Toxicology Review of Influenza A (H5N1) Monovalent, Adjuvanted Vaccine

Reviewer: Andrew O'Carroll, DVM

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File: BLA 125692/0

Product: Influenza A (H5N1) Monovalent, Adjuvanted Vaccine

Subject: Toxicology study review

Reviewer: Andrew O'Carroll, DVM

Reference: BLA Sections reviewed

- 4.2.3.2 Repeat-Dose Toxicity
- 4.2.3.5 Reproductive and Developmental Toxicity

Sponsor: Seqirus Inc.

Contents

EXECUTIVE SUMMARY

Influenza A (H5N1) Monovalent, Adjuvanted Vaccine was evaluated in a pair of toxicology studies in this BLA submission: one repeat-dose and one developmental and reproductive toxicology (DART) study, both in (b) (4) rabbits. No clinically-significant toxicological findings were found which would preclude the use of this vaccine in its intended human population at doses up to (b) (4) antigen plus (b) (4) MF59™ adjuvant up to (b) (4) times intramuscularly.

In the repeat-dose toxicology study, study rabbits received either saline control or the test vaccine at the entire human dose (b) (4) 3 times, each separated by 2 weeks followed by a 2-week recovery period. There was no treatment-related toxicity or any clinical evidence of toxicity in the study rabbits. Treatment-related findings were limited to mild increases to fibrinogen and globulins on clinical pathology assessments, minimal to mild splenic follicular hyperplasia and minimal evidence of inflammation at injection sites. These findings were all considered anticipated sequelae of the intended immune response rather than as a sign of frank toxicity and none had any clinical effect on the study rabbits.

In the DART study included in this submission, virgin rabbit does received 2 doses of saline control or aH5N1c prior to mating and twice while pregnant at the entire human dose (7.5 μg antigen plus (b) (4) MF59™). One subset of does from both groups had terminal caesarean sections on gestation day 29 (near term) to assess uterine effects and fetal development while another subset proceeded to parturition with both the does and F1 kits followed until 29 days postpartum. There was no treatment-related mortality or any treatment-related effects on female fertility and mating performance, fetal development, parturition or postnatal development up until 29 days postpartum. Treatment-related effects were limited to a low incidence of very slight, reversible edema at the injection sites of does. Serology assessment from this study confirmed passive transfer of antibodies to fetuses from vaccinated does. The formulation of Influenza A (H5N1) Monovalent, Adjuvanted Vaccine containing (b) (4) was not assessed in this study and this should be noted in the Prescribing Information (PI) for this product.

INTRODUCTION

BLA: 125692/0

Sponsor: Seqirus Inc

Product: Influenza A (H5N1) Monovalent Vaccine, Adjuvanted

Proposed use: "AUDENZ is a vaccine indicated for active immunization for the prevention of disease caused by the influenza A virus H5N1 subtype contained in the vaccine. AUDENZ is approved for use in adults and pediatric persons 6 months of age and older."

Introduction: Seqirus is submitting an original biologics licensing application (BLA) for consideration of licensure in the US for their developed vaccine Influenza A (H5N1) Monovalent Vaccine, Adjuvanted but shortened to "aH5N1c" in the submission. This is a vaccine containing the purified hemagglutinin (b) (4) antigens from the H5N1 strain A/turkey/Turkey/1/2005 in the form of the NIBRG-23 virus: "a reverse genetics-derived reference strain supplied by NIBSC UK." This virus is manufactured in a process based on that used for Flucelvax®, also produced by the sponsor: the virus is manufactured on Madin Darby Canine Kidney (MDCK) cells and inactivated via beta-propiolactone treatment. The vaccine adjuvanted using MF59™: an oil-in-water emulsion containing 9.75 mg squalene, 1.175 mg polysorbate 80, 1.175 mg sorbitan triolate, 0.66 mg sodium citrate dihydrate and 0.04 mg citric acid monohydrate. This vaccine adjuvant is found in the licensed vaccine Fluad® and in the same volume and dose. Per prior discussions with CBER, this submission does not contain any nonclinical studies on MF59™ alone or with other products.

The intended clinical dosing regimen of aH5N1c is for recipients to receive a 0.5 mL dose twice, separated by 21 days and is intended for adults and pediatrics 6 months of age and older. The vaccine comes in (b) (4) manufactured (b) (4): single-dose, 0.5 mL prefilled syringes containing 7.5 µg HA (b) (4) MF89 without any preservative, (b) (4) The sponsor state's that this product will not be commercialized "…until requested to do so by U.S. governmental authorities, anticipated upon pandemic declaration or sustained human-to-human transmission of A/H5N1 virus."

The nonclinical toxicology program for this submission includes one repeat-dose toxicology study to establish general safety as well as one developmental and reproductive toxicology (DART) study to develop a safety profile should this vaccine be administered to pregnant women (though not on the label indication) as well as support the claims in section 8 of the Prescribing Information (PI). The other nonclinical studies submitted under sections 4.2.1 (pharmacology) and 4.2.2 (pharmacokinetics) are not included in this review.

6-WEEK TOXICITY STUDY WITH H5N1 FCC + MF59 (b) (4) VACCINE BY 3 INTRAMUSCULAR INJECTIONS IN (b) (4) RABBITS

Repeat dose toxicology study number: 466122
Performing laboratory: (b) (4)
Study initiation date: January 8th, 2007
Final Report date: December 10th, 2007
Test article batch/lot: all manufactured by Novartis Vaccines & Diagnostics GmbH

- *H5N1 FCC + MF59™ Vaccine*: test article batch number 545001011
- *MF59™ Adjuvant*: adjuvant article batch number "Adjuvant(b) (4)"
- (b) (4) control article batch number "Control(b) (4)"

Animal species and strain: (b) (4) rabbits
Breeder/supplier: (b) (4)
Number of animal per group and sex: 4
Age: 12-14 weeks at initiation of treatment
Body weight range: 1971 to 2691 grams
Means of administration: Intramuscular injection, needle and syringe
Site of administration: Alternating hind leg, starting with the right (specific muscle body not provided)
Volume of injection: 0.5 mL
Frequency of administration and study duration: A total of 3 biweekly administrations occurred followed by a 14-day recovery phase for a total study duration of 43 days
Dose: 15 μg H5N1 FCC, (b) (4) MF59™, (b) (4)
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use vials (one vial per dose). Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study (see Stability Report and Certificate of Analysis appendices).
Report status: Final

Experimental design: Animals were acclimated for a minimum of 5 days, randomized and assigned to 1 of 3 groups according to table 1 below. Administration of test and control articles were administered on study days 1, 15 and 29. Half of the study animals were humanely euthanized on study day 31 while the other half was humanely euthanized after a recovery phase on study day 43.

Group	*Treatment*	*Treatment phase*	*Recovery phase*
1	(b) (4)	4	**4**
2	MF59™ (b) (4)	4	**4**
3	H5N1 FCC + MF59™ (b) (4)	4	**4**

Table 1: Group assignments – values represent number of rabbits per sex assigned to that phase of the study

Methods for blood collection: Unfasted blood samples were drawn from ear arteries without any sedative or anesthetic assistance
Randomization procedure: Yes, computer-assisted procedure based on body weight
Statistical analysis plan: Yes, Dunnett, Steel and Fisher-exact tests

The following parameters were evaluated:

Parameters	*Frequency of Testing*
Mortality check	**Twice daily**
Clinical observations[1]	**Daily**
Body weight	**Weekly**
Food consumption	**Twice weekly**
Body temperature (rectal)	**Pre-test, pre-dose, 2 hours post-dose**
Ophthalmologic exam	**Pre-test, SD 29 and 39**
Heart rate & respiratory rate	**Pre-test, pre-dose, 2 hours post-dose**
Clinical chemistry*	**Pre-test, SD 8, 17, 31 and 43**
Hematology*	**Pre-test, SD 8, 17, 31 and 43**
Coagulation*	**Pre-test, SD 8, 17, 31 and 43**
Immunological response*	**Pre-test, SD 15, 29, 31 and 43**
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	**24- and 48-hours post-dose**
Necropsy	**Treatment phase: SD 31** **Recovery phase: SD 43**
Organ weights	**Treatment phase: SD 31** **Recovery phase: SD 43**
Tissues for histopathology	**Treatment phase: SD 31** **Recovery phase: SD 43**

Table 2: Experimental design – SD = study day; *blood collected from ear arteries

Postmortem procedures: The following tissues were collected at necropsy.

