A₄₅₀₋₅₄₀ were ≥ 0.200. O.D. values < 0.200 were arbitrarily given a value of 1.000.

^bRabbits were immunized at weeks 0, 3, and 6. Sera were collected pretest and 2 weeks post immunization. The log₁₀ Ab titer mean ± SEM was 2.241 ± 0.060 (5 determinations) for the positive control, rabbit anti-MVA-BN antiserum. Pooled normal rabbit serum, used as the negative control, had a log₁₀ Ab titer mean ± SEM 1.000 ± 0.000 (5 determinations). Assays were performed in triplicate.

Test article-related effects:

Table 14: Test article-related effects (study #1)

Test article related effects	Effects considered incidental
↑ CPK in females ↓ Eosinophils ↓ Basophils ↓ Adrenal weight ↑ Spleen weight ↓ Thymus weight ↓ Ovary weight Immune responses	Injection site irritation

Assessment:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), food consumption, body temperature, urinalysis, ophthalmoscopic parameters, gross pathology, or microscopic anatomy were reported.

Creatine kinase (CK) also known as creatine phosphokinase (CPK) or phospho-creatine kinase is an enzyme expressed by various tissues and cell types. CK catalysis the conversion of creatine and utilizes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). This CK enzyme reaction is reversible and thus ATP can be generated from PCr and ADP. Clinically, creatine kinase is assayed in blood tests as a marker of damage of CK-rich tissue such as in myocardial infarction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, the autoimmune myositides and in acute renal failure². The increase in creatine

² https://en.wikipedia.org/wiki/Creatine_kinase

phosphokinase (CPK) activity values for the test article-treated groups might be a reflection of muscle degeneration subsequent to the inflammatory response to intramuscular injection of the vaccine.

Eosinophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. They are granulocytes that develop during hematopoiesis in the bone marrow before migrating into blood.

Basophils play a role in both parasitic infections and allergies. Basopenia has been reported in association with autoimmune urticaria.

Adrenal glands are responsible for releasing hormones in response to stress through the synthesis of corticosteroids such as cortisol and catecholamines such as adrenaline (epinephrine) and noradrenaline. They also produce androgens in their innermost cortical layer. The adrenal glands affect kidney function through the secretion of aldosterone.

Spleen weight increase might be related to the intended immune response. The spleen plays important roles in regard to red blood cells and the immune system³. It removes old red blood cells and holds a reserve of blood in case of hemorrhagic shock while also recycling iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes. The globin portion of hemoglobin is degraded to its constitutive amino acids, and the heme portion is metabolized to bilirubin, which is subsequently shuttled to the liver for removal⁴. It synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation.

The thymus is a specialized primary lymphoid organ of the immune system. Within the thymus, T cells or T lymphocytes mature. T cells are critical to the adaptive immune system, where the body adapts specifically to foreign invaders. The thymus is composed of two identical lobes and is located anatomically in the anterior superior mediastinum, in front of the heart and behind the sternum. In males, at study day 57, the increase in thymus weight might be related to the immune responses due to test article-treatment. However, at study day 29, female's thymus weight was decreased significantly. As the thymus is the organ of T-cell development, any congenital defect in thymic genesis or a defect in thymocyte development can lead to a profound T cell deficiency in primary immunodeficiency disease. Defects that affect both the T cell and B cell lymphocyte lineages result in severe combined immunodeficiency syndrome (SCIDs). Acquired T cell deficiencies can also affect thymocyte development in the thymus.⁵

Even though no microscopic findings associated with the decrease in ovary weight, the changes were significant (21% and 36% in groups 2 and 3) and might be related to the test article treatment.

Immune responses due to test article treatment were reported.

Because the injections site findings were also reported in the control group, it is not considered test article-related and might be related to the injection procedure.

³ Spleen, Internet Encyclopedia of Science.

⁴ Mebius RE, Kraal G. (2005). Structure and function of the spleen. *Nat Rev Immunol.* 5(8):606-16.

⁵ <https://en.wikipedia.org/wiki/Thymus>.

Based on the overall findings in this study, it can be concluded that in rabbits, administration of MVA-BN vaccine had no adverse effects in terms of systemic toxicity.

GLP study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

Conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues were reported in this study.

Internal communication:

CRP levels were not measured in this study. CRP are important in determining the cause of inflammation, if any, caused by the test article.

Study # 2: Safety evaluation study of subcutaneously administered MVA-BN vaccine in male and female rabbits (study number M254-03).

Performing laboratory: (b) (4)

Initiation date: August 14, 2003

Final report date: October 21, 2003

Batch/lot number of test article:

Test Article

MVA-BN vaccine (MV A-BN); 1 x 10⁸ TCID₅₀/vial

Sterile water

Dilution buffer [10 mM TRIS, 140 mM NaCl,

(b) (4)

Lot No.

130303

(b) (4)

(b) (4)

Animal species and strain: (b) (4) rabbits

Breeder/supplier: (b) (4)

Number of animal per sex per group: 10 per sex per group

Age: 7-8.5 months

Body weight range: 3.3 to 4.6 kg

Route and site of administration: Subcutaneous injections were made to the dorsal area on the left side of the spine and were rotated to a different site on the same side for each injection.

Volume of administration: 0.5 (±10%, or ±0.05) mL/dose

Frequency of administration and study duration: Rabbits were treated at weeks 0, 3, and 6 and study duration was 56 days

Dose/animal: See study design

Stability: The stability analysis provided by the applicant is presented in appendix B1-3. The stability of the test article, when reconstituted with water, was 1 hr when stored on wet ice (2-8°C).

Means of administration: Subcutaneous (SC)

Report status: Final

Methods:

Study design

Animals were randomized and assigned to 3 different groups. Each group consisted of 10 animals/sex/group. Animals were dosed by subcutaneous route. The details of the study design are listed in the following table:

Table 15: Experimental design (study #2)

Group	Dose Route	MVA-BN Dose Levels (TCID ₅₀ /Immunization) ^A	Number of Immunizations	Number of Animals	
				Main Study ^B	Recovery Group ^C
1	SC	Vehicle Control	3	5M + 5F	5M + 5F
2	SC	1 x 10 ⁷	3	5M + 5F	5M + 5F
3	SC	1 x 10 ⁸	3	5M + 5F	5M + 5F

SC = subcutaneous

^A Rabbits were immunized with the MVA-BN vaccine or control article at weeks 0, 3, and 6 (days 0, 21, and 42).

^B Main Study: Five animals per sex per group were sacrificed 3 days after the last immunization & day 45).

^C Recovery group: Five animals per sex per group were sacrificed 2 weeks after the last immunization (week 8; day 56).

Randomization procedure: Yes.

Statistical analysis plan: Yes.

The following parameters were evaluated: Cage side observations (once daily), clinical observations (pre-injection and approximately 2-4 hr after injection on days of dosing, then once daily throughout the study), injection site evaluations (pre-injection and approximately 2-4, 24, 48, and 72 hr after each injection), body weights (before initiation of treatment on day 0 and weekly thereafter), food consumption (daily qualitatively and twice weekly quantitatively), body temperature (pre-injection and approximately 2-4, 24, 48, 72 and 96 hr after each injection), ophthalmoscopy (pretest and once during the final week), clinical chemistry, hematology, and coagulation (pretest, and then 3 days after each immunization [days 3, 24, and 45] and at necropsy), serology (pretest, 2 weeks after the first and second immunizations [days 14 and 35], and at necropsy 2 weeks after the third [final] immunization), urinalysis (once at scheduled necropsy). Gross anatomy at termination and organ weights and histopathology were evaluated/determined on selected tissues.

Table 16: Parameters evaluated (study #2)

Parameters	Frequency of Testing
Cage-side observations	Once daily
Clinical observations	Pre-injection and approximately 2-4 hr after injection on days of dosing, then once daily throughout the study
Injection site evaluations	Pre-injection and approximately 2-4, 24, 48, and 72 hr after each injection
Body weight	Before initiation of treatment on day 0 and weekly thereafter

Parameters	Frequency of Testing
Food consumption	Daily qualitatively and twice weekly quantitatively
Body temperature	Pre-injection and approximately 2-4, 24, 48, 72, and 96 hr after each injection
Ophthalmoscopy	Pretest and once during the final week
Clinical chemistry*	Pretest, 3 days after each immunization [days 3, 24, and 45], and at necropsy
Hematology*	Pretest, 3 days after each immunization [days 3, 24, and 45], and at necropsy
Coagulation*	Pretest, 3 days after each immunization [days 3, 24, and 45], and at necropsy
Urinalysis	Once at scheduled necropsy
Serology*	Pretest, 2 weeks after the first and second immunizations [days 14 and 35], and at necropsy 2 weeks after the third [final] immunization
Necropsy	Days 45 and 56
Tissues for histopathology	Days 45 and 56

*Ear blood vessel

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Table 17: Tissues collected at necropsy (study #2)

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	!	
Bone marrow smear		X
Bone marrow (histology and cytology, sternum)	!	
Bone (femur with distal metaphysis and epiphysis)	!	
Bone (sternum [examined along with the bone marrow section])	!	
Brain	!*	
Cecum	!	
Cervix	!	
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes	!	
Fallopian tubes		X
Gall bladder	!	

Organ/Tissue	Collected	Not collected
Gut Associated Lymphoid Tissue (GALT) (Peyer's patches)		X
Gross lesions	!	
Harderian gland		X
Heart	!*	
Ileum	!	
Injection site	!	
Jejunum	!	
Kidneys	!*	
Liver	!*	
Lungs	!	
Lymph nodes (inguinal, iliac, mandibular, and mesenteric)	!	
Mammary gland	!	
Optic nerve	!	
Ovaries	!*	
Pancreas	!	
Parathyroid glands	!	
Pituitary gland	!	
Prostate	!	
Rectum	!	
Salivary glands	!	
Sciatic nerve	!	
Seminal vesicle	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (thoracolumbar junction) with spinal column	!	
Spleen	!*	
Stomach	!	
Testes	!*	
Thymus	!*	
Thyroid	!	
Tongue	!	
Trachea	!	
Ureters		X
Uterus	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland		X

Results:

Morbidity and mortality:

No test article-related morbidity or mortality was reported.

Clinical Chemistry, hematology, and coagulation:

Table 18: Clinical chemistry results (study #2)

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if Greater than 1.5 so Indicated Otherwise ≤ 1.5)	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, potassium, sodium, phosphorus
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR B) HEPATOBILIARY	Aspartate aminotransferase (AST or SGOT) SD24 F $\downarrow \leq 0.2$ G2 (very high SD in G1) SD24 F $\downarrow \leq 0.1$ G3 (very high SD in G1) Alanine aminotransferase (ALT or SGPT) SD24 F $\downarrow \leq 0.6$ G2 (very high SD in G1) SD24 F $\downarrow \leq 0.4$ G3 (very high SD in G1)	
		Total bilirubin Alkaline phosphatase (ALP)
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation) C-reactive protein*
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Cholesterol SD24 M $\uparrow \geq 1.7$ G3 CPK SD3 M $\uparrow \geq 1.8$ G3 SD45 M $\uparrow \geq 1.9$ G3 SD24 F $\downarrow \leq 0.6$ G3 SD56 F $\downarrow \leq 0.5$ G3 GGT SD3 M $\uparrow \geq 1.6$ G2 SD56 F $\uparrow \geq 1.6$ G2 Fasting Triglycerides SD24 M $\uparrow \geq 1.8$ G3	Albumin (A) Total protein Carbon dioxide Globulin A/G ratio Lactate dehydrogenase

* Not measured. SD = Standard deviation.

Clinical chemistry results show a decrease in AST and ALT levels in groups 2 and 3 females at study day 24. However, the standard deviation for the control group (group 1) was very high. Thus, these decreases are considered of no toxicological importance. Cholesterol levels were increased in group 3 males at study day 24. Cholesterol levels were decreased in group 3 males at study day 24.

Creatinine phosphokinase (CPK) levels were increased in group 3 males at study days 3 and 45. CPK levels were decreased in group 3 females at study days 24 and 56. GGT levels were increased in group 2 males and females at study days 3 and 56, respectively. Triglyceride levels were increased in group 3 males at study day 24.

Table 19: Hematology results (study #2)

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if Great or Less than 1.56, ie, ≥ 1.6 or ≤ 1.6)	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	White Blood Cells (WBC) SD3 F $\uparrow \geq 1.7$ G3 Monocyte count: SD3 F $\uparrow \geq 5.6$ G3 SD56 M $\downarrow \leq 0.5$ G2 SD56 M $\downarrow \leq 0.4$ G3 Eosinophils count SD3 M $\downarrow \leq 0.4$ G2 SD3 M $\downarrow \leq 0.5$ G3 SD24 M $\downarrow \leq 0.6$ G2 SD24 M $\downarrow \leq 0.6$ G3 SD3 F $\downarrow \leq 0.6$ G2 SD45 F $\uparrow \geq 5.1$ G2 SD45 F $\uparrow \geq 5.8$ G3 SD56 M $\downarrow \leq 0.5$ G2 SD56 M $\downarrow \leq 0.3$ G3 SD56 F $\uparrow \geq 1.9$ G2 Basophils SD3 M $\downarrow \leq 0.5$ G2 SD3 F $\uparrow \geq 1.9$ G3 SD24 M $\downarrow \leq 0.3$ G2 SD24 M $\downarrow \leq 0.5$ G3 SD24 F $\downarrow \leq 0.6$ G3 SD45 F $\downarrow \leq 0.2$ G3 SD56 M $\downarrow \leq 0.6$ G2 SD56 F $\downarrow \leq 0.5$ G2 SD56 F $\downarrow \leq 0.3$ G3	Macrophage Leukocytes Large unstained cells (LUC) Segmented neutrophil (ANS)

⁶ With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if Great or Less than 1.56, ie, ≥ 1.6 or ≤ 1.6)	NOT of NOTE
	Lymphocyte count SD3 F $\uparrow \geq 1.8$ G3	
Clotting potential	Platelet count (PLC) SD3 F $\downarrow \leq 0.6$ G2	Prothrombin time Activated partial-thromboplastin time clotting time Fibrinogen
Others		Bone marrow cytology

Hematology results show an increase in WBC levels in group 3 females at study day 3. Monocyte levels were increased in group 3 females at study day 3. Monocyte levels were decreased in groups 2 and 3 males at study day 56.

Eosinophil levels were decreased in groups 2 and 3 males at study days 3 and 24. Eosinophil levels were decreased in group 2 females at study day 3. Eosinophil levels were increased in groups 2 and 3 females at study day 45. Eosinophil levels were decreased in groups 2 and 3 males at study day 56. Eosinophil levels were increased in group 2 females at study day 56.

Basophil levels were decreased in group 2 males at study days 3, 24, and 56. Basophil levels were decreased in group 3 males at study day 24. Basophil levels were increased in group 3 females at study day 3. Basophil levels were decreased in group 3 females at study days 24, 45, and 56. Basophil levels were decreased in group 2 females at study day 56. Lymphocyte levels were increased in group 3 females at study day 3. PLC levels were decreased in group 2 females at study day 3.

Systemic toxicity:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), food consumption, urinalysis, ophthalmoscopic parameters, gross pathology, or microscopic anatomy were reported.

Organ Weight:

Table 20: Male's organ weight (study #2)

SEX	Males		
GROUPS	1 Day 45/56	2 Day 45/56	3 Day 45/56
NUMBER OF ANIMALS	5/5	5/5	5/5
BODY WEIGHT (terminal)	4100/3800	3800/3800	3900/3700
BRAIN	9.80/9.72	9.82/9.62	9.66/10.19
ADRENALS	0.55/0.46	0.73/0.55	0.60/0.63*
EPIDIDYMIDES	NC	NC	NC
HEART	10.06/9.46	8.71/8.86	8.69/9.43
KIDNEYS Right	11.08/9.76	9.48/9.85	9.74/9.71

SEX		Males		
GROUPS		1 Day 45/56	2 Day 45/56	3 Day 45/56
NUMBER OF ANIMALS		5/5	5/5	5/5
	Left	10.68/9.86	9.51/10.10	9.90/9.88
LIVER		116.1/104.6	98.12/104.3	112.0/92.32
LUNGS		NC	NC	NC
ILLIAC LYMPH NODES	Right	NC	NC	NC
	Left	NC	NC	NC
PROSTATE		NC	NC	NC
SPLEEN		1.46/1.40	1.14/1.21	1.42/1.82
TESTES		6.41/8.11	6.87/6.65	7.50/6.86
PITUITARY		NC	NC	NC
THYROID and PARATHYROID		NC	NC	NC
THYMUS		4.02/4.89	5.48/4.50	2.84/4.32
OVARIES				
UTERUS				

NC = Not collected. *Significant difference from control, $P \leq 0.05$.

Table of male's organ weight: Absolute weights are expressed as mean (grams). Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and at the end of the recovery phase (days 45/56).

At study day 45, adrenal weight was increased 33% in group 2 males. At study day 56, adrenal weight was increased 20% and 37% in groups 2 and 3 males, respectively. At study day 45, heart weight was decreased 13% and 14% in groups 2 and 3 males, respectively. At study day 45, right kidney weight was decreased 14% and 12% in groups 2 and 3, respectively. Liver weight was decreased 15% in group 2 males at study day 45. Liver weight was decreased 12% in group 3 males at study day 56. Spleen weight was decreased 22% in group 2 males at study day 45. Spleen weight was decreased 14% in group 2 males at study day 56. Spleen weight was increased 30% in group 3 males at study day 56. Testes weight was increased 17% in group 3 at study day 45. At study day 56, testes weight was decreased 18% and 15% in groups 2 and 3 males, respectively. Thymus weight was increased 36% in group 2 at study day 45. Thymus weight was decreased 29% in group 3 at study day 45. Thymus weight was decreased 12% in group 3 at study day 56.

Table 21: Female's organ weight (study #2)

SEX		Females		
GROUPS		1 Day 45/56	2 Day 45/56	3 Day 45/56
NUMBER OF ANIMALS		5/5	5/5	5/5
BODY WEIGHT (terminal)		4100/4100	4000/3900	4200/4300
BRAIN		10.15/9.76	9.62/9.95	9.56/9.79
ADRENALS		0.54/0.50	0.56/0.53	0.47/0.62
EPIDIDYMIDES				

SEX		Females		
GROUPS		1 Day 45/56	2 Day 45/56	3 Day 45/56
NUMBER OF ANIMALS		5/5	5/5	5/5
HEART		9.84/8.19	8.65/8.61	9.11/9.84
KIDNEYS	Right	9.61/9.31	8.96/9.88	10.29/10.39
	Left	9.81/9.70	9.27/10.40	10.35/10.82
LIVER		96.96/90.97	98.39/95.07	100.7/102.8
LUNGS		NC	NC	NC
ILLIAC LYMPH NODES	Right	NC	NC	NC
	Left	NC	NC	NC
PROSTATE AND SEMINAL VESICLE				
SPLEEN		2.00/1.64	1.83/2.05	1.66/2.23
TESTES				
PITUITARY		NC	NC	NC
THYROID and PARATHYROID		NC	NC	NC
THYMUS		5.68/6.03	3.93*/4.32	4.15/5.56
OVARIES		0.57/0.59	0.50/0.83	0.72/0.74
UTERUS		NC	NC	NC

NC = Not collected. *Significant difference from control, $P \leq 0.05$.

Table of female's organ weight: Absolute weights are expressed as mean (grams). Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and at the end of the recovery phase (days 45/56).

Adrenal weight was decreased 13% in group 3 females at study day 45. Adrenal weight was increased 24% in group 3 females at study day 56. Heart weight was decreased 12% in group 2 females at study day 45. Heart weight was increased 20% in group 3 females at study day 56. Right kidney's weight was increased 12% in group 3 females at study day 56. Left kidney's weight was increased 12% in group 3 females at study day 56. Liver weight was increased 13% in group 3 females at study day 56. Spleen weight was decreased 17% in group 3 females at study day 45. At study day 56, spleen weight was increased 25% and 36% in groups 2 and 3 females, respectively. At study day 45, thymus weight was decreased 31% and 27% in groups 2 and 3 females, respectively. At study day 56, thymus weight was decreased 28% in group 2 females. At study day 45, ovary weight was decreased 12% and increased 26% in groups 2 and 3, respectively. At study day 56, ovary weight was increased 41% and 25% in groups 2 and 3, respectively.

Injection Site Irritation

Slight irritation was reported for some control animals following injection. However, there was a dose-related increase in the incidence, severity, and persistence of injection site irritation with increasing dose of vaccine. The details of the injection site findings are listed in the tables below:

Table 22: Injection site irritation in males and females (study #2)

Dose Group (TCID ₅₀ /imm)	Males		
	Vehicle	1 × 10 ⁷	1 × 10 ⁸
Main Study			
Number of animals	5	5	5
Normal (no irritation)	4	0	0
Erythema	1 (44-45)	5 (0-19, 21-36, 43-45)	5 (0-15, 22-35, 38-39, 43-45)
Edema	0	5 (0-7, 21, 23-28, 36, 43-45)	5 (1-5, 16-36, 38-45)
Recovery Group			
Number Of Animals	5	5	5
Normal (No Irritation)	1	1	0
Erythema	2 (21-30)	4 (0-4, 22-31, 43-45, 47-48)	5 (0-19, 22-34, 43-45, 47-49, 51-52)
Edema	4 (21-30)	4 (1, 23-26, 28, 44-45)	5 (2-16, 18-36, 38-45, 47-49, 51-56)

TCID₅₀/imm = TCID₅₀/immunization.
 Numbers in parentheses () are day(s) of occurrence.
 Animals were dosed on Days 0, 21, and 42.

Dose Group (TCID ₅₀ /imm)	Females		
	Vehicle	1 × 10 ⁷	1 × 10 ⁸
Main Study			
Number Of Animals	5	5	5
Normal (No Irritation)	3	0	0
Erythema	0	5 (1-12, 21-28, 43-45)	5 (0-35, 37-41, 43-45)
Edema	2 (21)	2 (3-5, 21-27)	5 (1-45)
Recovery Group			
Number Of Animals	5	5	5
Normal (No Irritation)	2	0	0
Erythema	3 (0-2, 21-24)	5 (0-7, 22-33, 42-45)	5 (0-16, 21-34, 43-45)
Edema	0	5 (2-4, 22-31)	5 (0-30, 43-45)

TCID₅₀/imm = TCID₅₀/immunization.
 Numbers in parentheses () are day(s) of occurrence.
 Animals were dosed on Days 0, 21, and 42.

Gross pathology:

Macroscopic findings are listed below:

Table 23: Male's and female's macroscopic findings (study #2)

Group	Males			Females		
	1	2	3	1	2	3
Animals examined	5	5	5	5	5	5
Prostate						
Moderately enlarged	1	0	0	0	0	0
Iliac lymph node						
Moderately discolored red	0	1	0	0	0	0
Discolored red	0	0	0	1	0	0
Slightly discolored red	0	0	0	0	0	1
Inguinal lymph node						
Slightly enlarged	0	0	0	0	1	1
Injection site 3						
Slight edema	0	2	0	0	0	1
Slight erythema	0	2	0	0	0	0
Heart						
Right atrium discolored pink	0	0	1	0	0	0
Left atrium discolored pale	0	0	1	0	0	0
Spleen						
Slightly small	0	0	1	0	0	0
Pancreas						
Two red nodule 1-2 mm	0	0	1	0	0	0
Ovaries						
Slightly red foci 2-3 mm				1	0	1
Slightly black foci 2-3 mm				0	0	1
Slightly red foci				0	0	1
Multiple slightly black foci				1	2	1
Multiple markedly black foci				1	0	2
Moderately black foci				0	0	1

No test article-related macroscopic findings were reported.

Microscopic findings are listed below:

Table 24: Males' microscopic findings (study #2)

GROUP:	1M (1)		1M (2)		2M (3)		2M (4)		3M (5)		3M (6)	
NUMBER OF ANIMALS:	5		5		5		5		5		5	
	#	%	#	%	#	%	#	%	#	%	#	%
ADRENAL GLANDS	5		5		0		0		5		5	
ACCESSORY CORTICAL NODULE	0	0.0	0	0.0	0	0.0	0	0.0	2	40.0	2	40.0
HEART	5		5		0		0		5		5	
DEGENERATION, MYOFIBER	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0
INFLAMMATION	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0
INFLAMMATION, PERICARDIUM	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0
INJECTION SITE 1	5		5		5		5		5		5	
MIXED INFLAMMATION	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
DERMIS, CHRONIC INFLAMMATION	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	40.0
INJECTION SITE 2	5		5		5		5		5		5	
DERMIS, CHRONIC INFLAMMATION	0	0.0	0	0.0	3	60.0	0	0.0	3	60.0	1	20.0
HEMORRHAGE	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0	0	0.0
DERMAL KERATIN GRANULOMA	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0
INJECTION SITE 3	5		5		5		5		5		5	
DERMIS, CHRONIC INFLAMMATION	1	20.0	0	0.0	5	100.0	3	60.0	5	100.0	4	80.0
ACUTE INFLAMMATION	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0	1	20.0
HEMORRHAGE	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0	1	20.0
ABSCESSTION	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0
FIBROSIS	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0
KIDNEYS	5		5		5		5		5		5	
FATTY CHANGE	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
LIVER	5		5		5		5		5		5	
FATTY CHANGE	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0	0	0.0
LUNGS WITH BRONCHI	5		5		0		0		5		5	
INFLAMMATION, CHRONIC	0	0.0	0	0.0	0	0.0	0	0.0	2	40.0	0	0.0
BRONCHUS, CHRONIC INFLAMMATION	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0
LYMPH NODE, ILIAC	5		5		5		5		5		5	
HEMORRHAGE	0	0.0	0	0.0	1	20.0	1	20.0	1	20.0	0	0.0
LYMPHOID HYPERPLASIA	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0

Incidence Calculated by No. of Tissues Scored

(1) - VEHICLE CONTROL MAIN STUDY

(2) - VEHICLE CONTROL RECOVERY GROUP

(3) - MVA-BN 1×10^7 /IMMUNIZATION SC MAIN STUDY

= # of tissues evaluated.

