Toxicology Review of BLA 125020 supplement 1668

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Sponsor: MedImmune LLC
One MedImmune Way
Gaithersburg, MD 20878

Product: Quadrivalent live attenuated influenza vaccine, intranasal

Cross references: BLA 125020/90.1, 38.0

Proposed use: immunization of individuals 2 to 49 years of age against influenza disease caused by influenza virus subtypes A and both influenza B lineages contained in the vaccine

Reviewer: Steven C. Kunder, Ph.D, DABT

Précis

MedImmune conducted an intranasal toxicology study in ferrets to support the supplemental BLA for Quadrivalent FluMist (MEDI3250). The toxicology study used 3 doses of 10^8 FFU, 4 strains (approximately 10^7 FFU/strain) at a volume of 0.2 ml, the same as the intended clinical dose. No treatment related effects were observed in this study. An intranasal reproductive and developmental toxicology study in rats used the same dose with either 3 pre-mating or 3 pre- and 3 post-mating doses. No treatment-related findings were observed affecting fertility or mating performance, maternal health, gestation or post-natal development. No soft tissue or skeletal anomalies or variations were noted due to treatment. Pup survival, weight, sex were unaffected from birth through weaning. F_1 generation physical and behavioral development appeared unaffected.

Introduction

The purpose of this supplemental Biologics License Application (sBLA) is to seek regulatory approval for Q/LAIV (MEDI3250) for the active immunization of individuals 2 to 49 years of age against influenza disease caused by influenza virus subtypes A and both influenza B lineages contained in the vaccine. Due to the frequent mismatches that have occurred between the B lineage selected for inclusion in the annual trivalent influenza vaccines and the wild-type B lineage that has subsequently circulated, the sponsor developed a Quadrivalent influenza vaccine containing, in addition to the 2 influenza A subtypes, 2 type B strains, one from each B lineage (B/Victoria/02/87-like and B/Yamagata/16/88-like). Quadrivalent live attenuated influenza vaccine (Q/LAIV) is an intranasally administered, live attenuated influenza vaccine intended for the prevention of seasonal influenza illness. As the Quadrivalent and trivalent formulations of the vaccine are nearly identical, a bridging strategy was used by the sponsor for the clinical development of Q/LAIV so that the immunogenicity and safety profile of Q/LAIV were directly compared to two formulations of the currently approved trivalent vaccine, one with a B strain from the B/Victoria lineage and one with a B strain from the B/Yamagata lineage. FluMist is the proprietary name for the MedImmune trivalent live attenuated influenza vaccine that contains A/H1N1, A/H3N2, and one B strain.

For the nonclinical support of this new vaccine, an intranasal toxicology study in ferrets and an intranasal reproductive/developmental toxicology study in rats were conducted. Similar nonclinical studies were used to support the safety of the trivalent vaccine.

Clinical study: A multicenter, randomized, double-blind, active-controlled, non-inferiority study (MI-CP208) was performed to assess the immunogenicity of FluMist Quadrivalent compared to FluMist (active control) in children and adolescents 2 through 17 years of age. A total of 2312 subjects were randomized by site at a 3:1:1 ratio to receive either FluMist Quadrivalent or one of two formulations of comparator vaccine FluMist, each containing a B strain that corresponded to one of the two B strains in FluMist Quadrivalent (a B strain of the Yamagata lineage or a B strain of the Victoria lineage). Children 2 through 8 years of age with no history of influenza vaccination received 2 doses (0.2 ml each, $10^{7.5}$ FFU per strain, 4 strains) approximately 30 days apart. Children 2 through 8 years of age with a history of influenza vaccination and children 9 years of age and older received 1 dose.

Studies reviewed within this submission:
1. Intranasal dose toxicology study of MED13250 (CAIV-Q) influenza vaccine in ferrets, SVT08-18
2. Reproductive and Developmental Toxicology Study of MEDI3250 in Rats, 20001854

Toxicology Study Review
Title and study number: Intranasal dose toxicology study of MED13250 (CAIV-Q) influenza vaccine in ferrets, (b)(4)T08-18
Performing laboratory: ----------(b)(4)----------
Study initiation date: June 2, 2008
Final Report date: December 10, 2008
Test article batch/lot: lot no. 052908
Animal species and strain: --------*(b)(4)*---------- (ferret)
Breeder/supplier: ----------(b)(4)----------
Number of animal per group and sex: 15
Age: 7 weeks
Body weight range: 235-426 grams for females and 313-495 grams for males
Route and site of administration: intranasal, nares
Volume of inhalation: 0.1 mL (0.05 mL per nare)
Frequency of administration and study duration: days 0, 14, 28; 31 days treatment to sacrifice, 56 days for treatment, recovery and sacrifice
Dose: $10^{7.5}$ FFU per strain, 4 strains
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was 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 appendix).

The egg produced CAIV-Q material, lot # 052908 was used for the inhalation toxicology study. The assay dates for the stability data for this lot were coordinated with the animal dosing dates. Samples were assayed approximately 1 week before dosing per study calendar. Storage of Lot 052908 is at (b)(4) from date of manufacture.

Stability of CAIV-Q

Antigen	-19 days	0 days	14 days	28 days	56 days
	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD
H1-A/South Dakota	7.4 0.08	7.4 0.07	7.4 0.07	7.6 0.07	7.7 0.10
H3-A/ Uruguay	7.2 0.08	7.1 0.00	7.1 0.00.	7.2 0.00	7.4 0.00
IY-B/ Florida	7.5 0.08	7.4 0.00	7.4 0.00	7.6 0.07	7.6 0.13
BN-B/ Malaysia	7.6 0.06	7.0 0.07	7.8 0.00	7.8 0.14	7.7 0.12

Titers are reported in log10 FFU/mL SD=study day

Antigens showed no potency loss during the study period. The CAIV-Q quadrivalent vaccine potency appears to be stable at (b)(4) storage.

Means of administration: Up to three (3) intranasal administrations were given at weeks 0, 4, and 14 (study days 0, 28, and 98, respectively) in an inoculation volume of 0.2 ml (0.1 per nare). The AccuSpray clinical delivery device was pre-loaded by the sponsor with an inoculation volume of 0.2 ml and is designed with a stop device that allows for 0.1 ml of material to be delivered to each nostril of the ferret. Animals were briefly, but deeply, anesthetized with 5.0% isofluorane during the intranasal administration procedure.
Report status: final

Experimental design

group	Test/control article	Dose (FFU)	Day 0 dose	Day 14 dose	Day 28 dose	Necropsy days	Total # animals
1	saline	0	15/sex	10/sex	10/sex	3, 31, 56 (5/sex/group/interval)	30
2	MEDI3250 CAIV-Q	$10^{7.5}$	15/sex	10/sex	10/sex	3, 31, 56 (5/sex/group/interval)	30

Methods:

Endpoint	Methodology
Hematology	----------(b)(4)---------- Blood samples were analyzed for the following parameters: erythrocyte count (RBC), total leukocyte count (WBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT) and platelet count (PLT). The differential white cell count included counts of neutrophils (NEUT), lymphocytes (LYM), basophils (BAS), eosinophils (EOS) and monocytes (MONO).
Clinical chemistry	----------(b)(4)---------- The following serum chemistry parameters were determined: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN), calcium (CA), cholesterol (CHOL), chloride (CL), creatine phosphokinase (CK), creatinine (CREAT), direct bilirubin (DBILI), gamma glutamyl transferase (GGT), globulin (GLOB), total protein (TP), glucose (GLU), potassium (K), lactate dehydrogenase (LDH), sodium (NA), phosphorus (PHOS) and total bilirubin (TBILI).
Coagulation	Not performed

Randomization procedure: Following the quarantine period ferrets were randomized by assignment to groups using a computer-generated randomization program (----------(b)(4)----------).