Organ/Tissue	*Collected*	*Not collected*
Adrenal glands	!*	
Aorta	!	
Bone (sternum & femur)	!	
Bone marrow (sternum)	!	
Brain (cerebellum, mid-brain, cortex)	!*	
Cecum	!	
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes (with optic nerve)	!	
Fallopian tubes (oviduct)		**NC**
Gall bladder	!	
Gross lesions (if any)	!	
Harderian gland	!	
Heart	!*	

[1] Clinical observation parameter details not provided.

Organ/Tissue	*Collected*	*Not collected*
Ileum	!	
Inguinal gland	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!*	
Lacrimal glands		**NC**
Larynx	!	
Liver	!*	
Lung	!	
Lymph nodes (iliac)	!	
Lymph nodes (cervical)	!	
Lymph nodes (mesenteric)	!	
Mammary glands	!	
Naso-oropharyngeal cavity		**NC**
Ovaries	!*	
Pancreas	!	
Peyer's patch (if detectable)	!	
Pituitary gland	!	
Prostate	!	
Rectum	!	
Salivary glands (mandibular and parotid)	!	
Sciatic nerve	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	!*	
Stomach	!	
Testes	!*	
Thymus	!*	
Thyroid (with parathyroid glands)	!	
Tongue	!	
Trachea	!	
Ureters	!	
Urinary bladder	!	
Uterus (with cervix)	!	
Vagina	!	
Zymbal's gland (if applicable)		**NA**

Table 3: Histology – tissues examined for histology are marked with an '!'; tissues marked with an asterisk were weighed; NC = not collected; NA = not applicable

At scheduled terminations, animals were anesthetized with a combination of medetomidine, ketamine and fentanyl, followed by exsanguination. Tissues were first examined macroscopically for gross findings, then embedded in paraffin wax, sectioned at 2-4 μm and stained with hematoxylin and eosin. The tissues listed in table 3 above were collected from all animals of both sexes from all groups in this study.

RESULTS

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

Clinical observations: No treatment-related clinical signs were observed in this study. The only abnormality observed was a single control group female with a single incidence of diarrhea.

Ophthalmologic observations: There were no treatment-related abnormalities noted on ophthalmologic examinations in this study. A single group 2 male was found to have a focal area of corneal edema on study day 30 and a single group 3 female was found to have a focal corneal opacity, but this was present prior to treatment. These are both considered incidental to the study due to sporadic incidence and/or presence prior to treatment.

Heart rate & respiratory rate:

Study Day	1M	2M	3M	1F	2F	3F
Pre-test	218	239	**250****	225	220	225
1 (pre-dose)	250	**219****	243	242	234	241
1 (post-dose)	224	**241***	**240***	236	243	245
15 (pre-dose)	232	232	248	228	247	249
15 (post-dose)	212	221	**235***	233	243	247
29 (pre-dose)	225	**240***	**248****	248	243	237
29 (post-dose)	260	250	260	254	264	254

Table 4: Heart rate – values presented as mean beats/minute; *p<0.05; **p<0.01

Study Day	1M	2M	3M	1F	2F	3F
Pre-test	179	191	**201***	170	171	185
1 (pre-dose)	211	199	220	222	216	203
1 (post-dose)	184	**207***	200	205	200	204
15 (pre-dose)	195	200	209	109	212	214
15 (post-dose)	184	188	201	180	189	**215****
29 (pre-dose)	220	220	229	229	223	**203****
29 (post-dose)	208	232	**233***	211	**236***	225

Table 5: Respiratory rate – values presented as mean breaths/minute; *p<0.05; **p<0.01

There were no treatment-related, toxicologically-relevant effects on heart rate and respiratory rate observed in this study. Those changes observed, including those with statistical significance, are considered incidental because they varied in trend between study points, were present pre-test and/or are considered within the normal bounds of biologic variation.

Body temperature:

Group	*Males*	*Females*
1 (Control)	1	**0**
2	0	**0**
3	0	**0**

Table 6: Body temperature – values represent number of occurrences for body temperature ≥40° C

There were no treatment-related effects on body temperature observed in this study, but it should be noted that readings were only taken 2 hours post-dose.

Body weight:

Study Day	1M	2M	3M	1F	2F	3F
1	2362	2206	2399	2353	2364	2347
8	2564	2427	2612	2568	2651	2574
15	2757	2666	2813	2747	2858	2807
22	2944	2811	2969	2972	3117	2942
29	3100	2950	3133	3095	3257	3129
36	3022	3343	3303	3177	3431	3298
43	3011	3438	3414	3380	3562	3459

Table 7: Body weight – values presented in mean grams (g); no statistically-significant differences observed

There were no treatment-related effects on body weight gain in this study. Those differences observed in table 7 above are considered incidental to the study because of being within the bounds of normal biologic variation.

Food consumption:

Study Days	1M	2M	3M	1F	2F	3F
1-5	148	141	162	161	170	161
5-8	150	135	167	164	165	157
8-12	147	145	160	160	164	158
12-15	151	149	162	148	167	164
15-19	148	140	149	152	163	157
19-22	160	154	169	171	184	169
22-36	155	147	152	160	161	161
26-29	164	157	176	162	174	173
29-33	129	162	168	157	174	169
33-36	152	165	169	155	160	115
36-40	139	167	165	173	166	141
40-43	137	163	167	175	183	194

Table 8: Food consumptions – values presented in grams (g) consumed per animal per day; no statistically-significant differences observed

There were no treatment-related effects on food consumption in this study. Those differences observed in table 8 above are considered incidental to the study because of being within the bounds of normal biologic variation.

Clinical chemistry:

MEASUREMENT RELATED TO	*END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE (if greater or less than 1.5, i.e. ↑ 1.6 or ↓ 1.6)*	*NOT OF NOTE*
ELECTROLYTE BALANCE		Calcium Phosphorus Sodium Potassium Chloride
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: **A) HEPATOCELLULAR**		Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) Glutamate dehydrogenase (ND) Sorbitol dehydrogenase (ND) Total bile acids (ND)
B) HEPATOBILIARY		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids (ND) Total bilirubin
ACUTE PHASE REACTANTS	Fibrinogen (see table10)	C-reactive protein (ND)
KIDNEY FUNCTION		Creatinine Blood urea nitrogen (BUN)
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Total protein Albumin (A) Globulin (G, calculated) A/G ratio Total cholesterol Cholinesterase (ND) Creatine kinase (CK) Lactate dehydrogenase (LDH) Fasting triglycerides Phospholipids

Table 9: Clinical chemistry results – ND = not determined

There were no toxicologically-relevant changes in clinical chemistry parameters observed during this study. The only treatment-related observations were a reversible, approximately 20% increase in globulins ($p<0.01$) and a subsequent equivalent decrease in albumin to globulin ratio which can be attributed to the intended immune response to vaccination.