(4) - MVA-BN 1×10^7 /IMMUNIZATION SC RECOVERY GROUP(5) - MVA-BN 1×10^8 /IMMUNIZATION SC MAIN STUDY(6) - MVA-BN 1×10^8 /IMMUNIZATION SC RECOVERY GROUP

Male's microscopic findings (continue)

GROUP:	1M	1M	2M	2M	3M	3M
	(1)	(2)	(3)	(4)	(5)	(6)
NUMBER OF ANIMALS:	5	5	5	5	5	5
	# %	# %	# %	# %	# %	# %
LYMPH NODE, INGUINAL	3	4	3	5	3	5
HEMORRHAGE	0 0.0	0 0.0	1 33.3	0 0.0	0 0.0	0 0.0
R-E CELL HYPERPLASIA	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 20.0
LYMPHOID HYPERPLASIA	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	2 40.0
LYMPH NODE, MANDIBULAR	4	5	5	3	5	4
ERYTHROPHAGOCYTOSIS	0 0.0	1 20.0	0 0.0	0 0.0	0 0.0	0 0.0
HEMORRHAGE	0 0.0	0 0.0	0 0.0	1 33.3	0 0.0	1 25.0
PANCREAS	5	5	0	0	5	5
ACCESSORY SPLEEN	0 0.0	1 20.0	0 0.0	0 0.0	0 0.0	1 20.0
SALIVARY GLAND	5	4	0	0	5	5
CYST(S)	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 20.0
TESTES	5	5	5	5	5	5
SEMINIFEROUS TUBULAR ATROPHY	1 20.0	1 20.0	1 20.0	0 0.0	1 20.0	0 0.0
THYMUS	5	5	5	5	5	5
HEMORRHAGE	0 0.0	1 20.0	0 0.0	0 0.0	0 0.0	0 0.0
INVOLUTION	5 100.0	3 60.0	5 100.0	3 60.0	5 100.0	2 40.0
THYROID GLANDS	5	4 75.0	0	0	5	5
CYST(S)	1 20.0	3 60.0	0 0.0	0 0.0	3 60.0	2 40.0
FOLLICULAR DILATION	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 20.0

Incidence Calculated by No. of Tissues Scored

(1) - VEHICLE CONTROL MAIN STUDY

(2) - VEHICLE CONTROL RECOVERY GROUP

(3) - MVA-BN 1×10^7 /IMMUNIZATION SC MAIN STUDY(4) - MVA-BN 1×10^7 /IMMUNIZATION SC RECOVERY GROUP(5) - MVA-BN 1×10^8 /IMMUNIZATION SC MAIN STUDY(6) - MVA-BN 1×10^8 /IMMUNIZATION SC RECOVERY GROUP

= # of tissues evaluated.

Table 25: Female's microscopic findings (study #2)

GROUP:	1F		1F		2F		2F		3F		3F	
	(1)		(2)		(3)		(4)		(5)		(6)	
NUMBER OF ANIMALS:	5		5		5		5		5		5	
	#	%	#	%	#	%	#	%	#	%	#	%
ADRENAL GLANDS	5		5		0		0		5		5	
ACCESSORY CORTICAL NODULE	0	0.0	2	40.0	0	0.0	0	0.0	0	0.0	1	20.0
BRAIN	5		5		5		5		5		5	
CHOROID PLEXUS, NODULAR LIPIDOSIS	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0
INJECTION SITE 1	5		5		5		5		5		5	
HEMORRHAGE	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0
DERMIS, CHRONIC INFLAMMATION	0	0.0	0	0.0	1	20.0	1	20.0	3	60.0	2	40.0
INJECTION SITE 2	5		5		5		5		5		5	
DERMIS, CHRONIC INFLAMMATION	0	0.0	0	0.0	4	80.0	2	40.0	4	80.0	1	20.0
ACUTE INFLAMMATION	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0	0	0.0
HEMORRHAGE	0	0.0	1	20.0	1	20.0	0	0.0	0	0.0	0	0.0
INJECTION SITE 3	5		5		5		5		5		5	
DERMIS, CHRONIC INFLAMMATION	0	0.0	0	0.0	5	100.0	1	20.0	5	100.0	1	20.0
ACUTE INFLAMMATION	0	0.0	0	0.0	0	0.0	0	0.0	2	40.0	0	0.0
HEMORRHAGE	1	20.0	1	20.0	0	0.0	0	0.0	1	20.0	0	0.0
SURFACE EXUDATE	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
HAIR FOREIGN BODY IN INFLAMMATORY SITE	0	0.0	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0
NECROSIS	0	0.0	0	0.0	0	0.0	0	0.0	2	40.0	0	0.0
KIDNEYS	5		5		5		5		5		5	
CALCULUS, MICROSCOPIC OBSERVATION	0	0.0	1	20.0	1	20.0	0	0.0	0	0.0	0	0.0
DILATATION, COLLECTING DUCTS	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0
CYST	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0	0	0.0
PELVIS, LEUKOCYTE INFILTRATION	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0
LIVER	5		5		5		5		5		5	
FIBROSIS	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0
PIGMENT DEPOSITION	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0
LUNGS WITH BRONCHI	5		5		0		0		5		5	
INFLAMMATION, CHRONIC	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	1	20.0
BRONCHUS, CHRONIC INFLAMMATION	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0

Incidence Calculated by No. of Tissues Scored

(1) - VEHICLE CONTROL MAIN STUDY

(2) - VEHICLE CONTROL RECOVERY GROUP

(3) - MVA-BN 1 X 10⁷ /IMMUNIZATION SC MAIN STUDY

= # of tissues evaluated.

(4) - MVA-BN 1 X 10⁷ /IMMUNIZATION SC RECOVERY GROUP(5) - MVA-BN 1 X 10⁸ /IMMUNIZATION SC MAIN STUDY(6) - MVA-BN 1 X 10⁸ /IMMUNIZATION SC RECOVERY GROUP

Female's microscopic findings (continue)

GROUP:	1F (1)	1F (2)	2F (3)	2F (4)	3F (5)	3F (6)
NUMBER OF ANIMALS:	5	5	5	5	5	5
	# %	# %	# %	# %	# %	# %
LYMPH NODE, ILIAC HEMORRHAGE	5 0 0.0	4 1 25.0	5 0 0.0	5 0 0.0	4 2 50.0	5 1 20.0
LYMPH NODE, INGUINAL HEMORRHAGE	3 0 0.0	5 0 0.0	5 1 20.0	5 0 0.0	5 1 20.0	5 0 0.0
R-E CELL HYPERPLASIA	0 0.0	0 0.0	1 20.0	0 0.0	0 0.0	0 0.0
LYMPHOID HYPERPLASIA	0 0.0	0 0.0	1 20.0	4 80.0	2 40.0	1 20.0
ABSCESSTION	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 20.0
LYMPH NODE, MANDIBULAR NECROSIS	5 1 20.0	5 0 0.0	5 0 0.0	5 0 0.0	5 0 0.0	4 0 0.0
CHRONIC INFLAMMATION	1 20.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0
ACUTE INFLAMMATION	1 20.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0
OVARIES CORPORA HEMORRHAGICA	5 2 40.0	5 2 40.0	5 2 40.0	5 5 100.0	5 3 60.0	5 3 60.0
PANCREAS ACCESSORY SPLEEN NECROSIS	5 1 20.0 1 20.0	5 0 0.0 0 0.0	0 0 0.0 0 0.0	0 0 0.0 0 0.0	5 0 0.0 0 0.0	5 0 0.0 0 0.0
SPINAL CORD MINERALIZATION	5 0 0.0	5 0 0.0	0 0 0.0	0 0 0.0	5 0 0.0	5 1 20.0
THYMUS HEMORRHAGE INVOLUTION	5 0 0.0 4 80.0	5 0 0.0 2 40.0	5 0 0.0 4 80.0	5 1 20.0 4 80.0	5 0 0.0 3 60.0	5 0 0.0 3 60.0
THYROID GLANDS CYST(S)	5 2 40.0	5 2 40.0	0 0 0.0	0 0 0.0	5 3 60.0	5 3 60.0
FOLLICULAR DILATION	0 0.0	1 20.0	0 0.0	0 0.0	0 0.0	0 0.0
EPIDERMAL REST CYST(S)	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 20.0
URINARY BLADDER ACUTE INFLAMMATION, SUBMUCOSA	5 0 0.0	4 0 0.0	0 0 0.0	0 0 0.0	5 0 0.0	5 1 20.0
UTERUS LUMEN DILATION	5 0 0.0	5 0 0.0	0 0 0.0	0 0 0.0	5 1 20.0	5 0 0.0

Incidence Calculated by No. of Tissues Scored

(1) - VEHICLE CONTROL MAIN STUDY

(2) - VEHICLE CONTROL RECOVERY GROUP

(3) - MVA-BN 1×10^7 /IMMUNIZATION SC MAIN STUDY(4) - MVA-BN 1×10^7 /IMMUNIZATION SC RECOVERY GROUP(5) - MVA-BN 1×10^8 /IMMUNIZATION SC MAIN STUDY(6) - MVA-BN 1×10^8 /IMMUNIZATION SC RECOVERY GROUP

= # of tissues evaluated.

An extensive number of tissues were examined for histology. Other than the injection site findings, no test article-related microscopic findings were reported.

At study day 45, mild to moderate lymphoid hyperplasia was present in the inguinal lymph nodes of 1/5 (20%) and 2/5 (40%) of groups 2 and 3 females, respectively. At study day 56, the same change was present in 2/5 (40%) of group 3 males and in 4/5 (80%) and 1/5 (20%) of groups 2 and 3

females, respectively. The inguinal lymph node of one group 3 females had lymphoid abscessation at study day 56. This might be related to a localized bacterial infection and not related to the test article.

Body temperature:

No test article-related effects on body temperature were reported in group 2 males and in groups 2 and 3 females. Body temperatures fell within the normal range for laboratory rabbits (101.5 - 104.2°F; average 103.1°F). Following each of the three injections, mean and individual body temperatures for group 3 males were generally higher than pre-injection values. This increase might be related to treatment with the high dose of the vaccine, although a similar trend was not reported in the high-dose females.

Statistically significantly lower mean body temperatures were reported in group 2 males on day 0 and in groups 2 and 3 females on days 3 and 42. Higher mean body temperatures were reported in low-dose males on days 21, 22, 25, and 42. However, in these groups, individual and mean body temperatures did not vary meaningfully from pre-injection body temperatures. For group 3 males, mean post-injection body temperatures were statistically significantly higher than those of the controls and/or meaningfully higher than pre-injection values on days 1, 2, 3, 21, 22, 25, 42, and 43. Body temperature repeatedly exceeded 104.2°F (the upper limit of the normal range for laboratory rabbits), following one or more injections, were reported in three animals (#s 41, 53, and 54).

Serology:

The Anti-MVA and Anti-CEF antibody response in rabbits were quantified using ELISA assay.

All rabbits in groups 2 and 3 responded with a strong antibody (Ab) responses at 2 weeks post-immunization. The mean anti-MVA responses (\log_{10} titer \pm SEM) for groups 2 and 3 were 2.572 ± 0.171 and 3.656 ± 0.081 , respectively. The anti-MVA response continued to increase for both dose groups two weeks after the second injection at week 3. At week 6 of the study, the magnitude of the tertiary response was only slightly elevated compared to the secondary response for the same dose groups. At the 5- and 8-week time points, the mean anti-MVA response in group 2 were 4.258 ± 0.144 and 4.589 ± 0.128 , respectively. In group 3, the mean anti-MVA response at the 5- and 8-week time points were 5.100 ± 0.156 and 5.221 ± 0.135 , respectively. Responses in group 3 were significantly greater ($p < 0.01$) than those in group 2.

For groups 2 and 3, the response to the CEF component of the vaccine mirrored that of the MVA response but was significantly lower at all time points ($p < 0.01$). Only two of the 10 rabbits in group 2 elicited a primary response to CEF. However, all animals in group 3 generated Ab to CEF. The \log_{10} titer \pm SEM was 1.200 ± 0.133 and 2.903 ± 0.078 for groups 2 and 3, respectively. For groups 2 and 3, responses continued to increase for both treatment groups two weeks after the second injection. In group 2, the magnitude of the tertiary response was only slightly elevated compared with the secondary response. In group 3, the tertiary response was significantly higher ($p < 0.05$) than the secondary response. In group 2, the mean anti-CEF response at the 5- and 8-week time points was 3.114 ± 0.180 and 3.565 ± 0.161 , respectively. In group 3, the mean anti-CEF response at the 5- and 8-week time points was 4.107 ± 0.110 and 4.619 ± 0.127 , respectively. All stages of the anti MVA responses (primary, secondary, and tertiary) in group 3 were significantly higher ($p < 0.01$) than those in group 2.

There was no statistically significant difference in the responses of males compared with females at each time point. Anti-MVA and Anti-CEF antibody responses in rabbits by sex are listed in the tables below:

Table 26: Anti-MVA antibody response in rabbits by sex^a (study #2)

Treatment	Sex	ELISA Titer at Week ^b			
		Pretest	2	5	8
Vehicle	M	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
	F	1.260 ± 0.260	1.260 ± 0.260	1.200 ± 0.200	1.000 ± 0.000
10 ⁷ TCID ₅₀ MVA-BN	M	1.000 ± 0.000	2.602 ± 0.269	4.227 ± 0.262	4.649 ± 0.241
	F	1.000 ± 0.000	2.542 ± 0.241	4.288 ± 0.153	4.528 ± 0.120
10 ⁸ TCID ₅₀ MVA-BN	M	1.000 ± 0.000	3.686 ± 0.120	4.950 ± 0.221	5.191 ± 0.225
	F	1.000 ± 0.000	3.625 ± 0.120	5.251 ± 0.221	5.251 ± 0.176

^aSerum samples were analyzed for Ab reactivity to MVA by ELISA. Data presented as mean ± SEM of the log₁₀ Ab titer. Antibody titers are reported as the log₁₀ of the reciprocal of the largest serum dilution in which the O.D. values at A₄₅₀₋₅₄₀ were ≥ 0.200. O.D. values ≤ 0.200 were arbitrarily given a value of 1.000.

^bRabbits were immunized at weeks 0, 3, and 6. Sera were collected pretest and 2 weeks post immunization. The log₁₀ Ab titer mean ± SEM was 4.451 ± 0.043 (7 determinations) for the positive control, rabbit anti-MBA-BN antiserum. Pooled normal rabbit serum, used as the negative control, had a log₁₀ Ab titer mean ± SEM 1.000 ± 0.000 (7 determinations). Assays were performed in triplicate.

Table 27: Anti-CEF antibody response in rabbits by sex^a (study #2)

Treatment	Sex	ELISA Titer at Week ^b			
		Pretest	2	5	8
Vehicle	M	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
	F	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
10 ⁷ TCID ₅₀ MVA-BN	M	1.000 ± 0.000	1.200 ± 0.200	3.023 ± 0.364	3.505 ± 0.330
	F	1.000 ± 0.000	1.200 ± 0.200	3.204 ± 0.095	3.625 ± 0.074
10 ⁸ TCID ₅₀ MVA-BN	M	1.000 ± 0.000	2.963 ± 0.113	3.926 ± 0.120	4.709 ± 0.233
	F	1.000 ± 0.000	2.843 ± 0.113	4.288 ± 0.153	4.528 ± 0.120

^aSerum samples were analyzed for Ab reactivity to CEF by ELISA. Data presented as mean ± SEM of the log₁₀ Ab titer. Antibody titers are reported as the log₁₀ of the reciprocal of the largest serum

dilution in which the O.D. values at $A_{450-540}$ were ≥ 0.200 . O.D. values < 0.200 were arbitrarily given a value of 1.000.

^bRabbits were immunized at weeks 0, 3, and 6. Sera were collected pretest and 2 weeks post immunization. The \log_{10} Ab titer mean \pm SEM was 2.301 ± 0.000 (4 determinations) for the positive control, rabbit anti-MBA-BN antiserum. Pooled normal rabbit serum, used as the negative control, had a \log_{10} Ab titer mean \pm SEM 1.000 ± 0.000 (4 determinations). Assays were performed in triplicate.

Test article-related effects:

Table 28: Test article-related effects (study #2)

Test article related effects	Effects considered incidental
↓ Eosinophils ↓ Basophils ↑ Adrenal weight ↓ Thymus weight Injection site irritation Immune responses	↓ Heart weight

Assessment:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), food consumption, body temperature, urinalysis, ophthalmoscopic parameters, gross pathology, or microscopic anatomy were reported.

Eosinophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. They are granulocytes that develop during hematopoiesis in the bone marrow before migrating into blood.

Basophils play a role in both parasitic infections and allergies. Basopenia has been reported in association with autoimmune urticaria.

Adrenal glands are responsible for releasing hormones in response to stress through the synthesis of corticosteroids such as cortisol and catecholamines such as adrenaline (epinephrine) and noradrenaline. They also produce androgens in their innermost cortical layer. The adrenal glands affect kidney function through the secretion of aldosterone.

The thymus is a specialized primary lymphoid organ of the immune system. Within the thymus, T cells or T lymphocytes mature. T cells are critical to the adaptive immune system, where the body adapts specifically to foreign invaders. The thymus is composed of two identical lobes and is located anatomically in the anterior superior mediastinum, in front of the heart and behind the sternum. As the thymus is the organ of T-cell development, any congenital defect in thymic genesis or a defect in thymocyte development can lead to a profound T cell deficiency in primary immunodeficiency disease. Defects that affect both the T cell and B cell lymphocyte lineages result in severe combined immunodeficiency syndrome (SCIDs). Acquired T cell deficiencies can also affect thymocyte development in the thymus.⁷

⁷ <https://en.wikipedia.org/wiki/Thymus>.

Since the heart weight decrease was not associated with any microscopic findings it is considered incidental.

Injection site findings and immune responses due to test article treatment were reported.

Based on the overall findings in this study, it can be concluded that in rabbits, administration of MVA-BN vaccine subcutaneously had no adverse effects in terms of systemic toxicity.

GLP study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

Conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues were reported in this study.

Internal communication:

CRP levels were not measured in this study. CRP are important in determining the cause of inflammation, if any, caused by the test article.

Study # 3: Repeat dose toxicity study in (b) (4) rabbit following two subcutaneous administrations (study number HPA0006/074055).

Performing laboratory: (b) (4)

Initiation date: July 26, 2007

Final report date: May 30, 2008

Batch/lot number of test article:

<u>Test Article</u>	<u>Lot No.</u>	<u>Expiry date</u>	<u>Purity/potency</u>
IMVAMUNE	0061205	NR	4.9x10 ⁸ TCID 50/ml
Sterile physiological saline ((b) (4) grade)	NR	NR	NR

Animal species and strain: (b) (4) rabbits

Breeder/supplier: Not reported

Number of animal per sex per group: 13 per sex per group

Age: 10-14 weeks

Body weight range: 2.87 to 3.31 kg for males and 2.89 to 3.36 kg for females

Route and site of administration: Subcutaneous injections

Volume of administration: 0.5 (±10%, or ±0.05) mL/dose

Frequency of administration and study duration: Rabbits were treated at study days 1 and 8 and study duration was 36 days

Dose/animal: 0.5 mL/dose

Means of administration: Subcutaneous (SC)

Report status: Final

Methods:

Study design

Animals were randomized and assigned to 2 different groups. Each group consisted of 13 animals/sex/group. Animals were dosed by subcutaneous route. The details of the study design are listed in the following table:

Table 29: Study design (study #3)

Group	Treatment	Number of animals and their identity					
		Sacrifice (Day 11)		Sacrifice (Day 22)		Sacrifice (Day 36)	
		Male	Female	Male	Female	Male	Female
1	Saline Control	5 (1-5)	5 (27-31)	5 (6-10)	5 (32-36)	3 (11-13)	3 (37-39)
2	IMVAMUNE	5 (14-18)	5 (40-44)	5 (19-23)	5 (45-49)	3 (24-26)	3 (50-52)

Randomization procedure: Yes.

Statistical analysis plan: Yes.

The following parameters were evaluated: Cage side observations (once daily), clinical observations (twice daily), injection site evaluations (daily for three days after each injection and weekly throughout the study), body weights (days -7 and 1 and weekly throughout study), food consumption (week -1 and each week throughout the study), body temperature (not measured), ophthalmoscopy (not tested), clinical chemistry, hematology, and coagulation (day 1 before dosing and on days 2, 3, 4, 11, 22 and 36), serology (before dosing on days 1 and 8 and at termination on days 11, 22, and 36). Gross anatomy at termination (days 11, 22, and 36) and organ weights and histopathology were evaluated/determined on selected tissues.

Table 30: Parameters evaluated (study #3)

Parameters	Frequency of Testing
Cage-side observations	Once daily
Clinical observations	Twice daily
Injection site evaluations	Daily for three days after each injection and weekly throughout the study
Body weight	Days -7 and 1 and weekly throughout study
Food consumption	Week -1 and each week throughout the study
Body temperature	Not measured
Ophthalmoscopy	Not tested
Clinical chemistry*	Day 1 before dosing and on days 2, 3, 4, 11, 22 and 36
Hematology*	Day 1 before dosing and on days 2, 3, 4, 11, 22 and 36
Coagulation*	Day 1 before dosing and on days 2, 3, 4, 11, 22 and 36

Parameters	Frequency of Testing
Serology*	Before dosing on day 1 and day 8 and at termination on days 11, 22, and 36
Necropsy	Days 11, 22, and 36
Tissues for histopathology	Days 11, 22, and 36

* Central auricular artery

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Table 31: Tissues collected at necropsy (study #3)

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	!	
Bone marrow smear		X
Bone marrow (histology and cytology, sternum)		X
Bone (femur with distal metaphysis and epiphysis)		X
Brain	!*	
Cecum	!	
Cervix	!	
Colon		X
Duodenum	!	
Epididymides	!*	
Esophagus	!	
Eyes	!	
Fallopian tubes		X
Femur	!	
Gall bladder	!	
Gut Associated Lymphoid Tissue (GALT) (Peyer's patches)		X
Gross lesions	!	
Harderian gland	!	
Head	!	
Heart	!*	
Ileum	!	
Injection site	!	
Jejunum	!	
Kidneys	!*	
Lachrymal glands	!	
Larynx	!	
Liver	!*	
Lungs with mainstem bronchi	!*	

Organ/Tissue	Collected	Not collected
Lymph nodes (inguinal, axillary, mandibular, and mesenteric)	!	
Mammary gland	!	
Optic nerve	!	
Ovaries	!*	
Pancreas	!	
Parathyroid glands	!*	
Pituitary gland	!*	
Prostate	!*	
Rectum	!	
Salivary glands	!*	
Sciatic nerve	!	
Seminal vesicle	!*	
Skeletal muscle	!	
Skin	!	
Spinal cord	!	
Spleen	!*	
Sternum	!	
Stomach	!	
Testes	!*	
Thymus	!*	
Thyroid	!*	
Tongue	!	
Trachea	!	
Ureters		X
Uterus and cervix	!*	
Urinary bladder	!	
Vagina	!	
Zymbal's gland		X

Those tissues subject to histological processing included the following regions:

Brain - cerebellum, cerebrum, midbrain and medulla

Heart - included auricular and ventricular regions

Ileum - including Peyer's patches

Liver - section from two main lobes

Lungs - section from two major lobes, to include bronchi

Skin - treated (both injection sites) and naïve sites

Spinal cord - transverse and longitudinal section at the cervical, thoracic and lumbar levels

Sternum - including bone marrow

Thyroid - included parathyroids in section where possible

Uterus - uterus section separate from cervix section

For bilateral organs, sections of both organs were prepared. A single section was prepared from each of the remaining tissues required for microscopic pathology.

Results:

Morbidity and mortality:

No test article-related morbidity or mortality was reported.

Clinical Chemistry, hematology, and coagulation:

Table 32: Clinical chemistry results (study #3)

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if Greater than 1.5 so Indicated Otherwise ≤ 1.5)	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, potassium, sodium, phosphorus
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR B) HEPATOBILIARY	Aspartate aminotransferase (AST or SGOT) SD2 F $\downarrow \leq 0.6$ G2 (high SD in G1) SD11 F $\downarrow \leq 0.6$ G2 (high SD in G1) SD22 F $\downarrow \leq 0.6$ G2 SD36 F $\downarrow \leq 0.5$ G2 Alanine aminotransferase (ALT or SGPT) SD36 F $\downarrow \leq 0.6$ G2	
		Total bilirubin Alkaline phosphatase (ALP)
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation) C-reactive protein*
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Cholesterol SD22 F $\uparrow \geq 1.6$ G2 SD36 M $\uparrow \geq 1.7$ G2	Albumin (A) Total protein Carbon dioxide Globulin A/G ratio Lactate dehydrogenase CPK GGT Fasting Triglycerides

* Not measured.

Clinical chemistry results show a decrease in AST levels in group 2 females at study days 2, 11, 22, and 36. ALT levels were decreased in group 2 females at study day 36. Cholesterol levels were increased in group 2 females and males at study days 22 and 36, respectively.

Table 33: Hematology results (study #3)

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if Great or Less than 1.58, ie, ≥ 1.6 or ≤ 1.6)	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	Large unstained cells (LUC) SD-1 M $\uparrow \geq 1.7$ G2 SD4 F $\uparrow \geq 1.8$ G2 SD11 F $\uparrow \geq 2.0$ G2 SD36 F $\uparrow \geq 2.0$ G2 Monocyte count: SD3 M $\uparrow \geq 2.3$ G2 SD36 M $\uparrow \geq 1.7$ G2 SD2 F $\uparrow \geq 1.6$ G2 SD3 F $\uparrow \geq 2.4$ G2 SD36 F $\uparrow \geq 2.3$ G2 Eosinophils count SD2 F $\uparrow \geq 1.6$ G2 SD22 M $\downarrow \leq 0.6$ G2 SD36 M $\uparrow \geq 2.3$ G2 Basophils SD36 M $\downarrow \leq 0.5$ G2	Macrophage Leukocytes Neutrophil White Blood Cells (WBC) Lymphocyte count
Clotting potential		Prothrombin time Activated partial-thromboplastin time clotting time Platelet count Fibrinogen
Others		Bone marrow cytology

Hematology results show an increase in LUC levels in group 2 males at study day -1 and in group 2 females at study days 4, 11, and 36. Monocyte levels were increased in group 2 females at study days 2, 3, and 36. Monocyte levels were increased in group 2 males at study days 3 and 36.

Eosinophil levels were increased in group 2 females at study day 2. Eosinophil levels were decreased in group 2 males at study day 22. Eosinophil levels were increased in group 2 males at study day 36. Basophil levels were decreased in group 2 males at study day 36.

