

Toxicology Review of Influenza A (H5N1) virus monovalent vaccine, adjuvanted

BLA: 125419

Sponsor: ID Biomedical Corporation of Quebec dba GlaxoSmithKline
Biologicals

Product: Influenza A (H5N1) virus monovalent vaccine, adjuvanted

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TABLE OF CONTENTS:

PRODUCT:	1
PROPOSED USE:	4
INTRODUCTION:	4
CLINICAL STUDIES SUBMITTED TO SUPPORT THIS BLA:	5
TOXICITY STUDIES SUBMITTED TO SUPPORT THIS BLA:	6
General toxicology studies:.....	6
Reproductive studies:	7
Safety pharmacology studies:	7
Gene toxicology studies:	7
STUDIES REVIEWED PREVIOUSLY:	8
SUMMARY OF TOXICOLOGY STUDIES:	8
Study # 1 [1536-06196]: (Pandemic influenza candidate vaccine with AS03B adjuvant [Pan2 (Q-Flu/AS03B)]: A local tolerance study in New Zealand White rabbits).	8
Summary and conclusion:	8
Study # 2 [2990/355]: (Quebec seasonal and pandemic influenza candidate vaccines: Intramuscular single dose toxicity and local tolerance study in the rabbit).	9
Summary and conclusion:	9
Study # 3 [1536-06194 (Complete review is available in EDR, IND 13413 amendment 77)]: Pandemic influenza vaccines with AS03B adjuvant: A 115-day repeat dose intramuscular toxicity study in New Zealand White rabbits. ..	10
Summary:	10

Test article related effects:.....	11
Assessment:.....	11
Conclusions:.....	12
Study # 4 [TNO V 8550]: Repeated-dose toxicity study with a Quebec pandemic influenza candidate vaccine (Flu Q-pan3.8/AS03A) administered intramuscularly (three times) to male and female rabbits.....	12
Experimental design:	13
Summary and conclusion.....	13
Study # 5 [2990-356]: Quebec seasonal and pandemic influenza candidate vaccines: Repeated (3 occasions) intramuscular administration toxicity study in the rabbit with a 4 week recovery period.....	15
Experimental design:	15
Summary	15
Conclusion.....	17
Study # 6 [1990/956]: Pandemic influenza candidate vaccines (split H5N1 and split H5N1/AS03): Repeated (4 occasions) intramuscular administration toxicity study in the rabbit with a 4 week recovery period.....	18
Product:.....	18
Experimental design:	18
Summary	18
Conclusion.....	20
SUMMARY OF REPRODUCTION TOXICITY STUDIES	21
Study # 7 [HEY0001 (Complete review is available in EDR, IND 13413 amendment 123)]: AS03A and H1N1v/AS03A: Investigative study of pre-implantation loss in the CD rat by intramuscular administration.....	21
Key study findings:.....	21
Study design:	21
Route, formulation, volume, and infusion rate:.....	21
Cohabitation procedures:.....	21
Summary	21
Conclusions.....	22
Study # 8 [1536-08129 (Complete review is available in EDR, IND 13413 amendment 083)]: Pandemic influenza vaccine with AS03 adjuvant (Q-Pan): Study for effects on pre- and post-natal development in pregnant Sprague Dawley rats.....	22
Key study findings:.....	22
Study design:	23
Cohabitation procedures:.....	23
Summary	23
Conclusions.....	25
Study # 9: Pandemic influenza candidate vaccines (whole H5N1/AI and split H5N1/AS03): Study of effects on embryo-fetal, pre- and post-natal development study in CD rats by intramuscular administration (including pre-mating immunization phase).	25
Key study findings:.....	25

Study design:	25
Summary	26
Results	26
Conclusion.....	27
Study # 10 [GVB0009/064374]: Influenza vaccines (Fluarix and FluLaval) : Study of effects on embryo-fetal, pre- and post-natal development in CD rats by intramuscular administration (including pre-mating immunization phase)...	27
Key study findings:.....	28
Study design:	28
Summary	28
Results	29
Conclusion.....	30
SUMMARY OF SAFETY PHARMACOLOGY STUDIES:.....	30
Study # 11 [GVB 0016/072148]: Pan2 (Q-Flu/AS03B) cardiovascular and respiratory evaluation in the anaesthetized rat.	30
Key study findings:.....	30
Study no.:.....	30
Summary and conclusion.....	30
Study # 12 [AS03A]: Adjuvant-effects on cardiovascular and respiratory functions, following intramuscular administration in the conscious Beagle dog monitored by telemetry.....	31
Key study findings:.....	31
Study no.:.....	31
Summary and conclusion.....	31
SUMMARY OF GENE TOXICOLOGY STUDIES:.....	32
Study # 13: SB62 Batches SB621011 and SB621017 CUPAC1 testing for mutagenic activity with Salmonella typhimurium TA 1535, TA 1537, TA 98, and TA 100 and Escherichia coli WP2UVRA.....	32
Key study findings:.....	32
Study no.:.....	32
Summary and conclusion.....	32
Study # 14: SB62 new SB62 old SB62/Alpha-tocopheryl –b(4)-- in vitro mutation test using mouse lymphoma L5178Y cell.	32
Key study findings:.....	32
Study no.:.....	32
Summary and conclusion.....	32
Study # 15: SB62 new SB62 old SB62/Alpha-tocopheryl –b(4)--- rat micronucleus test.	33
Key study findings:.....	33
Study no.:.....	33
Summary and conclusion.....	34
OVERALL SUMMARY:	35

General Toxicology: 35
Reproductive toxicology: 36
Safety pharmacology toxicology: 37
Gene toxicology: 37
Pregnancy category: 37
Internal communication: 38
Proposed label wording: 38

OVERALL CONCLUSION: 39

Proposed use: Prophylaxis of influenza A (H5N1) in persons 18 years of age and older.

Introduction: Influenza is a contagious respiratory illness caused by influenza viruses. Symptoms of the influenza are; fever (usually high), headache, extreme tiredness, dry cough, sore throat, runny or stuffy nose, muscle aches, and stomach symptoms such as nausea, vomiting, and diarrhea, also can occur but are more common in children than adults. It can cause mild to severe illness and at times can lead to death.

Previous preclinical studies using split virus antigen demonstrated the utility of AS03 adjuvant to augment the immune response to both H5 and non-H5 influenza strains through enhancement of humoral (HAI) and cellular (cytokine-secreting CD4+ cells) immune responses, suggesting that this adjuvant would be valuable in an antigen-sparing pandemic approach. Immunogenicity, efficacy, and toxicology studies were being performed to develop preclinical experience with detergent-split H5N1 antigen prepared according to the Quebec process administered in combination with AS03 adjuvant. GSK is proposing to use the heterologous challenge model to establish the neutralization response as a reasonable surrogate for protective immunity.