Hematology and coagulation:

MEASUREMENT RELATED TO	*END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE (if greater or less than 1.5[2], i.e. ↑ 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) Red cell distribution weight (RDW) Reticulocytes
WHITE BLOOD CELLS	Eosinophil count (%) SD31 M G2 ↓ 1.6 SD31 F G3 ↓ 1.9* SD43 F G2 ↑ 2.6 Monocyte count (%) SD17 F G3 ↑ 1.6 SD43 M G3 ↓ 1.7	Total leukocytes (WBC) Neutrophil count (%) Lymphocyte count (%) Basophil count (%) Large unstained cells (LUC)
CLOTTING POTENTIAL	Fibrinogen SD17 M G3 ↑ 2.0** SD17 F G3 ↑ 2.0** SD31 M G2 ↑ 1.8** SD31 M G3 ↑ 1.7** SD31 F G3 ↑ 1.7**	Activated partial-thromboplastin clotting time (APTT) Prothrombin time (PT) Platelet count Mean platelet volume (ND)
OTHER		Bone marrow cytology (ND)

Table 10: Hematology and coagulation results – ND = not determined; *p<0.05; **p<0.01

The only treatment-related, toxicologically relevant changed observed among the hematology and coagulation parameters were statistically significant ($p<0.01$) but reversible increases in fibrinogen. This is an acute phase reactant and elevates as a response to the intended immune response to vaccination. The changes in eosinophil and monocyte counts are considered incidental due to a lack of consistency in trend and observation across sexes.

Systemic toxicity: No treatment-related mortality or any toxicologically relevant changes in clinical signs, ophthalmoscopic examinations, body temperature, heart rate, respiratory rate, body weight gain, relative food consumption and hematology parameters were found. Overall, the test vaccine was generally well tolerated during the in-life phase of the study without any evidence of adversity. Treatment-related changes were limited to reversible increases in fibrinogen and globulins. These are considered anticipated sequelae of the intended immune response to vaccination rather than considered signs of toxicity.

[2] 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:

Parameter	1M	2M	3M	1F	2F	3F
NUMBER OF ANIMALS	4/4	4/4	4/4	4/4	4/4	4/4
BODY WEIGHT (gram)	3214/ 3020	**2657****/ 3407	3149/ 3372	3216/ 3363	3228/ 3528	3095/ 3418
Adrenals	0.224/ 0.192	0.178/ 0.208	0.181/ 0.219	0.208/ 0.236	0.208/ 0.218	0.200/ 0.211
Brain	9.4/ 9.6	9.2/ 10.2	9.4/ 9.5	9.2/ 9.5	9.3/ 9.2	9.0/ 9.6
Heart	8.02/ 7.01	**6.30****/ 8.12	7.56/ 7.75	7.00/ 7.31	7.40/ 8.43	7.28/ 7.43
Kidneys	18.64/ 15.92	15.39/ 19.34	18.94/ 19.78	17.61/ 18.02	17.35/ 18.75	17.32/ 18.06
Liver	110.5/ 92.3	**66.0***/ 110.3	108.8/ 114.5	96.0/ 115.3	105.8/ 118.3	97.8/ 111.7
Ovaries	NA	NA	NA	0.317/ 0.373	0.291/ 0.663	0.383/ 0.285
Spleen	1.38/ 1.01	1.28/ 0.98	1.24/ 1.05	1.21/ 1.39	1.41/ 1.52	1.35/ 1.55
Testes	3.35/ 4.14	2.22/ 4.46	3.82/ 4.33	NA	NA	NA
Thymus	4.48/ 4.11	3.89/ 4.00	3.86/ 4.92	4.15/ 4.29	3.78/ 4.03	4.17/ 4.07

Table 11: Organ weights – absolute organ weights presented in mean grams (g); NA = not applicable; *p<0.05; **p<0.01; values from treatment phase and recovery phase animals separated by a '/'

Parameter	1M	2M	3M	1F	2F	3F
NUMBER OF ANIMALS	4/4	4/4	4/4	4/4	4/4	4/4
BODY WEIGHT (gram)	3214/ 3020	**2657****/ 3407	3149/ 3372	3216/ 3363	3228/ 3528	3095/ 3418
Adrenals	0.007/ 0.006	0.007/ 0.006	0.006/ 0.007	0.006/ 0.007	0.006/ 0.006	0.006/ 0.006
Brain	0.3/ 0.3	**0.3****/ 0.3	0.3/ 0.3	0.3/ 0.3	0.3/ 0.3	0.3/ 0.3
Heart	0.25/ 0.23	0.24/ 0.24	0.24/ 0.23	0.22/ 0.22	0.23/ 0.24	0.24/ 0.22
Kidneys	0.58/ 0.53	0.58/ 0.57	0.60/ 0.58	0.55/ 0.53	0.54/ 0.53	0.56/ 0.53
Liver	3.4/ 3.1	2.5/ 3.2	3.4/ 3.2	3.0/ 3.4	3.2/ 3.3	3.1/ 3.2
Ovaries	NA	NA	NA	0.010/ 0.011	0.009/ 0.018	0.012/ 0.008
Spleen	0.04/ 0.03	0.05/ 0.03	0.04/ 0.03	0.04/ 0.04	0.04/ 0.04	0.04/ 0.05
Testes	0.10/ 0.14	0.08/ 0.13	0.12/ 0.13	NA	NA	NA
Thymus	0.14/ 0.14	0.15/ 0.12	0.12/ 0.14	0.13/ 0.13	0.12/ 0.11	0.13/ 0.12

Table 12: Organ weight to body weight ratios – d values presented as percentage (%) organ weight to body weight; NA = not applicable; *p<0.05; **p<0.01; values from treatment phase and recovery phase animals separated by a '/'

There were no treatment-related effects on organ weights or weight ratios observed in this study. These differences between treated animals and controls observed in tables 11 and 12 above are considered incidental because they did not correlate with any gross or histopathological findings, were observed with adjuvant alone and not the vaccine (e.g. reduced cardiac weights in treatment phase males) and/or were minimal in degree and within the normal bounds of biologic variation.

Gross Pathology:

Treatment Phase Finding	*1M*	*2M*	*3M*	*1F*	*2F*	*3F*
Injection site, focus/foci	0	1	1	0	0	0
Jejunum, nodule(s)	0	0	0	0	1	0
Lymph node (iliac), enlarged	0	0	1	0	1	0

Table 13: Treatment phase macroscopic pathology findings

Recovery Phase Finding	*1M*	*2M*	*3M*	*1F*	*2F*	*3F*
Jejunum, diverticulum (10x50 mm)	0	0	0	0	1	0
Liver, nodule(s)	0	0	0	1	0	0
Liver, enlarged	0	0	0	1	0	0

Table 14: Recovery phase macroscopic pathology findings

Histopathology:

Treatment Phase Finding	*1M*	*2M*	*3M*	*1F*	*2F*	*3F*
Adrenals, cortical hemorrhagic degeneration	1	0	1	0	0	0
Epididymides, aspermia	2	4	3	NA	NA	NA
Epididymides, hypospermia	1	0	1	NA	NA	NA
Epididymides, one missing	1	0	0	NA	NA	NA
Esophagus, focal parakeratosis	0	0	0	1	0	0
Eyes, limbal keratitis	0	0	0	0	0	1
Femur and articulation, hypercellular marrow	0	1	0	0	2	2
Heart, mononuclear cell infiltration	0	1	0	0	1	0
Ileum, Eimeria infestation	0	0	0	0	0	1
Inguinal glands, interstitial lymphocytic infiltration	2	0	0	2	0	2
Inguinal glands, focal purulent infiltration	1	0	0	0	0	0
Inguinal glands, intraductular acute inflammation	0	0	0	1	0	2
Injection site (left), intermuscular connective tissue macrophages (1)	0	0	2	0	0	0
Injection site (left), dermal hemorrhage (1)	0	1	0	0	0	0
Injection site (left), dermal hemorrhage (2)	0	0	0	0	1	0
Injection site (left), panniculus muscle fiber degeneration (1)	0	0	0	1	3	0
Injection site (left), panniculus muscle fiber degeneration (2)	0	2	0	0	0	0
Injection site (left), deep muscle focal degeneration (1)	0	1	0	0	0	0
Injection site (left), deep muscle focal degeneration (3)	0	1	0	0	0	0
Injection site (left), deep muscle hemorrhage (2)	0	1	0	0	0	0
Injection site (right), intermuscular connective tissue macrophages (2)	0	1	0	0	1	0
Injection site (right), dermal hemorrhage (2)	0	0	1	1	0	0
Injection site (right), panniculus muscle fiber degeneration (1)	0	2	0	1	0	0
Injection site (right), panniculus muscle fiber degeneration (2)	0	0	0	0	1	0