Statistical analysis plan: Group means and standard deviations were determined separately for males and females for all measured clinical parameters (hematology, serum chemistry, body weights, body temperatures, organ weights and organ-to-body weight ratios) using an Excel spread sheet. Statistical significance was determined using a ----------(b)(4)----------. Group data was analyzed for normality and equal variance. If the data set passed these tests the data was analyzed by one-way ANOVA for statistical differences between the means of the groups.

When a significant difference between the means of one or more of the groups occurred, a Dunnett's or Dun's test, whichever was appropriate, was performed using Group 1 as the control group. If the data set failed to pass the normality or equal variance tests the data set was analyzed using a nonparametric Kruskal-Wallis Analysis of Variances on Ranks. If there was a significant difference between the medians of any of the groups a Dunnett's or Dunn's test was performed, whichever was appropriate, using Group 1 as the control group. Organ to body weight ratios were analyzed using a nonparametric Kruskal-Wallis Analysis of Variances on Ranks. If there was a significant difference between the medians of any of the groups a Dunnett's or Dun's test was performed,

whichever was appropriate, using Group 1 as the control group. For all data sets, statistical comparisons were made at the $p < 0.05$ level (2- tailed).

The following parameters were evaluated:

Parameters	Frequency of Testing
Dose	Day 0 Dose 1 Day 14 Dose 2 Day 28 Dose 3
Cageside observation[1]	daily
Physical examination	0-7, 14-21, 28-35, 56
Body weight	Days 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 49, 52, 56
Body temperature	0-7, 14-21, 28-35, 56
Urinalysis	Day -4 or -1, 2, 30, 55
Clinical chemistry*	0, 3, 28, 31, 56
Hematology*	0, 3, 28, 31, 56
Immunological response*	0, 3, 28, 31, 56
Necropsy	3, 31, 56 (5/sex/group)

*(vena cava days 0, 28; vena cava or cardiac puncture, days 3, 31)

Postmortem procedures: The following tissues were collected at necropsy. Weighing and histopathology are noted.

Organ/Tissue	Collected	Weighed	Histopathology
Adrenal glands	X	x	x
Aorta	x		
Bone (sternum & femur)	x		
Bone marrow (sternum & femur)	X, smear from femur		x
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	x	x	x
Cervix	x		
Colon	x		
Duodenum	x		
Epididymides	x		
Esophagus	x		x
Eyes (optic nerve)	x		
Fallopian tubes (oviduct)	x		
Gall bladder	x		
Gross lesions (if any)	x		x
Heart	x	x	x
Ileum	x		
Injection site(s)			
Jejunum	x		

[1] Cageside observations include mortality, morbidity, general health and signs of toxicity.

Organ/Tissue	Collected	Weighed	Histopathology
Kidneys	x	x	x
Lacrimal glands	x		
Larynx	x		x
Liver	x	x	x
Lung (main-stem; bronchi)	x	x	x
Lymph nodes (cervical)	x	x	x
Lymph nodes (mandibular)	x		
Lymph nodes (mesenteric)	x		
Mammary glands			
Naso-oropharyngeal cavity (turbinates, nares, soft palate)	x		x
Ovaries	x	x	x
Pancreas	x		
Peyer's patch (if applicable)	x		
Pituitary gland	x		
Prostate	x		
Rectum	x		
Salivary glands (mandibular)	x		
Sciatic nerve	x		
Skeletal muscle	x		
Skin	x		
Spinal cord (cervical, lumbar, thoracic)	x		
Spleen	x	x	x
Stomach (squamous and glandular)	x		x
Testes	x	x	x
Thymus	x	x	x
Thyroid (w/ parathyroid glands)	x		
Tongue	x		
Trachea	x		x
Ureters	x		
Uterus (w/ cervix)	x		
Urinary bladder	x		
Vagina	x		

Table of Histology – Tissues examined, all dose group and control

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

Table of 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 great than 1.5 so indicated otherwise ≤0.7)**)**	NOT OF NOTE
ELECTROLYTE BALANCE	Magnesium, M day 3, ↑1.2x F, day 3, ↑1.3x Calcium, F day 28, ↑1.1x, Day 31, ↑1.1x Sodium, F day 31, ↑1.1x Chloride, F day 31, ↑1.1x F, ↑1.1x Phosphorus, M, day 31, ↑1.2x F, day 31, ↑1.1x	Potassium
CARBOHYDRATE METABOLISM	Glucose, M, day 28, ↑1.1x Lactate dehydrogenase, F, day 31, ↓, 0.65x	
LIVER FUNCTION: A) HEPATOCELLULAR	Alkaline phosphatase (ALP), M , day 31,↑ 1.2x	Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase, ND Sorbitol dehydrogenase, ND Total bile acids, ND
B) HEPATOBILIARY		Gamma-glutamyl transferase (GGT) ND Total bile acids ND Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein, fibrinogen (also under coagulation), ND
KIDNEY FUNCTION		Creatinine Blood urea nitrogen

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤0.7)**)**	NOT OF NOTE
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase, ND Total protein,ND Fasting triglycerides, ND
MUSCLE INJURY	Creatine kinase, m, day 28, ↓1.14x	Creatine phosphokinase (CPK)[2]

[2] Serum CPK activities in the range of 2000 to 3000 IU/liter following intramuscular dosing should be considered to have significant potential for human toxicity (Gray, Fundamental and Applied Tox 1:290, 1981). Minor increases in CPK serum levels (2 to 3 fold elevations) may be indicative of a febrile response (Mukhutdinova, Bulletin of Experimental Bio Med: 128: 674, 1999)

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 1.5[3], ie, ≥1.6 or ≤ 0.7	NOT OF NOTE
RED BLOOD CELLS	RBC day 3, m, ↓; f, ↓ HCT, day 3, m, ↓ MCH, day 3, f, ↑ MCHC, day, 3, ↑ day 28, f, ↓ day 31, f, ↑ Mean Corp. Volume (MCV), day 31, f, ↓	Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Reticulocytes, ND
WHITE BLOOD CELLS	Lymphocyte count, day 28, f, ↑ Neutrophil count, day 28, f, ↓	Basophils, eosinophils count Macrophage/monocyte count Total leukocytes (WBC) Large unstained cells (LUC)
CLOTTING POTENTIAL	Platelet vol, day 28, m, ↓; f, ↑	Activated partial-thromboplastin time clotting time, ND Prothrombin time, ND Fibrinogen, ND
OTHERS		Bone marrow cytology ND

Table of Hematology Results. ND = not determined)

Systemic toxicity:
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight, clinical chemistry, gross anatomy or organ weight were observed. Hematology findings included decreased RBC on day 3, males and females, decreased HCT, day 3 males, increased MHC in females on day 3, increased MCHC in females on day 3 and 31 but decreased on day 28, decreased MCV in females day 31, increased lymphocyte count on day 28 in females, decreased neutrophil count on day 28 in females, and increased platelet volume in male, decreased in females on day 28. Other than the RBC effects on day 3, no consistent hematologic effect appears related to treatment. The day 3 RBC effects may be related to initial inflammation and tissue injury following the first dose of vaccine.

[3] 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.