Clinical studies submitted to support this BLA:

Study Identifier (Identifier of Study Report)	Study Objective(s)	Study Design	Healthy Subjects or Diagnosis of Patients	Treatment Details (Test Product(s); Dosage Regimen; Route; Duration)	Total No. of Subjects by Group Entered (Completed)	Study Reporting Status (Type of Report and Date)
Q-Pan-H5N1-001 (core treatment groups)	Pivotal immunogenicity and safety	Randomized, observer-blind, parallel group, active control	Healthy 18 – 64 year old adults	2 IM doses, 21-day interval, either: Core Groups: Q-Pan H5N1: 3.8 µg HA alone Q-Pan H5N1: 3.8 µg HA; AS03A Q-Pan H5N1: 3.8 µg HA; AS03B D-Pan H5N1: 3.8 µg HA; AS03A D-Pan H5N1: 3.8 µg HA; AS03B Contingency Groups: Q-Pan H5N1 1.9µg HA; AS03A Q-Pan H5N1 1.9µg HA; AS03B Study duration 6 months	680 (673) 78 (76) 152 (150) 151 (151) 151 (151) 148 (145) 100 (99) 50(50) 50 (49) Number of subjects completed based on Day 42 analysis	m5.3.5.1 Q-Pan-001 Report Amendment 1 (Core, D42; 14 JUL 2008) Q-Pan-001 Annex Report (Core, D182; 18 JUL 2008) Q-Pan-001 Annex Report 2 (Core, MN; 29 SEP 2008) Q-Pan-001 Annex Report 3 (Contingency Arms; 20 APR 2010) Q-Pan-001 Annex Report 4 (Core, MN, drifted strains; 18 JUL 2011)
Q-Pan-H5N1-002	Pivotal immunogenicity and safety	Randomized, placebocontrolled, observer-blind, parallel group	Adults ≥ 18 years old	2 IM doses, 21-day interval, either: Q-Pan H5N1 HA 3.8µg; AS03A Saline placebo Study duration initially 6 months; amended to add a 12 month evaluation	4561 (4457) 3422 (3343) 1139 (1114) Number of subjects completed based on Day 42 analysis	m5.3.5.1 Q-Pan-002 Report Amendment 1 (D42; 01 DEC 2008) Q-Pan-002 Annex Report (D182; 18 MAR 2009) Q-Pan-002Annex Report 2 (D364; 25 JAN 2010)
Q-Pan-H5N1-005	Immunogenicity and safety of heterologous booster dose	Randomized, observer-blind, parallel group, placebo controlled	Adults ≥ 18 years old	IM doses given as described below. Group A (AS03_B): Day 0-3.8µg A/Indonesia D182-3.8µg A/turkey D549-saline placebo Group B (AS03_B): Day 0-7.5µg A/Indonesia D182-7.5µg A/turkey D549-saline placebo Group C (AS03_A): Day 0-3.8µg A/Indonesia D182-saline placebo D549-3.8µg A/turkey Group D (AS03_B): Day 0-3.8µg A/Indonesia D182- saline placebo D549-3.8µg A/turkey Group E (AS03_A): Day 0-7.5µg A/Indonesia D182- saline placebo D549-7.5µg A/turkey Group F (AS03_B): Day 0-7.5µg A/Indonesia D182- saline placebo D549-7.5µg A/turkey Group G (AS03_A): Day 0-saline placebo D182- 3.8µg A/turkey D549-3.8µg A/turkey Study duration 909 days	841 (678) 120 (95) 121 (101) 119 (103) 119 (99) 122 (98) 120 (89) 120 (93) Number of subjects completed based on D591 analysis	m5.3.5.1 Q-Pan-005 Report (D591; 02 FEB 2011) Q-Pan-005 Annex Report1 (D909; 14 JUL 2011) Q-Pan-005Annex Report 2 (MN; 15 NOV 2011)
Q-Pan-H5N1-010	Immunogenicity and safety of heterologous booster dose	Randomized, placebocontrolled, observer-blind, parallel group	Adults with primary vaccination in Q-Pan-001	Single H5N1 A/turkey/Turkey/1/2005 IM booster dose, 3.8µg HA unadjuvanted or unadjuvanted, as described below. 1. Q-Pan-001 Group A veterans: A/Turkey booster with AS03A 2. Q-Pan-001 Group B veterans (B1): A/Turkey booster with AS03A 3. Q-Pan-001 Group B veterans (B2): A/Turkey booster unadjuvanted 4. Q-Pan-001 Group C veterans (C1): A/Turkey booster with AS03A 5. Q-Pan-001 Group C veterans (C2): A/Turkey booster unadjuvanted 6. Q-Pan-001 Group D veterans (D1): A/Turkey booster with AS03A 7. Q-Pan-001 Group D veterans (D2): A/Turkey booster unadjuvanted 8. Q-Pan-001 Group E veterans (E1):	469 (467) 49 (49) 72 (72) 41 (40) 60 (59) 40 (40) 61 (61) 46 (46) 59 (59)	m5.3.5.1 Q-Pan-010 Report (D42; 08 SEP 2009) Q-Pan-010 Annex Report 1 Amendment 1 (D182; 15 NOV 2011) Q-Pan-010 Annex Report 2 (D364; 20 MAY 2010) Q-Pan-010 Annex Report 3 (001:010 enrollment; 23 JAN 2012) An additional annex report summarizing corrected drift variant MN assay results is in preparation but not

Study Identifier (Identifier of Study Report)	Study Objective(s)	Study Design	Healthy Subjects or Diagnosis of Patients	Treatment Details (Test Product(s); Dosage Regimen; Route; Duration)	Total No. of Subjects by Group Entered (Completed)	Study Reporting Status (Type of Report and Date)
				A/Turkey booster with AS03A 9. Q-Pan-001 Group E veterans (E2): A/Turkey booster unadjuvanted Study duration 6 months	41 (41) Number of subjects completed based on Day 42 analysis	complete
[D-Pan] H5N1-007 (Belgium)	Immunogenicity and safety	Randomized, observer-blind, parallel group, active control	Healthy 18 – 60 year old adults	2 IM doses, 21-day interval, either: [D-Pan] H5N1 HA 30µg [D-Pan]H5N1 (HA 15µg) [D-Pan]H5N1 (HA 7.5µg) [D-Pan]H5N1 (HA 3.8µg) [D-Pan]H5N1 (HA 30µg /AS03) [D-Pan]H5N1 (HA 15µg /AS03) [D-Pan]H5N1 (HA 7.5µg /AS03) [D-Pan]H5N1 (HA 3.8µg /AS03) Study duration 51 days	400 (400) 50 (50) 50 (50) 50 (50) 50 (50) 49 (49) 50 (50) 50 (50) 51 (51) Number of subjects completed based on Nov 2006 (Day 51) analysis	m5.3.5.1 H5N1-007 Report (D51; NOV-2006) H5N1-007 Annex Report (D180; 21-AUG-2007) H5N1-007 (106750) Annex Report 2 (antibody against drifted strains; 07-MAR-2008)
Q-Pan-H5N1-009	Immunogenicity and safety of compressed dosing schedules	Randomized, open-label, parallel group	Healthy 18 – 64 year old adults	2 IM doses of Q-Pan H5N1 3.8µg HA: AS03A Group A: Schedule Day 0, 21 Group B: Schedule Day 0, 14 Group C: Schedule Day 0, 7 Group D: Schedule Day 0 (2 doses, one in each arm) Study duration 6 months	312 (304) 78 (74) 78 (76) 78 (78) 78 (76) Number of subjects completed based on Day 51 analysis	m5.3.5.1 Q-Pan-009 Report (D51; 16 MAR 2009) Q-Pan-009 Annex Report 1 (D182; 11 SEP 2009)
Van Buynder et al., 2010	Pivotal effectiveness study	Retrospective cohort, communitybased, casecontrol, testnegative	Children 6 months to 9 years old with medicallyattened ILI for whom pandemic H1N1 influenza testing was sought in New Brunswick, Canada	1 or 2 IM doses of: Q-Pan H1N1pdm HA 1.9µg; AS03B	28 cases 63 controls	m5.3.5.4 Q-Pan-H1N1-AS03-049 Report