Treatment Phase Finding	*1M*	*2M*	*3M*	*1F*	*2F*	*3F*
Injection site (right), panniculus muscle fiber degeneration (3)	0	1	0	0	0	0
Injection site (right), deep epidermal acute inflammation (1)	0	1	0	0	0	1
Injection site (right), deep epidermal acute inflammation (2)	0	0	0	0	0	1
Injection site (right), deep dermal diffuse macrophage infiltration (1)	0	0	0	0	0	3
Injection site (right), deep dermal diffuse macrophage infiltration (2)	0	1	0	0	2	0
Injection site (right), panniculus focal macrophage infiltration (2)	0	1	0	0	0	0
Injection site (right), intermuscular acute inflammation (2)	0	2	0	0	0	0
Injection site (right), deep muscle focal degeneration (1)	0	0	0	0	1	0
Injection site (right), deep muscle focal degeneration (2)	0	0	0	1	0	0
Injection site (right), deep muscle hemorrhage (1)	0	0	0	1	0	0
Injection site (right), deep muscle macrophage infiltration (2)	0	0	0	1	0	0
Jejunum, Eimeria infestation	0	0	0	0	0	1
Jejunum, intramural cyst ("Meckl's diverticulum?")	0	0	0	0	1	0
Kidneys, basophilic tubules (1)	0	0	3	0	1	0
Kidneys, cortical intratubular mineral deposits (1)	0	0	1	2	1	1
Kidneys, cortical intratubular mineral deposits (2)	0	0	2	0	2	3
Kidneys, dilated cortical tubules (1)	0	1	0	1	1	1
Kidneys, interstitial mononuclear cell infiltration	0	0	0	0	1	0
Liver, periacinar hepatocytic pallor	3	1	4	2	4	2
Liver, periacinar hepatocytic hypertrophy	0	1	0	0	0	0
Lung, terminal alveolar hemorrhage	0	0	1	0	1	0
Lung, pneumonitis	0	1	0	0	0	0
Lymph node (cervical), sinus erythrophagia	0	0	1	1	0	0
Lymph node (iliac), sinus erythrophagia	0	1	2	1	0	0
Lymph node (iliac), medullary polymorphonuclear leukocyte infiltration	0	0	0	0	0	1
Lymph node (mesenteric), sinus erythrophagia	0	0	1	0	0	0
Mammary gland, no acinar tissue	0	0	0	2	1	3
Mammary gland, ductular dilation	0	0	0	2	2	1
Mammary gland, acinar hypertrophy	0	0	0	2	1	1
Ovaries, tertiary follicles predominate	NA	NA	NA	1	0	0
Ovaries, secondary follicles predominate	NA	NA	NA	3	4	4
Prostate, focal luminal acute inflammation	1	0	0	NA	NA	NA
Prostate, juvenile	1	0	0	NA	NA	NA
Sciatic nerve, adjacent subacute inflammation	0	1	0	0	0	0
Spinal cord (midthoracic), enlarged central canal	0	1	0	0	0	0
Spleen, follicular center hyperplasia (1)	0	1	0	2	1	3
Spleen, follicular center hyperplasia (2)	0	0	3	1	1	0
Sternum with bone marrow, hypercellular marrow	0	1	0	0	0	0
Stomach (fundus), mucosal cysts	0	0	0	0	1	0
Testes, juvenile	3	4	4	NA	NA	NA
Testes, single aplastic tubules	0	0	1	NA	NA	NA
Thymus, atrophy (1)	0	1	1	0	2	2
Thymus, atrophy (2)	0	0	0	1	1	1
Ureters, unilateral dilation	0	0	0	2	0	0
Vagina, mucinous epithelium	NA	NA	NA	1	1	2

Treatment Phase Finding	*1M*	*2M*	*3M*	*1F*	*2F*	*3F*
Vagina, columnar epithelium	NA	NA	NA	2	0	2
Vagina, squamous epithelium	NA	NA	NA	1	3	0
Vagina, submucosal edema	NA	NA	NA	1	0	0
Vagina, intramucosal acute inflammatory cysts	NA	NA	NA	0	0	1

Table 15: Treatment phase microscopic pathology findings – parenthetical values represent severity grading as 1 = minimal, 2 = mild, 3 = moderate, 4 = marked; some values were not graded for severity; NA = not applicable

Recovery Phase Finding	*1M*	*2M*	*3M*	*1F*	*2F*	*3F*
Epididymides, aspermia	0	0	3	NA	NA	NA
Epididymides, hypospermia	2	2	1	NA	NA	NA
Eyes, limbal keratitis	0	0	1	0	1	0
Femur and articulation, hypercellular marrow	0	0	0	3	1	1
Inguinal glands, interstitial lymphocytic infiltration	0	2	0	0	1	2
Inguinal glands, intraductular acute inflammation	0	2	0	0	1	1
Injection site (left), panniculus muscle fiber degeneration (1)	0	0	0	1	0	0
Injection site (left), panniculus muscle fiber degeneration (2)	0	0	1	0	0	1
Injection site (right), dermal hemorrhage (1)	0	0	0	1	1	0
Injection site (right), intermuscular connective tissue macrophages (1)	0	0	1	0	0	0
Injection site (right), panniculus muscle fiber degeneration (1)	0	1	0	1	1	0
Injection site (right), panniculus muscle fiber degeneration (2)	0	1	1	0	0	2
Injection site (right), panniculus muscle fiber degeneration (3)	0	1	0	0	0	0
Injection site (right), follicular adnexa deficit (2)	0	1	0	0	0	0
Injection site (right), superficial pustular dermatitis (1)	0	0	0	1	0	0
Jejunum, intramural cyst ("Meckl's diverticulum?")	0	0	0	0	1	0
Kidneys, basophilic tubules (1)	1	0	0	0	0	0
Kidneys, cortical intratubular mineral deposits (1)	0	0	1	0	1	1
Kidneys, cortical intratubular mineral deposits (2)	0	0	1	1	1	0
Liver, periacinar hepatocytic pallor	1	4	2	1	2	1
Liver, mineralized biliary granuloma	0	0	0	1	0	0
Lung, pneumonitis	0	0	0	4	3	3
Lung, mineralized biliary granuloma	0	0	0	1	0	0
Lung, terminal alveolar hemorrhage	0	0	1	0	0	0
Lymph node (iliac), sinus erythrophagia	0	0	0	1	0	0
Mammary gland, no acinar tissue	0	0	0	0	2	0
Mammary gland, ductular dilation	0	0	0	2	0	3
Mammary gland, acinar hypertrophy	0	0	0	2	0	1
Ovaries, tertiary follicles predominate	NA	NA	NA	0	1	0
Ovaries, secondary follicles predominate	NA	NA	NA	3	3	3
Ovaries, corpora albicantes predominate	NA	NA	NA	1	0	0
Pancreas, focal mononuclear cell infiltration	0	0	1	0	0	0
Pancreas, splenule	0	0	0	0	1	0
Peyer's patches, sinus erythrophagia	0	1	0	0	0	0
Sciatic nerves, adjacent subacute inflammation	0	0	0	0	0	1
Spleen, follicular center hyperplasia (1)	0	0	1	1	3	3
Spleen, follicular center hyperplasia (2)	0	0	1	0	0	0
Stomach (cardia), submucosal edema	0	0	0	1	0	0