⁸ With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

Systemic toxicity:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), food consumption, gross pathology, or microscopic anatomy were reported.

Organ Weight:

Table 34: Male's organ weights (study #3)

SEX		Males	
GROUPS		1 Day 11/22/36	2 Day 11/22/36
NUMBER OF ANIMALS		5/5/3	5/5/3
BODY WEIGHT (terminal)		3239/3337/3450	3107/3311/3468
BRAIN		10.0/10.1/10.0	10.2/10.8/10.5
ADRENALS		0.211/0.234/0.167	0.170/0.233/0.285
EPIDIDYMIDES		1.48/1.46/1.95	1.55/1.48/1.74
HEART		7.1/7.3/7.5	6.5/7.0/7.7
KIDNEYS		17.2/17.9/18.9	16.4/19.0/17.9
LIVER		75/90/82	72/87/97*
LUNGS		14.1/17.5/15.4	15.0/13.1/13.9
ILLIAC LYMPH NODES	Right	NC	NC
	Left	NC	NC
PROSTATE		0.797/0.683/0.597	0.603/0.503/1.115
Salivary gland (mandibular)		1.25/1.25/1.30	1.34/1.35/1.49
Seminal vesicles		1/1/1	1/1/1
SPLEEN		1.2/1.2/1.0	1.3/1.2/1.1
TESTES		4.2/4.0/5.2	3.5/4.3/5.3
PITUITARY		0.026/0.023/0.026	0.027/0.026/0.026
THYROID and PARATHYROID		0.281/0.252/0.251	0.233/0.251/0.232
THYMUS		4.66/5.89/4.45	4.04/4.78/4.48
OVARIES			
UTERUS			

NC = Not collected. *Significant difference from control, $P \leq 0.05$.

Table of male's organ weight: Absolute weights are expressed as mean (grams). Entries in table are expressed as organ weight from animals taken at study days 11, 22, and 36.

At study day 11, adrenal weight was decreased 19% in group 2 males. At study day 36, adrenal weight was increased 71% in group 2 males. At study day 36, liver weight was increased 18% in group 2 males. At study day 22, lungs weight was decreased 25% in group 2 males. In group 2, prostate weight was decreased 24% and 26% at study days 11 and 22, respectively. Prostate weight was increased 87% at study day 36. Salivary gland weight was increased 15% at study day 36. Testes weight was decreased 17% in group 2 at study day 11. Pituitary weight was increased 13% in group 2 males at study day 22. Thyroid weight was decreased 17% in group 2 males at study day 11. In group 2 males, thymus weight was decreased 13% and 19% at study days 11 and 22, respectively.

Table 35: Female's organ weights (study #3)

SEX		Females	
GROUPS		1 Day 11/22/36	2 Day 11/22/36
NUMBER OF ANIMALS		5/5/3	5/5/3
BODY WEIGHT (terminal)		3275/3435/3728	3272/3452/3580
BRAIN		10.2/10.5/10.8	10.2/10.3/10.2
ADRENALS		0.249/0.237/0.287	0.256/0.282/0.370
EPIDIDYMIDES			
HEART		7.0/7.2/7.2	6.6/6.9/7.0
KIDNEYS		20.2/17.6/18.9	17.2/17.4/17.6
LIVER		83/78/88	78/84/84
LUNGS WITH BRONCHI		11.9/12.9/12.3	12.6/12.7/12.2
ILLIAC LYMPH NODES	Right	NC	NC
	Left	NC	NC
PROSTATE AND SEMINAL VESICLE			
Salivary gland (mandibular)		1.42/1.53/1.52	1.37/1.40/1.57
SPLEEN		1.5/1.3/1.6	1.4/1.4/1.6
TESTES			
PITUITARY		0.032/0.030/0.028	0.029/0.028/0.030
THYROID and PARATHYROID		0.230/0.232/0.222	0.226/0.202/0.279
THYMUS		5.13/6.38/4.66	4.97/5.91/6.00
OVARIES		0.25/0.24/0.29	0.21/0.22/0.25
UTERUS + CERVIX		1.7/2.8/3.6	2.2/2.2/3.8

NC = Not collected.

Table of female's organ weight: Absolute weights are expressed as mean (grams). Entries in table are expressed as organ weight from animals taken at study days 11, 22, and 36.

In group 2 females, adrenal weight was increased 19% and 29% at study days 22 and 36, respectively. kidney weight was decreased 15% in group 2 females at study day 11. In group 2 females, thyroid weight was decreased 13% and increased 26% at study days 22 and 36, respectively. Thymus weight was increased 29% in group 2 females at study day 36. In group 2, ovary weight was decreased 16% and 14% at study days 11 and 36, respectively. In group 2, uterus weight was increased 29% and decreased 21% at study days 11 and 36, respectively.

Injection Site Irritation

Study day 11: At treated site 1, an increased incidence of dark areas was reported ten days after treatment with IMVAMUNE. At treated site 2, a similar response was reported three days after treatment with IMVAMUNE. In one male and one female of group 2, thickened or edematous areas were reported three days after treatment with IMVAMUNE. The details of the injection site findings are listed in the tables below:

Table 36: Injection site irritation in males and females at study day 11 (study #3)
Treated site 1 (day 1 administration)

Group/Sex	1M	2M	1F	2F
Treatment	Control	INVAMUNE	Control	INVAMUNE
Dark area(s)	3	5	1	2
Number of animals examined	5	5	5	5

Treated site 2 (day 8 administration)

Group/Sex	1M	2M	1F	2F
Treatment	Control	INVAMUNE	Control	INVAMUNE
Dark area(s)	2	5	2	4
Thickened / edematous	0	1	0	1
Number of animals examined	5	5	5	5

Study day 22: At treated site 1, increased incidence of dark areas was reported 21 days after treatment with INVAMUNE. At treated site 2, a similar response was reported 14 days after treatment with INVAMUNE. In one male and one female of group 2, thickened or edematous areas were reported 14 days after treatment with INVAMUNE.

Table 37: Injection site irritation in males and females at study day 22 (study #3)
Treated site 1 (day 1 administration)

Group/Sex	1M	2M	1F	2F
Treatment	Control	INVAMUNE	Control	INVAMUNE
Dark area(s)	0	2	1	3
Number of animals examined	5	5	5	5

Treated site 2 (day 8 administration)

Group/Sex	1M	2M	1F	2F
Treatment	Control	INVAMUNE	Control	INVAMUNE
Dark area(s)	1	2	1	3
Thickened / edematous	0	1	0	1
Number of animals examined	5	5	5	5

Study day 36: No test article-related findings were reported at the injection sites on day 36. Dark area(s) were reported at treated sites 1 and 2 were similar in both groups 1 and 2.

Gross pathology:

Macroscopic findings are listed below:

Table 38: Male's and female's macroscopic findings (study #3)

Group	Males		Females	
	1	2	1	2
Animals examined	5/5/3*	5/5/3	5/5/3	5/5/3
Adrenal				
Small	0/0/1	0/0/0	0/0/0	0/0/0
Thyroids				
Cyst	1/1/0	0/0/0	0/0/0	0/0/0
Dark area	0/1/0	0/0/0	0/0/0	0/1/0
Parathyroids				
Cyst	0/0/0	1/0/0	0/0/0	0/0/0
Axillary lymph node				
Congested	0/0/0	1/0/0	0/1/0	3/0/0
Enlarged	0/0/0	1/0/0	0/1/0	0/1/0
Skin				
Pinna (e) bruised	2/1/1	3/0/0	1/2/1	1/0/1
Scab	0/0/0	0/0/0	0/0/0	1/0/0
Cecum				
Nematodes present	2/0/0	0/0/0	0/0/0	0/0/0
Kidneys				
Pale	0/0/0	0/0/0	1/0/0	0/0/0
Vascular prominent	0/1/0	0/0/0	0/1/0	0/0/0
Lung and bronchi				
Dark area	0/0/0	0/0/0	1/0/0	1/0/0
Misshapen	0/0/0	0/1/0	0/0/0	0/0/0
Skeletal muscle				
Dark area	0/0/0	1/0/0	0/0/0	0/0/0
Spleen				
Irregular surface	0/0/0	0/0/1	0/0/0	0/0/0
Misshapen	0/0/0	0/0/0	0/0/1	0/0/0
Swollen	0/0/0	0/0/0	0/0/0	0/0/1
Treated site 1				
Dark area	3/0/0	5/2/1	1/1/3	2/3/2
Treated site 2				
Dark area	2/1/2	5/2/2	2/1/3	4/3/2
Edematous	0/0/0	0/1/0	0/0/0	1/0/0
Thickened	0/0/0	1/0/0	0/0/0	0/1/0
Naïve skin				
Dark area	1/1/1	0/1/1	1/0/0	2/1/1
Scab	0/0/0	0/0/0	0/0/0	1/0/0
Gall bladder				
Distended	0/0/0	0/0/0	0/0/0	1/0/0
Fur stained	3/5/3	5/5/3	4/3/2	2/3/3
Adipose tissue				
Dark	0/0/0	0/0/0	0/0/0	2/0/0
Edematous	0/0/0	0/0/0	0/0/0	2/0/0
Splenulus	0/0/0	0/0/0	1/0/0	0/0/1
Thymus				
Dark area	0/1/0	0/0/0	0/0/0	0/0/0

*Data presented for study days 11/22/36

No test article-related macroscopic findings were reported.

Microscopic findings are listed below:

Table 39: Male's and female's microscopic findings at study day 11 (study #3)

Tissue and Finding	Group/Se x:	1M 5	2M 5	1F 5	2F 5
Lungs + Bronchi	Number Examined:	5	5	5	5
Alveolitis		4	4	2	3
Congestion		0	0	0	1
Perivascular Inflammatory Cells		0	2	0	0
Pancreas	Number Examined:	5	5	4	5
Lymphoid Aggregation(s)		0	1	0	0
LN Mesenteric	Number Examined:	5	5	5	5
Sinus Erythrocytosis/Erythrophagocytosis		1	0	0	0
Liver	Number Examined:	5	5	5	5
Centrilobular Rarefaction		0	1	0	0
Periportal Inflammatory Cell Foci		4	2	4	5
Kidneys	Number Examined:	5	5	5	5
Basophilic Cortical Tubules		1	0	1	3
Cortical Interstitial Inflammatory Cell Infiltrate		1	0	1	0
Dilated Cortical Tubules		0	0	4	0
Medullary Mineralization		1	0	2	1
Thyroids	Number Examined:	5	5	5	5
Cyst(s)		1	0	0	0
Ectopic Thymic Tissue		0	0	0	1
Parathyroids	Number Examined:	5	5	4	5
Cyst(s)		0	1	0	0
Ectopic Thymus		0	2	1	1
LN Mandibular	Number Examined:	5	5	5	5
Dilated/Cystic Sinuses		1	2	0	0
Sinus Erythrocytosis/Erythrophagocytosis		0	0	1	0
LN Inguinal	Number	5	5	5	5
Sinus Erythrocytosis/Erythrophagocytosis		1	1	0	0
LN Axillary	Number Examined:	5	5	4	5
Hemorrhage		0	0	0	2
Paracortex - Increased Cellularity		0	0	0	1
Prominent Germinal Centers		0	2	0	4
Sinus Erythrocytosis/Erythrophagocytosis		2	3	0	3
Mammary	Number	5	5	5	5
Site Only		4	5	3	3
Skeletal Muscle	Number Examined:	5	5	5	5
Hemorrhage (Peripheral to Muscle)		0	1	0	0
Myofiber Degeneration		0	1	0	0
Pituitary	Number Examined:	5	5	5	5
Inflammatory Cell Infiltrate		1	0	0	0

Tissue and Finding		Group/Se x:	1M 5	2M 5	1F 5	2F 5
Testes	Number Examined:		5	5	-	-
Immataturity			5	5	-	-
Epididymides	Number Examined:		5	5	-	-
Underdeveloped			0	1	-	-
Ovaries	Number Examined:		-	-	5	5
Mineralization			-	-	2	4
Harderian Glands	Number Examined:		5	5	5	5
Acinar Atrophy			1	0	0	0
Lymphoid Aggregates			0	0	1	0
Parotid S.G.	Number Examined:		5	5	5	5
Basophilic Hypertrophic Foci			1	0	0	0
Treated Site 1	Number Examined:		5	5	5	5
Dermal Mixed Inflammatory Cell Infiltrate			0	1	0	1
Inflammation/Myofiber Necrosis-Panniculus Muscle			0	1	0	0
Subcutaneous Fibrosis			0	1	0	1
Subcutaneous Hemorrhage			3	2	0	0
Subcutaneous Mixed Inflammatory Cell Infiltrate			1	4	0	4
Tissue and Finding		Group/Sex: Number:	1M 5	2M 5	1F 5	2F 5
Treated Site 2	Number Examined:		5	5	5	5
Dermal Hemorrhage			1	1	0	2
Dermal Mixed Inflammatory Cell Infiltrate			0	4	0	3
Dermal/Subcutaneous Fibrosis+Inflammatory Cells			1	0	0	0
Subcutaneous Hemorrhage			0	3	0	1
Subcutaneous Mixed Inflammatory Cell Infiltrate			0	4	0	3
Skin	Number		2	3	1	0
Congestion			0	0	1	0
Perivascular Hemorrhage			1	3	0	0
Vascular Needle Tract Damage			0	1	0	0
Adipose Tissue	Number Examined:		0	0	1	2
Accessory Splenic Tissue			0	0	1	0
Hemorrhage			0	0	0	2
Inflammatory Cell Infiltrate			0	0	0	2
Naïve skin	Number Examined:		5	5	5	5
Dermal Hemorrhage			0	0	0	2
Dermal Mixed Inflammatory Cell Infiltrate			0	0	0	2
Epidermal Hyperplasia			0	0	0	1
Scab(s)			1	0	0	1
Subcutaneous Mixed Inflammatory Cell Infiltrate			0	0	0	2

Table 40: Male's and female's microscopic findings at study day 22 (study #3)

Tissue and Finding	Group/Se x:	1M 5	2M 5	1F 5	2F 5
Heart	Number Examined:	5	5	5	5
Endocarditis		0	0	0	1
Myocardial Inflammatory Cell Infiltrate		0	2	0	1
Lungs + Bronchi	Number Examined:	5	5	5	5
Aggregations of Alveolar Macrophages		0	1	0	0
Alveolitis		2	2	0	1
Congestion		2	0	0	0
Granuloma(ta)		0	1	0	0
Perivascular Inflammatory Cells		0	0	1	1
Vascular Endothelial Inflammatory Cell Infiltrate		0	0	1	0
Pancreas	Number Examined:	5	5	5	5
Ectopic Spleen		0	1	0	0
LN Mesenteric	Number Examined:	5	5	5	5
Sinus Erythrocytosis/Erythrophagocytosis		1	0	0	0
Liver	Number Examined:	5	5	5	5
Periportal Inflammatory Cell Foci		1	2	4	2

Tissue and Finding	Group/Se x:	1M 5	2M 5	1F 5	2F 5
Kidneys	Number Examined:	5	5	5	5
Basophilic Cortical Tubules		3	3	2	3
Cortical Interstitial Inflammatory Cell Infiltrate		0	2	1	1
Dilated Cortical Tubules		3	2	3	4
Focal Cortical Scarring		0	0	0	1
Medullary Mineralization		3	2	0	1
Peripelvic Lymphoid Aggregate		1	0	0	0
Thyroids	Number Examined:	5	5	5	5
Cyst(s)		1	1	1	0
Parathyroids	Number Examined:	5	5	5	5
Ectopic Thymus		0	1	0	1
LN Mandibular	Number Examined:	5	5	5	5
Sinus Erythrocytosis/Erythrophagocytosis		2	1	1	2
LN Inguinal	Number	5	5	5	5
Sinus Erythrocytosis/Erythrophagocytosis		1	0	0	0
LN Axillary	Number Examined:	5	5	5	5
Paracortex - Increased Cellularity		0	1	0	0
Prominent Germinal Centers		0	4	0	4
Sinus Erythrocytosis/Erythrophagocytosis		1	1	2	3
Caecum	Number Examined:	5	5	5	5
Submucosal Inflammatory Cell Infiltrate		0	0	0	1

Tissue and Finding	Group/Sex:	1M 5	2M 5	1F 5	2F 5
Mammary Site Only	Number	5 1	5 2	5 0	5 0
Femur inc. Joint Connective Tissue Mineralization	Number Examined:	5 0	5 0	5 0	5 1
Testes Immaturity	Number Examined:	5 2	5 2	- -	- -
Ovaries Mineralization	Number Examined:	- -	- -	5 3	5 2
Parotid S.G. Lymphoid Cell Foci	Number Examined:	5 0	5 0	5 0	5 1
Treated Site 1 Subcutaneous Fibrosis Subcutaneous Hemorrhage Subcutaneous Mixed Inflammatory Cell Infiltrate	Number Examined:	5 0 0 0	5 1 2 1	5 0 0 0	5 0 4 2
Treated Site 2 Dermal Mixed Inflammatory Cell Infiltrate Epidermal Hyperplasia Scab(s) Subcutaneous Hemorrhage Subcutaneous Mixed Inflammatory Cell Infiltrate	Number Examined:	5 0 0 1 0 0	5 1 0 0 2 1	5 0 1 1 0 0	5 0 1 0 3 3
Skin Perivascular Hemorrhage Vascular Intimal Proliferation Vascular Needle Tract Damage	Number	1 0 0 0	0 0 0 0	2 2 1 1	0 0 0 0
Adipose Tissue Accessory Splenic Tissue	Number Examined:	1 1	0 0	0 0	0 0
Naïve skin Epidermal Hyperplasia Subcutaneous Hemorrhage	Number Examined:	5 1 0	5 0 0	5 0 0	5 0 1

Table 41: Male's and female's microscopic findings at study day 36 (study #3)

Tissue and Finding	Group/Sex :	1M 3	2M 3	1F 3	2F 3
Heart Adipose Tissue-Inflammatory Cell Infiltrate Myocardial Inflammatory Cell Infiltrate	Number Examined:	3 1 0	3 0 0	3 0 0	3 0 1
Lungs + Bronchi Alveolitis Congestion	Number Examined:	3 1 1	3 2 0	3 1 0	3 2 0
Pancreas Lymphoid Aggregation(s)	Number Examined:	3 0	3 0	3 0	3 1

Tissue and Finding		Group/Sex	1M	2M	1F	2F
		:	3	3	3	3
LN Mesenteric	Number Examined:		3	3	3	3
Increased Pigmented Macrophages			0	1	0	0
Liver	Number Examined:		3	3	3	3
Periportal Inflammation/Fibrosis			0	0	0	1
Periportal Inflammatory Cell Foci			1	1	1	1
Tissue and Finding		Group/Se	1M	2M	1F	2F
		x:	3	3	3	3
Kidneys	Number Examined:		3	3	3	3
Basophilic Cortical Tubules			1	1	3	3
Cortical Interstitial Inflammatory Cell Infiltrate			1	1	0	1
Cortical Mineralization			1	1	0	0
Dilated Cortical Tubules			1	1	2	2
Medullary Mineralization			1	0	2	3
Thyroids	Number Examined:		3	3	3	3
Cyst(s)			0	1	0	0
Ectopic Thymic Tissue			1	0	0	0
Parathyroids	Number Examined:		3	3	3	3
Ectopic Thymus			0	0	1	1
Adrenals	Number Examined:		3	3	3	3
Inflammatory Cell Infiltrate			0	1	0	0
Tissue and Finding		Group/Sex	1M	2M	1F	2F
		:	3	3	3	3
LN Mandibular	Number Examined:		3	3	3	3
Dilated/Cystic Sinuses			0	0	1	0
Sinus Erythrocytosis/Erythrophagocytosis			0	0	2	0
LN Axillary	Number Examined:		3	3	3	3
Prominent Germinal Centers			0	3	0	3
Sinus Erythrocytosis/Erythrophagocytosis			0	0	0	2
Ileum	Number		3	3	3	3
Inflammatory Cell Infiltrate			1	0	0	0
Caecum	Number Examined:		3	3	3	3
Submucosal Inflammatory Cell Infiltrate			0	0	1	0
Colon	Number Examined:		3	3	3	3
Submucosal Inflammatory Cell Infiltrate			0	0	1	0
Mammary	Number		3	3	3	3
Dermal Inflammatory Cell Infiltrate			0	1	0	0
Site Only			3	3	1	0
Eyes	Number		3	3	3	3
Cornea-Inflammatory Cell Infiltrate			0	0	0	1
Ovaries	Number Examined:		-	-	3	3
Mineralization			-	-	3	2
Harderian Glands	Number Examined:		3	3	3	3

Lymphoid Aggregates		1	0	0	0
Treated Site 1	Number Examined:	3	3	3	3
Subcutaneous Hemorrhage		0	0	0	2
Subcutaneous Mixed Inflammatory Cell Infiltrate		0	0	0	1
Treated Site 2	Number Examined:	3	3	3	3
Dermal Hemorrhage		0	0	0	1
Dermal Mixed Inflammatory Cell Infiltrate		0	1	0	1
Epidermal/Dermal Inflammatory Cell Focus		0	1	0	0
Parakeratosis		0	1	0	0
Subcutaneous Hemorrhage		0	2	0	2
Subcutaneous Mixed Inflammatory Cell Infiltrate		0	2	0	0
Skin	Number Examined:	1	0	1	1
Perivascular Hemorrhage		1	0	1	1
Adipose Tissue	Number Examined:	0	0	0	1
Accessory Splenic Tissue		0	0	0	1
Naïve skin	Number Examined:	3	3	1	3
Dermal Mixed Inflammatory Cell Infiltrate		0	1	0	0
Subcutaneous Hemorrhage		1	0	0	0
Subcutaneous Mixed Inflammatory Cell Infiltrate		0	0	0	1

An extensive number of tissues were examined for histology. Other than the axillary lymph node and injection site findings, no test article-related microscopic findings were reported. The following findings were reported at days 11, 22, and 36:

Prominent germinal centers in the axillary lymph nodes were reported in group 2. The axillary lymph nodes drain the sites treated with IMVAMUNE, therefore, this finding might be related to the immune response to the test article.

At the injection site number 1 of group 2, dermal and subcutaneous mixed inflammatory cell infiltrate, subcutaneous hemorrhage, subcutaneous fibrosis and inflammation with myofiber necrosis of the panniculus muscle were reported.

At the injection site number 2 of group 2, dermal and subcutaneous mixed inflammatory cell infiltrate were reported. The incidence of dermal hemorrhage was increased in group 2 females when compared to controls.

At the Naïve skin, dermal and subcutaneous mixed inflammatory cell infiltrate and subcutaneous hemorrhage were reported in group 2 females. These findings were considered to be the result of spread along the tissue plane from the treated sites.

The findings at injection site 1, injection site 2, and the naïve skin might be associated with the subcutaneous administration of the vaccine and were not considered adverse.

Body temperature:
Not measured

Serology:

An ELISA was performed detecting vaccinia-specific IgG bound to antigen by a HRP-conjugated secondary antibody against rabbit IgG. The amount of HRP-antibody is read out by a substrate reaction as OD values.

No vaccinia-specific antibody responses were reported in the control group. Both male and female rabbits immunized with IMVAMUNE® showed a vaccinia-specific antibody response. No titers were reported after the first administration of IMVAMUNE®. Ten days after the first administration (three days after the second immunization, day 11) all males and four out of five females had seroconverted. Increased titers in females were reported at the last two-time points (day 22 and day 36). Male rabbits showed only a slight trend to higher titers. Vaccinia-specific IgG in rabbits by sex are listed in the figure and the table below:

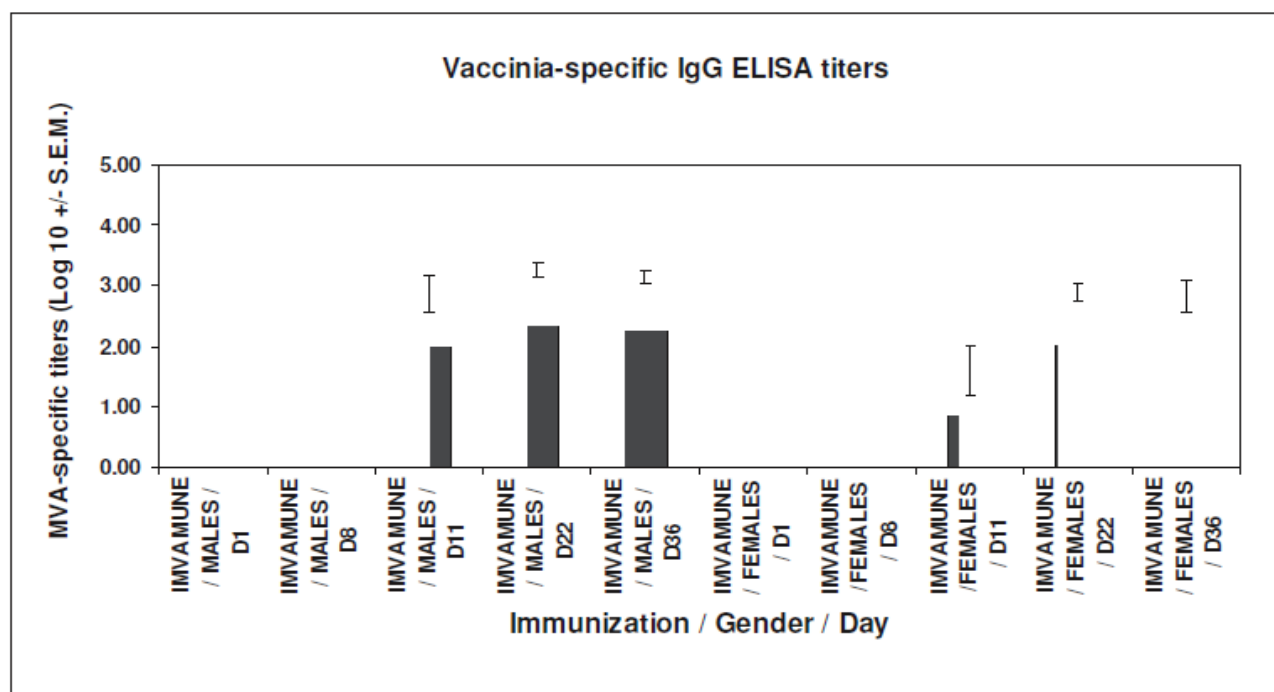


Figure 1: Mean vaccinia-specific IgG titer (Log 10) of group 2, bled on (pre-bleed) day 1, day 8, day 11, day 22, and day 36 after the second administration of MVA-BN®. Mean values are from 13 (days 1 and 8), 5 (days 11 and 22) or 3 (day 36) animals each. Error bars depict +/- SEM.