Tables of Organ Weights, day 31, 56

Summary of Organ Weight Data Day 31 sacrifice after 3rd dose

Group	Stat	B Wt.[1] (g)	ADR[2] (g)	BRA (g)	HEA (g)	KID[2] (g)	LIV (g)	LUN[2] (g)	LCLN22 (g)	RCLN[2] (g)	OVA (g)	TES[2] (g)	SPL (g)	THM (g)
GROUP 1 FEMALE	AVG	703	0.113	6.95	3.56	4.48	20.97	5.81	0.047	0.074	0.083	N/A	5.26	4.028
	STDEV	74	0.035	0.25	0.22	0.38	1.87	1.02	0.013	0.029	0.021	N/A	0.90	1.142
	NUM	5	5	5	5	5	5	5	5	5	5	N/A	5	5
MALE	AVG	812	0.245	8.07	4.16	5.69	26.47	7.60	0.071	0.065	N/A	0.28	5.78	3.564
	STDEV	121	0.235	0.32	0.44	0.66	3.45	1.06	0.036	0.029	N/A	0.06	0.74	0.863
	NUM	5	5	5	5	5	5	5	5	5	N/A	5	5	5
GROUP 2 FEMALE	AVG	663	0.123	6.81	3.35	4.05	18.35 *	5.91	0.063	0.055	0.085	N/A	6.06	4.232
	STDEV	76	0.033	0.15	0.23	031	1.70	0.43	0.024	0.033	0.040	N/A	1.03	1.244
	NUM	5	5	5	5	5	5	5	5	5	5	N/A	5	5
MALE	AVG	863	0.310	7.87	4.10	5.31	24.52	8.22	0.055	0.048	N/A	0.36 *	7.02	3.665
	STDEV	95	0.397	0.29	0.51	0.48	2.02	0.67	0.024	0.016	N/A	0.03	1.28	2.024
	NUM	5	5	5	5	5		5	5	5	N/A	5	5	5

[1] Fasted body weight.
[2] Paired organs weighed together.
Statistical key: *p<0.050 (all statistical comparisons made against Group 1 Control)

Summary of Organ Weight Data Day 56 following recovery

Group	Stat	B Wt.[1] (g)	ADR[2] (g)	BRA (g)	HEA (g)	KID[2] (g)	LIV (g)	LUN[2] (g)	LCLN (g)	RCLN (g)	OVA[2] (g)	TES[2] (g)	SPL (g)	THM (g)
GROUP 1 FEMALE	AVG	917 62	0.117	6.59	4.24	4.68	23.92	6.17	0.050	0.076	0.077	N/A	5.74	5.33
	STDEV	5	0.014	0.41	0.61	0.39	4.83	0.63	0.022	0.015	0.010	N/A	1.40	1.12
	NUM		5	5	5	5	5	5	5	5	5	N/A	5	5
MALE	AVG	1311	0.153	8.26	5.06	6.64	30.85	8.80	0.068	0.089	N/A N/A	0.76	7.07	7.01
	STDEV	117	0.020	0.80	0.48	0.42	1.23	0.68	0.008	0.033	N/A	0.20	0.80	0.58
	NUM	5	5	5	5	5	5	5	5	5		5	5	5
GROUP 2 FEMALE	AVG	868 119	0.107	6.74	3.87	4.44	20.63	5.92	0.057	0.063	0.081	N/A	5.73	4.06
	STDEV	5	0.013	0.52	0.51	0.65	2.96	0.60	0.012	0.020	0.013	N/A	1.34	1.31
	NUM		5	5	5	5	5	5	5	5	5	N/A	5	5
MALE	AVG	1147	0.153	7.82	4.93	6.09	29.0	9.57	0.064	0.123	N/A N/A	0.84	8.92	4.55*
	STDEV	175	0.021	0.17	0.34	0.58	3.97	2.82	0.019	0.053	N/A	0.36	1.64	1.01
	NUM	5	5	5	5	5	5	5	5	5		5	5	5

[1] Fasted body weight.
[2] Paired organs weighed together. Statistical key:
*p<0.050 (all statistical comparisons made against Group 1 Control)

Organ weight tables based upon those provided by sponsor.

Following treatment (3 doses), study day 31 and recovery, study day 56, the following significant (p<0.050) organ weight changes were observed:
Day 31: liver, treated females, decreased
Testes, treated males, increased
Day 56 thymus, treated males, decreased
No histopathologic findings correlated with these differences in organ weight

Gross Pathology:

Table of Gross Pathology Findings

GROUP	FINDINGS DAY 3	FINDINGS DAY 31	FINDINGS DAY 56
1M	NF	Adrenal and renal cysts 4/5	NF
2M	NF	Adrenal and renal cysts 5/5	Enlarged LN 1/5 Lung foci correlated w Inflammatory exudate, 1/5
1F	NF	Adrenal and renal cysts 5/5	NF
2F	NF	Adrenal and renal cysts 5/5	Thickened gall bladder 1/5
NF= no findings			

Histopathology:

Table of Histopathology Findings:

GROUP	FINDINGS DAY 3	FINDINGS DAY 31	FINDINGS DAY 56
1M CONTROL	Nasal turbinate: Inflammation: nasal epithelium 5/5	Nasal turbinate: Inflammation: Nasal epithelium 4/5 Vomeronasal organ 4/5	Nasal turbinate: Hyperplasia, Nasal epithelium, 4/5 Inflammation: Nasal epithelium 5/5 Vomeronasal organ 5/5 Cervical LN, hyperplasia 1/5
2M	Nasal turbinate: Inflammation: Nasal epithelium 5/5 Exudate, cellular 5/5	Nasal turbinate: Inflammation: Nasal epithelium 5/5 Vomeronasal organ 5/5 Nasal epithelium erosion 1/5 Exudate, cellular 5/5	Nasal turbinate: Hyperplasia, nasal epithelium 2/5 Inflammation, nasal epithelium 5/5 Vomeronasal organ 4/5
1F control	Nasal turbinate Inflammation, nasal epithelium 2/5	Nasal turbinate: Inflammation: nasal epithelium 5/5 Vomeronasal organ 4/5 Exudate, cellular 2/5	Nasal turbinate: Inflammation, nasal epithelium 5/5 Vomeronasal organ 4/5 Cervical LN, hyperplasia 2/5
2F	Nasal turbinate Inflammation: Nasal epithelium 5/5 Exudate, cellular 5/5	Nasal turbinate: Inflammation: Nasal epithelium 5/5 Vomeronasal organ 5/5 Exudate, cellular 3/5	Hyperplasia, Nasal epithelium, 1/5 Inflammation, Nasal epithelium 5/5 Vomeronasal organ 4/5

Day 3 Sacrifice**:** Microscopic findings considered to be test article-related were in the nasal turbinates. The most pronounced of these findings was inflammatory cell exudate in all Group 2 males and females. The exudate was in the nasal cavity, usually in the folds of the turbinates (meatus). Inflammatory exudate was not seen in control males and females.
There was an increased mean severity of acute inflammation of the nasal epithelium for Group 2 males and an increased incidence and mean severity of acute inflammation of the nasal epithelium for Group 2 females compared to the control groups. Inflammation of the vomeronasal organ was also observed.

Day 31 Sacrifice: Microscopic findings considered to be test article-related were in level 1 of the nasal turbinates. These findings, however, were not as clearly different between control and treated groups as they were at Day 3.
The primary difference between the control and the treated groups was an increased mean severity of acute inflammation of the nasal epithelium in Group 2 animals, males more so than females. In contrast to Day 3, cellular exudate was not as consistently present in Group 2 animals although two Group 2 males had minimal exudate compared to the lack of exudate in the control male group; both control and Group 2 females had comparable incidences and severity for cellular exudate. Erosion, hyperplasia, squamous metaplasia and syncytial formation of the nasal epithelium were seen in Group 2 males and not in the control males. However, since control females also had similar findings (other than erosion) that were not seen in the Group 2 females, these changes were of questionable significance with respect to test article exposure.
Male and female treated and control groups had focal inflammation of the nasal mucosa at the inoculation site at roughly comparable incidences and at comparable severities; this was not considered a test article-related finding.
All other changes, including differences in the incidences of occasional background findings were considered incidental and not related to treatment.
Inflammation of the vomeronasal organ also appeared not related to test to test article-exposure due to similar incidence and severity in treated and control males and females.