Recovery Phase Finding	*1M*	*2M*	*3M*	*1F*	*2F*	*3F*
Testes, juvenile	2	0	3	NA	NA	NA
Testes, single aplastic tubules	2	0	0	NA	NA	NA
Thymus, atrophy (1)	0	2	2	2	3	0
Thymus, atrophy (2)	0	0	1	0	1	0
Thymus, atrophy (3)	0	0	0	0	1	0
Vagina, mucinous epithelium	NA	NA	NA	2	0	0
Vagina, columnar epithelium	NA	NA	NA	1	1	1
Vagina, squamous epithelium	NA	NA	NA	1	3	2

Table 16: Recovery phase microscopic pathology findings – parenthetical values represent severity grading as 1 = minimal, 2 = mild, 3 = moderate, 4 = marked; some values not graded for severity; NA = not applicable

An extensive number of tissues were examined for histology. Aside from injection site findings discussed below, the only treatment-related change observed post-mortem was splenic follicular center hyperplasia in males during the treatment phase and in females after the recovery phase. Overall, no increased incidence of histological findings indicative of potential adverse events was observed in the treated groups relative to the controls.

The rest of the findings described in tables 13 – 16 above are considered incidental either because they were sporadic, at similar incidence between treated and control animals, and/or are considered recognized background changes in laboratory rabbits. This includes the incidence of thymic atrophy observed in this study. The pathologist states that "…these thymic results are considered as a secondary effect of 'stress' of treatment." Additionally, there appeared to be an increased incidence of renal basophilic tubules in treated males and cortical intratubular mineral deposits. These are considered recognized background changes in laboratory rabbits and did not present with a similar trend after the recovery phase[1]. Lastly, while it would appear there was a treatment-related trend towards hypospermia/aspermia in males, but it should be noted that the rabbits in this study were not sexually mature and the first spermatids do not begin to appear until 14-15 weeks of age [2]. Lastly, it is not clear from the pathologist's report why some of the findings were subject to severity grading while others were not.

Local toxicity: There was no evidence of erythema, edema or eschar formation to register a Draize scoring of the injection sites[3] after any of the administrations in any of the animals in this study.

Histologically the injection site in treated animals at the end of the treatment phase showed only a minimally increased incidence of macrophages in the deep dermis of females and the connective tissue of males in the 2nd injection site only, as well as an increased incidence of mild panniculus muscle degeneration in the right injection site after the recovery period. These findings were not further characterized in the pathologist's report.

Serology: Analysis of serum samples for neutralizing antibodies to H5N1 were analyzed through a hemagglutination inhibition assay (HAI). Results are provided below:

Group	Sex	Pre-test	SD 15	SD 29	SD 31	SD 43
1	**Combined**	**<10**	**<10**	**<10**	**<10**	**<10**
1	Male	<10	<10	<10	<10	<10
1	Female	<10	<10	<10	<10	<10
2	**Combined**	**<10**	**<10**	**11.39**	**<10**	**<10**
2	Male	<10	<10	<10	<10	<10
2	Female	<10	<10	12.97	<10	<10
3	**Combined**	**<10**	**12.42**	**226.27**	**226.27**	**348.96**
3	Male	<10	<10	207.49	269.09	380.55
3	Female	<10	15.42	246.75	190.27	320.00

Table 17: Serology results – values presented as geometric mean titers (GMT); SD = study day; values below the lower limit of quantification were used as 10 during GMT calculations

Analysis of serum samples for neutralizing antibodies to H5N1 revealed minimal immunogenic response in only four female animals treated with the vaccine 14 days after the first dosing. However, demonstrable titers were observed in all vaccine treated animals from day 29 onwards. This correlates with the increased globulin levels and decreased albumin to globulin ratios observed on clinical chemistry assessments. There also appeared to be a stronger immunogenic response in females at the end of the treatment phase, but then in males by the end of the recovery phase. In animals treated with either (b) (4) control or adjuvant control a titer of <10 was noted throughout the study period, except for three females in the adjuvant control group. An explanation for this is not provided in the study report, but the minimal detection of antibodies was insufficient to imply cross-contamination or exposure to the test vaccine.

Assessment: There was no treatment-related mortality or any treatment-related changes among clinical signs, ophthalmoscopic examinations, body temperatures, heart rates, respiratory rates, body weight gain, relative food consumption and hematology parameters observed in this study. Overall, the vaccine was well tolerated by animals of both sexes and treatment-related changes during the in-life portion of the study were limited to increases in clinical pathology parameters which are considered anticipated sequelae of the intended systemic immune response rather than as a sign of frank toxicity. There were other changes of statistical significance (data not shown) among the clinical pathology parameters considered incidental that were not included in this review because they were minimal in degree and within the normal bounds of biologic variation and/or trended in a direction without any clinical significance or explanation.

During post-mortem assessments, treatment-related changes were limited to splenic follicular hyperplasia and inflammatory changes at injection sites. These changes were not consistently observed across sexes and study phases and are considered anticipated sequelae of the intended systemic and local immune response to vaccination, respectively. The differences in organ weights and the rest of the findings on post-mortem examinations were considered incidental to the study and not related to the test article.

Immunology performed in this study verified that an active dose was administered. Males appeared to have a stronger immunogenic response from day 29 onward.

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

AH5N1C – DEVELOPMENTAL TOXICITY STUDY (INCLUDING TERATOGENICITY AND POSTNATAL INVESTIGATIONS) BY INTRAMUSCULAR ADMINISTRATIONS IN THE RABBIT

Developmental and reproductive toxicology study number: AB20852
Performing laboratory: (b) (4)
Study initiation date: September 19th, 2016
Final Report date: December 27th, 2017
Test article batch/lot:

- *aH5N1c*: test article batch number 181053
- *Sterile physiological saline (NaCl 0.9%)*: control article batch numbers (b) (4) manufactured by (b) (4)

Animal species and strain: (b) (4) rabbit (b) (4))
Breeder/supplier: (b) (4)
Number of animal per group and sex: 55 females per group (stud males used for mating purposes only and were otherwise not a part of the study)
Age: 16 to 19 weeks at study initiation, 19 to 22 weeks at start of the mating period
Body weight range: 3.2 to 5.0 kg
Means of administration: Intramuscular injection, needle and syringe
Site of administration: Lumbar epaxial musculature, 4 different injection sites
Volume of injection: 0.5 mL
Frequency of administration: 2 pre-mating doses, 2 gestational doses
Dose: 7.5 µg HA, (b) (4) MF59™
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use, pre-filled syringes (one syringe per dose). Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study (see addendum 1).
Report status: Final

Experimental design: Animals were acclimated for at least 11 days, randomized then assigned to 1 of 2 groups according to table 18 below. Administration of saline control and aH5N1c occurred on premating days (PMD) -21 and -7 and gestation days (GD) 7 and 20. Does were assigned to have terminal caesareans on GD 29 or to be humanely terminated on postnatal day (PND) 29.