AS03A = Adjuvant System containing 11.86 mg tocopherol per dose

AS03B = Adjuvant System containing 5.93 mg tocopherol per dose

CSR = Clinical Study Report

D = Day

D-Pan = Dresden manufactured pandemic vaccine antigen

H1N1pdm = H1N1 pandemic vaccine

HA = Hemagglutinin

IM = Intramuscular

MN = Microneutralization assay results

Q-Pan = Québec-manufactured pandemic vaccine antigen

Toxicity studies submitted to support this BLA:

General toxicology studies:

- 1- Study no. 2990-355 “Quebec seasonal and pandemic influenza candidate vaccines: Intramuscular single dose toxicity and local tolerance study in the rabbit”

- 2- Study no. 1536-06196 “Pandemic influenza candidate vaccine with AS03B adjuvant [Pan2 (Q-Flu/AS03B)]: A local tolerance study in New Zealand White rabbits”
- 3- Study no. 1536-06194 “Pandemic influenza vaccines with AS03B adjuvant: A 115-day repeat dose intramuscular toxicity study in New Zealand White rabbits”
- 4- Study no. TNO V 8550 “Repeated-dose toxicity study with a Quebec pandemic influenza candidate vaccine (Flu Q-pan3.8/AS03A) administered intramuscularly (three times) to male and female rabbits”
- 5- Study no. 2990-356 “Quebec seasonal and pandemic influenza candidate vaccines: Repeated (3 occasions) intramuscular administration toxicity study in the rabbit with a 4 week recovery period”
- 6- Study no. 1990-956 “Pandemic influenza candidate vaccines (split H5N1 and split H5N1/AS03): Repeated (4 occasions) intramuscular administration toxicity study in the rabbit with a 4 week recovery period”

Reproductive studies:

- 1- Study no. 1536-08129 “Pandemic influenza vaccine with AS03 adjuvant (Q-Pan): Study for effects on pre- and post-natal development in pregnant Sprague Dawley rats”
- 2- Study no. HEY0001 “Flu D-H1N1 pandemic project AS03A and H1N1v/AS03A: Investigative study of pre-implantation loss in the CD rat by intramuscular administration”
- 3- Study no. GVB-0007-063710 “Pandemic influenza candidate vaccines (whole H5N1/AI and split H5N1/AS03) study of effects on embryo-fetal, pre- and post-natal development study in CD rats by intramuscular administration (including pre-mating immunization phase)”
- 4- Study no. 0009-064374 “Influenza vaccines (Fluarix and FluLaval): Study of effects on embryo-fetal, pre- and post-natal development in CD rats by intramuscular administration (including pre-mating immunization phase)”

Safety pharmacology studies:

- 1- Pan2 (Q-Flu/AS03B) cardiovascular and respiratory evaluation in the anaesthetized rat.
- 2- AS03A adjuvant-effects on cardiovascular and respiratory functions, following intramuscular administration in the conscious Beagle dog monitored by telemetry.

Gene toxicology studies:

- 1- SB62 batches SB621011 and SB621017 –b(4)-- testing for mutagenic activity with Salmonella typhimurium TA 1535, TA 1537, TA 98, and TA 100 and Escherichia coli WP2UVRA.

- 2- SB62 new SB62 old SB62/alpha-tocopheryl –b(4)- in vitro mutation test using mouse lymphoma L5178Y cell.
- 3- SB62 new SB62 old SB62/alpha-tocopheryl –b(4)- rat micronucleus test.

Studies reviewed previously:

- 1- Study no. 1536-06194 [IND 13413 amendment 77] “Pandemic influenza vaccines with AS03B adjuvant: A 115-day repeat dose intramuscular toxicity study in New Zealand White rabbits”
- 2- Study no. HEY0001 [IND 13413 amendment 123] “Flu D-H1N1 pandemic project AS03A and H1N1v/AS03A: Investigative study of pre-implantation loss in the CD rat by intramuscular administration”
- 3- Study no. 1536-08129 [IND 13413 amendment 083] “Pandemic influenza vaccine with AS03 adjuvant (Q-Pan): Study for effects on pre- and post-natal development in pregnant Sprague Dawley rats”.

Summary of Toxicology Studies:

Study # 1 [1536-06196]: (Pandemic influenza candidate vaccine with AS03B adjuvant [Pan2 (Q-Flu/AS03B)]: A local tolerance study in New Zealand White rabbits).

Summary and conclusion:

The purpose of this study was to determine the local tolerance of the detergent-split (inactivated) pandemic influenza vaccine antigen with adjuvant (AS03B) when administered by single IM injection to male and female New Zealand White rabbits.

Eighteen rabbits were assigned to one of three groups (3/sex/group) and received either phosphate buffered saline (control; group 1), adjuvant (0.25 mL of AS03B; group 2), or Pan2 vaccine (15 µg Q-Flu/AS03B; group 3). Animals were treated with a single 0.5 mL of the appropriate article via an IM injection into the thigh muscle on study day 1. Clinical observations, body weight, and injection site evaluation (Draize scores) data were collected. Animals were euthanized and necropsied on study day 4. Tissues were collected and the injection site and any gross lesions were evaluated microscopically.

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

Minimal or mild subacute inflammation of the subcutaneous and/or epimysial tissues was reported in animals receiving the adjuvant. There were no microscopic findings associated with the injection of the vaccine antigen (Q-Flu/AS03B).

In conclusion, no adverse effects related to the single IM injection of Pan2 vaccine (Q-Flu/AS03B) in New Zealand White rabbits were reported.

Study # 2 [2990/355]: (Quebec seasonal and pandemic influenza candidate vaccines: Intramuscular single dose toxicity and local tolerance study in the rabbit).

Summary and conclusion:

The objective of this study was to evaluate the acute toxicity and local tolerance of three Quebec seasonal and pandemic influenza candidate vaccines (FluQIV60/-b(4)-, FluQIV60 ---b(4)-----, and FluQ-PAN30/AS03) after a single IM injection in the rabbit. Animals treated with a single IM injection to the right anterior thigh muscle. The study duration was 4 days.

This study supports the concept of a Quebec seasonal quadrivalent influenza vaccine (2 “A” strains + 2 “B” strains) however, the influenza strains were not available, thus, 2 trivalent influenza candidate vaccines were tested (2 “A” strains + 1 “B” strain at double antigen amount, in order to mimic the total amount of antigen included in the quadrivalent vaccine).

The details of the study design are listed in the following table:

Group #	Test article	Dose volume (mL)	Number of Males/group	Number of Females/group
1	Saline (Control)	0.5	3	3
2	Flu QIV60/-b(4)---- (Vaccine 1)	0.5	3	3
3	Flu QIV60 -b(4)---- (Vaccine 2)	0.5	3	3
4	Flu Q-PAN30/AS03+ (Vaccine 3)	0.5	3	3

---b(4)-----
+ AS03 = **250** µL SB62 per 250 µL

The test articles were administered via IM injection into the right anterior thigh (IJ1). Animals were dosed once on day 1.

The following parameters were evaluated: In-life animal observations, injection site reactions, body weight, macroscopic observations, and microscopic observations (injection sites only). Animals were sacrificed on day 4 (three days post injection).

No test article-related mortality, clinical observations, body weight, body weight gain, or macroscopic findings were reported during the study. Very slight erythema at the dose site was reported in one group 3 male 72 hours after dosing.

Microscopically, minor inflammation was noted at the injection site in all groups, including controls. The incidence and severity of fasciitis and cellulitis was similar in animals given adjuvanted vaccines (group 2 or group 4) and generally higher

than in group 3 animals (Flu QIV60—b(4)----) or controls. The incidence and severity of these findings in groups 1 and 3 animals was similar.