Table 42: Average vaccinia specific IgG Log₁₀ titer of analyzed sera from MVA-BN® immunized rabbits [group 2] (study #3)

Immunization / Gender / Day	Average Log Titer	S.E.M.	N
IMVAMUNE / MALES / D1	0.00	0.00	13
IMVAMUNE / MALES / D8	0.00	0.00	13
IMVAMUNE / MALES / D11	2.86	0.30	5
IMVAMUNE / MALES / D22	3.25	0.11	5
IMVAMUNE / MALES / D36	3.15	0.10	3
IMVAMUNE / FEMALES / D1	0.00	0.00	13

Immunization / Gender / Day	Average Log Titer	S.E.M.	N
IMVAMUNE / FEMALES / D8	0.00	0.00	13
IMVAMUNE / FEMALES / D11	1.60	0.41	5
IMVAMUNE / FEMALES / D22	2.91	0.14	5
IMVAMUNE / FEMALES / D36	2.83	0.27	3

Test article-related effects:

Table 43: Test article-effects (study #3)

Test article related effects	Effects considered incidental
↓ AST ↑ LUC ↑ Monocyte ↑ Adrenal weight ↓ Prostate weight ↓ Thyroid weight ↑ Uterus weight Injection site findings Prominent germinal centers in axillary lymph nodes Immune responses	

Assessment:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), food consumption, gross pathology, or microscopic anatomy were reported.

The hepatocellular leakage enzyme (AST) are useful in detecting injury to liver parenchymal cells. Generally, increased serum activity represents enzyme leakage from cells through damaged cell membranes. AST is useful as an indicator of liver and/or muscle injury in large and small animals.

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

Monocytosis could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair.

Adrenal glands are responsible for releasing hormones in response to stress through the synthesis of corticosteroids such as cortisol and catecholamines such as adrenaline (epinephrine) and noradrenaline. They also produce androgens in their innermost cortical layer. The adrenal glands affect kidney function through the secretion of aldosterone.

The prostate is a walnut-sized gland located between the bladder and the penis. The prostate secretes fluid that nourishes and protects sperm. The milky fluid produced by the prostate (prostatic fluid) makes up around 30 percent of the total fluid ejaculated (the rest is sperm and fluid from the

seminal vesicles). Prostatic fluid contains a number of ingredients, including enzymes, zinc, and citric acid. One of the enzymes in prostatic fluid is prostate-specific antigen (PSA). After ejaculation, PSA makes thickened semen runnier, helping sperm travel through it more easily, increasing their likelihood of successfully fertilizing an egg.⁹

The thyroid gland controls how quickly the body makes proteins and uses energy. And, controls how sensitive the body is to other hormones. It produces the thyroid hormones [triiodothyronine (T₃) and thyroxine (sometimes referred to as tetraiodothyronine (T₄)]. These hormones regulate the growth and rate of function of many other systems in the body. T₃ and T₄ are synthesized from iodine and tyrosine. The thyroid also produces calcitonin, which plays a role in calcium homeostasis. Hormonal output from the thyroid is regulated by thyroid-stimulating hormone (TSH) produced by the anterior pituitary. TSH is regulated by thyrotropin-releasing hormone (TRH) produced by the hypothalamus.

The uterus is a major female hormone-responsive secondary sex organ of the reproductive system in humans and most other mammals. During gestation, the fetus develops in the uterus. In the human embryo, the uterus develops from the paramesonephric ducts which fuse into the single organ known as a simplex uterus. The uterus has different forms in many other animals and in some it exists as two separate uteri known as a duplex uterus.¹⁰

The axillary lymph nodes drain the sites treated with IMVAMUNE, therefore, this finding might be related to the immune response to the test article.

Injection site findings and immune responses due to test article treatment were reported.

Based on the overall findings in this study, it can be concluded that in rabbits, administration of IMVAMUNE vaccine subcutaneously had no adverse effects in terms of systemic toxicity.

GLP study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

Conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues were reported in this study.

Internal communication:

CRP levels were not measured in this study. CRP are important in determining the cause of inflammation, if any, caused by the test article.

Study # 4: (b) (4) and MVA-BN®: A 3 week repeat dose subcutaneous administration toxicity and local tolerance study in the adult rat followed by a 2 day and a 28-day treatment-free period (study number 2699/011).

Performing laboratory: (b) (4)

⁹ https://www.malecontraceptive.org/2018/09/14/what-is-the-prostate/?gclid=EAlaIQobChMIiKi-6NDy3wIVEZ-fCh0KVwqcEAAYAAAEgIeM_D_BwE

¹⁰ <https://en.wikipedia.org/wiki/Uterus>

Initiation date: April 8, 2008

Final report date: September 7, 2009

Batch/lot number of test article:

Table 44: Batch/lot number of test article (study #4)

Test article	(b) (4) lot number	Batch number	Quantity supplied	Expiry Date	Date of receipt at (b) (4)
(b) (4)	1	(b) (4)	222 vials	28 February 2010	27 March 2008
MVA-BN® (IMVAMUNE®)	1	0061205	200 vials	#2 years post filling	28 February 2008

Animal species and strain: (b) (4) rats

Breeder/supplier: (b) (4),

Number of animal per sex per group: 10 per sex per group

Age: 9 weeks

Body weight range: 276.6 to 369.6 g for males and 196.4 to 235.8 g for females

Route and site of administration: Subcutaneous injections in the left and right scapular region

Volume of administration: 1 mL per animal (0.5 mL to each of the two sites) for the control (group 1) and high dose (groups 3 and 4), and 0.2 mL per animal (single injection site) for the low dose (group 2) groups.

Frequency of administration and study duration: Rats were treated at study days 1, 8, 15, and 22 and the study duration was 50 days.

Dose/animal: See study design

The high dose level of (b) (4) (i.e. a dose of (b) (4) TCID₅₀) is the highest dose intended for use in the clinic, although, on a body weight basis, it represents a large multiple of the human dose. The low dose level of (b) (4) (i.e. a dose of (b) (4) TCID₅₀) was included as a potential clinical dose in case adverse effects were determined in this study with the high dose of (b) (4). The high dose level of MVA-BN® (i.e. a nominal dose of (b) (4) TCID₅₀) was comparable to the high dose level of (b) (4).

Stability: A provisional expiry date for the MVA-BN and MVA-mBN157B mixtures has been set for 2 years post filling.

Means of administration: Subcutaneous (SC)

Report status: Final

Methods:

Study design

Animals were randomized and assigned to 4 different groups. Each group consisted of 10 animals/sex/group. Animals were dosed by subcutaneous route on study days 1, 8, 15, and 22. The details of the study design are listed in the following table:

Table 45: Study design (study #4)

Group Number	Group Description	Dose level ¹ (TCID ₅₀) per animal	Number of animals in group			
			2 day treatment-free		28 day treatment-free	
			Male	Female	Male	Female
1	Reference control (TBS)	n.a.	5	5	5	5
2	(b) (4)	(b) (4)	5	5	5	5
3			5	5	5	5
4	MVA-BN [®] (High)		5	5	5	5

¹ based on the release titres; MVA-BN (b) (4) TCID₅₀ and (b) (4) TCID₅₀

Randomization procedure: Yes.

Statistical analysis plan: Yes.

The following parameters were evaluated: Cage side observations (twice daily), clinical observations (weekly intervals), injection site evaluations (on dosing days 1, 8, 15 and 22 observations were made and recorded pre-dose and at 4 hours post-dose), body weights (day -7, before treatment on the first day of dosing, at weekly intervals and before necropsy), food consumption (weekly), body temperature (not measured), ophthalmoscopy (days -6, 23, and 46), clinical chemistry, hematology, and coagulation (days 24 and 50), serology (days -1, 24, and 50). Gross anatomy at termination (days 24 and 50) and organ weights and histopathology were evaluated/determined on selected tissues.

Table 46: Parameters evaluated (study # 4)

Parameters	Frequency of Testing
Cage-side observations	Twice daily
Clinical observations	Weekly intervals
Injection site evaluations	On dosing days 1, 8, 15 and 22 observations were made and recorded pre-dose and at 4 hours post-dose
Body weight	Day -7, before treatment on the first day of dosing, at weekly intervals, and before necropsy
Food consumption	Weekly
Body temperature	Not measured
Ophthalmoscopy	Days -6, 23, and 46
Clinical chemistry*	Days 24 and 50
Hematology*	Days 24 and 50
Coagulation*	Days 24 and 50
Urinalysis	Days 23 and 49
Serology*	Days -1, 24, and 50
Necropsy	Days 24 and 50
Tissues for histopathology	Days 24 and 50

* Lateral caudal vein

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Table 47: Tissues collected at necropsy (study #4)

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	!	
Bone marrow smear (femur) (a) (c)	!	
Brain	!*	
Cecum	!	
Colon	!	
Duodenum	!	
Epididymides	!*	
Esophagus	!	
Eyes (b)	!	
Fallopian tubes		X
Femur with bone marrow and stifle joint	!	
Gall bladder		X
Gut Associated Lymphoid Tissue (GALT) (Peyer's patches)		X
Gross lesions	!	
Harderian gland (d)	!	
Head	!	
Heart	!*	
Ileum	!	
Injection site	!	
Jejunum	!	
Kidneys	!*	
Lachrymal glands (d)	!	
Larynx	!	
Liver	!*	
Lungs with mainstem bronchi	!	
Lymph nodes (mandibular, mesenteric, and popliteal)	!*	
Mammary gland	!	
Muscle	!	
Nasal cavity	!	
Nasopharynx/nares	!	
Optic nerve	!	
Ovaries	!*	

Organ/Tissue	Collected	Not collected
Oviducts	!	
Pancreas	!	
Peyer's patch	!	
Pituitary gland	!*	
Prostate	!*	
Rectum	!	
Salivary glands (mandibular, sublingual, and parotid)	!	
Sciatic nerve	!	
Seminal vesicles	!	
Skeletal muscle		X
Skin	!	
Spinal cord (cervical, lumbar, and thoracic)	!	
Spleen	!*	
Sternum with bone marrow	!	
Stomach	!	
Testes (e)	!*	
Thymus	!*	
Thyroid and parathyroids	!*	
Tongue	!	
Trachea	!	
Ureters	!	
Uterus and cervix	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland (d)	!	

Fixative = 10% neutral buffered formalin except where indicated by: a- methanol, b- Davidson's fluid e – testes and epididymides into Bouin's fixative and processed to block stage

c- See clinical pathology section

d- Preserved with the head in situ

Bone designated for histopathological examination was decalcified using Kristenson's fluid.

Results:

Morbidity and mortality:

No test article-related morbidity or mortality was reported.

Clinical Chemistry, hematology, and coagulation:

Table 48: Clinical chemistry results (study #4)

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G) , DIRECTION, FOLD CHANGE if Greater than 1.5 so Indicated Otherwise ≤ 1.5)	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, potassium, sodium, phosphorus
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Aspartate aminotransferase (AST or SGOT) Alanine aminotransferase (ALT or SGPT)
B) HEPATOBILIARY		Total bilirubin Alkaline phosphatase (ALP)
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation)
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin (A) Total protein Carbon dioxide Globulin A/G ratio Lactate dehydrogenase CPK GGT Fasting Triglycerides Cholesterol

Potassium levels were decreased ($P < 0.05$) in group 3 males at study day 24. Inorganic phosphate levels were decreased ($P < 0.05$) in group 3 at study day 24. An increase in inorganic phosphate ($P < 0.05$) and decreases in albumin ($P < 0.01$) and AG ratio ($P < 0.01$) were reported in group 3 females at study day 50. An increase in creatinine ($P < 0.001$) levels were reported in group 2 females at study day 50. A decrease in AG ratio ($P < 0.05$) were reported in group 4 females at study day 50. These changes were minor and within the historical background values.

Table 49: Hematology results (study #4)

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if Great or Less than 1.511, ie, ≥ 1.6 or ≤ 1.6)	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	Monocyte count: SD24 F $\downarrow \leq 0.5$ G3 SD50 F $\downarrow \leq 0.5$ G2 SD50 F $\downarrow \leq 0.5$ G4 Eosinophils count SD24 M $\uparrow \geq 2.0$ G3 SD24 M $\uparrow \geq 4.0$ G4 SD50 F $\downarrow \leq 0.5$ G2 SD50 F $\downarrow \leq 0.5$ G4 Neutrophil SD24 F $\downarrow \leq 0.6$ G4 SD50 M $\uparrow \geq 1.6$ G4 White Blood Cells (WBC) SD50 F $\downarrow \leq 0.3$ G4	Macrophage Leukocytes White Blood Cells (WBC) Lymphocyte count Large unstained cells (LUC) Basophils
Clotting potential		Prothrombin time Activated partial-thromboplastin time clotting time Platelet count Fibrinogen
Others		Bone marrow cytology

In females, a decrease in monocyte levels were reported in group 3 at study day 24 and in groups 2 and 4 at study day 50. Eosinophil levels were increased in groups 3 and 4 males at study day 24. Eosinophil levels were decreased in groups 2 and 4 females at study day 50. Neutrophil levels were decreased in group 4 females at study day 24. Neutrophil levels were increased in group 4 males at study day 50. WBC levels were decreased in group 4 females at study day 50.

Systemic toxicity:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), food consumption, ophthalmology, gross pathology, or microscopic anatomy were reported.

¹¹ With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

Organ Weight:

Table 50: Male's organ weights (study #4)

SEX	Males			
GROUPS	1 Day 24/50	2 Day 24/50	3 Day 24/50	4 Day 24/50
NUMBER OF ANIMALS	5/5	5/5	5/5	5/5
BODY WEIGHT (terminal)	426/508	442/466*	438/484	443/478
BRAIN	1.977/2.137	2.101/2.165	2.022/2.130	2.008/2.143
ADRENALS	0.069/0.070	0.066/0.058	0.063/0.066	0.065/0.069
HEART	1.335/1.458	1.321/1.486	1.286/1.479	1.606/1.491
KIDNEYS	2.680/2.687	2.456/2.782	2.497/2.745	2.465/2.643
LIVER	11.96/12.18	11.16*/12.51	11.18*/11.60	11.08*/12.12
LUNGS	NC	NC	NC	NC
MANDIBULAR LYMPH NODE	0.337/0.446	0.331/0.449	0.272/0.371	0.337/0.486
MESENTERIC LYMPH NODE	0.340/0.369	0.298/0.454	0.351/0.402	0.374/0.410
POPLITEAL LYMPH NODE	0.029/0.018	0.025/0.026	0.023/0.025	0.037/0.028
PROSTATE	1.129/1.395	0.933/1.286	0.963/1.185	1.083/1.146
SALIVARY GLAND	NC	NC	NC	NC
SEMINAL VESICLES	NC	NC	NC	NC
SPLEEN	0.787/0.862	0.684/0.857	0.752/0.817	0.803/0.759
TESTES	4.693/5.233	4.867/5.527	4.658/5.022	4.673/5.137
PITUITARY	0.012/0.013	0.012/0.014	0.012/0.013	0.013/0.013
THYROID and PARATHYROID	0.022/0.021	0.017/0.021	0.018/0.024	0.020/0.020
THYMUS	0.418/0.338	0.498/0.415	0.494/0.369	0.446/0.425
OVARIES				
UTERUS				

NC = Not collected. * $P < 0.05$

Table of male's organ weight: Absolute weights are expressed as mean (grams). Entries in table are expressed as organ weight from animals taken at study days 24/50.

At study day 50, adrenal weight was decreased 17% in group 2 males. At study day 24, heart weight was increased 20% in group 4 males. At study day 24, mandibular lymph node weight was decreased 19% in group 3 males. At study day 50, mandibular lymph node weight was decreased 17% in group 3 males. At study day 24, mesenteric lymph node weight was decreased 12% in group 2 males. At study day 50, mesenteric lymph node weight was increased 23% and 11% in groups 2 and 4 males, respectively. At study day 24, mesenteric lymph node weight was decreased 12% in group 2 males. At study day 24, popliteal lymph node weight was decreased 14% and 21% in groups 2 and 3 males, respectively. At study day 24, popliteal lymph node weight was increased 28% in group 4 males. At study day 50, popliteal lymph node weight was increased 44%, 39%, and 56% in groups 2, 3, and 4 males, respectively. At study day 24, prostate weight was decreased 17% and 15% in groups 2 and 3, respectively. At study day 50, prostate weight was decreased 15% and 18% in groups 3 and 4, respectively. Spleen weight was decreased 13% in group 2 at study day 24. Spleen weight was decreased 12% in group 4 at study day 50. At study day 24, thyroid weight was decreased 23% and

18% in groups 2 and 3 males, respectively. Thyroid weight was increased 14% in group 3 males at study day 50. At study day 24, thymus weight was increased 19% and 18% in groups 2 and 3, respectively. At study day 50, thymus weight was increased 23% and 26% in groups 2 and 4, respectively.

Table 51: Female's organ weights (study #4)

SEX	Females			
GROUPS	1 Day 24/50	2 Day 24/50	3 Day 24/50	4 Day 24/50
NUMBER OF ANIMALS	5/5	5/5	5/5	5/5
BODY WEIGHT (terminal)	261/256	254/267	261/258	236/271
BRAIN	1.866/1.859	1.889/1.944	1.872/1.882	1.841/1.971
ADRENALS	0.075/0.059	0.067/0.063	0.077/0.065	0.069/0.074
EPIDIDYMIDES				
HEART	0.907/0.884	0.903/0.880	0.874/0.884	0.959/0.863
KIDNEYS	1.478/1.500	1.465/1.610	1.513/1.558	1.490/1.551
LIVER	6.773/6.706	6.917/6.955	7.349/6.844	7.154/7.009
LUNGS	NC	NC	NC	NC
MANDIBULAR LYMPH NODE	0.244/0.263	0.219/0.224	0.223/0.294	0.212/0.228
MESENTERIC LYMPH NODE	0.276/0.314	0.319/0.285	0.305/0.316	0.297/0.274
POPLITEAL LYMPH NODE	0.023/0.018	0.028/0.023	0.025/0.022	0.032/0.022
PROSTATE AND SEMINAL VESICLE				
SALIVARY GLAND	NC	NC	NC	NC
SPLEEN	0.521/0.485	0.504/0.490	0.595/0.474	0.452/0.530
TESTES				
PITUITARY	0.017/0.014	0.015/0.019	0.015/0.016	0.017/0.020*
THYROID and PARATHYROID	0.020/0.016	0.014/0.014	0.015/0.021	0.015/0.018
THYMUS	0.408/0.297	0.367/0.271	0.318/0.334	0.395/0.318
OVARIES	0.082/0.087	0.079/0.077	0.098/0.088	0.086/0.087
UTERUS + CERVIX	NC	NC	NC	NC

NC = Not collected. * $P < 0.05$

Table of female's organ weight: Absolute weights are expressed as mean (grams). Entries in table are expressed as organ weight from animals taken at study days 24/50.

In group 4 females, adrenal weight was increased 25% at study day 50. Mandibular lymph node weight was decreased 13% in group 4 females at study day 24. At study day 50, mandibular lymph node weight was decreased 15% and 13% in groups 2 and 4 females, respectively. Mandibular lymph node weight was increased 12% in group 3 females at study day 50. At study day 24, mesenteric lymph node weight was increased 16% and 11% in groups 2 and 3 females, respectively. Mesenteric lymph node weight was decreased 13% in group 4 females at study day 50. At study day 24, popliteal lymph node weight was increased 22% and 39% in groups 2 and 4 females, respectively. At study day 50, popliteal lymph node weight was increased 28%, 22%, and 22% in groups 2, 3, and 4 females, respectively. At study day 24, spleen weight was increased 14% and decreased 13% in

groups 3 and 4 females, respectively. At study day 24, pituitary weight was decreased 12% in groups 2 and 3 females. At study day 50, pituitary weight was increased 36%, 14%, and 43% in groups 2, 3, and 4 females, respectively. At study day 24, thyroid weight was decreased 30%, 25%, and 25% in groups 2, 3, and 4 females, respectively. At study day 50, thyroid weight was decreased 12% in group 2 females. At study day 50, thyroid weight was increased 31% and 13% in groups 3 and 4 females, respectively. At study day 24, thymus weight was decreased 22% in group 3 females. At study day 50, thymus weight was decreased 12% in group 3 females. At study day 24, ovary weight was increased 20% in group 3 females.

Injection site irritation:

Skin reactions at the injection sites included very slight erythema, scabbing, desquamation, and slight or very slight edema. All the signs were resolved during the 28-day treatment-free period (days 22 to Day 50). Animals in group 2 (3/10 males and 2/10 females) or group 3 (1/10 females) showed edema on some occasions between days 16-18.

Animals in group 4 showed a slight increase in the incidence of skin reactions in comparison to control group. However, the incidence of skin reactions in groups 2 and 3 animals was generally similar to control group.

Gross pathology:

Macroscopic findings are listed below:

Table 52: Male's and female's macroscopic findings at day 24 (study #4)

Group	Males				Females			
	1	2	3	4	1	2	3	4
Animals examined	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Skin and subcutis								
Sore	1	0	0	0	0	0	0	0
Muscle								
Red area	0	0	0	0	0	0	1	0
Liver								
Mottled	2	3	3	3	2	0	1	1
Large	1	0	0	0	0	0	0	0
Pale	0	2	2	1	2	0	1	1
Pancreas								
Small	1	0	0	0	0	0	0	0
Kidney								
Depressed area	1	0	1	0	0	0	0	0
Pelvic dilatation	0	1	0	0	0	0	0	0
Depressed focus	0	0	1	0	0	0	0	0
cyst	0	0	0	0	0	0	1	0
Epididymis								
Raised area	0	0	1	0				
Uterus								
Distension					0	0	0	1
Mandibular lymph node								
Large	2	2	0	2	2	1	1	1
Red focus	2	1	0	2	3	1	0	0
Red	0	0	0	0	0	0	1	0
Lung								
Dark focus	1	0	1	0	0	0	0	0

Group	Males				Females			
	1	2	3	4	1	2	3	4
Animals examined	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Red focus	0	0	0	1	0	0	0	0
Thyroid								
Pale	0	0	0	0	0	1	0	0
Small	0	0	0	0	0	0	0	1
Large	0	0	0	0	1	0	0	0
Pituitary								
Large	0	0	0	0	1	1	0	1
Left scapular								
Red focus	0	1	1	0	0	0	0	0
Red	0	0	1	0	0	0	0	0
Sore	0	0	0	0	0	0	1	0
Left scapular								
Red focus	0	0	1	0	0	0	0	0
Red	0	0	1	0	0	0	0	0
Red area	0	0	1	0	0	0	0	0
mass	0	0	0	0	0	0	1	0
Popliteal lymph node								
Large	1	1	0	0	0	1	0	0
Red focus	0	1	0	0	0	0	0	0
Small	0	1	0	0	0	0	0	0
Red	0	0	0	0	0	1	0	0

No test article-related macroscopic findings were reported in males.

Table 53: Male's and female's macroscopic findings at day 50 (study #4)

Group	Males				Females			
	1	2	3	4	1	2	3	4
Animals examined	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Skin and subcutis								
Mass 1	1	0	0	0	0	0	0	0
Fur loss	0	0	0	0	0	1	0	0
Liver								
Mottled	0	1	1	1	1	2	0	0
Dark	0	0	0	0	1	0	0	0
Spleen								
Large	1	0	0	0	0	0	0	0
Small	0	0	0	0	0	0	1	0
Stomach								
Pale	0	1	0	0	0	0	1	0
Yellow	0	0	0	0	1	0	0	0
Adrenal								
Large	0	0	0	1	0	0	0	0
Kidney								
Pelvic dilatation	0	0	0	0	0	1	0	1
Depressed focus	0	0	1	0	0	0	0	0
Dark	0	0	0	0	1	0	0	0
Testes								
Dark	1	0	0	0				
Ovary								
Red					0	0	0	1
Uterus								

Group	Males				Females			
	1	2	3	4	1	2	3	4
Animals examined	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Distension					0	0	2	1
Salivary gland								
Red focus	0	0	0	1	0	0	0	0
Mandibular lymph node								
Large	3	1	1	4	0	1	2	0
Red focus	1	0	0	0	1	1	1	0
Red	1	0	0	1	0	0	1	0
Thymus								
Red focus	0	0	3	1	1	0	2	0
Large	0	0	0	1	0	0	0	0
Lungs								
Dark focus	1	0	0	0	0	0	0	0
Red focus	0	1	0	0	0	0	0	0
Pales focus	0	0	0	0	0	1	0	0
Pituitary								
Large	0	0	0	0	0	4	1	4
Popliteal lymph node								
Large	0	1	1	3	0	0	0	0
Small	0	0	0	0	0	0	0	1
Red	0	0	0	1	0	0	0	0
Abdominal cavity								
Mass multiple	1	0	0	0	0	0	0	0
Oral cavity								
Abnormal teeth	0	0	0	0	0	1	0	0

No test article-related macroscopic findings were reported in females.