Group	*Treatment*	*Caesarean Subset*	*Littering Subset*
1	Saline control	25	**30**
2	aH5N1c	25	**30**

Table 18: Group assignments – values represent number of female rabbits assigned to group subsets

Methods for blood collection: Blood from unfasted does was drawn from ear arteries without anesthetic or sedative assistance. Blood samples from fetuses and kits were collected via intracardiac puncture and pooled per litter following sedation oral sodium pentobarbitone (fetuses) or intramuscular administration of ketamine and xylazine (pups)
Randomization procedure: Yes, computer-generated random number assignment
Statistical analysis plan: Yes, with assistance from the (b) (4) data acquisition system
The following parameters were evaluated:

Parameters	*Frequency of Testing*
Cageside observation[3]	**Twice daily**
Clinical observations[4]	**Daily, twice on administration days**
Injection site observations	**Pre-dose, 24 and 48 hours post-dose**
Body weight	**PMD: -21 and -7** **GD: 0, 6, 9, 13, 16, 20, 24, 27, 29 and 34** **PND: 4, 7, 11, 14, 17 21 and 28**
Food consumption	**Recorded daily but reported for:** **PMD: 1-8, 8-14 and 14-21** **GD: 0-6, 6-9, 9-13, 13-16, 16-20, 20-24, 24-27, 27-29** **PND: 0-4, 4-7, 7-11, 11-14, 14-17, 17-21, 21-28**
Body temperature	**Not collected**
Littering data:	
No. kits alive	**PND 0, 4, 7, 11, 14, 17, 21 and 28**
Live kit weight	**PND 4, 7, 11, 14, 17, 21 and 28**
Physical development	**PND 4-7**
Surface righting reflex	**PND 11**
Pupil reflex	**PND 22**
Auditory reflex	**PND 14**
Post-mortem examinations	**Caesarean subset: GD 29** **Littering subset: PND 29**
Immunological response*	**Does: PMD -21, -7; GD 7, 20, 29; PND 29** **Fetuses: GD 29** **Kits: PND 29**

Table 19: Experimental design – PMD = pre-mating day; GD = gestation day; PND = postnatal day; *blood collected from ear arteries from does and intracardiac puncture from fetuses and kits

Postmortem procedures: Does were humanely euthanized on their scheduled termination days (GD 29 and PND 29 for caesarean and littering subsets, respectively) via an intravenous injection of sodium pentobarbitone followed by exsanguination. The carcasses were then weighed and underwent a macroscopic necropsy examination. For caesarean subset females, the following were recorded: pregnancy status, number and distribution of corpora lutea, gravid uterine weight, pre- and post-implantation loss, number and distribution of implantations to correlate with live fetuses, dead fetuses, early resorptions and late resorptions, individual fetal weights and fetal sexing.

[3] Cageside observations include mortality, morbidity, general health and signs of toxicity.
[4] Clinical observations include "…any abnormalities in appearance, behavior or other signs of reaction to treatment."

Fetuses from the caesarean subset were humanely euthanized by oral administration of sodium pentobarbitone then one half were submitted for visceral tissue examinations while the other half processed for skeletal examinations via maceration in potassium hydroxide, staining with Alizarin red and passage into glycerol

Kits from the littering subsets were humanely euthanized in the same fashion and underwent a macroscopic necropsy examination in the same fashion as the does.

RESULTS

Morbidity and mortality: One control group doe (16) was euthanized on PMD 11 after a decline in clinical condition described as "markedly swollen anus, superficial necrosis of the vulva and anus mucosa and pyodermatitis of the tail". This doe had a dilated uterus and renal pelvis on necropsy. On the day of parturition, one control group doe (7) and one treated doe (47) were euthanized for ethical reasons after finding aborted material in their cages. Both had ovarian and oviduct cysts on necropsy. Lastly, one kit from a treated doe (48) was euthanized on PND 25 for declining condition including a swollen anus with soiled fur. None of these deaths were deemed treatment-related.

Clinical observations: Aside from injection site findings discussed below. There were no treatment-related clinical signs observed in either does or kits in this study. Those observed are considered incidental either because they were sporadic, of comparable incidence between treated and controls, or are recognized background findings in laboratory rabbits.

Injection site observations: There was a slightly increased incidence of reversible, very slight to well-defined edema in treated does after the premating phase (2 does) and gestation phase (4 does). Additionally, there was 1 treated doe which presented with very slight induration of the administration site after the first gestation phase administration.

Body weight change:

Study Day	Group 1 (C)	Group 2 (C)	Group 1 (L)	Group 2 (L)
Pre-test to PMD -21	274.4	294.2	275.2	246.2
PMD -21 to -7	301.2	249.3*	360.7	368.2
GD 0 to 6	113.0	122.0	161.2	144.4
GD 6 to 9	18.0	24.7	26.8	56.7**
GD 9 to 13	51.9	54.1	110.2	100.2
GD 13 to 16	25.1	35.0	70.6	67.3
GD 16 to 20	-44.7	-26.2	14.2	3.6
GD 20 to 24	33.9	82.6*	47.7	66.4
GD 24 to 27	7.2	32.0	72.5	70.3
GD 27 to 29	13.0	24.8	64.9	56.0
PND 4 to 7	NA	NA	54.5	64.5
PND 7 to 11	NA	NA	14.4	38.8
PND 11 to 14	NA	NA	51.3	53.7
PND 14 to 17	NA	NA	55.0	50.3
PND 17 to 21	NA	NA	-53.4	-36.0

Study Day	Group 1 (C)	Group 2 (C)	Group 1 (L)	Group 2 (L)
PND 21 to 28	NA	NA	-85.0	-109.0

Table 20: Body weight change – values presented as change in body weight in mean grams (g) between study days; (C) = caesarean subset; (L) = littering subset; PMD = premating day; GD = gestation day; PND = postnatal day; NA = not applicable (animals were terminated); *p<0.05; **p<0.01

There were no treatment-related, toxicologically-relevant differences in body weight gain in does during this study. The differences observed are considered incidental because they are minimal in nature and within the normal bounds of biologic variation.

Food consumption:

Study Day	Group 1 (C)	Group 2 (C)	Group 1 (L)	Group 2 (L)
PMD -21 to -14	185.18	181.22	178.91	177.04
PMD -14 to -7	188.88	181.81	183.44	181.71
PMD -7 to 0	186.92	179.17	182.40	179.91
GD 0 to 6	180.13	174.91	181.85	187.23
GD 6 to 9	179.38	175.45	186.99	194.83
GD 9 to 13	161.31	159.51	181.81	188.61
GD 13 to 16	120.05	123.26	173.95	173.51
GD 16 to 20	128.19	141.61	172.79	177.76
GD 20 to 24	113.15	138.42*	147.88	160.42
GD 24 to 27	77.17	110.67*	114.65	112.18
GD 27 to 29	81.34	107.87*	122.10	126.38
PND 0 to 4	NA	NA	241.14	250.92
PND 4 to 7	NA	NA	302.13	312.08
PND 7 to 11	NA	NA	314.09	337.10
PND 11 to 14	NA	NA	314.4	342.96*
PND 14 to 17	NA	NA	324.17	343.65
PND 17 to 21	NA	NA	328.73	343.97
PND 21 to 28	NA	NA	506.95	530.92

Table 21: Food consumption – values presented as mean grams/animal/day between study days; (C) = caesarean subset; (L) = littering subset; PMD = premating day; GD = gestation day; PND = postnatal day; NA = not applicable (animals were terminated); *p<0.05

There were no treatment-related, toxicologically-relevant differences in food consumption in does during this study. The differences observed are considered incidental because they are minimal in nature and within the normal bounds of biologic variation, or the treated does consumed more food than control does.