In groups 2 and 4 males, the incidence and severity of granulomatous myositis was higher than groups 1 and 3 animals. The incidence and severity of granulomatous myositis was generally similar in vaccinated females and higher than controls.

In conclusion, minor inflammation was seen in all treated groups with a slightly higher incidence and severity reported in groups 2 and 4 (adjuvanted vaccines). Minimal to slight fasciitis, cellulitis, and granulomatous myositis were reported in groups 2 and 4. No test article-related mortality, clinical observations, body weight, body weight gain, or macroscopic findings were reported in this study.

Study # 3 [1536-06194 (Complete review is available in EDR, IND 13413 amendment 77)]: Pandemic influenza vaccines with AS03B adjuvant: A 115-day repeat dose intramuscular toxicity study in New Zealand White rabbits.

Product: Pan1 (D-Flu/AS03B) and Pan2 (Q-Flu/AS03B)

Animal species and strain: New Zealand White rabbits

Dose: 60 µgHA/mL antigen, 3 mLs of AS03B and PBS

Means of administration: Intramuscular to the right thigh on SD's 1, 29 (only if sacrificed on SD 32 or 43), and 71 and to the left thigh on SD's 15 and 85.

Summary:

In this repeated dose toxicology study, rabbits were treated with PBS, AS03B, Pan 1, or Pan 2. Each 0.5 mL injection contained the standard human dose of trivalent vaccine (15 µg HA). Test articles contained 9-11% thimerosal as indicated in the certificate of analysis.

Seventy males and 70 females (age 14-15 weeks) were assigned to four different groups. Sixteen or ten animals per sex per group were treated with priming dose on SD's 1, 15, and 29 (G's 1, 2, and 3) and on SD's 1 and 15 (G 4). Animals were also treated with boost dose on SD's 71 and 85. Three rabbits/sex from G's 1-3 of terminal and recovery sacrifice 1 was euthanized on SD's 32 and 43, respectively. Five rabbits/sex from G's 1-4 of terminal and recovery sacrifice 2 was euthanized on SD's 88 and 115, respectively. The details of the study design are listed in the following table:

Group	Treatment-Priming	Treatment-Boost	Dose Level-Priming	Dose Level-Boost	Days of Dosing-Priming	Days of Dosing-Boost	Number of Animals-Male	Number of Animals-Female
1	PBS	PBS	0.5 mL	0.5 mL	SD 1, 15, and 29	SD 71 and 85	16	16
2	AS03B	AS03B	0.25 mL with PBS	0.25 mL with PBS	SD 1, 15, and 29	SD 71 and 85	16	16

Group	Treatment-Priming	Treatment-Boost	Dose Level-Priming	Dose Level-Boost	Days of Dosing-Priming	Days of Dosing-Boost	Number of Animals-Male	Number of Animals-Female
3	Pan 2	Pan 2	15 µg Q-Flu with AS03B	15 µg Q-Flu with AS03B	SD 1, 15, and 29	SD 71 and 85	16	16
4	Pan 1	Pan 2	15 µg D-Flu with AS03B	15 µg Q-Flu with AS03B	SD 1 and 15	SD 71 and 85	10	10

Note: SD 29 dosing was only for animals that were sacrificed on SD's 32 and 43.

Test article related effects:

Test article related effects	Effects considered incidental
Injection sites findings Increase in fibrinogen levels Increase in monocyte, neutrophil and eosinophil counts Increase in total protein and globulin Decrease in albumin to globulin ratio (A/G) Increase in bilirubin Minimal to slight differences in coagulation (except for fibrinogen) parameters	Increase in creatine kinase Increase in platelet count

Assessment:

Injection site reactions were attributable to recovery of trauma due to injection. The perineural connective tissue surrounding the sciatic nerves findings (inflammatory cell infiltrates, hemorrhage, necrosis, and fibrosis) were considered to be an inflammatory response to the vaccine adjuvant and to the dosing procedure. The increase in monocyte, neutrophil, and eosinophil counts could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair. The increases in fibrinogen levels were not considered frank toxicity but rather an anticipated effect associated with an immunological response.

The increase in mean and individual animal total protein and globulin values and the lower mean and individual animal albumin to globulin ratio (A/G) values for the adjuvant and vaccine treated groups might be the result of an increase in immunoglobulin synthesis due to polyclonal activation of B lymphocytes by the adjuvant. The significant increase in males' total bilirubin was seen after the first dose administration and in G4 only. This increase was not seen after the subsequent dose administrations in males and considered non-adjuvant related.

However, the increase in females' total bilirubin were seen at SD's 4 (G's 2 and 3), 32 (G3), and 88 (G4) which might indicate test article-related effect in the liver. The increase in bilirubin levels in G4 females at recovery (SD 88) might indicate a delayed type effect by the test article on the liver. Bilirubin testing is used to check liver function and watch for signs of liver disease, such as hepatitis or cirrhosis, effect of medicines that can damage the liver, bile ducts blockage, or increased destruction of red blood cells. The increase in mean and individual creatine kinase activity values for the adjuvant and vaccine treated groups observed might be a reflection of minimal muscle degeneration subsequent to the inflammatory response to intramuscular injection of the adjuvant and the vaccine antigen/adjuvant. The increase in creatine kinase activity for G's 2 and 3, males and females, had resolved by SD 32.

There were transient minimal increase in mean and individual animal platelet count for the adjuvant and vaccine treated groups on SD 4 which had returned to predose values by SD 32. The minimal to slight differences in coagulation (except for fibrinogen) parameters subsequent to adjuvant and vaccine administration were considered to be representative of release of acute inflammatory proteins from the liver.

Minor and transient changes in clinical pathology parameters suggesting an acute phase response were associated with the receipt of adjuvant, with or without antigen. A subacute inflammatory response at the injection site was induced by both the adjuvant alone or with influenza antigens. The presence of antigen was immaterial after the primary series, but appeared to increase lesion severity after the booster series. Microscopic findings tended to resolve at the recovery sacrifice after both immunization series, and there were no treatment-related effects outside the injection sites.

There were no treatment-related mortality nor were any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, gross anatomy, or organ weight found.

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

Conclusions: Based on nonclinical toxicity assessments of this study there are no significant safety issues were reported.

Study # 4 [TNO V 8550]: Repeated-dose toxicity study with a Quebec pandemic influenza candidate vaccine (Flu Q-pan3.8/AS03A) administered intramuscularly (three times) to male and female rabbits.

Product: Q-Pan, AS03A

Animal species and strain: -b(4)- New Zealand White albino rabbits

Dose: 500 µL per animal per injection

Means of administration: Intramuscular to the left hind leg (left hamstring muscle) on SD 0, left hind leg (left calf muscle) on SD 14, and right hind leg (right calf muscle) on SD 28.