Day 56 Sacrifice:. There were no microscopic findings at Day 56 that were clearly test article-related. Group 2 males had a very slight increased mean severity of acute inflammation of the nasal turbinates compared to the control males but overall the incidence and mean severity of acute inflammation was considered similar between control and treated groups. The control males had an increased incidence and/or mean severity of changes (nasal epithelial hyperplasia and nasal squamous metaplasia) compared to the Group 2 males that was considered to be secondary to inflammation.
Males overall had more changes in level 1 of the nasal turbinates than did the females.
The significance of single incidences of syncytial formation in a Group 2 male and nasal epithelial hyperplasia in a Group 2 female in the nasal turbinates was not clearly treatment related.
Two incidences of lymphoid follicular hyperplasia was observed in Group 2 males compared to no incidences in the control males. Since the hyperplasia was also present in one Group 1 (control) female and was seen in only one of a pair of draining lymph nodes, this change does not appear to be treatment-related.
One other notable change was moderately severe subacute exudative inflammation in the lung of one Group 2 male. Foreign material, granulomatous foci and some airway inflammation was found, with neutrophilic and histiocytic infiltrates in the alveolar spaces. It was not clear if the severity of inflammation in this animal's lung had a similar cause as the much milder inflammation seen in most other lungs from controls and treated animals.

Body temperature
Body temperatures did not exceed 40º C at any timepoint

Local toxicity: see gross pathology, histopathology for nasal findings.

Serology: Hemagglutination inhibition (HAI) assay was used to measure influenza vaccine induced serum antibody response. Results from the HAI assay showed all ferrets had undetectable antibody titer prior to treatment on Day 0 and that all Group 1

(PBS) animals had undetectable antibody titer on Days 0, 3, 28, 31 and 56. After 3 doses 29/30 animals in Group 2 (Q/CAIV-treated) had detectable antibodies on Days 28, 31 and 56 but not on Day 3. One animal in Group 2 had a low titer response (4) to one of the inoculated strains, *AlUruguay/716/07,* on Days 28 and 56 while this animal's responses to the other three strains, AlSouth *Dakotal6/07, B/Floridal4/06* and B/Maiaysial1506/04 were higher and more consistent with other Group 2 animals. Otherwise, a titer of 8-512 for each individual strain was observed in all vaccine treated animals from day 28 onwards. In animals treated with either PBS control a titer of <4 was noted throughout the study period, indicating control animals were not exposed to vaccine.

Serology results by hemagglutination inhibition (HAI) assay for the repeat intranasal dose toxicology study of MEDI3250 (Q/CAIV) in ferrets (----------(b)(4)---------- Study No. (b)(4)T08-18) confirm that animals in this toxicology study had the intended seroconversion indicative of antigenic exposure following treatment with three intranasal doses of quadrivalent influenza virus vaccine, MEDI3250.

TEST ARTICLE RELATED EFFECTS	EFFECTS CONSIDERED INCIDENTAL
nasal turbinates with cellular exudate and acute inflammation of the nasal epithelium for Group 2 animals at Day 3, 31 and 56 syncytium formation day 56 male, nasal epithelium nasal epithelial hyperplasia, treated female day 56	inflammation of the vomeronasal organ

Assessment:
There were no clear treatment-related effects on clinical pathology parameters including clinical chemistry and hematology, although there were a number of statistically significant differences (data not shown). Many of these differences were of a magnitude or nature that was not clinically significant or that remained within the normal range of values established for gender, laboratory or species.

The nasal turbinates received the inhaled vaccine resulting in cellular exudate and acute inflammation of the nasal epithelium for Group 2 animals at Day 3, 31 and 56, syncytium formation nasal epithelium on day 56 male, and nasal epithelial hyperplasia, treated female day 56. Inflammatory responses at the nasal turbinate are anticipated in response to an irritant such as the vaccine. Syncitial formation and epithelial hyperplasia are typical responses to tissue damage induced by treatment.

Other than the inflammation and injury responses noted, there were other no treatment-related effects on histopathology, and any histopathology findings were considered as incidental to the study and not related to the test article. The correlation of the Inflammation of the vomeronasal organ in ferrets to clinical exposure is unclear. The existence and role of the vomeronasal organ in humans is controversial. The location of the vomeronasal organ exposes it to irritants such as the vaccine of an inhalation toxicology study which makes inflammatory findings for this structure not unexpected.

These findings were similar to those seen in the toxicology study results for BLA 125020/90.1 for the trivalent Flumist vaccine. Nasal inflammation also was seen in the

toxicology studies supporting the trivalent vaccine. The addition of a fourth antigen might be anticipated to induce a greater immune response. The trivalent vaccine produced nasal inflammation mainly after the initial dose while the present quadrivalent vaccine produced a more prolonged inflammatory response during the second and third doses.

There were no treatment-related effects on body weights, organ weight, or gross pathology.

Immunology performed in this study verified that an active dose was administered. No differences were found between female and male animals.

The ferret is regarded as a well characterized animal model for inhaled influenza studies. As an animal model for toxicologic studies it is less well characterized than other rodents.

GLP Study Deviations or amendments: No protocol deviations were noted that impacted on the quality or integrity of the studies.

This nonclinical study supports the safety of this vaccine for its intended clinical application.

Reproductive and developmental toxicology

Study title: Reproductive and Developmental Toxicology Study of MEDI3250 in Rats (GLP)

Key study findings: MEDI3250 administered on GDs 6, 13, and 20 or weekly prior to mating on SD 1, 8, and 14 and weekly during gestation did not demonstrate maternal toxicity, embryofetal toxicity or affect growth and development of the F1 generation offspring through weaning.

Study no.:20001854
Conducting laboratory and location: ----------
---------(b)(4)----------

Date of study initiation: July 8, 2010
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: CAIV-Q-(b)(4) (MEDI3250), lot no 0141700024
Vehicle SPGAG buffer prepared from buffer 1 [sucrose phosphate, ----------(b)(4)----------] and buffer [concentrated gelatin arginine glutamate]
Methods:

Doses:

GROUP	DOSE PREMATING (FFU/STRAIN)	DOSES-POSTMATING (FFU/STRAIN)	# RATS *
GROUP 1 CONTROL	0	0	50
GROUP 2 MEDI3250	0	$10^{7.0 \pm 0.5}$	50
GROUP 3 CONTROL	0	0	50
GROUP 4 MEDI3250	$10^{7.0 \pm 0.5}$	$10^{7.0 \pm 0.5}$	50

* 25 rats per dosage group assigned to Cesarean-sectioning and 25 rats per dosage group assigned to natural delivery

Species/strain: rat, ----(b)(4)----
Number/sex/group: 50 females
Route, formulation, volume: intranasal, SPGAG buffer, 0.2ml
Study design: see dose chart above
Frequency of administration
F0 Generation Rats
Female rats assigned to Groups 1 and 2 were given the test article and/or the vehicle once on GDs 6, 13 and 20. Female rats assigned to Groups 3 and 4 were given the test article and/or vehicle once weekly beginning two weeks prior to mating (a total 3 doses administered on DS 1, 8 and 14) and once weekly during the gestation period for a total of 3 doses.
Rats were anesthetized using isofluorane/oxygen immediately prior to administration for a duration of less than 5 minutes.
The test article or vehicle was administered by intranasal instillation. A total volume of 200 µL was administered to each rat. The total volume was split between each nostril from a blunted needle or pipette tip for a total of 100 µL per nostril.