Microscopic findings are listed below:

Table 54: Male's and female's microscopic findings at study day 24 (study #4)

ORGAN AND FINDING DESCRIPTION	Males				Females			
	GROUP: -1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER: 5	5	5	5	5	5	5	5
TOP OF LIST								
SKIN+ SUBCUTIS.....NUMBER EXAMINED:	5	5	5	5	5	5	5	5
-FASCITIS/FIBROSIS	4	0	0	1	0	0	0	0
MUSCLE.....NUMBER EXAMINED:	5	5	5	5	5	5	5	5
-MYOPATHY/MYOSITIS	2	1	0	1	1	0	0	1
-HAEMORRHAGE	0	0	0	0	0	0	1	0
-FASCITIS	0	0	0	0	0	0	1	0
LIVER.....NUMBER EXAMINED:	5	5	5	5	5	5	5	5
-INFLAMMATORY CELL FOCI	5	5	5	5	5	5	5	5
-AGONAL CONGESTION/HAEMORRHAGE	2	3	3	3	2	0	1	1
-FOCAL NECROSIS	0	0	0	0	0	0	0	1
SPLEEN.....NUMBER EXAMINED:	5	5	5	5	5	5	5	5
-HAEMOPOIESIS	5	5	5	5	5	5	5	5
-PIGMENT	0	0	0	0	0	0	1	0
-LYMPHOID HYPERPLASIA	1	0	1	1	1	1	0	1
PANCREAS.....NUMBER EXAMINED:	5	5	5	5	5	5	5	5
-INFLAMMATORY CELL FOCI	2	0	3	0	0	0	0	0
-LOBULAR ATROPHY	0	0	0	0	0	1	0	1
STOMACH.....NUMBER EXAMINED:	5	5	5	5	5	5	5	5
-CYSTIC GLANDS	0	0	1	0	0	0	0	0
DUODENUM.....NUMBER EXAMINED:	5	5	5	5	5	5	5	5

-CYSTICGLANDS		0	1	0	0	0	0	0	0
ADRENALNUMBER EXAMINED:		5	5	5	5	5	5	5	5
-CORTICAL VACUOLATION		1	1	0	1	0	0	0	0
ORGAN AND FINDING DESCRIPTION	NUMBER:	5	5	5	5	5	5	5	5
		-	-	-	-	-	-	-	-
-EOSINOPHILIC FOCUS		1	0	0	0	0	0	0	0
-MINERALISATION		0	0	0	1	0	0	0	0
KIDNEYNUMBER EXAMINED:		5	5	5	5	5	5	5	5
-FOCAL NEPHROPATHY		2	1	1	2	3	0	1	0
-HYALINE DROPLETS		2	3	3	3	0	0	0	0
-PIGMENT		0	0	0	1	1	1	2	1
-HYDRONEPHROSIS		0	1	0	0	0	0	0	0
-INFLAMMATORY CELL FOCI		0	1	2	0	0	0	1	1
-PAPILLARY MINERALISATION		0	0	1	0	2	1	0	0
-CASTS		0	0	1	0	0	0	0	0
-CORTICOMEDULLARY MINERALISATION		0	0	0	0	0	0	0	1
EPIDIDYMISNUMBER EXAMINED:		5	5	5	5	0	0	0	0
-INFLAMMATORY CELL FOCI		0	2	2	1				
OVARYNUMBER EXAMINED:		0	0	0	0	5	5	5	5
-MINERALISATION						0	0	1	0
URINARY BLADDERNUMBER EXAMINED:		5	5	5	5	5	5	5	5
-INFLAMMATORY CELL FOCI		2	0	0	0	0	0	0	0
-DISTENSION		0	1	0	0	0	0	0	0
PROSTATENUMBER EXAMINED:		5	5	5	5	0	0	0	0
-PROSTATITIS		1	0	0	0	0	0	0	0
PROSTATENUMBER EXAMINED:		5	5	5	5	0	0	0	0
-INFLAMMATORY CELL FOCI		2	3	1	1				
UTERUSNUMBER EXAMINED:		0	0	0	0	5	5	5	5
-PRO-OESTRUS						1	0	2	0
-OESTRUS						2	2	0	1
-METOESTRUS						2	2	2	4
-DIOESTRUS						0	1	1	0
MANDIBULAR LNNUMBER EXAMINED:		5	5	5	5	5	5	5	5
-LYMPHOID HYPERPLASIA		3	4	5	4	4	4	4	5
-AGONAL CONGESTION/HAEMORRHAGE		2	1	0	2	3	1	1	0
THYMUSNUMBER EXAMINED:		5	5	5	5	5	5	5	5
-CYST		0	0	1	0	1	1	2	2
LUNGNUMBER EXAMINED:		5	5	5	5	5	5	5	5
-INFLAMMATORY CELL FOCI		3	1	2	1	0	1	0	2
-AGONAL CONGESTION/HAEMORRHAGE		1	0	1	1	0	0	0	0
-FOAMY MACROPHAGES		1	1	2	2	2	1	1	0
-GRANULOMA		0	0	0	1	0	0	0	0
-PNEUMONITIS		0	0	0	0	0	0	0	1
ORGAN AND FINDING DESCRIPTION	NUMBER:	5	5	5	5	5	5	5	5
		-	-	-	-	-	-	-	-
HEARTNUMBER EXAMINED:		5	5	5	5	5	5	5	5
-INFLAMMATORY CELL FOCI		0	1	1	1	0	0	0	0
-CARDIOMYOPATHY		0	1	2	1	0	0	0	1
THYROIDNUMBER EXAMINED:		5	5	5	5	5	5	5	5
-ECTOPIC THYMUS		1	1	1	0	0	1	0	1
-FOLLICULAR CELL HYPERTROPHY		1	0	0	2	0	0	0	0
-CYST		0	0	0	0	0	1	0	1
LARYNXNUMBER EXAMINED:		5	5	5	5	5	5	5	5
-LARYNGITIS		0	0	0	0	1	0	0	0
PITUITARYNUMBER EXAMINED:		5	5	5	5	5	5	5	5

-CYST	0	0	1	0	0	1	0	0
-CYSTICLEFT	0	0	0	0	1	0	0	0
LEFTSCAPULAR.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5
-CRUST	2	2	1	0	0	1	2	2
-CELLULITIS	3	5	5	5	4	5	5	5
-FOLLICULITIS	0	2	0	1	0	0	0	0
-DERMATITIS	0	0	3	2	0	0	2	0
-HAEMORRHAGE	0	0	1	1	0	0	1	0
-NEEDLE-TRACKLESION	0	0	0	0	0	0	1	1
RIGHTSCAPULAR.....NUMBEREXAMINED:	5	0	5	5	5	0	5	5
-CELLULITIS	2	0	5	5	4	0	5	5
-CRUST	0	0	0	0	0	0	3	0
-NEEDLE-TRACKLESION	0	0	0	1	0	0	0	0
-FOLLICULITIS	0	0	1	0	0	0	0	0
-DERMATITIS	0	0	2	0	0	0	1	0
<hr/>								
POPLITEAL LN NUMBER EXAMINED:	5	5	5	5	5	5	5	5
--LYMPHOID HYPERPLASIA	0	1	0	0	0	1	0	0
--AGONAL CONGESTION/HAEMORRHAGE	0	1	0	0	0	1	0	0
** END OF LIST **								

Table 55: Male's and female's microscopic findings at study day 50 (study #4)

ORGANANDFINDINGDESCRIPTION	NUMBER:	Males				Females			
		GROUP: -1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
EYE.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-RETINALFOLD	0	0	0	0	0	1	0	0	0
SKIN+ SUBCUTIS.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-CRUST	0	0	0	1	0	0	0	0	0
-DERMATITIS	0	1	0	0	0	0	0	0	0
MUSCLE.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-MYOPATHY/MYOSITIS	0	0	0	0	1	2	0	0	0
-INFLAMMATORYCELLFOCI	0	0	1	0	0	0	0	0	0
LIVER.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-INFLAMMATORYCELLFOCI	5	5	5	5	5	5	5	5	5
-AGONALCONGESTION/HAEMORRHAGE	0	1	1	1	1	2	0	0	0
-HEPATOCYTEVACUOLATION	0	1	0	0	0	0	0	0	0
SPLEEN.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-HAEMOPOIESIS	5	5	5	5	5	5	5	5	5
-PIGMENT	2	1	3	5	4	5	5	5	5
PANCREAS.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-INFLAMMATORYCELLFOCI	1	2	1	1	0	1	0	0	0
-LOBULARATROPHY	0	0	1	0	0	0	0	0	1
MESENTERICLN.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-LYMPHOIDHYPERPLASIA	0	0	1	0	0	0	0	0	0
<hr/>									
STOMACH.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-CYSTICGLANDS	1	1	0	1	0	0	0	0	0
COLON.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-NEVIATODE	0	0	1	1	1	0	0	0	0
RECTUM.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-PARASITE	2	0	2	2	2	0	0	0	0
ADRENAL.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-CORTICALVACUOLATION	1	0	0	1	0	0	0	0	0
-INFLAMMATORYCELLFOCI	0	0	0	0	0	0	1	0	0
KIDNEY.....NUMBEREXAMINED:	5	5	5	5	5	5	5	5	5
-FOCALNEPHROPATHY	2	0	0	2	0	0	1	1	1
-HYALINEDROPLETS	5	5	5	5	0	0	0	0	0
-PIGMENT	0	0	0	0	4	2	2	2	2
-HYDRONEPHROSIS	1	1	0	0	0	0	0	1	1
-INFLAMMATORYCELLFOCI	1	1	1	1	0	1	0	0	0

		GROUP: -1-				-2-				-3-				-4-			
ORGAN AND FINDING DESCRIPTION	NUMBER:	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
-PAPILLARY MINERALISATION		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-CYST		1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
-AGONAL CONGESTION/HAEMORRHAGE		0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
TESTIS.....NUMBER EXAMINED:		5	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
-AGONAL CONGESTION/HAEMORRHAGE		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-INFLAMMATORY CELL FOCI		1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
OVARY.....NUMBER EXAMINED:						5	5	5	5								
-AGONAL CONGESTION/HAEMORRHAGE						0	0	0	1								
URINARY BLADDER.....NUMBER EXAMINED:		5	5	5	5	5	5	5	5								
-INFLAMMATORY CELL FOCI		0	0	0	1	0	0	0	0								
PROSTATE.....NUMBER EXAMINED:		5	5	5	5												
-INFLAMMATORY CELL FOCI		0	1	2	1												
UTERUS.....NUMBER EXAMINED:		0	0	0	0	5	5	5	5								
-PRO-OESTRUS						1	0	2	1								
-OESTRUS						0	0	0	1								
-METOESTRUS						2	5	2	1								
-DIOESTRUS						2	0	1	2								
MANDIBULAR LN.....NUMBER EXAMINED:		5	5	5	5	5	5	5	5								
-LYMPHOID HYPERPLASIA		4	4	5	4	5	5	4	3								
-AGONAL CONGESTION/HAEMORRHAGE		2	0	0	1	1	1	2	0								
THYMUS.....NUMBER EXAMINED:		5	5	5	5	5	5	5	5								
-CYST		1	2	1	2	3	3	2	3								
-AGONAL CONGESTION/HAEMORRHAGE		0	0	3	1	1	0	2	0								
LUNG.....NUMBER EXAMINED:		5	5	5	5	5	5	5	5								
-INFLAMMATORY CELL FOCI		1	2	2	1	1	0	3	0								
-AGONAL CONGESTION/HAEMORRHAGE		1	1	0	0	0	0	0	0								
-FOAMY MACROPHAGES		0	1	2	1	0	0	0	1								
HEART.....NUMBER EXAMINED:		5	5	5	5	5	5	5	5								
-INFLAMMATORY CELL FOCI		1	0	1	0	0	1	0	0								
-CARDIOMYOPATHY		0	0	2	0	0	0	0	0								
ESOPHAGUS.....NUMBER EXAMINED:		5	5	5	5	4	5	5	5								
-MYOPATHY/MYOSITIS		0	0	0	0	0	0	1	0								
THYROID.....NUMBER EXAMINED:		5	5	5	5	5	5	5	5								
-ECTOPIC THYMUS		0	0	0	0	1	1	0	0								
-FOLLICULAR CELL HYPERTROPHY		0	1	1	2	0	0	0	0								
-CYST		1	1	1	3	0	0	0	0								
PITUITARY.....NUMBER EXAMINED:		5	5	5	5	5	5	5	5								
-CYSTIC CLEFT		0	0	0	0	1	1	1	3								
LEFT SCAPULAR.....NUMBER EXAMINED:		5	5	5	5	5	5	5	5								
-CELLULITIS		3	2	5	4	1	2	3	3								
-FOLLICULITIS		0	0	0	0	1	0	0	0								
-DERMATITIS		0	0	0	1	0	1	0	0								
RIGHT SCAPULAR.....NUMBER EXAMINED:		5	0	5	5	5	0	5	5								
-CELLULITIS		2	0	3	2	0	0	3	2								
RIGHT SCAPULAR.....NUMBER EXAMINED:		5	0	5	5	5	0	5	5								
-CRUST		0	0	0	2	1	0	0	2								
-FOLLICULITIS		0	0	0	0	2	0	0	1								
-DERMATITIS		0	0	1	0	0	0	0	0								
-INFLAMMATORY CELL FOCI		0	0	1	0	0	0	0	0								
-MYOPATHY/MYOSITIS		0	0	0	1	0	0	0	0								
POPULTEAL LN.....NUMBER EXAMINED:		5	5	5	5	5	5	5	5								
-LYMPHOID HYPERPLASIA		0	0	1	0	0	0	0	1								
-AGONAL CONGESTION/HAEMORRHAGE		0	0	0	1	0	0	0	0								

** End of list **

An extensive number of tissues were examined for histology.

Study day 24

Crust, folliculitis, dermatitis, and cellulitis were reported at the left scapular injection site (IJ1). Folliculitis and dermatitis were reported only in groups 2, 3, and 4 whereas cellulitis and crust were reported in all groups. There was an increased incidence and severity of cellulitis in groups given 2, 3, and 4. However, there was a marginally greater injection site reaction in group 3 animals. Cellulitis was characterized by a diffuse infiltration of the subcutaneous tissue with mixed inflammatory cells, with varying involvement of the overlying panniculus muscle. Folliculitis was characterized by localized inflammation of hair follicles, and adjacent dermis. Dermatitis was characterized by the presence of a variable inflammatory cell infiltrate within the dermis, not associated with the hair follicles.

Crust, and cellulitis were reported at the right scapular injection site (IJ2). Crust was reported in groups 2 and 3 females only. Cellulitis was reported in all treated groups but with increased incidence and severity in groups 2, 3, and 4. Overall, there was a marginally greater injection site reaction in group 3.

Study day 50

Recovery evidence were reported at study day 50. At both injection sites, the levels of cellulitis were reduced in all groups. However, the incidence of cellulitis was still marginally increased in groups 2, 3, and 4 compared with group 1. The overall injection site reaction was still marginally greater in group 3 animals.

Urinalysis

Mean urine volume was increased ($P < 0.05$) in group 4 males on day 23. This increase was correlated with a decrease in mean specific gravity. Because this increase was within the historical background data and was not correlated with histopathological changes in the kidney, it was considered of no toxicological significance.

Body temperature:

Not measured

Serology:

Immunogenicity results were not included in this report.

Test article-related effects:

Table 56: Test article-related effects (study #4)

Test article related effects	Effects considered incidental
↑ Popliteal lymph node weight ↓ Thyroid weight ↑ Thymus weight Injection site findings	↑ Urine volume

Assessment:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), food consumption, ophthalmology, gross pathology, or microscopic anatomy were reported.

The popliteal lymph nodes are embedded in the fat contained in the popliteal fossa. One lies immediately beneath the popliteal fascia and drains the region from which this vein derives its tributaries, such as superficial regions of the posterolateral aspect of the leg and the plantar aspect of the foot ¹². The second one is located between the popliteal artery and the posterior surface of the knee-joint. It receives afferents from the knee-joint, together with those that accompany the genicular arteries. The third one is located at the sides of the popliteal vessels and receive the trunks that accompany the anterior and posterior tibial vessels ¹³. The flow of lymph from the legs towards the heart is the result of the calf pump (during walking the calf muscle contracts, squeezing lymph out of the leg via the lymphatic vessels). When the muscle relaxes, valves in the vessels shut preventing the fluid from returning to the lower extremities ¹⁴.

The thyroid gland controls how quickly the body makes proteins and uses energy. And, controls how sensitive the body is to other hormones. It produces the thyroid hormones [triiodothyronine (T₃) and thyroxine (sometimes referred to as tetraiodothyronine (T₄)). These hormones regulate the growth and rate of function of many other systems in the body. T₃ and T₄ are synthesized from iodine and tyrosine. The thyroid also produces calcitonin, which plays a role in calcium homeostasis. Hormonal output from the thyroid is regulated by thyroid-stimulating hormone (TSH) produced by the anterior pituitary. TSH is regulated by thyrotropin-releasing hormone (TRH) produced by the hypothalamus.

The thymus is a specialized primary lymphoid organ of the immune system. Within the thymus, T cells or T lymphocytes mature. T cells are critical to the adaptive immune system, where the body adapts specifically to foreign invaders. The thymus is composed of two identical lobes and is located anatomically in the anterior superior mediastinum, in front of the heart and behind the sternum.^[13] As the thymus is the organ of T-cell development, any congenital defect in thymic genesis or a defect in thymocyte development can lead to a profound T cell deficiency in primary immunodeficiency disease. Defects that affect both the T cell and the B cell lymphocyte lineages result in severe combined immunodeficiency syndrome (SCIDs). Acquired T cell deficiencies can also affect thymocyte development in the thymus ¹⁵.

Injection site findings due to test article treatment were reported.

Because urine volume increase was within the historical background data and was not correlated with histopathological changes in the kidney, it was considered of no toxicological significance.

Based on the overall findings in this study, it can be concluded that in rats, administration of (b) (4) and MVA-BN® vaccine subcutaneously had no adverse effects in terms of systemic toxicity.

GLP study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

¹² The Lymphatic System. 5. The Lymphatics of the Lower Extremity, Gray, Henry (1918) Anatomy of the Human Body.

¹³ <http://www.bartleby.com/107/179.html>

¹⁴ Jarvis, C. (2004). Physical Examination and Health Assessment (fifth ed.). St. Louis, Missouri: Saunders Elsevier. pp. 530–553.

¹⁵ <https://en.wikipedia.org/wiki/Thymus>.

Conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues were reported in this study.

Reproductive studies:

Study # 1: Developmental toxicity (teratology) study of subcutaneously administered MVA-BN[®] vaccine in pregnant rabbits (study number M350-06).

Key study findings: No significant findings were reported.

Study no.: M350-06

Conducting laboratory and location: (b) (4)

Date of study initiation: 04/24/2006

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity:

<u>Test article</u>	<u>Batch/Lot #</u>	<u>Purity %</u>
MVN-BN [®] vaccine	0170505	NR*
TBS	(b) (4)	NR*
Chorionic gonadotropin human/pregnyl	(b) (4)	NR*
Human hormone	(b) (4)	
Sterile water for injection	(b) (4)	

* NR = Not reported

Stability Summary

The stability of one batch of IMVAMUNE (MVA-BN[®] batch # 170505) was tested by determination of the virus titer at two different time points (0 and 2 hours incubation on wet ice) when either undiluted or 10-fold diluted. Protocol BN-PD-2005-021 was used to run stability analysis. The undiluted and the 10-fold diluted suspension of MVA-BN[®] batch 170505 were stable over 2 hours when stored on wet ice.

Methods

Doses: Test article or control groups were dosed at 0.5 mL per subcutaneous (sc) injection. Rabbits were immunized 3 times at 2 weeks intervals. The first immunization occurred 14 days prior to mating, the second on the sperm positive day (gestation day 2 [GD 2]), and the third on GD 14.

Study design: Animals were assigned to 3 different groups. Twenty four animals per group were received 3 immunizations at 2 weeks intervals. Animals were treated at 14 days prior to mating, sperm negativity day (GD 0), and GD 14 for the 1st, 2nd, and 3rd immunizations, respectively. The details of the study design are listed in the following table:

Table 57: Study design (study #1 repro)

GROUP	TREATMENT	DOSE LEVEL (μ L)	DAYS OF DOSING (FEMALES ONLY)	NUMBER ASSIGNED PER GROUP
				FEMALES
1	PBS	500	2 weeks prior to cohabitation and on GD's 0 and 14	24
2	1X10 ⁷ TCID ₅₀ MVA-BN [®]	500	2 weeks prior to cohabitation and on GD's 0 and 14	24
3	1X10 ⁸ TCID ₅₀ MVA-BN [®]	500	2 weeks prior to cohabitation and on GD's 0 and 14	24

GD = Gestation day.

Species/strain: (b) (4) rabbits.

Number/group: 24 per group.

Age:

Males: 9 months

Females: 5 months

Weight:

Week 1 start: 2.6-3.4 kg

Week 2 start: 2.8-3.6 kg

Week 3 start: 2.8-3.6 kg

Week 4 start: 3.0-3.6 kg

Source: (b) (4)

Route, volume, and frequency of test article administration: Animals were treated with single sc injection of 500 μ L at 14 days prior to the day of mating, GD 0, and GD 14.

Parameters and endpoints evaluated:

The following parameters were evaluated: Clinical observations were performed twice a day and an individual examination was performed once a week during the treatment period. Body weight was recorded weekly prior to mating, and on GD's 0, 6, 9, 12, 15, 18, 21, 24, and 28. Corrected body weight was recorded for the 28th day of pregnancy (body weight on GD 28 minus weight of the gravid uterus). Body weight changes were calculated for GD's 0-6, 6-9, 9-12, 12-15, 15-18, 18-28, and 0-28. Food consumption was recorded daily. Blood samples for immunogenicity assays were collected from the ear vein prior to the first dose (SD -14), week 3 (mating), GD 14, and GD 28 (C-section).

Examination of F₀ females and fetuses at Cesarean section: Viscera of the does examined macroscopically. Histopathological examination was conducted on the organs with undiagnosed macroscopic alterations. Uterus was weighed and examined for early and late embryonic as well as fetal deaths and for the number of live fetuses. Live fetuses and placentas were weighed individually and litter means were calculated. Pre-implantation loss was calculated by subtracting the number of corpora lutea from implantations number. The crown-rump length of fetuses was measured and the litter mean was calculated.

Fetuses' evaluations:

External and visceral examination on all fetuses was conducted. The heads from half of each litter were removed and fixed and examined by Wilson's free-hand razor blade method. Skeletons were examined after cartilage and bone staining and abnormalities recorded.

Histological examination was performed on organs and tissues of dams with macroscopic findings.

Statistical methods

Bartlett's homogeneity of variance test, ANOVA, Dunnett test, Kruskal-Wallis ANOVA, Mann-Whitney U-test, Fisher's exact test, Pearson's chi-square were used for data evaluation using the program package SAS. Immunology data was analyzed using an unpaired, two-tailed t-test using (b) (4) software, version (b) (4). Antibody responses of the responders over time were analyzed by ANOVA followed by Tukey's multiple comparison posttest.

Results

F₀ generation females: After mating and applying all exclusions criteria, 12, 14, and 15 dams in the control, low, and high dose groups, respectively, were included in the final statistical evaluation.

Mortality/Clinical signs: No test article-related effects on mortality or clinical observations during the pre-mating period were reported.

Body weight changes: No test article-related effects on body weight changes, gravid uterine weight, or corrected body weight during the premating and the gestational periods were reported. Single increase in the maternal body weight was reported on GD 21.

Food consumption: No test article-related effects on food consumption during the pre-mating period were reported.

Table 58: body weight changes [kg, mean \pm SD] (study #1 repro)

Group	PBS	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
Gestation Period	n = 12	n = 14	n = 15
Day 0	3.2 \pm 0.2	3.3 \pm 0.2	3.3 \pm 0.2
Day 6	3.4 \pm 0.2	3.4 \pm 0.2	3.5 \pm 0.2
Day 9	3.4 \pm 0.2	3.5 \pm 0.2	3.6 \pm 0.2
Day 12	3.4 \pm 0.2	3.5 \pm 0.3	3.6 \pm 0.3
Day 15	3.5 \pm 0.2	3.6 \pm 0.3	3.7 \pm 0.2
Day 18	3.5 \pm 0.3	3.6 \pm 0.3	3.7 \pm 0.2
Day 21	3.6 \pm 0.2	3.7 \pm 0.3	3.8* \pm 0.3
Day 24	3.6 \pm 0.3	3.7 \pm 0.2	3.8 \pm 0.3
Day 28	3.6 \pm 0.3	3.8 \pm 0.3	3.8 \pm 0.3

n = group size. * Significantly different from control at P \leq 0.05. Not paired, sperm-negative, sperm-positive but not pregnant, or with \leq 3 implantations females, were excluded from this table.

Table 59: Corrected body weight and weight gain at day 28 [kg, mean \pm SD] (study #1 repro)

Group	PBS	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
	n = 12	n = 14	n = 15
Gravid Uterine Weight	0.45 \pm 0.08	0.49 \pm 0.07	0.48 \pm 0.05
Corrected Body Weight ^a	3.19 \pm 0.26	3.31 \pm 0.24	3.37 \pm 0.24
Corrected Body Weight Change ^b	-0.04 \pm 0.14	0.04 \pm 0.16	0.06 \pm 0.15

n = group size. ^a Corrected body weight = body weight on GD 28 – weight of gravid uterus.

^b Corrected body weight change = corrected body weight – body weight on GD 0.

Fetal and placental data:

No test article-related effects on litter averages for corpora lutea, implantations, post-implantation mortality (such as early and late embryonic resorption), dead and viable fetuses, male and female fetuses, and placental weight were reported. All placentas were normal. The values of pre-implantation loss and intrauterine mortality were higher in the control than treated groups. In addition, lower fetal weight and length were reported in the control group compared to treated groups. The above observations were not considered significant toxicology findings.

Table 60: Summary of fetal and uterine results [mean \pm SD] (study #1 repro)

Group	PBS	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
	n = 12	n = 14	n = 15
Pre-implantation loss %	9.6 \pm 15.8	6.9 \pm 13.8	3.3 \pm 8.1
Early embryonic death %	3.3 \pm 6.1	0.9 \pm 3.3	1.4 \pm 3.7
Late embryonic death %	0.0 \pm 0.0	0.8 \pm 3.0	0.0 \pm 0.0
Dead fetuses %	0.0	0.0	0.0
Post-implantation loss %	3.3 \pm 6.1	1.7 \pm 4.3	1.4 \pm 3.7
Total intra-uterine mortality %	12.4 \pm 16.9	8.2 \pm 15.2	2.5 \pm 6.7
Male fetuses %	48.1 \pm 18.1	50.4 \pm 19.3	46.7 \pm 16.5
Female fetuses %	51.9 \pm 18.1	49.6 \pm 19.3	53.3 \pm 16.5
Corpora lutea	9.8 \pm 1.7	9.8 \pm 2.2	9.5 \pm 1.3
Pre-implantation loss	1.0 \pm 1.7	0.9 \pm 1.8	0.3 \pm 0.8
Implantation	8.8 \pm 2.0	8.9 \pm 1.6	9.2 \pm 1.3
Early embryonic death	0.3 \pm 0.5	0.1 \pm 0.3	0.1 \pm 0.4
Late embryonic death	0.0 \pm 0.0	0.1 \pm 0.3	0.0 \pm 0.0
Dead fetuses	0.0	0.0	0.0
Post-implantation loss	0.3 \pm 0.5	0.1 \pm 0.4	0.1 \pm 0.4
Total intrauterine mortality	1.3 \pm 1.7	1.0 \pm 2.0	0.3 \pm 0.7

Group	PBS	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
	n = 12	n = 14	n = 15
Viable fetuses	8.6 ± 2.2	8.8 ± 1.7	9.1 ± 1.3
Male fetuses	4.2 ± 2.0	4.4 ± 1.8	4.1 ± 1.2
Female fetuses	4.4 ± 1.7	4.4 ± 1.8	4.9 ± 1.8

n = group size.