Experimental design:

Animals were divided to 2 treatment groups, one saline control group and one Flu Q-Pan3.8/AS03A group. Each group was divided into 2 subgroups. Animals were sacrificed on study days 31 (3 days post 3rd inoculation) and 57 (29 days post 3rd inoculation) for subgroups 1 and 2, respectively. The treatment groups were divided as follows:

Males:

Group	Treatment	Volume (mL)	Animal numbers of males S1 (n=5)	Animal numbers of males S2 (n=5)
8550/1	Saline	0.5	2-10	12-20
8550/2	Flu Q-pan3.8/AS03A	0.5	22-30	32-40

Females:

Group	Treatment	Volume (mL)	Animal numbers of females S1 (n=5)	Animal numbers of females S2 (n=5)
8550/1	Saline	0.5	1-9	11-19
8550/2	Flu Q-pan3.8/AS03A	0.5	21-29	31-39

S1 = subgroup 1 sacrificed on day 31 (3 days post 3rd inoculation)

S2 = subgroup 2 sacrificed on day 57 (29 days post 3rd inoculation)

Summary and conclusion

The purpose of this study was to examine the local and the general toxicity/reactogenicity of Quebec pandemic influenza candidate vaccine (Flu Q-Pan3.8/AS03A) after repeated intramuscular injections (three times, with a 2-week interval between inoculations) in male and female New Zealand White albino rabbits. The groups were divided into subgroups 1 and 2, sacrificed at 3 and 29 days after the third inoculation, respectively. The following parameters were examined: clinical signs, ophthalmoscopy, rectal body temperature, body weight, food intake, hematology, clinical chemistry, gross changes at necropsy, organ weights, and histopathological examinations.

No test article-related effects were reported on the general and on the local clinical signs, ophthalmoscopy, body weight, food intake, rectal body temperature, and organ weights.

An increase in fibrinogen and white blood cell (WBC) (mainly an increase in neutrophil) levels were reported in males and females after the first and the third inoculation. This increase was considered part of the inflammatory process upon vaccination.

Decrease in albumin/globulin (A/G) ratios was reported after the first and the third inoculation. This decrease was considered part of the immune response to vaccination.

No test article-related macroscopic changes were reported.

Slight widespread, mixed cell type inflammation at the right calf muscle on day 3 post inoculation was reported. This inflammation was associated with hemorrhages, edema, and disintegration of collagen/necrosis (presumably of the epimysium). This response was very slight at day 29 after the third inoculation. Associated with this inflammation was granulated tissue and fibrosis. No test article-related effects on the other organs histopathological changes were reported.

Inflammatory responses at the inoculation sites in all FluQ-Pan3.8/AS03A animals were reported. The responses consisted of a mixed inflammatory cell type (granulocytes, small- to medium-sized macrophages with occasionally vacuolated cytoplasm and lymphocytes) in 3 out of the 5 males and all females. It was widespread in 2 males and 4 females (extending along the epimysium and diffusely between the muscle fibers) and multifocal in 1 male and 1 female. The inflammation was associated with granulation tissue (fibrocytes and angiogenesis) or fibrosis (birefringent collagen), especially alongside the epimysium and graded as very slight to slight. One male exhibited fibrosis without inflammatory cells.

Increased germinal centre development of the spleens of the treated animals were reported. This is considered a physiological response against the inoculation with the FluQ-Pan3.8/AS03A candidate vaccine.

Immunological data provided evidence that the rabbits were experimentally exposed with the Flu Q-Pan vaccine and that anti-A/Indonesia/5/2005 IgG antibody was induced as demonstrated by seroconversion of all rabbits.

In conclusion, test article (Flu Q-Pan3.8/AS03A) treatment of rabbits caused a slight inflammation locally in the injected muscle. Systemically, a few hematology and clinical chemistry parameters related to the local inflammation were transiently affected. The inflammation was diminished in the recovery animals.

Study # 5 [2990-356]: Quebec seasonal and pandemic influenza candidate vaccines: Repeated (3 occasions) intramuscular administration toxicity study in the rabbit with a 4 week recovery period.

Product: Flu QIV60/-b(4)-, Flu QIV60 -b(4)-----, Flu Q-PAN30/AS03

Animal species and strain: -b(4)- New Zealand White rabbits

Dose: 500 µL per animal per injection

Means of administration: Intramuscular injection into the left anterior thigh on days 1 and 15 (Injection site 1 (IJ1)) and into the right anterior thigh on day 29 (Injection site 2 (IJ2)).

Experimental design:

This study supports the concept of a Quebec seasonal quadrivalent influenza vaccine (two “A” strains + two “B” strains). However, as all the influenza strains were not available, two trivalent influenza candidate vaccines were tested (two “A” strains + one “B” strain at double antigen amount, in order to mimic the total amount of antigen included in the quadrivalent vaccine).

Animals were divided to 4 treatment groups, one saline control group and 3 (Flu QIV60/-b(4)-, Flu QIV60 -b(4)-----, Flu Q-PAN30/AS03) treatment groups. Animals were sacrificed on study days 32 (3 days post 3rd inoculation) and 57 (28 days post 3rd inoculation). The treatment groups were divided as follows:

Group number	Group description	Test Article	Dose Volume (µL)	Animals per group (Male)	Animals per group (Female)
1	Control	Saline	500	10	10
2	Candidate vaccine 1	Flu QIV60/-b(4)-	500	10	10
3	Candidate vaccine 2	Flu QIV60 -b(4)-----	500	10	10
4	Candidate vaccine 3	Flu Q-PAN30/AS03	500	10	10

The test article was administered via intramuscular injection on days 1 and 15 into the left anterior thigh (injection site 1 (IJ1)) and on day 29 into the right anterior thigh (injection site 2 (IJ2)).

Summary

The purpose of this study was to evaluate the local and/or systemic toxic effects induced by multiple intramuscular injections of three Quebec seasonal and pandemic influenza candidate vaccines (FluQIV60/-b(4)----, FluQIV60 --b(4)----- and FluQ-PAN30/AS03) in the rabbit and to assess the reversibility of any changes over a period of four weeks after the last injection.

The following parameters were examined: clinical signs, ophthalmoscopy, body temperature, body weight, food consumption, hematology, clinical

chemistry, gross changes at necropsy, organ weights, histopathological examinations, and serology.

In animals given FluQIV60, lesions at the injection site were generally more severe than in controls. Lesions were generally similar in animals given FluQPAN30/AS03 and FluQIV60/-b(4)-----, but were more severe than in animals given FluQIV60.

In groups given the -b(4)----- vaccines, increases in fibrinogen and white blood cell count were reported. These increases are suggestive of an acute and transient inflammatory response and correlates with the higher weights of the lymphoid organs and the associated lymphoid hyperplasia noted microscopically. There were reductions in lymphoid hyperplasia in lymph nodes and spleen, coupled with reductions in organ weight of the recovery animals (indication of partial reversal). This is might be related to stimulation of the immune system by the vaccine.

Reticulocytes levels were lower in all treated female groups at days 2 and 4 (14 to 43%, up to $p < 0.01$). Lower reticulocytes levels (24%) were reported in group 4 males on day 4. Higher reticulocytes levles (18 to 25%, up to $p < 0.05$) were reported in the treated females on day 32. Females given the adjuvanted vaccines had lower reticulocytes than the control group (38%, $p < 0.001$ and 11% for FluQIV60/-b(4)---- and FluQ-PAN30/AS03 respectively) at the end of the recovery period. The levels at the recovery period and the pre-treatment phase were comparable.

Creatine phosphokinase levels were lower in groups 2 and 4 (33% and 40%, respectively) on day 2. Creatine phosphokinase levels were higher (25%) in group 2 females on day 28. Creatine phosphokinase levels were higher (29%) in group 3 males on day 28. In all male treated groups, creatine phosphokinase levels were lower than the control group on day 32. This decrease was related to one outlier animal and thus, considered not toxicologically significant.

Alkaline phosphatase levels were lower (19%) in group 3 males than the control group on day 4. Alkaline phosphatase levels were lower in all male groups and in groups 2 and 4 females than the control group on days 28, 30, and 32. Alkaline phosphatase levels were lower in all treated male and female recovery groups than the control group.