Rats administered the test article were administered the intended human dose of 10^7 ± 0.5 FFU/strain of Q/LAIV with a total virus content of approximately 10^8 FFU administered to each rat.
F1 generation pups were not directly given the test article or vehicle but may have been exposed to the test article or vehicle during maternal gestation (*in utero* exposure) or via maternal milk during the lactation period.

Parameters and endpoints evaluated:
Method of Study Performance

F0 Generation Rats

After acclimation, virgin female rats were cohabitated with breeder male rats, one male rat per female rat. The cohabitation period consisted of a maximum of 5 days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* were considered to be at GD 0 and assigned to individual housing. Rats were observed for viability twice each day of the study and for clinical observations and general appearance at least weekly during acclimation and on GD 0. The rats were also examined for clinical observations, abortions, premature deliveries and deaths weekly before dosage administration, within two hours after dosage administration, daily during non-dosing days and during the postdosage period.
Body weights for Group 1 and 2 rats were recorded at least weekly during the acclimation period, on GD 0, and GD 6, 13, 20, 21 and 25 and LD 1, 7, 10, 14 and 21. Body weights for Group 3 and 4 were recorded at least weekly during the acclimation period, daily during the gestation period and on LDs 1, 7, 10, 14 and 21. Feed consumption for Group 1 and 2 rats were recorded on GDs 0, 6, 13, 20, 21 and 25 and LDs 1, 7, 10 and 14. Feed consumption for Group 3 and 4 were recorded weekly prior to cohabitation, GDs 0, 6 through 21 and 25, LDs 1, 7, 10 and 14. Because pups begin to consume maternal feed on or about LD 14, feed consumption values were not tabulated after LD 14.
Rats assigned to natural delivery were evaluated for adverse clinical signs observed during parturition, duration of gestation (GD 0 to the day the first pup was observed), litter sizes (all pups delivered) and pup viability at birth. Maternal behavior was evaluated on LD 1, 4, 7, 14, 18 and 21 (rats assigned to natural delivery). Litters were examined after delivery to identify the number and sex of pups, stillbirths, live births and gross alterations.
Blood samples were collected before the first dosage was administered (0.4 mL to 0.7 mL) and on GD 21 [rats assigned to Caesarean-sectioning (0.5 mL to 0.6 mL)] or LD 21 [rats assigned to natural delivery (1.0 mL)] from all female rats for the Hemagglutination Assay (HAI).
Blood was collected via the lateral tail vein (in-life collections) or via vena cava after euthanasia (terminal). ----------(b)(4)----------.

On GD 21, fetal blood samples (0.1 mL to 0.45 mL) were collected via decapitation from 2 fetuses/sex/litter (where possible) from dams assigned to Caesarean-sectioning. Blood samples were pooled by sex and litter and processed as previously described for maternal blood samples.
On LD 21, individual pup blood samples (0.1 mL to 1.0 mL) were collected via vena cava following euthanasia from 2 pups/sex/litter from dams assigned to natural delivery. Blood samples were processed as previously described for maternal blood samples.

F1 Generation Pups/Litters

Lactation day 1 (postpartum) was defined as the day of birth and was also the first day on which all pups in a litter were individually weighed (pup body weights were recorded after all pups in a litter were delivered and groomed by the dam).

Each litter was evaluated for viability at least twice daily. The pups in each litter were counted daily. Clinical observations were recorded once daily during the preweaning period. Pup body weights were recorded on LDs 1, 4, 7, 14, 18 and 21.

The following reflex and developmental observations were recorded: surface righting reflex [from postpartum day 1 (PPD 1)], pinna unfolding (from PPD 2), incisor eruption (from PPD 9), eye opening (from PPD 12), acoustic (auditory) startle (from PPD 13), air righting reflex (from PPD 14) and pupil constriction (evaluated once on PPD 21). The number of pups meeting the criterion was recorded on each day of testing. Testing continued daily until the day the criterion was attained by all pups in the litter.

Gross Necropsy

Gross lesions were retained in neutral buffered 10% formalin for possible future evaluation, unless specifically cited below, all other tissues were discarded. Representative photographs of maternal gross lesions and fetal gross, soft tissue and skeletal alterations were retained by the sponsor.

F0 Generation Rats

Female rats assigned to Caesarean-sectioning observations were euthanized by carbon dioxide asphyxiation on GD 21, Caesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Uteri of apparently nonpregnant rats were examined while being pressed between two glass plates to confirm the absence of implantation sites. Uteri and ovaries of apparently nonpregnant rats were retained in neutral buffered 10% formalin and discarded upon authorization by the Study Director. Maternal and fetal blood samples were collected as described above.

The number and distribution of corpora lutea were recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantation sites, early and late resorptions and live and dead fetuses. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to stimuli and that was not markedly autolyzed; dead fetuses demonstrating marked to extreme autolysis were considered to be late resorptions. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption. Placentae were examined for size, color and shape.

Each fetus was removed from the uterus, placed in an individual container and individually identified with a tag noting the study number, litter number, uterine distribution and fixative. Each fetus was subsequently weighed and examined for sex and gross lesions. Live fetuses were euthanized by an intraperitoneal injection of sodium pentobarbital (fetuses selected for blood collection were euthanized by decapitation). Approximately one-half of the fetuses in each litter were examined for soft tissue alterations, using a variation of the microdissection technique of Staples. These fetuses were then fixed in Bouin's solution and the heads were subsequently examined by free-hand sectioning; head sections were stored in alcohol. The decapitated carcasses were discarded. The remaining fetuses (approximately one-half of the fetuses in each litter) were eviscerated, cleared, stained with alizarin red S and examined for skeletal alterations. The fetuses were initially fixed in alcohol. Skeletal preparations were retained in glycerin with thymol added as a preservative.

Female rats assigned to natural delivery were euthanized by carbon dioxide asphyxiation on LD 21. A gross necropsy of the thoracic, abdominal and pelvic viscera

was performed; the number and distribution of implantation sites were recorded. Maternal and pup blood samples were collected. Rats that did not deliver a litter were euthanized on GD 25 and examined for gross lesions. Uteri of apparently non-pregnant rats were examined while being pressed between glass plates to confirm the absence of implantation sites. Uteri and ovaries of apparently nonpregnant rats were retained in neutral buffered 10% formalin.

F1 Generation Pups

Pups that died before initial examination of the litter for pup viability were evaluated for vital status at birth. The lungs were removed and immersed in water. Pups with lungs that sank were considered stillborn; pups with lungs that floated were considered liveborn and to have died shortly after birth. Pups with gross lesions were preserved in Bouin's solution for possible future evaluation.

Pups that died were examined for gross lesions and the cause of death as soon as possible after the observation was made. Pups found on days 2 to 4 postpartum were preserved in Bouin's solution for possible future evaluation; pups found on days 5 to 21 postpartum were preserved in neutral buffered 10% formalin.

All surviving pups were euthanized by carbon dioxide asphyxiation after the 21-day postpartum period, and blood samples were collected from 2 pups/sex/litter and processed as previously described. Pups were examined for gross lesions. All gross lesions were preserved in neutral buffered 10% formalin. Necropsy included a single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly.

Preweaning Developmental Observations

The number of pups meeting the criterion is recorded on each day of testing. Testing continues until the day the criterion is attained by all pups in the litter.