External, visceral, and skeletal examination data:

Retarded growth was reported in 3/103 (2.9%), 1/123 (0.8%), and 3/136 (2.2%) fetuses in G's 1, 2, and 3, respectively. No test article-related effects on the fetuses were reported.

Gonad agenesis (microscopic examinations of the testicles indicate that the right testicle appears to be less than one-half the size of left testicle) was reported in 1 control fetus. Left common carotid artery branches off directly from the aortic arch were reported in 1 G2 fetus. Fused sternebra were reported in 2 G2 fetuses. In G3, 1 fetus with left common carotid artery branches off directly from the aortic arch and 1 fetus with ventricle septal defect were reported. Because of the low incidences of these observations they were considered of no toxicological value. No evidence for differences between G's 1 and 3 with respect to ventricle septal defect were shown.

No skeletal abnormalities were reported in G1. In G2, fused sternebra was reported in 2 fetuses. Irregular calcification of parietal bone was reported in 1 G3 fetus. In addition, bent hyoid alae and fused sternebra were reported in 5 and 1 fetuses of Gs 2 and 3, respectively. Malformed thoracic vertebrae and ribs and LVI-SI misshapen were reported in 1 G3 fetus.

Evidence for differences between G1 and G3 with respect to skeletal bent hyoid and skeletal abnormalities and variations, with the difference in abnormalities due principally to differences in the percent variations, were reported.

Table 61: Summary of fetal and uterine results [mean ± SD] (study #1 repro)

Group	PBS	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
	n = 12	n = 14	n = 15
External examination			
Fetuses examined	103	123	136
Fetuses with abnormalities	2.5 ± 8.7	0.6 ± 2.4	2.1 ± 4.5
Variation	2.5 ± 8.7	0.6 ± 2.4	2.1 ± 4.5
Malformation	0.0	0.0	0.0
Visceral examination			
Fetuses with abnormalities	1.2 ± 4.1	1.2 ± 4.5	1.3 ± 3.4
Variation	0.0 ± 0.0	1.2 ± 4.5	0.6 ± 2.4
Malformation	1.2 ± 4.1	0.0 ± 0.0	0.7 ± 2.6
Skeletal examination (without skull)			

Group	PBS	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
	n = 12	n = 14	n = 15
Fetuses with abnormalities	0.0 ± 0.0	1.9 ± 4.9	7.0 ± 8.3
Variation	0.0 ± 0.0	1.9 ± 4.9	5.5 ± 7.4
Malformation	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 3.7
Skeletal examination (skull only)			
Fetuses examined	47	58	65
Fetuses with abnormalities	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 6.5
Variation	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 6.5
Malformation	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

n = group size.

Table 62: Summary of types of external, visceral, and skeletal abnormalities results [sum/%] (study #1 repro)

Group	PBS	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
	n = 12 (N/%)	n = 14 (N/%)	n = 15 (N/%)
External abnormalities			
Retarded in weight	3/2.9	1/0.8	3/2.2
Visceral abnormalities			
Left common carotid artery branches off from aortic arch directly	0.0/0.0	1.0/0.8	1.0/0.7
Right testicle under microscope appears half the size of left testicle	1.0/1.0	0.0/0.0	0.0/0.0
Ventricle septal defect (VSD)	0.0/0.0	0.0/0.0	1.0/0.7
Skeletal abnormalities (without skull)			
Fetuses with abnormalities	0.0/0.0	2.0/1.6	9.0/6.6
Variations	0.0/0.0	2.0/1.6	7.0*/5.2*
Bent hyoid alae	0.0/0.0	0.0/0.0	6.0*/4.4*
Fused sternebrae	0.0/0.0	2.0/1.6	1.0/0.7
Malformations	0.0/0.0	0.0/0.0	2*/1.5**
Vertebral bodies and ribs-LVI-SI misshapen	0.0/0.0	0.0/0.0	1.0/0.7
Vertebral bodies and ribs-malformed	0.0/0.0	0.0/0.0	1.0/0.7

Group	PBS	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
	n = 12 (N/%)	n = 14 (N/%)	n = 15 (N/%)
thoracic vertebrae and ribs			
Skeletal abnormalities (skull only)			
Fetuses examined	47	58	65
Variations	0.0/0.0	0.0/0.0	1.0/1.5
Irregular calcification of parietal bone	0.0/0.0	0.0/0.0	1.0/1.5

n = group size. * Significantly different at ≤ 0.05 . ** Significantly different at ≤ 0.01 .

Differences between the control and high dose group with respect to skeletal bent hyoid (<0.05) and skeletal abnormalities (<0.01) and variations (<0.05), with the difference in abnormalities due principally to differences in the percent variations were reported.

Historical control data from (b) (4) and/or (b) (4) were used to examine the four malformations found. The spontaneous frequencies were as follows:

- _ Gonad agenesis ranged from 0.01% to a 1.27%.
- _ Ventricle septal defect ranged from 0.10% to 4.35%.
- _ Sacral vertebrae misshapen ranged from 0.011% to 1.11%.
- _ Vertebral anomaly with or without associated rib anomaly ranged from 0.4% to 2.3%.

The individual malformation frequencies found in this study were considered insignificant because all of them fell within the historical range of spontaneous abnormalities.

Immunology:

Blood samples for immunology were collected from the ear vein prior to the first dose (SD -14), week 3 (mating), GD 14, and GD 28 (C-section). Serum (about 1 mL) samples were obtained from all does (total 96 samples). To detect antibodies to Modified Vaccinia Ankara virus (MVA-BN[®]) in rabbit's serum, an enzyme linked immunosorbent assay (ELISA), were used. The group geometric mean antibody levels are summarized in the following table:

Table 63: Geometric mean of anti-MVA BN[®] antibody response^a (study #1 repro)

Treatment group ^b	Parameters	Antibody Titer (Study Days)			
		-14	0	14	28
Vehicle	Geo-Mean	< 2.00	< 2.00	< 2.00	< 2.00
	n	24	24	24	24
1X10 ⁷ TCID ₅₀ MVA-BN [®]	Geo-Mean	< 2.00	2.64	3.75	4.55
	n	24	15	24	24
1X10 ⁸ TCID ₅₀ MVA-BN [®]	Geo-Mean	< 2.00	3.31	4.96	4.99
	n	24	24	24	24

^a Samples yielding net OD values ≤ 0.139 at 1:100 dilution is reported as < 2.00 .

^b Dams were immunized at SD's -14, 0, and 14.

The mean net OD value of the pretest sera of the dams at 1:100 dilution (0.039), plus 0.1 OD units, resulting in 0.139 and used as a cut-off value. The positive control value was 4.99 and the negative control value was < 0.139 .

No anti-MVA-BN[®] antibodies were detectable in the serum collected pretest from all female rabbits. Antibody responses, after a single MVA-BN[®] immunization, were exhibited in 15 and 24 (out of 24/group) dams in G's 2 and 3, respectively. All dams in G2 had an antigen-specific antibody response after the second immunization. The secondary response in G2 at SD 14 was significantly higher than the primary response at SD 0. In addition, the tertiary response in G2 at SD 28 was significantly higher than the secondary response at SD 14. Group 3 followed the same trend, however, after the third immunization, the antibody titer remained constant in this group. Antibody responses in G3 were significantly higher than G2 at all time points. It can be concluded that the antibody responses were time and dose dependent.

Summary:

The objective of this study was to evaluate the effect of MVA-BN[®] smallpox vaccine on the fertility of the F₀ generation and the embryonic/fetal development of the F₁ generation and to measure anti-MVA-BN[®] antibodies in the F₀ female rats. Animals (24/group) were assigned to three different groups and treated by sc injections of control or test article. Test article was administered at 14 days prior to mating, and GD's 0 and 14.

Blood samples for immunology were collected prior to the first dose (SD -14), and on GD's 0, 14, and 28. Serum samples were obtained from all does and analyzed by an ELISA to detect antibodies to Modified Vaccinia Ankara virus (MVA-BN[®]).

Uterine contents were examined and number of corpora lutea and gross placental morphology were evaluated. Embryonic/fetal viability, fetal weights, sex ratios, and external, visceral, coronal, and skeletal morphology were assessed for developmental toxicity.

No test article-related effects on mortality, clinical observations, body weight, body weight changes, or food consumption during the pre-mating period were reported. No test article-related effects on fertility, embryonic/fetal survival, or gross examination (F₀ females) were reported. There was no evidence of treatment-related developmental toxicity in either G2 or G3.

There were no detectable anti-MVA-BN[®] antibodies in the serum collected pretest from all F₀ female rats. All dams in G's 2 and 3 had an antigen-specific antibody response after the second immunization. Antibody responses in test article treated animals were time and dose dependent.

Conclusions

The administrations of MVA-BN[®] vaccine on SD -14, and GD's 0 and 14 at 500 μ L via the sc route did not give an indication of F₀ generation toxicity. No test article-related effect on mating, pregnancy, or fetal examination data evaluations was reported. Serological analysis data indicated vaccine exposure of dams during pregnancy to anti-MVA-BN[®] antibodies.

Study # 2: Peri- and postnatal developmental toxicity study of subcutaneously administered MVA-BN® vaccine in pregnant rats (study number M351-06).

Key study findings: No significant findings were reported.

Study no.: M351-06

Conducting laboratory and location: (b) (4)

Date of study initiation: 02/08/2006

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity:

<u>Test article</u>	<u>Batch/Lot #</u>	<u>Purity %</u>
MVN-BN® vaccine	0170505	NR*
TBS	(b) (4)	NR*

* NR = Not reported

Stability Summary

The stability of one batch of IMVAMUNE (MVA-BN® batch # 170505) was tested by determination of the virus titer at two different time points (0 and 2 hours incubation on wet ice) when either undiluted or 10-fold diluted. Protocol BN-PD-2005-021 was used to run stability analysis. The undiluted and the 10-fold diluted suspension of MVA-BN® batch 170505 were stable over 2 hours when stored on wet ice.

Methods

Doses: Test article or control was dosed at 0.5 mL per subcutaneous (sc) injection. Rats were immunized 3 times at 2 weeks intervals. The first immunization occurred 14 days prior to mating, the second on the sperm positive day (gestation day [GD] 0), and the third on GD 14.

Study design: Animals were assigned to three different groups. Thirty two (G's 1 and 3) or 34 (G 2) animals per group were received 3 immunizations at 2 weeks intervals. The details of the study design are listed in the following table:

Table 64: Study design (study #2 repro)

GROUP	TREATMENT	DOSE LEVEL (µL)	DAYS OF DOSING (FEMALES ONLY)	NUMBER ASSIGNED PER GROUP
				FEMALES
1	PBS	500	2 weeks prior to cohabitation and on GD's 0 and 14	32
2	1X10 ⁷ TCID ₅₀ MVA-BN®	500	2 weeks prior to cohabitation and on GD's 0 and 14	34
3	1X10 ⁸ TCID ₅₀ MVA-BN®	500	2 weeks prior to cohabitation and on GD's 0 and 14	32

GD = Gestation day.

Species/strain: (b) (4) rats.
Number/group: 32 or 34 rats per group.
Age:

Males: 7 months

Females: 3 months

Weight: 213-244 grams

Source: (b) (4)

Route, volume, and frequency of test article administration: Animals were treated with single subcutaneous injection of 500 µL, of low (1×10^7 TCID₅₀ MVA-BN®) or high (1×10^8 TCID₅₀ MVA-BN®) test article dose, at 14 days prior to the day of mating, GD 0, and GD 14.

Parameters and endpoints evaluated:

The following parameters were evaluated: Clinical observations were performed once a day and an individual examination was performed at least once a week during the treatment period. Females body weight was recorded weekly prior to mating, and on GD's 0, 3, 6, 9, 13, 16, and 20, on the day of delivery (within the first 24 hr after delivery), and weekly thereafter up to the 28th postpartum (pp) day (0, 7, 14, 21, and 28). Body weight gain was calculated for GD's 0-6, 6-9, 9-13, 13-16, 16-20, and 0-20, and for postpartum days 0-7, 7-14, 14-21, and 21-28. Food consumption was calculated for GD's 0-6, 6-9, 9-13, 13-16, and 16-20 and for postpartum days 0-7, 7-14, 14-21, and 21-28. Blood samples for immunogenicity assays were collected from the dams (retro-orbital sinus) and the pups (by decapitation [pp 0 or pp 7] or from retro-orbital sinus [pp 28]). Serum samples for antibody response were collected from all dams prior to vaccination, GD's 0 and 14, and pp days 0, 7, and 28. Pups' blood samples were collected on pp days 0, 7, and 28. Milk samples (collected from the pups' stomach) for antibody response, pooled from all pups in a litter, were collected on pp days 0 and 7.

Pups' evaluations:

The litters were checked for viable and dead pups daily. Surface-righting reflex was examined on the first postnatal day. Pinna unfolding examined on the 1st and the 2nd postnatal days, and the observation was considered to be positive if at least one of the pinnas unfolded. Incisor eruption was examined on the 9th postnatal day. Eyes openings were examined on the 14th postnatal day.

Statistical methods

Bartlett's homogeneity of variance test, ANOVA, Dunnett test, Kruskal-Wallis ANOVA, Mann-Whitney U-test, Fisher's exact test, Pearson's chi-square were used for data evaluation using Stata SE version for Windows and (b) (4) version (b) (4). Immunology data was analyzed using an unpaired, two-tailed t-test using (b) (4) software, version (b) (4). Antibody responses of the responders over time were analyzed by ANOVA followed by Tukey's multiple comparison posttest.

Results

F₀ generation females: After mating and applying all exclusions criteria, 28, 27, and 28 dams in the control, low, and high dose groups, respectively, were included in the final statistical evaluation.

Mortality/Clinical signs: One dam died accidentally during terminal blood collection (pp 0) and another dam was found dead (pp 25) in the control group. One animal in the control group was sacrificed on GD 46 because of the failure to deliver. One animal in the low dose group was found dead on GD 7. One dam in the high dose group died accidentally during terminal blood collection on pp 28. No test article-related effects on mortality or clinical observations during the pre-mating period were reported.

Body weight changes: No test article-related effects on body weight changes or body weight gain during the pre-mating, gestation, and postpartum periods were reported.

Food consumption: No test article-related effects on food consumption during the gestation and postpartum periods were reported.

Table 65: Body weight changes at gestation period [grams, mean \pm SD] (study #2 repro)

Group	CONTROL	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
Gestation Period	n = 28	n = 27	n = 28
Day 0	254 \pm 16	259 \pm 17	260 \pm 18
Day 3	265 \pm 15	267 \pm 19	269 \pm 20
Day 6	277 \pm 15	279 \pm 19	279 \pm 22
Day 9	287 \pm 16	290 \pm 18	291 \pm 23
Day 13	311 \pm 17	314 \pm 20	312 \pm 23
Day 16	332 \pm 20	330 \pm 25	335 \pm 27
Day 20	394 \pm 28	394 \pm 27	393 \pm 39

n = group size. Non-pregnant females or females with ≤ 5 implantations were excluded from the statistical evaluation.

Table 66: Body weight changes at postpartum period [grams, mean \pm SD] (study #2 repro)

Group	CONTROL	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
Gestation Period	n = 28	n = 27	n = 27
Day 0	308 \pm 18	310 \pm 24	311 \pm 26
Day 7	326 \pm 16	329 \pm 22	327 \pm 28
Day 14	338 \pm 18	341 \pm 21	342 \pm 31
Day 21	327 \pm 16	332 \pm 19	332 \pm 26
Day 28	300 \pm 20	304 \pm 21	306 \pm 24

n = group size. Non-pregnant females or females with ≤ 5 implantations were excluded from the statistical evaluation.

Table 67: Male pup mean body weight summary [grams, mean \pm SD] (study #2 repro)

Group	CONTROL	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
Gestation Period	n = 28-22	n = 26-21	n = 28-22
Day 0	6.5 \pm 0.4	6.4 \pm 0.6	6.4 \pm 0.4
Day 7	14.9 \pm 1.9	14.6 \pm 2.2	15.6 \pm 5.4
Day 14	28.0 \pm 3.7	28.2 \pm 4.6	28.5 \pm 3.5
Day 21	43.8 \pm 7.5	45.5 \pm 7.8	46.0 \pm 6.2
Day 28	86.5 \pm 10.7	87.7 \pm 11.8	87.7 \pm 11.0

n = group size range, due to loss of few pups over time.

Table 68: Females pub mean body weight summary [grams, mean \pm SD] (study #2 repro)

Group	CONTROL	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
Gestation Period	n = 27-21	n = 27-22	n = 28-22
Day 0	6.1 \pm 0.3	6.1 \pm 0.6	6.0 \pm 0.4
Day 7	14.1 \pm 1.9	14.2 \pm 2.1	14.0 \pm 0.7
Day 14	26.4 \pm 3.4	27.3 \pm 3.7	27.5 \pm 0.9

Group	CONTROL	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
Gestation Period	n = 27-21	n = 27-22	n = 28-22
Day 21	41.8 ± 6.9	44.1 ± 6.4	44.2 ± 1.5
Day 28	77.5 ± 10.0	80.1 ± 8.3	80.2 ± 1.8

n = group size range, due to loss of few pups over time.

Fetal and pups data:

No test article-related effects on mortality, clinical observations, body weight, body weight changes, or food consumption during the pre-mating period were reported. No test article-related effects on gross examination, number of implantations, average litter size, number of viable pups and their sex ratio, percent intrauterine mortality, percent extrauterine mortality, and pup body weight were reported.

Percentage of positive surface-righting reflex on pp day 0 in Gs 2 and 3 (39% and 39.2%, respectively) were significantly different from G1 (46.2%). Percentage of pinna unfolding on pp day 1 was statistically lower (0.0%) in G3 when compared to G1 (2.0%). Percentage of pups with incisors erupted on pp day 9 was statistically increased in Gs 2 and 3 (62% and 61%, respectively) when compared to G1 (50%). Percentage of pups with eyes opening on pp day 14 was statistically increased in G3 (54%) when compared to G1 (40%).

Table 69: Summary of fetal and pups data [sum/%] (study #2 repro)

Group	PBS n = 28	1X10 ⁷ TCID ₅₀ MVA-BN n = 27	1X10 ⁸ TCID ₅₀ MVA-BN n = 28
Dams with viable pups	28	27	28
Number of implantations	397	394	391
Total births	357/90	359/91	364/93
Viable pups	352/99	350/98	363/93
No. of males	182/53	167/48	175/50
No. of females	170/47	183/53	188/50
Intrauterine mortality	40/11	35/9	27/8
Surface-righting reflex	164/46	129/39	132/39
Pinna unfolding (Day 1)	6/2	14/8	0/0
Pinna unfolding (Day 2)	172/63	215/71	199/70
Incisors eruption (Day 9)	135/50	179/62	170/61
Eyes opening (Day 14)	103/40	93/38	145/54

n = group size.

Table 70: Summary of gross necropsy findings (study #2 repro)

Group	PBS #observations/ %	1X10 ⁷ TCID ₅₀ MVA-BN #observations/ %	1X10 ⁸ TCID ₅₀ MVA-BN #observations/ %
Uterus			
# of observations	32	34	32
Discolored black	1/3	0	0
Slightly small	0	0	1/3
No gross findings	31/97	34/100	31/97

Group	PBS	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
	#observations/ %	#observations/ %	#observations/ %
Cervix			
# of observations	32	34	32
No gross findings	32/100	34/100	32/100
Ovaries			
# of observations	32	34	32
Discolored black	1/3	0	0
Discolored dark	2/6	2/6	1/3
Discolored red	0	1/3	2/6
Left ovary-cyst	1/3	0	0
Moderately fluid filled	1/3	0	0
Right ovary-discolored dark	0	0	1/3
No gross findings	27/84	31/91	28/88
Viscera			
# of observations	32	34	32
Adrenals-Discolored black	1/3	0	0
Adrenals-Discolored red	1/3	2/6	0
Adrenals-slightly enlarged	1/3	0	0
Armpit right side-moderate firm	1/3	0	0
GI tract-filled with air	1/3	0	0
Heart enlarged	0	1/3	0
Heart slightly enlarged	0	1/3	1/3
Kidneys-discolored dark	3/9	13/38	12/38
Kidneys-moderately mottled	1/3	0	0
Kidneys-slight foci	0	1/3	0
Kidneys-slightly mottled	0	1/3	0
Liver-discolored red	1/3	2/6	2/6
Liver-extra lobe	0	1/3	0
Liver- ~1cm mass above	1/3	0	0
Lungs-discolored dark	2/6	0	0
Lungs-discolored red	1/3	2/6	1/3
Lungs-moderate foci	0	1/3	2/6
Lungs-moderately mottled	4/13	4/12	7/22
Lungs mottled	0	1/3	0
Lungs-slight foci	3/9	4/12	4/13
Lungs-slightly mottled	8/25	9/26	8/25
Mammary-masses	0	0	1/3
Spleen-discolored dark	0	3/9	1/3

Group	PBS	1X10 ⁷ TCID ₅₀ MVA-BN	1X10 ⁸ TCID ₅₀ MVA-BN
	#observations/ %	#observations/ %	#observations/ %
Spleen-discolored red	0	1/3	0
Thymus-discolored red	0	1/3	1/3
No gross findings	13/41	2/6	5/16

Immunology:

Serum samples for antibody response were collected (from the retro orbital sinus) from all dams prior to vaccination, GD's 0 and 14, and pp days 0, 7, and 28. Pups' blood samples were collected (by decapitation) on pp days 0, 7, and (from the retro orbital sinus) 28. Milk samples for antibody response, pooled from all pups in a litter, were collected (from the pups' stomach) on pp 0 and pp 7. To detect antibodies against MVA-BN[®] in rats' serum, an enzyme linked immunosorbent assay (ELISA) was used. The groups mean antibody levels are summarized in the following table:

Table 71: Group mean of anti-MVA BN[®] antibody response^a in sera from dams (study #2 repro)

Treatment group ^b	Parameters	Antibody titer (study days) ^a , group mean ± SD					
		-14	0	14	21 (PP0)	28 (PP7)	49 (PP28)
Vehicle	Group mean	<2.0 ^b	<2.0	<2.0	<2.0	<2.0	<2.0
	Responders/ group	0/30	1/30	0/30	0/30	0/29	0/28
1X10 ⁷ TCID ₅₀ MVA-BN [®]	Group mean	<2.0	2.42 ± 0.4	2.98 ± 0.6	3.29 ± 0.7	3.60 ± 0.8	3.39 ± 0.7
	Responders/ group	0/27	5/27	22/27	27/27	27/27	27/27
1X10 ⁸ TCID ₅₀ MVA-BN [®]	Group mean	2.0	2.70 ± 0.6	3.93 ± 0.7	4.07 ± 0.4	4.35 ± 0.5	4.15 ± 0.5
	Responders/ group	1/29	21/29	29/29	29/29	29/29	29/29

^a Sera were collected on SDs -14, 0, and 14 (before immunization), and pp days 21, 28, and 49.

^b Samples yielding net OD values ≤0.147 is reported as < 2.00.

Table 72: Group mean of anti-MVA BN[®] antibody response^a in serum and milk from litters (study #2 repro)

Treatment group	Parameters	Antibody titer (study says), group mean ± SD				
		Serum samples ^a			Milk samples ^b	
		21 (PP0)	28 (PP7)	49 (PP28)	21 (PP0)	28 (PP7)
Vehicle	Group mean	<2.0 ^c	<2.0	<2.0	<2.0	<2.0
	Responders/ group	0/5	0/3	0/22	0/4	0/3

Treatment group	Parameters	Antibody titer (study says), group mean \pm SD				
		Serum samples ^a			Milk samples ^b	
		21 (PP0)	28 (PP7)	49 (PP28)	21 (PP0)	28 (PP7)
1X10 ⁷ TCID ₅₀ MVA-BN [®]	Group mean	2.30 \pm 0.0	3.00 \pm 0.9	3.14 \pm 0.6	<2.0	2.75 \pm 0.6
	Responders/group	2/2	3/3	19/22	0/2	2/3
1X10 ⁸ TCID ₅₀ MVA-BN [®]	Group mean	3.51 \pm 0.6	4.41 \pm 0.4	3.66 \pm 0.5	3.02 \pm 0.5	3.51
	Responders/group	5/5	2/2	22/22	5/5	1/2

^a Serum samples, pooled from all pups in the litter, were collected (from different litters) on SDs 21, 28, and 49, post-partum (pp) days 0, 7, and 28, respectively.

^b Milk samples, pooled from all pups in the litter, were collected (from different litters) on SDs 21 and 28, post-partum (pp) days 0 and 7, respectively.

^c Samples yielding net OD values ≤ 0.147 is reported as < 2.00.

Note: Due to missing geometric mean data and individual results tables, mean group results were reported. Please refer to communication to Sponsor section for details. The geometric mean is a useful summary (compared to traditional mean) when we expect that changes in the data occur in a relative fashion (changes in antibody titer levels is in proportion to dose treatment level). Individual animal data is used to determine if there any outliers within the group affecting the results and also used to determine the geometric mean.

The mean net OD value of the pretest sera of the dams at 1:100 dilution (0.047), plus 0.1 OD units, resulting in 0.147 and used as a cut-off value. The positive control value was 5.39 ± 0.24 and the negative control value was < 0.147.

No anti-MVA-BN[®] antibodies were detectable in the serum collected pretest from all female rabbits. The primary response at GD 0 was low in both dose groups, with 21/29 dams responding in the high dose group compared to only 5/27 in the low dose group. After the second immunization (GD 14) the number of responders and the antibody titers increased in the low (22/27) and the high (29/29) dose groups. All animals in Gs 2 and 3 had an antigen-specific antibody responses after the third immunization (GD 21). From GD 14 to SD 49, immunization at the high dose elicited a significantly greater antibody response than immunization with the low dose. Animals in G3 were having greater antibody responses, in serum and milk samples, than G2. The differences between the groups reached statistical difference only in the serum samples at SD 49. The difference may not be biologically important because the ranges of the responses overlap.