Large iliac lymph node was reported in group 2 males and in group 4 males and females at day 32. Fewer animals were reported with large iliac lymph node in the recovery groups (day 57) when compared to day 32. Red focus iliac lymph node was reported in groups 2 and 4 males and females and group 3 males. In group 3, treatment-related large inguinal lymph node was reported in one female.

Pale kidneys were reported in 4 group 4 females and in 1 of groups 2 and 3 females on day 57. This finding correlated with findings reported microscopically.

In groups 2, 3, and 4 females, an increase in severity of inflammatory cell foci in the liver was reported at day 32. In groups 3 and 4 females, this lesion was also reported in the heart. No correlating clinical chemistry or hematology changes were reported in these organs. These changes were not reported in the recovery groups.

At the injection site, lesions in group 3 animals were more severe than in the control group. In animals given FluQPAN30/AS03 and FluQIV60/-b(4)----, lesions were generally similar but were more severe than in animals given FluQIV60.

Treatment-related findings (myopathy/myositis, fasciitis/fibrosis, perineuritis, perivascular cuffing and cellulitis) at the injection site of animals treated 3 days before (right anterior injection site) were reported at day 32.

In recovery animals, similar lesions at the injection site were noted to those seen at day 32. Since these lesions were less pronounced, partial recovery was considered.

Encephalitozoon cuniculi

Changes consistent with chronic infection with *E cuniculi* were reported in 4 animals. By serology, another control animal was shown to be infected with *E cuniculi*.

Interstitial nephritis and encephalitis were reported in 2 group 4 males. Myocarditis, orchitis and/or inflammatory cell foci in multiple organs including liver, stomach, lung, heart, aorta, thyroid and spinal cord (multisystemic inflammation) was also present. In brain and kidney, gram-positive ovoid micro-organisms were reported.

Interstitial nephritis and encephalitis were also reported in 1 group 2 male and to a lesser extent, in 1 group 1 female. Inflammatory cells in the heart and lungs were also reported. In brain and kidney, gram-positive ovoid micro-organisms were also reported.

These findings are consistent with a natural background infection by *Encephalitozoon cuniculi*, which is spread horizontally through contaminated urine.

Serology data showed an immune responses to Flu QIV vaccines (--b(4)-----
-----) or the Flu Q-Pan vaccine. Anti-influenza antigen IgG antibodies were induced as demonstrated by seroconversions of all rabbits.

Conclusion

Inflammation was reported in a higher incidence and severity at the injection site in the groups administered the adjuvanted vaccine in comparison to the saline control group. This might be an adjuvant effect rather than the antigen. In recovery animals, partial reversal of findings were reported (i.e. fasciitis/fibrosis and myopathy/myositis of lesser grades and a lower grade of perivascular cuffing).

At day 32, effects were reported in lymphoid organs (higher weights and lymphoid hyperplasia) in groups given the adjuvanted vaccine (group 4). Liver findings (inflammatory cell foci) in groups 2, 3, and 4 females were also reported. These findings were reversible with no indication of liver function being affected. Treatment related-effects cannot be dismissed.

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

Study # 6 [1990/956]: Pandemic influenza candidate vaccines (split H5N1 and split H5N1/AS03): Repeated (4 occasions) intramuscular administration toxicity study in the rabbit with a 4 week recovery period.

Product: Split H5N1 and split H5N1/AS03

Animal species and strain: -b(4)- New Zealand White rabbits

Dose: 500 µL (groups 1, 2, and 3) or 1000 µL (group 4) per animal per injection

Means of administration: Intramuscular injection. For animals in groups 1, 2 and 3, the dose on days 1, 15 and 29 was administered into the left anterior thigh and on day 43 dose was administered into the right anterior thigh. For animals in group 4, the dose on days 1, 15 and 29 was administered into the left anterior and posterior thigh and the dose on day 43 into the right anterior and posterior thigh muscle.

Experimental design:

Animals were divided to 4 treatment groups, one saline control group and 3 (AS03, Split H5N1, and Split H5N1 / AS03) treatment groups. Animals were sacrificed on study days 46 (three days post 4th injection) and 71 after a recovery period of four weeks (28 days post 4th injection). The treatment groups were divided as follows:

Group number	Group description	Test article	Dose volume (µL)	Animals per group (Male)	Animals per group (Female)
1	Control	Saline	500	10	10
2	Adjuvant	AS03	500	10	10
3	Candidate vaccine 1	Split H5N1	500	10	10
4	Candidate vaccine 2	Split H5N1 / AS03	1000	10	10

Summary

The purpose of this study was to evaluate the local and/or the systemic toxic effects induced by 4 intramuscular injections of the candidate vaccines (split

H5N1 and split H5N1/AS03), in the rabbit and to assess the reversibility of any changes over a 4 week recovery period.

The following parameters were examined: clinical signs, ophthalmoscopy, body temperature, body weight, food consumption, hematology, clinical chemistry, gross changes at necropsy, organ weights, histopathological examinations, and serology.

Erythema and/or edema at the injection site (up to 48 hours after dosing) were reported in groups 2 and 4 on four occasions (days 1, 15, 29 and 43). Erythema and/or edema at the injection site were reported to a lesser extent in group 3. Thus, the adjuvant AS03 might played a bigger role in this effect than split H5N1.

Treatment related-increases in fibrinogen (up to 74% in males and 56% in females) and white blood cell count [heterophils in particular] (up to 38% in males and 56% in females) were reported in groups 2 and 4. This is suggest an acute and transient inflammatory response and might correlates with the erythema and/or edema and the inflammation reported microscopically at the injection site. The increase in fibrinogen levels was not reported in the recovery groups. The increase in total white blood cell and heterophil levels were still evident in the recovery groups of females at levels of up to 33% and 67%, respectively. This might be treatment related as part of the immune responses. Increases in platelet count in groups 2 (22% and 40%) and 4 (35% and 28%) on day 46 were reported in males and females, respectively. This increase (up to 35%) was also reported in the female's recovery groups.

An increase (up to 29%) in globulin levels were reported in groups 2 and 4 females on days 2 and 4. An increase (up to 30%) in globulin levels were reported in groups 2 and 4 males on day 46. A decrease in albumin/globulin ratio was reported at these time points in the same groups (up to 21% in females and up to 26% in females). No increases in globulin levels were reported in the recovery groups.

Spleen weight was increased 41%, 34%, and 25% in groups 2, 3, and 4 males, respectively. Spleen weight was increased 24%, 24%, and 7% in groups 2, 3, and 4 females, respectively. Heart weight was increased 31%, 16%, and 20% in groups 2, 3, and 4 males, respectively. Heart weight was decreased 29%, 9%, and 14% in groups 2, 3, and 4 females, respectively. Pituitary weight was increased 50% in group 4 males. Pituitary weight was decreased 40% and 32% in groups 2 and 3 females, respectively. Uterus weight was increased 30% and 54% in groups 2 and 3 females, respectively. Iliac lymph node weight was decreased 3.4-, 3- and 2.3-folds in groups 2, 3, and 4 males, respectively. Iliac lymph node weight was increased 137% and 52% in groups 2 and 3 females, respectively. Iliac lymph node weight was decreased 37% in group 4 females. Thymus weight was increased 14% and 21% in groups 2 and 4 (recovery) males, respectively. Thymus weight was decreased 14% and 22% in groups 2 and 3 (recovery) females, respectively.

No treatment-related macroscopic changes were reported.

Microscopic findings of lymphoid hyperplasia were consistent with the increases reported in the adjusted spleen weight of all treated groups at the day 46 sacrifice. These increases and the microscopic findings were not reported in the recovery group animals. Stimulation of the immune system by the test article-treatment might be the cause of these findings.