Surface Righting Reflex (ability to right in 5 seconds): From day 1 postpartum.

Pinna Unfolding: From day 2 postpartum.

Incisor Eruption: From day 9 postpartum.

Eye Opening: From day 12 postpartum.

Acoustic (Auditory) Startle: From day 13 postpartum.

Air Righting Reflex: From day 14 postpartum.

Pupil constriction is evaluated once, on day 21 postpartum.

Indices:

Mating Index: Number of confirmed matings/number of rabbits paired. Rats identified as pregnant at postmortem examination or upon parturition were included in the number of confirmed matings.

Fertility Index: Number of pregnancies /number of rats with a confirmed mating.

Gestation Index: Number does with live offspring/number of pregnancies (females assigned to natural delivery).

Viability Index: Number of live pups on PPD 4/number of liveborn pups on PPD 1

Lactation Index: Number of live pups on PPD 21/number of live pups on PPD 4.

Statistical methods Clinical observations and other proportion data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution.

Variables with interval or ratio scales of measurement, such as body weights, feed consumption values and percent mortality per litter were analyzed as described under the Parametric heading of the schematic. Bartlett’s Test of Homogeneity of Variances was used to estimate the probability that the dosage groups have different variances. A non-significant result ($p>0.001$) indicated that an assumption of homogeneity of variance was appropriate, and the data were compared using the Analysis of Variance. If that test was significant ($p\leq0.05$), the groups given the test article were compared with the

control group using Dunnett's Test. If Bartlett's Test was significant ($p \leq 0.001$), the Analysis of Variance Test was not appropriate, and the data were analyzed as described under the nonparametric heading. When 75% or fewer of the scores in all the groups were tied, the Kruskal-Wallis Test was used to analyze the data, and in the event of a significant result ($p \leq 0.05$), Dunn's Test was used to compare the groups given the test article with the control group. When more than 75% of the scores in any dosage group were tied, Fisher's Exact Test was used to compare the proportion of ties in the dosage group.

Results F0 Generation

Mortality/Clinical signs:

All rats survived until scheduled sacrifice. There were no overt treatment-related clinical signs observed.

EXAMINED F_0 PARENTS	PARAMETERS*
x	Appearance
	Abnormal Stool
	Deficiencies in Care**
x	Morbidity
x	Mortality
x	Neurotoxicity Screening

Body weight:

Observations during Gestation (female): comparable mean body weight and weight gain were observed in control and treated groups

Observations during Lactation (female): comparable mean body weight and weight gain were observed in control and treated groups

Comments: Body weights and body weight gain were unaffected by treatment.

Food consumption: Absolute and relative feed consumption values during the precohabitation (Groups 3 and 4 only), gestation and lactation periods were unaffected by the test article. Values were comparable to the control group values within each dosing replicate and did not significantly differ, with the exception of a significant ($p \leq 0.05$) reduction in relative feed consumption in Group 4 on days 0 to 7 of gestation (DGs 0 to 7). After day 7, Group 4 food consumption was not different from that of group 3.

Necropsy: There were no treatment related findings at necropsy.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

All mating and fertility parameters [numbers of days in cohabitation, rats that mated, Fertility Index (number of pregnancies per number of rats that mated), rats with confirmed mating dates and number of pregnancies per number of rats in cohabitation] were unaffected by the test article. Values were comparable among the two dosage groups and did not significantly differ.

Reproductive parameters examined (in F_0 animals):

EXAMINED F_0 PARENTS	PARAMETERS*
x	Female Fertility Index
x	Gestation Index
	Gestation Length
x	Live-born Index
x	Number (Total and Per Liter) of Stillbirths at Day 0
x	Number (Total and Per Liter) of Live Births at Day 0
x	Number of Implantation Sites
x	Number of Resorptions (Total, Early, and Late)

Caesarean and live birth data

PREGNANCY PAPRAMETER	CONTROL	MEDI3250 PREMATING	CONTROL	MEDI3250 PRE, POST MATING
PREGNANCY				
NUMBER OF FEMALES PAIRED	25	25	25	25
NUMBER OF FEMALES ACHIEVING PREGNANCY	25	24	24	25
FEMALE FERTILITY INDEX (%)	100	96	96	100
MATERNAL WASTAGE				
# DIED	0	0	0	0
# DIED PREGNANT	0	0	0	0
# DIED NONPREGNANT	0	0	0	0
# ABORTED	0	0	0	0
# PREMATURE DELIVERY	0	0	0	0
GESTATION				
GESTATION LENGTH MEAN (DAYS)	21	21	21	21
GESTATION INDEX (%)				
CORPORA LUTEA				
TOTAL # CORPORA LUTEA	373	395	389	408
CORPORA LUTEA/DAM MEAN±S.D.	14.9±2.3	15.8±2.2	16.2±2.4	16.3±2.3
IMPLANTATIONS				
TOTAL # IMPLANTATIONS	350	358	358	385
IMPLANTATIONS/DAM MEAN±S.D.	14.0±2.9	14.9±1.6	14.9±3.5	15.4±1.2
RESORPTIONS				
TOTAL # RESORPTIONS	13	23	18	15
RESORPTIONS/DAM MEAN±S.D.	0.5±0.9	1.0±1.0	0.8±1.0	0.6±0.8
TOTAL # EARLY RESORPTIONS	13	21	18	15
EARLY RESORPTIONS/DAM MEAN±S.D.	0.5±0.9	0.9±0.9	0.8±1.0	0.6±0.8
TOTAL # LATE RESORPTIONS	0	2	0	0

PREGNANCY PAPRAMETER	CONTROL	MEDI3250 PREMATING	CONTROL	MEDI3250 PRE, POST MATING
LATE RESORPTIONS/DAM MEAN±S.D.	0.0±0	0.1±0.3	0	0
BIRTHS – DAY 0				
NUMBER STILLBORNS – TOTAL PER LITER S.D.	0	1 0.0 0.2	1 0.0 0.2	2 0.1 0.3
NUMBER LIVE-BORN – TOTAL PER LITER S.D	329 13.7 2.4	330 14.3 2.0	231 13.6 2.3	317 14.4 1.8
LIVE-BORN INDEX (%)	100	99.7	99.6	989.3

Natural delivery F1 generation

F_1 physical development: litter size

LITTER TIMEPOINT	CONTROL	MEDI3250 PREMATING	CONTROL	MEDI3250 PRE, POST MATING
NUMBER BORN – TOTAL PER LITTER S.D.	329 13.7 2.4	331 14.4 2.0	231 13.6 2.4	317 14.5 1.8
DAY1– TOTAL PER LITTER S.D.	328 13.7 2.4	330 14.3 2.0	231 13.6 2.4	314 14.3 1.8
DAY 4 –TOTAL PER LITTER S.D.	327 13.6 2.4	330 14.3 2.0	229 13.5 2.3	312 14.2 1.8
DAY 7 – TOTAL PER LITTER S.D.	326 13.6 2.4	330 14.3 2.0	228 13.4 2.3	312 14.2 1.8
DAY 14 –TOTAL PER LITTER S.D.	324 13.5 2.3	330 14.3 2.0	228 13.4 2.33	312 14.2 1.8
DAY 21 – TOTAL PER LITTER S.D.	324 13.5 2.3	330 14.3 2.0	228 13.4 2.3	310 14.1 1.8

F_1 evaluation: litter weight (g)