Antibody titers in serum were somewhat greater than those in milk in litters. However, the results may not reveal the complete content of antibodies in milk. Potential pH-related stability problems, the effects of dilution by stomach contents, and difficulty in isolating liquid from milk samples may all contribute to an impaired ability to detect antibodies in milk samples. Caution should be taken when interpreting the relative antibody amount in serum and milk data. It can be concluded that the high dose produced a significantly higher antibody response than the low dose.

Summary:

The objective of this study was to evaluate the effect of MVA-BN[®] smallpox vaccine on pregnant and lactating rats and on the developing offspring during the first pregnancy after sc administration of IMVAMUNE[®] (Modified Vaccinia Ankara, MVA-BN[®]) vaccine. Animals (32 [Gs 1 and 3] or 34 [G 2]/group) were assigned to three different groups and treated by 0.5 mL sc injections of control or test article. Test article was administered at 14 days prior to the day of sperm positivity, and at GDs 0 and 14.

Serum samples for immunology were collected from dams prior to the first dose (SD -14), on GD's 0 and 14, and on pp days 0, 7, and 28. Serum samples were also collected from pups on pp days 0, 7, and 28. Milk samples were collected from the pups' stomachs on pp days 0 and 7. Samples were analyzed by an ELISA to detect antibodies to Modified Vaccinia Ankara virus (MVA-BN[®]).

Clinical symptoms, mortality, body weight, necropsy findings, characteristics of pregnancy and the delivery process, and the nursing instinct of the dams were evaluated. Pups were weighed and developmental landmarks (surface-righting reflex, pinna detachment, incisors' eruption, and eye opening) were evaluated. Dams were sacrificed on pp day 28 and implantation sites were counted.

No test article-related effects on mortality, clinical observations, body weight, body weight changes, or food consumption during the pre-mating period were reported. No test article-related effects on gross examination, number of implantations, litter size, number of viable pups and their sex ratio, percent intrauterine mortality, percent extrauterine mortality, pup body weight, and developmental landmarks were reported.

Percentage of positive surface-righting reflex on pp day 0 in Gs 2 and 3 were significantly different from G1. Percentage of pinna unfolding on pp day 1 was statistically lower in G3 when compared to G1. Percentage of pups with incisors erupted on pp day 9 was statistically increased in Gs 2 and 3 when compared to G1. Percentage of pups with eyes opening on pp day 14 was statistically increased in G3 when compared to G1.

There were no detectable anti-MVA-BN[®] antibodies in the serum collected pretest from all female rats. All dams in G's 2 and 3 had an antigen-specific antibody response after the second immunization. Animals in G3 were having greater antibody responses, in serum and milk samples, than G2.

Conclusions

The administrations of MVA-BN[®] vaccine on SD -14, and GD's 0 and 14 at 500µL via the sc route did not give an indication of F₀ generation toxicity. Serological analysis data indicated a robust antibody response in dams and conferred passive immunity to their litters.

Communication to Sponsor:

It has been reported in the results and discussion section of the antibody analysis report (page I-4) that "the antibody responses results are presented in figure I-1 and tables I-1 and I-2. In addition, the individual animal data are presented in tables I-3 and I-4". However, the figure and the tables were not found in this report.

Study # 3: Developmental toxicity (teratology) study of subcutaneously administered MVA-BN vaccine in pregnant (b) (4) rats (study number M349-05) [Reviewed by Marion Gruber].

Background: This submission contains the final study report of the study BN-PRE-05-021/M349-05 (Developmental toxicity study of subcutaneously administered MVA-BN vaccine in pregnant (b) (4) rats) and a nonclinical briefing document to support BN's position to establish ICH compliance with regard to the number of treated animals and obtaining a category B labeling statement because of the results of BNs developmental toxicity study that was conducted. On December 19, 2005, amendment 15 was submitted for study BN-PRE-05-021/M349-05 "Developmental toxicity study of subcutaneously administered MVA-BN vaccine in pregnant (b) (4) rats." On January 11, 2006, amendment 18 was submitted containing a protocol entitled "Pre- and postnatal developmental toxicity study of subcutaneously administered MVA-BN vaccine in pregnant rats (PRE-06-005-M351-05) and on March 31, 2006, amendment 23 was submitted containing a protocol entitled "Developmental toxicity study of subcutaneously administered MVA-BN vaccine in pregnant (b) (4) rabbits (BN-PRE-06-004/M350-06). According to the sponsor, the sponsor did receive verbal agreement from CBER, i.e., Dr. Sally Hargus, via Tim Nelle, CBER on June 2, 2006. This submission contains the final study report for study BN-PRE-05-021/M349-05 (Developmental toxicity study of subcutaneously administered MVA-BN vaccine in pregnant (b) (4) rats) and is summarized in the following.

Testing facility: (b) (4)

Study initiation date: December 16, 2005

Principal investigator: (b) (4), (b) (6)

Sponsor: Bavarian Nordic A/S
Kvistgard/Denmark

Sponsor study number: BN-PRE-05-021

Purpose: To assess the effects of MVA-BN vaccine in pregnant females and their developing conceptuses during the first pregnancy in (b) (4) rats after subcutaneous (sc) administration

Study conducted in compliance with US FDA GLP, 21 CFR, part 58

Vaccine: Modified Vaccinia Ankara (MVA; chick embryo fibroblast cells) Smallpox Vaccine, Live
Test article: MVA-BN vaccine, at least 1×10^8 TCID₅₀/vial, liquid frozen in TBS, pH (b) (4), supplied by (b) (4), lot number 170505

Control: Tris buffered saline, pH (b) (4), 10mM TRIS, 140 mM NaCL, pH (b) (4), supplied by (b) (4), lot number (b) (4)

The species chosen were (b) (4) rats, (b) (4), 13.5 – 16.5 weeks old at day -14, body weight range 215 to 256 g. The test article was administered SC (0.5 ml/rat/occasion) at days -14 (14 days prior to sperm positivity) and on gestation day (GD) 14. The vaccine dose was 0.5 ml of 1×10^7 TCID₅₀/ml (low dose) or 0.5 ml of a 1×10^8 TCID₅₀/ml solution (high dose).

Each week 30 females were randomized until 120 females were obtained. Females were randomly assigned to treatment groups using a computerized body weight stratification procedure, the day that a sperm positive vaginal smear was found was considered day 0 of pregnancy.

Table 73: Study design (study #3 repro)

Treatment/Group	Group size	Dose level (human dose/adm.)	Dose volume (ml/animal)
Control/Caesarean	24	TBS	1x 0.5
Treated/Caesarean	24	1 x 10 ⁷ TCID ₅₀ /ml (low dose)	1x 0.5
Treated/Caesarean	24	1 x 10 ⁸ TCID ₅₀ /ml (high dose)	1x 0.5

Observations:

1. Morbidity and mortality: Adults 2x daily
2. Clinical signs: 2x daily cage side observations, individual exam 1x weekly
3. Body weights weekly during premating, on GD's 0, 3, 6, 9, 13, 16, and 20
4. Food consumption: Days 0-6, 6-9, 9-13, 13-16, and 16-20
5. Blood samples obtained from all dams prior to initial vaccination, and in week 3 (mating sperm positivity), on day 7, and on day 20, IgG abs specific for MVA-derived antigen were determined (analysis not conducted under GLP)
6. Pregnancy and parturition: Gross pathology of dams viscera, number of corpora lutea in each ovary and implantation in each uterine horn, number of live fetuses, early and late embryonic deaths and fetal deaths, individual fetal weights, fetal sex, fetal external examination, one half of each litter subjected to visceral examination and the other half to skeletal examination

Organs and tissues of dams with macroscopic findings were examined histologically, all organs were fixed in 10% buffered formalin solution after necropsy.

Data evaluation

Data concerning the control pregnant females and historical data were used to evaluate the effects of the test item. Mean and standard deviation were calculated for all parameters were feasible, using the litter as the basic sample unit.

Results

Number of paired females, number of sperm positive females and pregnancy data are shown in the table below:

Table 74: Pregnancy data (study #3 repro)

Dose groups	TBS		1 x 10 ⁷ MVA-BN		1 x 10 ⁸ MVA-BN	
	No	%	No	%	No	%
Paired females	40		30		30	
Sperm positive females	24	60	24	80	24	80
Signs of implantations	21		21		21	
Dams dead	0		1		0	
Dams with clinical symptoms	3	17	2	9	4	19
Non-pregnant females	3	17	2	9	1	5
Dams with <5 implant.	2	11	0	0	1	5
Dams excluded due to day 21 C-section/high fetal body weights	1	6	0	0	1	5
Dams with complete intrauterine death	0		0		0	
Dams with macroscopic abnormalities	13	72	10	45	17	81
Dams evaluated	18		22		21	

Mortality/clinical signs

There were no treatment related deaths. One dam in the low dose MVA-BN dose groups died accidentally during blood collection at terminal sacrifice on day 20. Clinical symptoms recorded sporadically during premating and gestation included alopecia, discolored, reddish, ruffled fur, and soft stool. These occurred across all groups including the control. The body weight profiles of the female rabbits were essentially similar in the vaccinated and unvaccinated groups before mating and throughout gestation.

Table 75: Body weight gains [gram, prior to mating] (study #3 repro)

Group	Day 1	Day 8
TBS		
Mean	249	257
S.D.	18	18
N	40	40
1x10⁷ MVA		
Mean	247	258
S.D	13	14
N	40	40
1x10⁸ MVA		
Mean	247	257
S.D	16	17
N	40	40

Table 76: Gestation body weight gains [gram] (study #3 repro)

Group		0	3	6	9	13	16	20
TBS	Mean	265	273	287	294	318	337	406
	SD	18	19	20	22	25	27	37
	N	18	17	18	18	18	18	18
1x10⁷ MVA	Mean	258	270	281	287	312	329	396
	SD	15	21	18	18	20	22	26
	N	22	16	22	22	22	22	22
1x10⁸ MVA	Mean	257	269	280	287	310	331	399
	SD	12	11	11	12	12	13	18
	N	21	16	21	21	21	21	21

Food consumption

Raw data showed that maternal food consumption was not adversely influenced by vaccination throughout gestation (not shown in this review).

Necropsy findings of adult females

Macroscopic findings at day 20 occurred in 13/24, 10/24, and 17/24 in the control, the low dose and the high dose MVA groups, respectively. The findings included discoloration in some organs, in particular the lungs (but also kidney, uterus, ovary, and thymus) and was attributed by the sponsor to the euthanasia of the dams.

Caesarean data

Eighteen (18) pregnant control dams, 22 vaccinated dams in the low dose MVA group and 21 vaccinated dams in the high dose MVA group were submitted to Caesarean examination on day 20 of gestation.

Table 77: Caesarean data (study #3 repro)

Group		TBS	1x10 ⁷ MVA	1x10 ⁸ MVA
Corpora lutea	Mean	16.1	15.5	15.7
	SD	2.3	2.4	1.4
	N	18	22	21
Implan-tations	Mean	14.3	13.9	14.7
	SD	3.25	2.9	1.62
	N	18	22	21
Early re-sorptions	Mean	1.6	1.5	1.3
	SD	1.38	1.26	0.9
	%	12	10.7	8.8
Late re-sorptions	Mean	0	0	0
	SD	0	0	0
	N	0	0	0

Group		TBS	1x10 ⁷ MVA	1x10 ⁸ MVA
Dead fetuses				
	Mean	0	0.1	0
	SD	0	0.64	0
	%	0	1	0
% pre-impl.		10.7	10.7	6.1
	Mean loss	15.83	12.50	5.79
	SD	18	22	21
% post-impl.		12.0	11.8	8.8
	Mean loss	11.08	10.42	6.02
	SD	18	22	21
Viable fetuses				
	Mean	12.7	12.2	13.4
	SD	3.54	2.91	1.78
Total intrauterine mortality				
	Mean	3.3	3.3	2.2
	SD	2.83	2.23	1.0
	%	29	26	15.6
Male fetuses				
	% Mean	49.9	47.1	49.0
	SD	15.62	13.71	10.70
Female fetuses				
	% Mean	50.1	52.9	51.0
	SD	15.62	13.71	10.70
Fetal body weight	Mean	3.7	3.74	3.81
	SD	0.17	0.266	0.179
	N	18	22	21

1. Pre-implantation data and post-implantation data

The number of corpora lutea, number of uterine implantation sites and percentage pre-implantation loss were comparable in the treated and control groups

The incidences of early and late resorptions and resulting post-implantation loss were comparable in the vaccinated group and in the control group. Three fetuses were recorded as dead in litter number 5045 in the low dose MVA group. The examination revealed umbilical hernias in 2 fetuses. No alterations were found in the third fetus.

2. Fetal data: The proportion of male to female fetuses were comparable between groups. The mean fetal weight was comparable between groups.

Fetal examinations

External, visceral and skeletal examinations are summarized in table 78. For external and visceral examinations of the great vessels 229, 269 and 282, fetuses were examined from dams in the control group (18 litters), the low dose (1 x 10⁷ MVA-BN) (22 litters) and the high dose (1 x 10⁸ MVA-BN) (21 litters) groups, respectively. For complete visceral examinations, 121, 142, and 150 fetuses were examined from dams in the control group, the low dose (1 x 10⁷ MVA-BN) and the high dose (1 x 10⁸ MVA-BN) groups, respectively. For skeletal examinations, 108, 127 and 132 fetuses were

examined from dams in the control group, the low dose (1×10^7 MVA-BN) and the high dose (1×10^8 MVA-BN) groups, respectively.

Sponsor states that for all evaluation, i.e. external, visceral and skeletal, findings with a relative frequency of at least 4% were considered typical (in the author's laboratory?) and were therefore not considered to be abnormalities in the evaluation of the groups examined.

Comment 1: Sponsor includes reference Hood 2006, developmental and reproductive toxicology, 251-256, to support this statement. Sponsor should provide historical control data obtained in the laboratory in which the study was conducted to support this approach.

External findings: The body weight of a fetus was classified as retarded when it was below the average minus a 2-fold standard deviation of all control fetuses. This was the case for 3.2%, 3%, and 1.6% in the control, the low, and the high dose vaccinated groups, respectively. Thus, there were no significant differences in body weight across groups. In addition, there were no gross fetal abnormalities observed in viable fetuses in the control group or the treated groups.

Visceral findings: Direct origin of the subclavian and common carotid arteries from the aortic arch were in 1 fetus in the control group, 2 in the low dose, and 1 in the high dose group. A hydro-ureter with dilated renal pelvis was found in one fetus in the high dose MVA group. Variation has been observed in (b) (4) rats with an incidence of 5.9%.

Skeletal findings: The incidence of skeletal malformations was similar between the control and the treated groups. Overall, skeletal variations did not differ significantly between the 3 groups. Five fetuses were found with malformations, one in the control group (bifurcate rib, small vertebral arch), one in the low dose MVA group (small vertebral arch), and three in the high dose group (hemi centric vertebral body and irregular ossification of several vertebral bodies or spine on the upper surface of vertebral body). However, the difference was not significant and considered spontaneous.

Comment 2: Sponsor cites historical control data from (b) (4), e.g., cumulative data of (b) (4) rats 1994, from 120 litters with a total of 1,663 fetuses, in studies performed in (b) (4). In study BN-PRE-05-021/M349-05, hydroureter with dilated renal pelvis was found in one fetus in this study in the high dose MVA group. For comparison, this variation was observed with an incidence of 5.9% in the cumulative control data. The multiple skeletal anomalies found in one fetus in the high dose MVA group (2.3%) have an incidence of 0.5 % in the cumulative control data. In one fetus, os pubis and ischii were not ossified in the low dose MVA group. This abnormality was not seen in the control group, but sponsor states that this abnormality has been seen in (b) (4) rats with an incidence of 2.2% in the cumulative control data. It is conceivable that these abnormalities are spontaneous, however sponsor should comment on historical control data at the testing facility.

Table 78: External, visceral, and skeletal examination (study #3 repro)

Groups		Control	1×10^7 MVA	1×10^8 MVA
External examination				
Retarded in weight	Total %	8 3.4	8 3.4	5 1.8
Variation	Total %	8 3.4	8 2.9	5 1.8
Malformation	Total %	0 0	0 0	0

Groups		Control	1 x 10 ⁷ MVA	1 x 10 ⁸ MVA
External examination				
Visceral examination (great vessels)				
Variation	Total %	0	0 0	0 0
Malformation	Total %	1 ^b 0.4	2 ^b 0.7	1 ^b 0.4
Visceral examination (complete)				
Variation	Total %	0	0	1 ^a 0.7
Malformation	Total %	0 0	0	0
Skeletal Examination				
Variation	Total %	20 18.5	33 26	22 16.7
Malformation	Total %	1.0 0.9	1 ^c 0.8	3.0 ^c 2.3

- Hydroureter with dilated renal pelvis
- Direct origin of the subclavian and common carotid arteries from the aortic arch pelvis
- Bifurcate rib, small vertebral arch, vertebral bodies hemi centric + irregular ossification of several vertebral bodies, spine on the upper surface of vertebral body

Antibody assessments

In this study, an ELISA to measure rat antibodies against MVA-BN was developed to measure antibody titers in serum samples from pregnant rats. Overall, MVA-BN yielded an antibody response in pregnant rats. The higher dose produced a significantly greater antibody response than the lower dose.

Assessment

Caesarean data were normal and were similar in both groups. No dams were found with complete intrauterine death. The mean maternal body weight gain during gestation and food consumption was similar between groups. Individual fetal external examinations, visceral, and skeletal observations were similar in control and treated groups.

Control group: In the control group, weight retardation occurred in 3% of the fetuses. Visceral examination one malformation was observed (direct origin of right carotid and subclavian arteries from the aortic arch). In the skeletal examination, 2 types of malformations were observed in one fetus (small vertebral arch and bifurcate ribs).

Variations occurred as delayed development of the skeletal system (e.g. not ossified sacral vertebrae, irregular calcification of the skull).

Low dose group: In the 1 x 10⁷ MVA group, weight retardation occurred in 3% of the fetuses. In the visceral examination two malformation were observed (direct origin of right carotid and subclavian arteries from the aortic arch). In the skeletal examination, one malformation was observed in one fetus (small vertebral arch). Variations occurred as delayed development of the skeletal system (e.g. not ossified sacral vertebrae, irregular calcification of the skull).

High dose group: In the 1×10^8 MVA group, weight retardation occurred in 2% of the fetuses. External/visceral examinations revealed umbilical hernias in 2 fetuses. In the visceral examination one variation (hydroureter with dilated renal pelvis) and one malformation (direct origin of right carotid and subclavian arteries from the aortic arch) occurred. In the skeletal examination, two types of malformations were observed in three fetuses hemi centric vertebral body and irregular ossification of several vertebral bodies or spine on the upper surface of vertebral body (small vertebral arch). Variations occurred as delayed development of the skeletal system (e.g. not ossified sacral vertebrae, irregular calcification of the skull).

In summary, differences observed were not significant between groups, involved only few fetuses across different litters and occurred in the control as well as vaccinated groups. They can be considered isolated findings rather than vaccine related events.

In this particular animal model and under the test conditions studied, data derived from the Caesarean subgroup, i.e., body weights, Caesarean data and necropsy findings from adult females as well as fetal examinations, suggest that the MVA-BN vaccine does not affect pre-natal development and had no teratogenic effects.

Comments for the sponsor: Sponsor should clarify the following statement:

1. Sponsor should be asked to clarify the statement that “For all evaluations, i.e. external, visceral and skeletal, findings with a relative frequency of at least 4% were considered typical and were therefore not considered to be abnormalities in the evaluation of the groups examined.” Reference is made to a publication by Hood 2006, developmental and reproductive toxicology, 251-256, but sponsor should provide further rationale for this statement.
2. Sponsor cites historical control data from (b) (4), e.g., cumulative data of (b) (4) rats 1994, from 120 litters with a total of 1,663 fetuses, in studies performed in (b) (4) to put study results into perspective. Sponsor should comment on the availability of historical control data at the current testing facility from more recent studies conducted as incidence rates may change over time and may depend on testing facility.
3. Dams with five or fewer implantations were excluded from the statistical analysis, two dams in the control group and one dam in the high dose MVA group were excluded for this reason. Although this exclusion may not have affected the overall data outcome, since 2 controls were excluded, the sponsor should provide a rationale for this exclusion.
4. Three studies were conducted to assess the potential teratogenic effect of MVA- BN vaccine: A) Study BN-PRE-05-021/M349-5 entitled: Developmental toxicity study of sc administered MVA-BN vaccine in pregnant (b) (4) rats. This study is summarized in this review. B) Study PRE-06-005/M351-05 entitled: Peri and postnatal developmental toxicity study of sc administered MVA-BN vaccine in pregnant rats. Sponsor states that the final study report is pending and will be submitted. C) Study BN-PRE-06-004/M350-06 entitled: Developmental toxicity study of sc administered MVA-BN vaccine in pregnant (b) (4) rabbits.

Sponsor expresses concern study BN-PRE-06-004/M350-06 which was recently finished had only 12 animals in the control group, and 14 and 15 animals in the low and high dose group, respectively. Thus, the number of animals evaluated is below the number recommended in applicable guidance documents, i.e., ICH5a. Sponsor states that a preliminary review of study results revealed that observed malformations were within the historical control range. Sponsor requests CBER comment if the number of animals studied is sufficient.

Sponsor should provide an explanation/rationale for the low number of animals/group. However, since CBER only requires one study in one animal species and the sponsor has conducted three studies (2 in rodents, one in non-rodent), there would be no requirement to repeat the study, unless data from the study in rabbits raise concern regarding teratogenic effects. Study BN-PRE-06-004/M350-06 conducted in rabbits may be used as supportive data.

5. Sponsor asks if the results of these three reproductive toxicology studies will be sufficient to grant a category B label claim for MVA-BN. A number of clinical studies conducted in the 1950s and 1960s with then licensed smallpox vaccine, including prospective ones, have attempted to ascertain the fetal risk resulting from smallpox vaccination in pregnancy. These involved 8,613 vaccinated pregnant women and 6542 controls. Although some of the studies were of retrospective design and some were lacking appropriate control groups, review of these studies allows the following overall conclusion: Published data available from human pregnancy outcomes provide evidence that vaccination with vaccinia virus induces rare but well-documented cases of fetal vaccinia and miscarriage (Toendury and Foukas, 1964, Green, 1966). Results of studies evaluating the effect of vaccination during pregnancy on fetal development suggest that the incidence of congenital malformation was not higher in vaccinated women compared to unvaccinated controls (Greenberg et al 1947, Mac Arthur et al 1952, Abramowitz et al (1957), Bourke et al (Brit. Med Journ, 1964), Bellows et al (1949), Bieniarz and Dabrowski (1956), Saxen et al, Am. J. Pub. Health, 1968, Teodorescu et al Rev. Roum. Med., 1975, Topciu et al, Zbl. Bakt. Hyg., 1976). However, some studies suggest that vaccination of pregnant women during the first trimester significantly increases fetal mortality/spontaneous abortion (Mac Arthur et al 1952). Information about the vaccinia strain(s) used in these clinical study was not always available. However, it can be assumed that immunization occurred with vaccinia strains available in the various countries during the specified time periods.

Historical human experience with smallpox vaccine is critical information that would need to be prominently displayed in the label, and would form the basis for contraindicating vaccine in pregnancy under non-emergent conditions. "Negative" data in animals will not change the implications that the human data have for use of their vaccine. In summary, clinical studies in which pregnant women were vaccinated with smallpox vaccine suggested increase fetal mortality and spontaneous abortions, in particular in the first trimester, as well as rare cases of fatal fetal vaccinia as a result of vaccination. Thus, in light of these clinical data, the pregnancy category for MVA-BN vaccines would not be a category B, regardless of the nonclinical study outcomes.

Study # 4: Study for effects on embryo-fetal development by subcutaneous route in rats (study number 40400 RSR).

Key study findings: No significant findings were reported.

Study no.: 40400 RSR

Conducting laboratory and location: (b) (4)

Date of study initiation: 11/19/2013

Date of study completion: 07/10/2014

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity:

<u>Test article</u>	<u>Batch/Lot #</u>	<u>Purity %</u>
IMVAMUNE®	C00005 (F00104)	NR*
TRIS-buffered Saline (TBS)	(b) (4)	NR*

* NR = Not reported

Methods:

Doses: Test article or control groups were dosed at 0.5 mL per subcutaneous (sc) injection (resulting in at least a dose of 5.0×10^7 TCID₅₀/animal per treatment). Rats were immunized 2 times on study days 0 and 6 post-coitum (p.c.).

Study design: Animals were assigned to 2 different groups. Twenty five animals per group were received 2 immunizations on study days 0 and 6 post-coitum (p.c.). The details of the study design are listed in the following table:

Table 79: Study design (study #4 repro)

GROUP	TREATMENT	DOSE LEVEL (µL)	DAYS OF DOSING (FEMALES ONLY)	NUMBER ASSIGNED PER GROUP
				FEMALES
1	PBS	500	Study days 0 and 6	25
2	1X10 ⁷ TCID ₅₀ IMVAMUNE®	500	Study days 0 and 6	25

Species/strain: (b) (4) rats

Number/group: 25 per group.

Age:

Males: 12 weeks

Females: 10-11 weeks

Weight: 226 g to 313 g

Source: (b) (4)

Route, volume, and frequency of test article administration: Animals were treated with single sc injection of 500 µL at study days 0 and 6 post-coitum.

Parameters and endpoints evaluated:

The following parameters were evaluated: Clinical observations (once a day and at least twice a day during and after the treatment period and an individual examination was performed); clinical signs

(once a day); body weight (study days 0, 2, 4, 6, 9, 12, 15, 18, and 21 *p.c.*); food consumption (study days 0-2, 2-4, 4-6, 6-9, 9-12, 12-15, 15-18 and 18-21 *p.c.*); immunogenicity (pre-test and day 21); and blood samples from fetuses (day 21 *p.c.*) .

Post-mortem examination: The ovaries and uterus of the females were examined to determine; gravid uterus weight, number of *corpora lutea*, number and distribution of dead and live fetuses, number and distribution of early and late resorptions, number and distribution of uterine scars, number and distribution of implantation sites, fetus body weight, sex of fetuses.