At the injection sites of some animals (from all groups), granulomatous/needle track myositis was present with no clear difference in severity or incidence between rabbits in all groups. This was characterized by focal myofibre degeneration sometimes surrounding a hemorrhagic core (needle track) with a mixed inflammatory reaction of macrophages, heterophils, and multinucleate giant cells.

Fasciitis at the injection site was higher in incidence and severity in groups 2, 3, and 4 compared to control rabbits or rabbits given split H5N1 alone. This was characterized by a mixed inflammatory cell infiltration in fascial planes around muscle bundles and sometimes in deep subcutaneous tissue.

At the injection site of rabbits in groups 3 and 4, perivascular cuffing was reported. This was characterized by the presence of a predominantly lymphocytic cellular infiltrate centered around blood vessels.

At the injection site of groups 2 and 4, higher incidence and severity of inflammation was reported. In recovery animals, partial reversal of findings (i.e. chronic fasciitis/fibrosis of a lesser grade with a resolving of the inflammatory process and lower incidence of perivascular lymphoid cuffing) were reported. This is might be an indication of a recovery process.

Unilateral fasciitis/perivascular cuffing in the connective tissue surrounding the sciatic nerve were reported in group 4 animals.

Treatment with Flu H5N1 test vaccine or Flu H5N1/AS03 test vaccine induced A/Vietnam/1194/2004 H5N1 strain antibodies (seroconversion) in all vaccine recipient animals.

Conclusion

Erythema and/or edema (local effects) noted at the injection sites of some animals administered AS03 or split H5N1/AS03 up to 48 hours after dosing. Inflammation was noted at the dose injection site by the microscopical examination. No treatment related-systemic effects were reported in all treated groups. Serology testing showed an immune response in all vaccine recipient animals.

Summary of reproduction toxicity studies

Study # 7 [HEY0001 (Complete review is available in EDR, IND 13413 amendment 123)]: AS03A and H1N1v/AS03A: Investigative study of pre-implantation loss in the CD rat by intramuscular administration.

Vaccine: H1N1v/AS03A

Trade name: Pandemic influenza virus, vaccine, Quebec (Q-Pan).

Route of clinical administration: Intramuscular (IM) injection.

Indication: Active immunization against influenza disease caused by a pandemic influenza subtype using H1N1 as a developmental model.

Key study findings: Decrease in animal's body weight and food consumption and swollen area(s) at the injection site and limited movement of the hindlimbs in test article treated animals.

Doses: Test article or control was dosed daily from day 0 to day 6 after mating. Animals were treated with 100 µL/occasion, which represents one fifth of full human dose.

Study design: In this repeated dose reproductive toxicology study, Sprague Dawley rats (20/group/sex) were treated daily with 100 µL of PBS, AS03A, or H1N1V/AS03A vaccine on gestation days 0 to 6. Test article were administered intramuscularly into the anterior region of the left or right thigh muscle of each animal by alternating thighs. Terminal sacrifice necropsies were conducted on study day 14 after mating. The dose selected represents one fifth of full human dose. The details of the study design are listed in the following table:

Group	Treatment	Dose level (µL)	Days of dosing (females only)	Animals per group (Males ^a)	Animals per group (Females)
1	PBS	100 ^b	Daily GD 0- GD 6	20	20
2	AS03A	100	Daily GD 0- GD 6	20	20
3	H1N1v/AS03A	100	Daily GD 0- GD 6	20	20

^a Males were not dosed. ^b Volume/occasion.

Route, formulation, volume, and infusion rate: Intramuscular injections of 100 µL/occasion in the anterior region of the left or right thigh muscle. The opposite thigh muscle was used for each alternating injection occasion.

Cohabitation procedures: Each female was cohabited with one untreated male animal (1:1 basis) at 5 days after the pre-treatment blood sampling. The day of confirmation of mating (ejected copulation plugs in cage trays and the presence of sperm in vaginal smear) was designated as GD 0.

Summary

The objective of this study was to evaluate the influence of the H1N1v/AS03A candidate vaccine, when administered by IM injection during the early stage of

pregnancy, on pre-implantation loss in the CD rats. Animals were treated daily between mating and implantation (day 0 to day 6 after mating).

Animals (20 females/group) were assigned to 3 different groups and treated with one (100 µL) IM injection in the anterior region of the left or right thigh muscle. Each female was cohabited with one untreated male animal (1:1 basis) at 5 days after the pre-treatment blood sampling. The day of confirmation of mating was designated as GD 0.

No test article-related effects on mortality or clinical observations were reported. No test article-related effects on corpora lutea, implantations, resorptions, live embryos, and pre- and post-implantation losses were reported.

Increased incidences of swollen area(s) at the injection site in the treated groups (2 and 3) were reported. Group 2 showed greater recovery of this sign than group 3. Limited movement of the hindlimbs (this was considered to be associated with the presence of swelling) was reported in groups 2 and 3. This sign was no longer apparent at day 9 of gestation.

Significant decrease in body weight gain in groups 2 (days 3-6 of gestation) and 3 (days 0-6 of gestation) was reported. Following cessation of treatment (days 6-14 of gestation), body weight gain was similar to controls in both treated groups. Significant decrease in groups' 2 (days 0-7) and 3 (throughout the study) food consumption was also reported.

Vaccine exposure of dams during pregnancy to anti-H1N1 antibodies was indicated by the serological analysis data.

Conclusions

The administrations of H1N1v/AS03A vaccine, daily for 6 days, at 100 µL/occasion via the IM route did not give an indication of developmental toxicity. No test article-related effect on mortality or clinical observations was reported. Test article treatment caused decrease in animal's body weight and food consumption. Swollen area(s) at the injection site and limited movement of the hindlimbs in test article treated animals were also reported. Serological analysis data indicated vaccine exposure of dams during pregnancy to anti-H1N1 antibodies.

Study # 8 [1536-08129 (Complete review is available in EDR, IND 13413 amendment 083)]: Pandemic influenza vaccine with AS03 adjuvant (Q-Pan): Study for effects on pre- and post-natal development in pregnant Sprague Dawley rats.

Key study findings: No significant findings were reported.

Study no.: 1536-08129 under IND 13413 amendment 083.

Vaccine: Q Pan (H5N1)/AS03A

Doses: Test article or control was dosed at 2 IM injections of 100 µL each.

Study design: In this repeated dose reproductive toxicology study, Sprague Dawley rats (48/group/sex) were treated daily with 200 µL of PBS, AS03A followed by AS03/PBS, PBS followed by Q_Pan/AS03, or Q_Pan/AS03 vaccine on study day 28 (pre-mating), gestation days (GD's) 7, 9, 12, 16, and postnatal day (PND) 7. Animals were treated with two intramuscular injections of 100 µL each in the rear limbs. Females were divided into 2 cohort per group after mating confirmation. The first cohort was terminated by C-section on GD 21 and the remaining cohort was allowed to deliver naturally. The latter dams and their surviving pups were terminated on PND 25. The details of the study design are listed in the following table:

Group	Treatment	Dose level (µL)	Days of dosing (females only)	Animals per group (Males ^a)	Animals per group (Females)
1	PBS	200 ^b	Once 28 days pre-mating, GD's 7, 9, 12, 16, and PND 7	48	48
2	AS03	200	Once 28 days pre-mating	48	48
2	AS03/ PBS	200	GD's 7, 9, 12, 16, and PND 7		
3	PBS	200	Once 28 days pre-mating	48	48
3	Q-Pan/AS03	200 (1.5 µg HA)	GD's 7, 9, 12, 16, and PND 7		
4	Q-Pan/AS03	200 (1.5 µg HA)	Once 28 days pre-mating, GD's 7, 9, 12, 16, and PND 7	48	48

GD = Gestation day. PND = Postnatal day.