TIMEPOINT		CONTROL	MEDI3250 PREMATING	CONTROL	MEDI3250 PRE, POST MATING
DAY 0	N	329	331	231	314
	MEAN	6.5	6.4	6.5	6.6
	S.D.	0.5	0.5	0.6	0.4
DAY 4	N	327	330	229	312
	MEAN	9.2	9.1	9.2	9.1
	S.D.	0.9	0.9	0.8	1.0
DAY 7	N	326	330	228	312
	MEAN	13.4	13.1	12.8	12.8
	S.D.	1.4	1.5	1.2	1.7
DAY 14	N	324	330	228	312
	MEAN	23.7	22.8	25.4	24.8
	S.D.	3.1	3.3	2.6	3.2
DAY 21	N	324	330	228	310
	MEAN	35.6	34.6	41.0	40.4
	S.D.	5.7	5.9	4.6	5.3

F_1 reproductive indices

REPRODUCTIVE INDEX	CONTROL	MEDI3250 PREMATING	CONTROL	MEDI3250 PRE, POST MATING
VIABILITY INDEX DAY 0-4 ALIVE/BORN (%)	327/329 (99.4)	329/330 (99.7)	229/231 (99.1)	312/317 (98.4)
LACTATION INDEX DAY4 / 21 (%)	324/327 (99.1)	329/329 (100)	228/229 (99.6)	310/312 (99.4)
SEX RATIO M/F				
DAY 1	45.8/54.2	43.5/56.5	54.0/46.0	47.8/52.2
DAY 21	46.4/43.7	43.7/56.3	53.9/46.1	47.6/52.4

F_1 evaluation: developmental endpoints for criterion day (50% response)

DEVELOPMENTAL ENDPOINTS:	CONTROL	MEDI3250 PREMATING	CONTROL	MEDI3250 PRE, POST MATING
DETACHMENT OF PINNA:	3.2±0.4	3.3±0.5	3.2±0.7	3.1±0.4
EYE OPENING:	14.8±0.7	14.9±0.7	14.4±0.6	14.6±0.5
TOOTH	11.8±0.8	11.9±1.0	11.5±1.0	11.2±0.8

DEVELOPMENTAL ENDPOINTS:	CONTROL	MEDI3250 PREMATING	CONTROL	MEDI3250 PRE, POST MATING
ERUPTION:				

Means ±S.D

F1 neurologic endpoints for criterion day (50% response)

GROSS NEUROTOXICITY OBSERVATIONS	CONTROL	MEDI3250 PREMATING	CONTROL	MEDI3250 PRE, POST MATING
PUPIL REFLEX:	21.0±0.00	21.0±0.00	21.0±0.00	21.0±0.00
STARTLE REFLEX:	13.0±0.00	13.0±0.00	13.0±0.00	13.0±0.00
SURFACE REFLEX	3.6±1.6	3.4±1.1	3.0±1.8	3.1±1.5
MID-AIR REFLEX:	16.2±1.4	15.9±1.3	15.0±1.3	15.4±0.8

Means ±S.D

Caeserian groups

Fetal Alterations in F_1 animals

GROUP	CONTROL	PREMATING MEDI3250	CONTROL	PRE-, POST MATING MEDI3250
LITTERS EVALUATED/ FETUSES EVALUATED	25/338	24/335	24/340	25/369
LITTERS WITH FETUSES WITH ANY ALTERATION	6	5	9	8
FETUSES WITH ALTERATION	9	5	12	10
% FETUSES WITH ALTERATION/LITTER	2.4±4.9	1.5±2.9	4.1±8.7	2.7±4.3

Fetal gross external alteration in F_1 animals

GROUP	CONTROL	PREMATING MEDI3250	CONTROL	PRE-, POST MATING MEDI3250
LITTERS EVALUATED/	25/338	24/335	24/340	25/369

GROUP	CONTROL	PREMATING MEDI3250	CONTROL	PRE-, POST MATING MEDI3250
FETUSES EVALUATED				
JAW, MICROGNATHIA	1/1	0	0	0

Values expressed as Litter incidence/fetal incidence

Fetal soft tissue alteration in F_1 animals

GROUP	CONTROL	PREMATING MEDI3250	CONTROL	PRE-, POST MATING MEDI3250
LITTERS EVALUATED/ FETUSES EVALUATED	25/338	24/335	24/340	25/369
PALATE INVAGINATED	1/1	0	0	0
TONGUE, SMALL	1/1	0	0	0
URINARY BLADDER CYST	0	0	1/1	0

Values expressed as Litter incidence/fetal incidence

Fetal skeletal alterations in F_1 animals

GROUP	CONTROL	PREMATING MEDI3250	CONTROL	PRE-, POST MATING MEDI3250
LITTERS/FETUSES EXAMINED	25/172	24/173	24/177	25/191
SKULL, NASAL FRONTAL SUTURE	1/1	0	0	0
SKULL, SQUAMOSAL, INCOMPLETE OSSIFICATION	1/1	0	0	0
ZYGOMATIC, INCOMPLETE OSSIFICATION	1/1	0	0	0
CERVICAL VERTEBRAE, CERV RIB AT 7^{TH} CERV VERT	2/1	1/1	5/5	2/2
CERVICAL VERTEBRAE, 6^{TH} ARCH LIKE 7TH	0	1/1	0	2/2
CERVICAL VERTEBRAE, ARCH	2/2	0	1/1	0

GROUP	CONTROL	PREMATING MEDI3250	CONTROL	PRE-, POST MATING MEDI3250
INCOMPLETE OSSIFICATION				
THORACIC VERT, CENTRUM, BIFID	3/3	1/1	2/2	6/5
RIBS, SHORT	1/1	0	2/2	0
STERNAL CENTRA, INCOMPLETE OSSIFICATION	1/1	0	1/1	0

Fetal ossification sites in F_1 animals

GROUP	CONTROL	PREMATING MEDI3250	CONTROL	PRE-, POST MATING MEDI3250
LITTERS/FETUSES EXAMINED	25/172	24/173	24/177	25/191
HYOID	0.99±0.04	1.00±0.00	1.00±0.00	0.99±0.04
VERTEBRAE				
CERVICAL	7.00±0.00	7.00±0.00	7.00±0.00	7.00±0.00
THORACIC	13.02±0.05	13.09±0.19	13.08±0.15	13.08±0.14
LUMBAR	5.97±0.07	5.90±0.20	5.91±0.15	5.92±0.14
SACRAL	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
CAUDAL	7.45±0.88	7.33±0.66	7.40±0.76	7.49±0.36
STERNUM				
MANUBRIUM	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
STERNAL CENTERS	4.00±0.00	4.00±0.00	4.00±0.00	3.99±0.04
XIPHOID	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
FORELIMB				
CARPALS	0.00	0.00	0.00	0.00
METACARPALS	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
DIGITS	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
PHALANGES	6.16±0.88	5.94±0.90	8.17±0.78	8.43±0.42
HINDLIMB				
TARSALS	0.03±0.08	0.00±	0.04±0.11	0.02±0.10
METATARSALS	4.81±0.22	4.83±0.25	4.83±0.27	4.88±0.15
DIGITS	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
PHALANGES	6.16±0.88	5.94±0.90	6.14±0.94	6.37±0.97

Ossification sites/fetus/litter ± S.D.

8.6. Caesarean-Sectioning and Litter Observations

Pregnancy occurred in 25 (100.0%). 24 (96.0%), 25 (100.0%) and 24 (96.0%) of the rats assigned to Caesarean-sectioning in Groups 1 through 4, respectively.