Fetuses' evaluations:

External, soft tissue, and skeletal examination on all fetuses was conducted. External examination includes observation of all visible structures, surfaces, and orifices. Soft tissue examination included the observation of all the organs and structures of the neck, thorax, and abdomen. The fetuses were then eviscerated and were fixed with (b) (4) for examination of the structures of the head. Skeletal examination included a detailed examination of the skeleton (bones) after (b) (4). This examination included the observation of all the bone structures of the head, spine, rib cage, pelvis, and limbs.

Statistical methods

Mean values were compared by one-way analysis of variance and Dunnett test (mean values being considered as normally distributed and variances being considered as homogeneous). Percentage values were compared by Fisher exact probability test.

Results:

Clinical observation:

Pregnancy status:

At termination on day 21 *p.c.*, there were 21 and 22 rats with live fetuses and 4 and 3 non-pregnant females in the control and test article-treated groups, respectively.

Mortality: No test article-related premature deaths were reported.

Clinical signs: No test article-related effects on clinical signs were reported. One group 2 female (B26437) had a cutaneous lesion on abdomen (a common finding in this species and strain).

Body weight

Mean body weight and mean body weight changes are summarized in the following table:

Table 80: Mean body weight and body weight changes (study #4 repro)

Group	Control item	Test item
Body weight (g)		
. Day 0 <i>p.c.</i>	263	262
. Day 6 <i>p.c.</i>	294	294
. Day 21 <i>p.c.</i>	439	439
Body weight change (g)		

Group	Control item	Test item
. Days 0 - 6 <i>p.c.</i>	+31	+33
. Days 6 - 21 <i>p.c.</i>	+146	+145

No test article-related effects on mean body weight and mean body weight gains were reported. Mean carcass weights and mean net body weight changes are summarized in the following table:

Table 81: Mean carcass weights and mean net body weight changes (study #4 repro)

Group	Control item	Test item
Carcass weight (g)	333.2	338.7
Net body weight change from day 6 <i>p.c.</i>	39.4	44.3

No test article-related effects on mean carcass weight and mean net body weight change were reported.

Food consumption

Mean food consumptions (g/animal/day) are summarized in the following table:

Table 82: Mean food consumption [g/animal/day] (study #4 repro)

Group	Control item	Test item
Day 0 - 2 <i>p.c.</i>	25	25
Days 6 - 9 <i>p.c.</i>	28	27
Days 18 - 21 <i>p.c.</i>	31	33

No test article-related effects on mean food consumption were reported.

Immunogenicity evaluation

In the control group, no vaccinia-specific Ig antibodies were reported in the fetus serum samples (pooled per litter) from pregnant females. False positive (approximately 16%) with titers between 50 and 227 were reported in females before treatment and control group. In group 2, 100% of the females had high vaccinia-specific Ig antibody with a geometric mean titer (GMT) of 7522.6 and titers ranging from 1435.00 to 42410.50. In the sera of their fetuses, the antibody levels (pooled per litter) were a little lower with a GMT of 2575.21. However, 100% sero-conversion was reported and titers were ranging from 1212.00 to 18465.00.

Table 83: Seroconversion rates and GMTs determined by ELISA (study #4 repro)

	Group 1			Group 2		
	Control Item (TBS)			Test Item (IMVAMUNE)		
Day	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³
Pre-test Females	16	1,9	25	12	1,7	25
Day 21 <i>p.c.</i> Females	20	2,4	25	100	7522,6	25
Day 21 <i>p.c.</i> fetuses pooled per litter	0	1,0	20	100	2575,2	22

¹% Sero-conversion

²Geometric mean titer

³Number of animals

Maternal terminal examinations

Gravid uterus weight

Mean gravid uterus weights are summarized in the following table:

Table 84: Mean gravid uterus weights (study #4 repro)

Group	Control item	Test item
Gravid uterus weight (g)	106.3	100.5

No test article-related effects on mean gravid uterus were reported

Macroscopic post-mortem examination

No test article-related effects on macroscopic evaluation were reported. One finding (dilated renal pelvis) was reported in one group 2 female (B26418).

Hysterectomy data

Hysterectomy data are presented in the following table:

Table 85: Hysterectomy data (study #4 repro)

Group	Control item	Test item
Number of females with live fetuses at termination	21	22
Mean number of <i>corpora lutea</i> per animal	16.6	16.4
Mean number of implantations per animal	14.6	14.2
Mean pre-implantation loss (%)	11.8	13.5
Mean number of fetuses per animal	14.0	13.5
Dead fetuses (%)	0	0
Mean number of implantation scars per animal	0	0
Mean number of early resorptions per animal	0.7	0.7
Mean number of late resorptions per animal	0	0
Mean post-implantation loss (%)	4.2	4.6

No test article-related effects on mean hysterectomy data were reported.

Fetal examinations

Fetal sex

Mean percentages of male fetuses are presented in the following table:

Table 86: Mean percentage of male fetuses [%] (study #4 repro)

Group	Control item	Test item
Mean percentage of male fetuses (%)	48.3	44.5

No test article-related effects on sex ratio were reported.

Fetal weights

Mean fetal body weights are presented in the following table:

Table 87: Mean fetal body weight [g] (study #4 repro)

Group	Control item	Test item
Mean fetal body weight (g)	5.56	5.45

No test article-related effects on mean fetal body weight were reported.

External examination

No external variations and no external malformations were reported.

Table 88: Fetal external malformation (study #4 repro)

		Control item	Test item
Litters Evaluated	N	21	22
Fetuses Evaluated	N	293	297
Live	N	293	297
Dead	N	0	0
Total fetal external malformations			
Fetal Incidence	N	0	0
	%	0.0	0.0
Litter Incidence	N	0	0
	%	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0	0.0
	S.D.	0.0	0.0

Statistical key: f=Fishers exact test

Table 89: Fetal external variations (study #4 repro)

		Control item	Test item
Litters Evaluated	N	21	22
Fetuses Evaluated	N	293	297
Live	N	293	297
Dead	N	0	0
Total fetal external variations			
Fetal Incidence	N	0 f	0
	%	0.0	0.0
Litter Incidence	N	0 f	0
	%	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0	0.0
	S.D.	0.0	0.0

Statistical key: f=Fishers exact test

Soft tissue examination

No visceral malformations and no test article-related visceral variations were reported. The recorded variations (dilated cerebral ventricle, liver with colored focus and short innominate artery) were of common background in (b) (4) fetuses.

Table 90: Fetal soft tissue malformations (study #4 repro)

		Control item	Test item
Litters Evaluated	N	21	22
Fetuses Evaluated	N	140	145
Total fetal soft tissue malformations			
Fetal Incidence	N	0 f	0
	%	0.0	0.0
Litter Incidence	N	0 f	0
	%	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0	0.0
	S.D.	0.0	0.0

Statistical key: f=Fishers exact test

Table 91: Fetal soft tissue variations (study #4 repro)

		Control item	Test item
Litters Evaluated	N	21	22
Fetuses Evaluated	N	140	145
BRAIN			
Litter Incidence	N	1	0
Fetal Incidence	N	1	0
DILATED CEREBRAL VENTRICLE	N	1 f	0
	%	0.7	0.0
Litter Incidence	N	1 f	0
	%	4.8	0.0
Affected Fetuses/Litter	MEAN%	0.8 d	0.0
	S.D.	3.6	0.0
LIVER			
Litter Incidence	N	1	0
Fetal Incidence	N	1	0
LIVER: COLORED			
Fetal Incidence	N	1 f	0
	%	0.7	0.0
Litter Incidence	N	1 f	0
	%	4.8	0.0
Affected Fetuses/Litter	MEAN%	0.6 d	0.0
	S.D.	2.7	0.0
VESSELS			
Litter Incidence	N	2	2
Fetal Incidence	N	2	2
SHORT INNOMINATE			
Fetal Incidence	N	2 f	2
	%	1.4	1.4
Litter Incidence	N	2 f	2
	%	9.5	9.1
Affected Fetuses/Litter	MEAN%	1.5 d	1.1
	S.D.	4.7	3.7
TOTAL FETAL SOFT VARIATIONS			
Fetal Incidence	N	4 f	2
	%	2.9	1.4
Litter Incidence	N	4 f	2
	%	19.0	9.1
Affected Fetuses/Litter	MEAN%	2.9 d	1.1
	S.D.	6.1	3.7
Litters Evaluated	N	21	22
Fetuses Evaluated	N	140	145

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

Skeletal examination

No test article-related effects on skeletal malformations and skeletal variations were reported. Variations recorded were of common background in (b) (4) fetuses.

Fetal skeletal malformations (summary table)

Table 92: Fetal skeletal malformations (study #4 repro)

		Control	Test item
Litters Evaluated	N	21	22
Fetuses Evaluated	N	153	152
Total fetal skeletal malformations			
Fetal Incidence	N	0f	0
	%	00	00
Litter Incidence	N	0f	0
	%	00	00
Affected Fetuses/Litter	MEAN%		0.0
	S.D.		0.0

Statistical key: f=Fishers exact test

Conclusion

Vaccinia-specific Ig antibody responses were reported in pregnant females on day 21 p.c. as well as in their fetuses. IMVAMUNE® administered at a standard dose of at least 5.0×10^7 TCID₅₀/animal during early gestation in rats was not associated with maternal or developmental toxicity.

OVERALL SUMMARY:

General toxicology:

Four general toxicology studies were submitted to support this BLA. All studies were repeat dose studies. Adequate nonclinical toxicology data were included in these studies. Based on nonclinical toxicity assessments of the above mentioned studies there were no significant safety issues to preclude the BLA from approval. The delivery of an active dose of the product was verified.

Reproductive studies:

In four developmental toxicity studies, the effect of smallpox vaccine (live, attenuated) on embryo-fetal and post-natal development was evaluated in pregnant rabbits and rats.

Rabbits and rats were administered a full human dose [0.5 mL] of smallpox vaccine by subcutaneous injection 3 times or 2 times. No vaccine-related fetal malformation or variations and adverse effects on pre-weaning development were reported in these studies. These findings are described in section 8.1 of the PI.

Genotoxicology studies:

No genotoxicology studies were submitted to support this BLA.

Package Insert Part Review

In compliance with PLLR, section 8.1 is recommended to revise as marked below:

USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk summary

A developmental and reproductive toxicity study was performed in female rabbits and rats given a full human dose (0.5 mL) of JYNNEOS (live, attenuated) by subcutaneous injection on three or two occasions. The study showed no teratogenic potential, and no effect on pre- or post-natal development. [see *Animal Data (8.1)*]

Clinical Considerations

Maternal Adverse Reactions

Because systemic and local adverse reactions with immunotherapy may be poorly tolerated during pregnancy, JYNNEOS should be used during pregnancy only if clearly needed.

Animal data

In four developmental toxicity studies, the effect of JYNNEOS (live, attenuated) on embryo-fetal and post-natal development was evaluated in pregnant rabbits and rats.

Rabbits and rats were administered a full human dose [0.5 mL] of JYNNEOS by subcutaneous injection 3 times or 2 times. No vaccine-related fetal malformation or variations and adverse effects on pre-weaning development were reported in these studies.

13.1 Genotoxicity data:

Carcinogenesis, Mutagenesis, Impairment of Fertility

JYNNEOS has not been evaluated for carcinogenic or mutagenic potential.

Overall conclusion:

Based on the nonclinical toxicity assessments of the smallpox vaccine (live, attenuated) submitted in this BLA, there were no significant safety issues to preclude the BLA from approval.

Concurrence: Martin D. Green

References:

- 1- Fenner F, Henderson DA, Arita I, Ježek Z, Ladnyi ID. Smallpox and its eradication. World Health Organization, Geneva 1988;1–1460.

Historical data

(b) (4) REFERENCE CONTROL DATA

Species : (b) (4) Rats
 Breeder : (b) (4)

This document presents:

- . Food consumption
- . Body weight
- . Hysterectomy data
- . Fetal body weights
- . External, soft tissue, skeletal examination data

Seven studies cover a period ranging from February 2008 to March 2012

(end of in vivo phase of studies is considered).

	1	2	3	4
Study Number	33992	34110	35878	36618
Vehicle	Mannitol in water for injection	Carboxymethyl- cellulose 0.5% + Tween 80 0.5% in purified water	20% hydroxypropyl -β-cyclodextrin in purified water	Methylcellulose 0.5% in purified water
Administration route	Gavage	Gavage	Gavage	Gavage
Administration volume	5 mL/kg/day	10 mL/kg/day	5 mL/kg/day	5 mL/kg/day
Study Number	5 37158	6 37178	7 38586	
Vehicle	Hydroxypropyl- methylcellulose 0.5% in purified water	10% Trehalose dehydrate in sterile water for injection	Purified water	
Administration route	Gavage	Subcutaneous	Gavage	
Administration volume	5 mL/kg/day	1 mL/kg/day	5 mL/kg/day	

The experimental conditions were set as follows:

Species: Rat
 Strain: (b) (4)
 Sanitary Status: (b) (4)
 Age: 9-12 weeks old
 Breeder: (b) (4)
 Environmental conditions:
 . temperature : 22 ± 2°C
 . relative humidity : 50 ± 20%
 . light/dark cycle : 12h dark/12h light
 . ventilation : about 12 cycles/hour of filtered, non-recycled air.
 Diet: (b) (4)
 (b) (4) *ad libitum*
 Water: Filtered tap water *ad libitum*

STRAIN ALL
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REPRODUCTION DATA

			RANGE OF STUDY MEANS	
			MINIMUM	MAXIMUM
Inseminated females	N	162		
Pregnant	N	160		
	%	98.8	95.8	100.0
Aborted	N	0		
Premature delivery	N	2		
Dead	N	0		
Alive, pregnant at term	N	150		
-with all resorptions	N	0		
-with all dead fetuses	N	0		
-with live fetuses	N	150		
Corpora lutea				
Total		2225		
Mean		14.9	14.0	15.5
Implantation Sites				
Total		2023		
Mean		13.5	12.8	14.0
Preimplantation Loss	MEAN%	9.3	7.2	13.9
Dead fetuses				
Total		11		
Mean		0.07	0.00	0.38
Resorptions				
Total		111		
Mean		0.74	0.30	1.00
Postimplantation Loss	MEAN%	6.5	2.0	8.7
Live fetuses				
Total		1901		
Mean		12.7	12.0	13.2
Implantation sites	MEAN%	93.5	91.3	98.0
Male fetuses				
Total		921		
%		48.4	46.0	54.5
Female Fetuses				
Total		980		
%		51.6	45.5	54.0

STRAIN ALL
SUPPLIER ALL

FETAL EXTERNAL MALFORMATIONS

	FETAL INCIDENCE (%)				LITTER INCIDENCE (%)			
	OVERALL		BY STUDY		OVERALL		BY STUDY	
	N	MEAN	MIN	MAX	N	MEAN	MIN	MAX
NUMBER EVALUATED	1824				143			
Live	1813							
Dead	11							
M ASTOMIA	1	0.1	0.0	0.3	1	0.7	0.0	4.2
M OPEN EYE	1	0.1	0.0	0.3	1	0.7	0.0	4.2
TOTAL FETAL EXTERNAL MALFORMATIONS	1	0.1			1	0.7		

OBSERVATION CODE: M=Malformation V=Variation

STRAIN ALL
SUPPLIER ALL

FETAL EXTERNAL VARIATIONS

	FETAL INCIDENCE (%)				LITTER INCIDENCE (%)			
	OVERALL N	MEAN	BY STUDY MIN	MAX	OVERALL N	MEAN	BY STUDY MIN	MAX
NUMBER EVALUATED	1824				143			
Live	1813							
Dead	11							
V AUTOLYSIS	11	0.6	0.0	2.8	3	2.1	0.0	8.3
V PALE FETUS	5	0.3	0.0	1.6	1	0.7	0.0	4.2
TOTAL FETAL EXTERNAL VARIATIONS	16	0.9			3	2.1		

OBSERVATION CODE: M=Malformation V=Variation

STRAIN ALL
SUPPLIER ALL

FETAL SOFT TISSUE MALFORMATIONS

	FETAL INCIDENCE (%)				LITTER INCIDENCE (%)			
	OVERALL N	MEAN	BY STUDY MIN	MAX	OVERALL N	MEAN	BY STUDY MIN	MAX
NUMBER EVALUATED	861				140			
Live	861							
Dead	0							
M RIGHT SIDED AORTIC ARCH	1	0.1	0.0	0.8	1	0.7	0.0	5.0
M RIGHT SIDED PULMONARY TRUNK	1	0.1	0.0	0.8	1	0.7	0.0	5.0
M INTERRUPTED AORTIC ARCH	1	0.1	0.0	0.8	1	0.7	0.0	5.0
M TRANSPOSITION OF GREAT VESSELS	1	0.1	0.0	0.8	1	0.7	0.0	5.0
TOTAL FETAL SOFT TISSUE MALFORMATIONS	2	0.2			2	1.4		

OBSERVATION CODE: M=Malformation V=Variation

STRAIN ALL
SUPPLIER ALL

FETAL SOFT TISSUE VARIATIONS

	FETAL INCIDENCE (%)				LITTER INCIDENCE (%)			
	OVERALL N	MEAN	BY STUDY MIN	MAX	OVERALL N	MEAN	BY STUDY MIN	MAX
NUMBER EVALUATED	861				140			
Live	861							
Dead	0							
V DILATED CEREBRAL VENTRICLE	1	0.1	0.0	0.7	1	0.7	0.0	4.3
V DILATED RENAL PELVIS	11	1.3	0.0	4.3	10	7.1	0.0	20.0
V SMALL KIDNEY	1	0.1	0.0	0.7	1	0.7	0.0	4.2
V SHORT INNOMINATE ARTERY	3	0.3	0.0	2.0	2	1.4	0.0	8.3
V DILATED URETER	12	1.4	0.0	8.6	8	5.7	0.0	30.0
TOTAL FETAL SOFT TISSUE VARIATIONS	24	2.8			17	12.1		

OBSERVATION CODE: M=Malformation V=Variation

STRAIN ALL
SUPPLIER ALL

FETAL SKELETAL MALFORMATIONS

	FETAL INCIDENCE (%)				LITTER INCIDENCE (%)			
	OVERALL N	MEAN	BY STUDY MIN	MAX	OVERALL N	MEAN	BY STUDY MIN	MAX
NUMBER EVALUATED	930				141			
Live	930							
Dead	0							
M MANDIBLE: ABSENT	1	0.1	0.0	0.6	1	0.7	0.0	4.2
M THORACIC VERTEBRA(E): UNOSSIFIED CENTRUM	2	0.2	0.0	0.8	2	1.4	0.0	5.0
M LUMBAR VERTEBRA(E): ABSENT	2	0.2	0.0	0.8	2	1.4	0.0	5.0
TOTAL FETAL SKELETAL MALFORMATIONS	5	0.5			5	3.5		

OBSERVATION CODE: M=Malformation V=Variation

STRAIN ALL
SUPPLIER ALL

FETAL SKELETAL VARIATIONS

	FETAL INCIDENCE (%)				LITTER INCIDENCE (%)			
	OVERALL N	MEAN	BY STUDY MIN	MAX	OVERALL N	MEAN	BY STUDY MIN	MAX
NUMBER EVALUATED	930				141			
Live	930							
Dead	0							
V INTERPARIETAL: INCOMPLETE OSSIFICATION	70	7.5	1.5	12.9	45	31.9	10.0	40.0
V PARIETAL: INCOMPLETE OSSIFICATION	34	3.7	0.0	11.0	22	15.6	0.0	37.5
V SUPRAOCCIPITAL: INCOMPLETE OSSIFICATION	11	1.2	0.0	3.7	7	5.0	0.0	8.7
V FRONTAL: INCOMPLETE OSSIFICATION	4	0.4	0.0	0.8	4	2.8	0.0	5.0
V PARIETAL: SPLIT	2	0.2	0.0	0.8	2	1.4	0.0	5.0
V INTERPARIETAL: BIPARTITE OSSIFICATION	1	0.1	0.0	0.6	1	0.7	0.0	4.2
V FONTANEL: ENLARGED	1	0.1	0.0	0.6	1	0.7	0.0	4.2
V HYOID: INCOMPLETE OSSIFICATION	39	4.2	0.0	9.0	23	16.3	0.0	40.0
V HYOID: UNOSSIFIED	1	0.1	0.0	0.6	1	0.7	0.0	4.2
V HYOID: INCOMPLETE OSSIFICATION OF CENTRUM	14	1.5	0.0	8.6	6	4.3	0.0	25.0
V CERVICAL VERTEBRA(E): INCOMPLETE OSSIFICATION OF CENTRUM	102	11.0	3.0	19.0	57	40.4	15.0	66.7
V CERVICAL VERTEBRA(E): UNOSSIFIED CENTRUM	38	4.1	1.5	6.1	26	18.4	10.0	29.2
V CERVICAL VERTEBRA(E): INCOMPLETE OSSIFICATION OF ARCH	1	0.1	0.0	0.7	1	0.7	0.0	5.0
V THORACIC VERTEBRA(E): DUMBBELL OSSIFICATION CENTRUM	25	2.7	1.2	4.8	20	14.2	8.3	21.7
V THORACIC VERTEBRA(E): INCOMPLETE OSSIFICATION OF CENTRUM	66	7.1	0.0	11.7	39	27.7	0.0	41.7
V THORACIC VERTEBRA(E): BIPARTITE OSSIFICATION OF CENTRUM	15	1.6	0.0	4.0	11	7.8	0.0	20.0
V THORACIC VERTEBRA(E): INCOMPLETE OSSIFICATION OF HEMICENTRUM	1	0.1	0.0	0.8	1	0.7	0.0	5.0

STRAIN ALL
SUPPLIER ALL

FETAL SKELETAL VARIATIONS

	NUMBER EVALUATED	FETAL INCIDENCE (%)			LITTER INCIDENCE (%)		
		OVERALL N	MEAN	BY STUDY MIN MAX	OVERALL N	MEAN	BY STUDY MIN MAX
	Live	930			141		
	Dead	0					
V LUMBAR VERTEBRA(E): INCOMPLETE OSSIFICATION OF CENTRUM		1	0.1	0.0 0.7	1	0.7	0.0 5.0
V LUMBAR VERTEBRA(E): OSSIFICATION POINT		1	0.1	0.0 1.5	1	0.7	0.0 10.0
V CAUDAL VERTEBRA(E): INCOMPLETE OSSIFICATION OF ARCH		11	1.2	0.0 3.7	8	5.7	0.0 12.5
V CAUDAL VERTEBRA(E): UNOSSIFIED ARCH		7	0.8	0.0 2.5	5	3.5	0.0 8.3
V CAUDAL VERTEBRA(E): UNOSSIFIED CENTRUM		11	1.2	0.0 3.7	7	5.0	0.0 12.5
V CAUDAL VERTEBRA(E): INCOMPLETE OSSIFICATION OF CENTRUM		9	1.0	0.0 2.5	7	5.0	0.0 12.5
V UNOSSIFIED 5th STERNEBRA		7	0.8	0.0 1.6	7	5.0	0.0 10.0
V INCOMPLETE OSSIFICATION OF 1st TO 4th STERNEBRA(E)		6	0.6	0.0 2.4	5	3.5	0.0 10.0
V INCOMPLETE OSSIFICATION OF 6th STERNEBRA		22	2.4	0.0 7.4	16	11.3	0.0 25.0
V EXTRA STERNEBRAL OSSIFICATION SITE		1	0.1	0.0 0.6	1	0.7	0.0 4.2
V MISSHAPEN STERNEBRA(E)		1	0.1	0.0 1.5	1	0.7	0.0 10.0
V OSSIFICATION POINT ON 14th THORACIC VERTEBRA		30	3.2	1.2 7.5	24	17.0	8.3 30.0
V KNOBBY RIB(S)		8	0.9	0.0 2.5	7	5.0	0.0 12.5
V SHORT SUPERNUMERARY 14th RIB(S)		2	0.2	0.0 0.6	2	1.4	0.0 4.2
V SHORT RIB(S)		6	0.6	0.0 2.0	5	3.5	0.0 13.0
V WAVY RIB(S)		17	1.8	0.6 3.4	13	9.2	4.2 13.0
V THICKENED RIB(S)		13	1.4	0.0 3.4	11	7.8	0.0 17.4

STRAIN ALL
SUPPLIER ALL

FETAL SKELETAL VARIATIONS

	NUMBER EVALUATED	FETAL INCIDENCE (%)			LITTER INCIDENCE (%)		
		OVERALL N	MEAN	BY STUDY MIN MAX	OVERALL N	MEAN	BY STUDY MIN MAX
	Live	930			141		
	Dead	0					
V INCOMPLETE OSSIFICATION OF RIB(S)		4	0.4	0.0 1.2	4	2.8	0.0 8.3
V SUPERNUMERARY 14th RIB(S)		1	0.1	0.0 0.6	1	0.7	0.0 4.2
V INCOMPLETE OSSIFICATION OF METACARPAL(S)		14	1.5	0.0 4.3	8	5.7	0.0 12.5
V UNOSSIFIED METACARPAL(S)		2	0.2	0.0 1.2	1	0.7	0.0 4.2
V FOREPAW: UNOSSIFIED PROXIMAL PHALANX		34	3.7	0.0 17.2	19	13.5	0.0 58.3
V FOREPAW: UNOSSIFIED DISTAL PHALANX		48	5.2	0.0 29.4	11	7.8	0.0 45.8
V UNOSSIFIED 1st METATARSAL		34	3.7	0.0 12.3	19	13.5	0.0 37.5
V HINDPAW: UNOSSIFIED DISTAL PHALANX		33	3.5	0.0 20.2	9	6.4	0.0 37.5
V ISCHIUM: INCOMPLETE OSSIFICATION		3	0.3	0.0 1.8	1	0.7	0.0 4.2
V PUBIS: INCOMPLETE OSSIFICATION		3	0.3	0.0 1.8	1	0.7	0.0 4.2
V PRESENCE OF 25 PRE-SACRAL VERTEBRAE		2	0.2	0.0 0.8	2	1.4	0.0 5.0
V PRESENCE OF 27 PRE-SACRAL VERTEBRAE		1	0.1	0.0 0.6	1	0.7	0.0 4.2
TOTAL FETAL SKELETAL VARIATIONS		377	40.5		122	86.5	

OBSERVATION CODE: M=Malformation V=Variation

For more historical data, see appendix 17 on page 193 of study number 40400 RSR.