Mating performance:

Parameter (unit)	Group 1	Group 2
Does died before pairing (N)	1	0
Does paired (N)	54	55
Does failed to mate (N)	0	1
Does inseminated (N)	0	1
Does not pregnant (N)	4	6

Parameter (unit)	Group 1	Group 2
Does pregnant (N)	50	48
Inseminated caesarean subset does with viable fetuses (N)	22	23
Inseminated littering subset does that aborted (N)	1	1
Pregnant littering subset does that littered (N)	27	24
Pregnant littering subset does that reared kits to weaning (N)	27	24
Pre-coital interval (mean days)	1.46	1.17
Copulation index (%)	100	98
Fertility index (%)	93	89

Table 22: Mating performance – copulation index = (no. inseminated females/no. paired females) x 100; fertility index = (no. pregnant females/no. inseminated females) x 100

Caesarean examinations:

Parameter (unit)	Presented As	Group 1	Group 2
Females pregnant (N)	Total	22	23
Does with viable fetuses (N)	Total	22	23
Gravid uterus weight (g)	Mean	559.49	551.97
Corpora lutea (N)	Mean	10.2	9.8
Implantations (N)	Mean	9.1	8.7
Pre-implantation loss (N)	Mean	1.1	1.1
Pre-implantation loss (%)	Mean	10.56	13.38
Early resorptions (N)	Mean	0.1	0.1
Early resorptions (%)	Mean	1.17	1.16
Late resorptions (N)	Mean	0.1	0.3
Late resorptions (%)	Mean	1.34	3.41
Dead fetuses (N)	Mean	0.0	0.0
Post-implantation loss (N)	Mean	0.3	0.5
Post-implantation loss (%)	Mean	2.51	4.57
Live fetuses per doe (N)	Mean	8.8	8.2
Female fetuses/litter (N)	Mean	3.8	4.3
Male fetuses/litter (%)	Mean	57.82	44.58
Total litter weight (g)	Mean	380.94	366.40
Fetal weight (g)	Mean	43.49	45.91
Male fetal weight (g)	Mean	43.79	46.01
Female fetal weight (g)	Mean	42.32	45.18

Table 23: Caesarean examination results

There were no treatment-related, toxicologically-relevant differences in both mating performance and caesarean examination results. The differences observed are considered incidental because they are minimal in nature and within the normal bounds of biologic variation, and/or are within the historical reference range for the testing facility.

Fetal examinations:

Examination Finding	Group 1	Group 2
Forepaw, unossified metacarpal 1st digit (V)	4/4	2/1
Forepaw, unossified middle phalanx (V)	1/1	4/3
Gall bladder, bilobed (A)	1/1	0/0
Gall bladder, small (A)	4/4	6/3
Hindpaw, unossified phalanx (A)	1/1	1/1
Hindpaw, unossified tarsal bone (A)	2/2	0/0
Liver, misshapen (A)	1/1	0/0
Lung, small lobe (A)	0/0	2/2
Major blood vessel, absent common carotid trunk (V)	96/21	90/22
Ovary, cyst (A)	2/2	2/2
Pelvic girdle, malpositioned (A)	1/1	3/3
Pelvic girdle, unossified pubis (A)	1/1	0/0
Ribs, detached (A)	0/0	3/3
Ribs, interrupted (A)	4/2	0/0
Ribs, number of full ribs = 12/12 (V)	105/20	78/19
Ribs, number of full ribs = 12/13 (V)	33/18	34/20
Ribs, short (A)	24/15	18/13
Ribs, supernumerary cervical (A)	0/0	1/1
Sibs, supernumerary lumbar (A)	31/17	17/9
Skull, incomplete presphenoid ossification (A)	1/1	2/1
Sternebra, asymmetric (A)	0/0	1/1
Sternebra, bipartite ossification (A)	0/0	1/1
Sternebra, minor fusion (A)	2/2	3/3
Sternebra, unossified, 5th (V)	33/11	39/12
Sternebra, unossified, 6th (V)	1/1	6/5
Testis, cyst (A)	1/1	0/0
Ureter, retrocaval (A)	1/1	1/1
Vertebra (caudal), malpositioned (A)	2/2	1/1
Vertebra (cervical), hemicentric centrum (A)	0/0	1/1
Vertebra (lumbar), number = 6 (V)	63/18	70/20
Vertebra (lumbar), number = 8 (V)	1/1	3/2
Vertebra (thoracic), multiple abnormalities (M)	2/2	0/0
Vertebra (thoracic), number = 12 (V)	106/20	78/19

Table 24: Fetal examination results – total kits affected and total litters affected separated by a '/'; findings classified as (A) = anomaly, (V) = variation and (M) = malformation

There were no treatment-related fetal anomalies, variations or malformations observed in this study. The findings in table 24 above are considered incidental because they were either sporadic in incidence, of comparable incidence between treated and control groups, were within the historical reference range for the facility or did not result in any observable change in kits allowed to rear in the littering subsets.

Delivery and Littering results:

Parameter (unit)	Presented As	Group 1	Group 2
Does Completing Delivery (N)	Total	25	24
Does with liveborn kits (N)	Total	25	24
Does with stillborn kits (N)	Total	4	5
Does with all stillborn kits (N)	Total	0	0
Does with all dead pups on PND 29 (N)	Total	0	0
Gestation length (days)	Mean	31.4	31.3
Number of implantation sites (N)	Mean	8.8	9.5
Pre-birth loss (%)	Mean	5.19	4.15
Pups delivered/litter (N)	Mean	8.3	9.1
Live pups on PND 0 (N)	Mean	8.1	8.8
Live pups on PND 4 (N)	Mean	7.8	8.5
Live pups on PND 7 (N)	Mean	7.8	8.5
Live pups on PND 11 (N)	Mean	7.6	8.4
Live pups on PND 14 (N)	Mean	7.6	8.4
Live pups on PND 17 (N)	Mean	7.5	8.3
Live pups on PND 21 (N)	Mean	7.5	8.3
Live pups on PND 28 (N)	Mean	7.5	8.2
Live pups on PND 29 (N)	Mean	7.5	8.2
Pups dead, missing or cannibalized on PND 0	Sum	5	7
Pups dead, missing or cannibalized on PND 1-7	Sum	7	7
Pups dead, missing or cannibalized on PND 8-14	Sum	7	3
Pups dead, missing or cannibalized on PND 15-21	Sum	1	2
Pups dead, missing or cannibalized on PND 22-28	Sum	0	2
Pups dead, missing or cannibalized on PND 1-29	Sum	15	14
Live birth index (%)	%	97.6	98.8
Viability index for PND 0-1 (%)	%	100.0	99.5
Lactation index for PND 4-28 (%)	%	93.5	96.1
Sex ratio on PND 29 (% males)	Mean	48.5	57.7

Table 25: Littering results – PND = postnatal day; pre-birth loss = ((implantation site scars - pups born)/implantation site scars) x 100; live birth index = (pups born alive/pregnant females) x 100; viability index = (pups alive on PND 1/pups alive at birth) x 100

There were no treatment-related, toxicologically-relevant differences in delivery and littering parameters in this study. The differences observed in table 25 above are considered incidental because they are within the normal bounds of biologic variation and/or within the historical reference range established by the test facility.

Clinical observations of kits: There were no treatment-related effects on littering subset kits observed during the postnatal period. Abnormal clinical signs observed in this study (e.g. thin, scabs, weak) were considered incidental because they occurred in a comparable rate between kits born from treated and control does.

Body weight (kits):

Postnatal Day	Group 1	Group 2
4	92.15	91.32
7	123.71	124.03
11	165.11	167.99
14	197.50	201.05
17	232.46	234.04
21	282.88	278.90
28	480.96	481.40

Table 26: Kit body weights – values presented in mean grams (g)

There were no treatment-related, toxicologically-relevant differences on kit body weight and growth. The differences observed in table 26 above are considered incidental because they are minimal in nature and within the normal bounds of biologic variation.