No Caesarean-sectioning or litter parameters were affected by MEDI3250. Litter averages for corpora lutea, implantations, litter sizes, live and dead fetuses, early and later resorptions, fetal body weights, percent live male fetuses and percent dead or resorbed conceptuses were comparable among the four dosage groups and did not significantly differ. All placentae appeared normal. A significant ($p \leq 0.01$) increase in the number of dams with resorbed conceptuses occurred in Group 2; this change was not considered test article related by the sponsor as the values were within those observed historically at the ------(b)(4)------ facility. The increased resorptions observed in Group 2 were not replicated by Group 4 rats that were administered the test article both prior to and during gestation.

Fetal alterations may be defined as malformations (irreversible changes that occur at low incidences in this species and strain); or variations (common findings in this species and strain and reversible delays or accelerations in development). Litter averages were calculated for specific fetal ossification sites as part of the evaluation of the degree of fetal ossification.
Fetal evaluations were based on 338, 335, 340 and 369 live, DG 21 Caesarean-delivered fetuses in 25, 24, 24, and 25 litters in Groups 1 through 4, respectively. Each of these fetuses was examined for gross external alterations. Soft tissue examination was conducted on 166, 162, 163 and 178 fetuses and skeletal alterations and fetal ossification site averages were conducted on 172, 173, 177 and 191 fetuses in the four respective groups. It was also possible to examine the dead fetus in Group 4. This fetus appeared normal for its developmental age at gross external and soft tissue examination.
No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by the test article. All fetal alterations were considered unrelated to the test article because: 1) the incidences were not dosage dependent; 2) the incidences were within the historical ranges of the testing facility; and/or 3) the observation occurred in a single litter in a group.

In Groups 1 through 4, litters with fetuses with alterations numbered 6 (24.0%), 5 (20.8%), 9 (37.5%) and 8 (32.0%), respectively. The numbers of fetuses with any alteration observed were 9 (2.7%), 5 (1.5%), 12 (3.5%) and 10 (2.7%), and the percentages of fetuses per litter with any alteration per litter were 2.4, 1.5, 4.1 and 2.7 in these same respective dosage groups.

Soft tissue alterations included one fetus in Group 1 with micrognathia. At soft tissue evaluation, this fetus had a small tongue and an invaginated palate at soft tissue examination and micrognathia at gross external examination.
One fetus in Group 3 had a clear fluid-filled cyst on the urinary bladder. This fetus had no additional alterations. No additional soft tissue alterations occurred.

No skeletal malformations occurred.

Variations included a Group 1 fetus with incompletely ossified squamosal and zygomatic bones and a large nasal frontal suture of the skull. This fetus also had an incompletely ossified cervical arch.
One fetus in Group 2 and two fetuses in Group 4 had a 6^{th} cervical arch that had the appearance of the 7th. These fetuses had no additional alterations.
Two fetuses in Group 1 and one fetus in Group 3 had one or more incompletely ossified cervical arches.

Bifid centra in one or more thoracic vertebrae occurred in 3, 1, 2 and 6 fetuses from 3, 1, 2 and 5 litters in Groups 1 through 4, respectively. These fetuses had no additional alterations.
A cervical rib at the 7th cervical vertebra, occurred in 2, 1, 5 and 2 fetuses from 1, 1, 5 and 2 litters in Groups 1 through 4,respectively. These fetuses had no additional alterations.
One fetus in Group 1 and two fetuses in Group 3 had short ribs. Fetus 8508-5 also had a segmented right 13th rib. These fetuses had no additional alterations.
Two fetuses in Group 2 and one fetus in Group 3 had incompletely ossified sternal centra. These fetuses had no additional alterations.
The average number of ossification sites in the hyoid, vertebrae (cervical, thoracic, lumbar, sacral and caudal), ribs, sternum (manubrium, sternal centers and xiphoid), forelimbs (carpals, metacarpals and phalanges) and hindlimbs (tarsals, metatarsals and phalanges) occurred at similar incidences in litters in all dosage groups and did not significantly differ.

Reflex and Physical Development - F1 Generation Pups
There were no biologically important differences among the four dosage groups in the measures of reflex and physical development (surface righting, pinna unfolding, incisor eruption, eye opening, acoustic startle, air righting or pupil constriction). Criterion grading (timepoint for 50 % of group to achieve a response) was similar for all group for all assays.

Serology
Hemagglutination inhibition (HAI) assay was used to measure influenza vaccine induced serum antibody response. Two control group 3 mothers and 3 offspring seroconverted to one virus strain. All treated mothers and their offspring seroconverted, ranging from 96-100% in mothers to 12-100% in offspring.

HAI response to Q-LAIV antigens, mothers and offspring

GROUP DAY OF ASSAY	A SOUTH DAKOTA F0	A URU-GUAY F0	B FLOR IDA F0	B MALAY-SIA F0	SERO-CONVERTED F0	A SOUTH DAKOTA F1	A URU-GUAY F1	B FLORIDA F1	B MALAY SIA F1	SERO-CONVERTED F1
CONTROL GD 21	0/22	0/22	0/24	0/24	No	0/44	1/44	0/44	0/44	No (3 strains)
CONTROL LD 21	0/23	0/23	0/21	0/21	No	0/96	0/96	0/96	0/96	No
VACCINE POST MATING DAYS 6, 13, 20 GD 21	25/25	24/25	24/25	23/25	Yes	24/39	5/41	14/41	1/41	Yes
VACCINE POST MATING DAYS 6, 13, 20 LD 21	23/23	23/23	23/23	23/23	Yes	90/90	90/90	90/90	83/90	Yes
CONTROL GD 21	1/25	0/25	1/25	0/25	No (3strains)	0/43	0/43	2/42	0/42	No (3 strains)
CONTROL LD 21	0/17	0/17	0/17	0/17	No	0/68	0/68	0/68	0/68	No
VACCINE DOSED PRE, POST MATING GD 21	25/25	25/25	25/25	25/25	Yes	38/43	39/43	38/45	35/45	Yes
VACCINE DOSED PRE, POST	22/22	21/21	21/21	2222	Yes	79/87	87/87	87/87	87/87	Yes

GROUP DAY OF ASSAY	A SOUTH DAKOTA F0	A URU-GUAY F0	B FLOR IDA F0	B MALAY-SIA F0	SERO-CONVERTED F0	A SOUTH DAKOTA F1	A URU-GUAY F1	B FLORIDA F1	B MALAY SIA F1	SERO-CONVERTED F1
MATING LD 21										

GD= gestation day LD=lactation day F0= maternal F1= pups

Pregnancy Category B
The developmental and reproductive toxicology study performed in pregnant female rats at a dose approximately 150 times the human dose (based on FFU/kg body weight, otherwise an identical dose in terms of viral antigen FFU and volume) showed no evidence of impaired fertility or harm to the fetus due to Quadrivalent FluMist (MEDI 3250) treatment. There are no adequate and well controlled studies in pregnant women. Because animal studies are not always predictive of human response, Quadrivalent FluMist should be used during pregnancy only if clearly needed.

CONCLUSION
Based on the intranasal reproductive and developmental toxicology study in rats, MEDI3250 administered once on GDs 6, 13 and 20 (Groups 1 and 2) or once weekly beginning two weeks prior to mating (a total 3 doses administered on DS 1, 8 and 14) and once weekly during the gestation period for a total of 6 doses (Groups 3 and 4) did not demonstrate maternal toxicity, embryo-fetal toxicity or affect the growth and development of the F1 generation offspring when evaluated up to weaning. The results of this study are similar to those of the previous trivalent influenza intranasal FluMist vaccine. The addition of another influenza strain did not adversely affect reproductive and developmental toxicology for the submitted quadrivalent product

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 and reproductive/developmental assessments of this submission, there are no significant nonclinical safety issues which prevent the approval of this BLA supplement.

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