Physical development (kits):

Parameter (unit)	Group 1	Group 2
Incisor eruption on PND 4 (% kits positive)	100	100
Fur growth on PND 4 (% kits positive)	76	77
Fur growth on PND 5 (% kits positive)	100	100
Eyes open on PND 7 (% kits positive)	1	0
Eyes open on PND 8 (% kits positive)	1	0
Eyes open on PND 9 (% kits positive)	12	7
Eyes open on PND 10 (% kits positive)	49	38
Eyes open on PND 11 (% kits positive)	86	78
Eyes open on PND 12 (% kits positive)	95	94
Eyes open on PND 13 (% kits positive)	99	100
Eyes open on PND 14 (% kits positive)	100	100
Surface right reflex on PND 11 (% kits positive)	100	100
Pupillary reflex on PND 22 (% kits positive)	100	99
Auditory reflex on PND 14 (% kits positive)	100	100

Table 27: Physical development of kits – PND = postnatal day

There were no treatment-related, toxicologically-relevant differences on kit development. The differences observed in table 26 above are considered incidental because they are minimal in nature and within the normal bounds of biologic variation.

Necropsy results (does):

Caesarean Subset Finding	Group 1 (N=25)	Group 2 (N=25)
Oviduct (right), several cysts	1	1
Oviduct (right), single cyst	2	5
Oviduct (left), several cysts	0	1

Caesarean Subset Finding	Group 1 (N=25)	Group 2 (N=25)
Oviduct (left), single cyst	1	4
Oviducts (bilateral), single cyst	1	1
Skin/subcutis, single dark focus	1	0

Littering Subset Finding	Group 1 (N=25)	Group 2 (N=24)
Oviduct (right), several cysts	3	2
Oviduct (right), single cyst	4	2
Oviduct (left), many cysts	1	0
Oviduct (left), several cysts	1	2
Oviduct (left), single cyst	2	1
Oviducts (bilateral), several cysts	3	1
Oviducts (bilateral), single cyst	2	2

Interim Sacrifice Finding	Group 1 (N=3)	Group 2 (N=5)
Ovaries (bilateral), several dark foci	0	1
Oviduct (right), single cyst	0	1
Skin/subcutis, single sore/crust	1	0
Vagina, dark	0	1
Vagina, abnormal vulvar shape	0	1

Moribund Sacrifice Finding	Group 1 (N=1)	Group 2 (N=0)
Kidney (right), dilated pelvis	1	0
Skin/subcutis (anogenital region), swelling	1	0
Skin/subcutis (near 1st injection site), dark focus	1	0

Unplanned Terminal Sacrifice Finding	Group 1 (N=1)	Group 2 (N=1)
Oviduct (right), single cyst	1	0
Oviduct (left), several cysts	1	0
Oviducts (bilateral), several cysts	0	1

Table 28: Macroscopic pathology findings

There were no treatment-related, toxicologically relevant findings on post-mortem necropsy examinations of does in this study. While there was a slightly increased incidence of oviduct cysts in treated does observed during the gestation phase of the study, this was not observed after the littering phase. The rest of the findings are considered incidental to the study due to being sporadic in incidence (i.e. in a single doe).

Serology: Analysis of serum samples for neutralizing antibodies to H5N1 were analyzed through an enzyme-linked immunosorbent assay (ELISA).

Group	PMD -21	GD 29	PND 29	SC Rate (%)
1 (caesarean)	NE	<606	<606	0
1 (littering)	NE	<606	<606	0
2 (caesarean)	<606	304,258	NA	100
2 (fetuses)	NA	602,304	NA	100
2 (littering)	<606	NE	78,122	100

Table 29: Serology results – values presented in geometric mean titers as the inverse serum diltion at EC50; a titer of 606 was the lower limit of quantitation; PMD = premating day, GD = gestation day, PND = postnatal day, SC rate = seroconversion rate; NE = not evaluated, NA = not evaluated

Analysis of serum samples for neutralizing antibodies to H5N1 revealed a robust immunogenic response was observed in treated does by the end of the gestation phase, though this began to attenuate by the end of the postnatal period in this study. Evidence of passive transfer of antibodies was demonstrated through an even higher titer in caesarean subset fetuses. Seroconversion occurred on 100% of vaccinated does and litters from caesarean subset litters. No detectable antibodies were observed in control does at the end of the gestation and postnatal phases in this study.

Assessment: Aside from a slight incidence of reversible injection site edema in does, there was no treatment-related mortality or any treatment-related effect on the clinical presentation of the dams (including food consumption and body weights), mating performance, female fertility, gestational development of fetuses, parturition, or development of F1 kits. In all, the vaccine was well tolerated by the does in this study and there were no treatment-related adverse effects observed in any of the included parameters in this study. Immunology performed in this study verified that an active dose was administered and confirmed passive transfer of antibodies to F1 generation kits.

CONCLUSIONS

Test article related effects	*Effects considered incidental*
• **Mild, reversible increases in fibrinogen and globulins** • **Splenic follicular hyperplasia** • **Minimal to mild microscopic evidence of inflammatory changes at injection sites**	• **Thymic atrophy** • **Renal basophilic tubules and cortical intratubular mineral deposits** • **Aspermia and hypospermia**

Table 30: Summary of observations

Overall assessment: One repeat-dose toxicology study and one DART study were included in this submission to assess the safety and tolerability of aH5N1c in (b) (4) rabbits. In both of these studies, aH5N1c was well tolerated by the rabbits and there were no treatment-related adverse effects beyond those which are considered anticipated sequelae of the intended immune response. Serology assessments in these studies confirmed the administration of the test articles as well as passive transfer of antibodies to fetuses in the DART study.

In the repeat-dose toxicology study, the rabbits thrice received the test vaccine formulation which contains the (b) (4) (and double the antigen dose intended for human). There was no evidence of clinically-relevant adverse events observed in the treated rabbits in the repeat-dose toxicology study beyond effects which are considered anticipated sequelae of the intended local and systemic immune response to vaccination. Mild, reversible elevations in fibrinogen and blood globulins are expected during the systemic immune response. Fibrinogen is a glycoprotein that is converted to fibrin to aid in clot formation after vascular injury but is also a recognized acute phase protein that rises in blood concentration in response to systemic inflammation. Splenic hyperplasia is also a non-adverse change which occurs in response to systemic inflammation and is not considered a sign of frank toxicity. The presence of inflammation or inflammatory infiltrates at the sites of administration are commonly seen as the local immune response to the vaccine components, the repair response to the injection procedure, or both.

In the DART study, pregnant does received the entire human dose (0.5 mL) of the vaccine formulation which does not contain the (b) (4) formulation 4 times: twice prior to mating and twice while pregnant. There was no evidence of treatment-related, clinically relevant adverse events in either the does or the F1 generation kits. The only treatment-related finding was a low incidence of reversible, very slight edema at injection sites in the does which is a commonly observed finding in nonclinical toxicology studies for investigational vaccines. There were no treatment-related effects on female fertility and mating performance, fetal development, parturition or postnatal development up until 29 days postpartum. Male fertility was not assessed in either study, though there were no treatment-related effects observed on histologic examinations of male reproductive organs in the repeat-dose toxicology study.

Conclusions: This submission is acceptable with regards to nonclinical toxicology and adequate data has been presented demonstrating safety and tolerability of aH5N1c when administered with the adjuvant MF59™. Evidence of systemic inflammation and positive titers on serology adequately demonstrate the immunomodulatory effect of the vaccine. There are no toxicologic issues identified which would preclude approval of the BLA in the intended human population. However, the vaccine formulation with (b) (4) was not assessed in the DART study in this submission and the Prescribing Information (PI) label, section 8, should reflect this study design.

Concurrence: Martin D. Green, PhD, Supervisory Toxicologist

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