^a Males were not dosed and were euthanized by CO₂ inhalation and discarded without necropsy after confirmation of mating.

^b Two IM injections of 100 µL each in the rear limbs.

Route, formulation, volume, and infusion rate: Two intramuscular injections of 100 µL/each rear limbs. Total dose volume per animal was 200 µL.

Cohabitation procedures: Each female was cohabited with one untreated male animal at 28 days after the initial dose. The day of confirmation of mating was designated as GD 1.

Summary

The objective of this study was to evaluate the developmental effect of A/Indonesia/5/05 H5N1 antigen with or without AS03, when administered by IM injection in each (100 µL) rear limb once 28 days pre-mating and/or gestation days (GD's) 7, 9, 12, 16, and postnatal day (PND) 7 in Sprague Dawley rats.

Animals (48/sex/group) were assigned to four different groups and treated with two (100 µL each) IM injections of control or test article. Females were divided into 2 cohort per group after mating confirmation. The first cohort was terminated by C-section on GD 21 and the remaining cohort was allowed to deliver naturally.

The latter dams and their surviving pups were terminated on PND 25. All males and the unconfirmed females were euthanized and discarded after the mating period was over.

No test article-related effects on mortality, clinical observations, body weight, body weight changes, or food consumption during the pre-mating period were reported. No test article-related effects on mating or pregnancy data were reported. No test article-related effects on body weight, body weight changes, or food consumption during the gestation period were reported. No test article-related effects on mortality, clinical observations, body weight, body weight changes, or food consumption during the postnatal period were reported. Slight decrease (9% and 6%) in mean gravid uterine weights was reported in G4 when compared to G's 1 and 2, respectively. No changes were reported in gravid uterine weights, when adjusted to mean total body weight changes.

No treatment-related effects on gross pathology were reported in this study. No test article-related changes were reported in the mean number of corpora lutea and implantations per female. Thus, the increase in percent loss reported in G's 2, 3, and 4 was considered incidental. Slight decrease of live fetuses and percent of implantations was reported for animals in G's 2, 3, and 4, when compared to G1.

No treatment-related effects on fetal weights were reported in this study. No external variations or malformations were observed. No visceral malformations were reported. Due to low frequency, presence in control group, presence in the historical control database of the laboratory conducted the study, and/or lack of any dose relationship, the visceral and skeletal findings were considered incidental.

No treatment-related effects on gross pathology were reported in the litter. Implant scars, live pups, and post-implantation loss per female in the treated groups were not different from the control group. Early deaths were reported in all groups for pups born alive. No treatment-related effects were reported on air righting, surface righting, auditory response, and pupil reflex in pups. No treatment-related effects on gross pathology were reported in pups.

After 5 or 6 IM injections of Q-pan vaccine, anti-H5N1 antibodies were induced in all pregnant rats (dams before, during and after pregnancy). Fetal samples analysis collected on GD 21, showed trans-placental transfer of anti-H5N1 antibodies from dams to fetuses during gestation. Pup samples analysis, collected on day 25 of lactation, showed increased antibody responses compared to fetuses suggesting a transfer of anti-H5N1 antibodies via the milk during the lactation period. However, pup samples that were collected on day 4 of lactation showed a similar antibody response in group 4 or slight increase of antibody response in group 3 when compared to fetal samples that were collected on GD21.

Serological analysis data above indicates vaccine exposure of dams during pregnancy and exposure of fetuses and offspring to anti-H5N1 antibodies.

Conclusions

The administrations of Q-pan vaccine, once 28 days pre-mating, on GD's 7, 9, 12, 16, and PND 7 (littering females only), at 1.5µg HA via the IM route did not give an indication of developmental toxicity. No test article-related effect on mating, pregnancy, C-section data, fetal examination data, or pup evaluations was reported. Serological analysis data indicated vaccine exposure of dams during pregnancy and exposure of fetuses and offspring to anti-H5N1 antibodies.

Study # 9: Pandemic influenza candidate vaccines (whole H5N1/AI and split H5N1/AS03): Study of effects on embryo-fetal, pre- and post-natal development study in CD rats by intramuscular administration (including pre-mating immunization phase).

Vaccine: Whole H5N1/AI and Split H5N1/AS03

Route of clinical administration: Intramuscular (IM) injection.

Indication: Active immunization against influenza disease caused by a pandemic influenza subtype (whole H5N1/AI and split H5N1/AS03).

Key study findings: Swollen area(s) at the injection site.

Study no.: GVB0007/063710

Doses: Test article or control was dosed on day -30 before pairing, then days 6, 8, 11, and 15 after mating. Animals were treated with 100 or 200 µl/occasion.

Study design: Animals, (CrI:CD® (SD) IGS BR rats, at age 28±2 days and weight 80-100 g) were assigned to 6 different groups. Forty eight females per group were treated with control, adjuvant, and/or test article on day -30 before pairing, then days 6, 8, 11, and 15 after mating. The details of the study design are listed in the following table:

Group Number	Treatment	Dose Volume (µL)	Treatment Days	Number of Females
1	Saline	200	Days -30 before pairing, then days 6, 8, 11, 15 after mating	48
2	AS03	200	Days -30 before pairing, then days 6, 8, 11, 15 after mating	48
3	Saline (PM) Split H5N1/AS03	200	Days -30 before pairing, then days 6, 8, 11, 15 after mating	48
4	Split H5N1 / AS03	200	Days -30 before pairing, then days 6, 8, 11, 15 after mating	48
5	Saline (PM) Whole H5N1/AI	100	Days -30 before pairing, then days 6, 8, 11, 15 after mating	48
6	Whole H5N1/AI	100	Days -30 before pairing, then days 6, 8, 11, 15 after mating	48

PM = Premating. Forty eight animals per group were allocated and treated, in order to obtain 44 females with a positive indication of mating. Forty four females per group were treated during gestation.

The F1 generation received no direct administration of the candidate vaccines or adjuvant. Any exposure to the test substance or metabolites was through the mother to the offspring in utero and/or through the milk.

Summary

The objective of this study was to evaluate the effect of 2 pandemic Influenza vaccines (whole H5N1/AI and split H5N1/AS03) with or without AS03 on embryo-fetal and pre- and post-natal development in CrI:CD® (SD) IGS BR rats, naïve or pre-immunized following intramuscular administration. For each group, 22 animals (F0) were sacrificed on day 20 after mating (embryo-fetal phase) and the remaining 22 animals were allowed to rear their young to day 25 of age (littering phase). Animals in groups 2, 3, and 4 were treated with 200 µl/occasion of AS03, saline (pre-mating) and then split H5N1/AS03 (candidate vaccine), and split H5N1/AS03, respectively. Animals in groups 5 and 6 were treated with 100µl/occasion of saline (pre-mating) and then whole H5N1/AI (candidate vaccine) and whole H5N1/AI, respectively. A similarly constituted group acted as control (group 1) and received 200 µl of saline on the same occasions before pairing and after mating.

On day 20 after mating, embryo-fetal phase F0 animals were sacrificed for reproductive assessment and fetal examination. On day 25 of lactation, littering phase F0 animals were (allowed to litter and rear their offspring to weaning) sacrificed. The F1 offspring received no direct administration of the test article (any exposure was in utero or via the milk).

The following parameters were examined: clinical condition, bodyweight, food consumption, gestation length and parturition observations, and macroscopic pathology evaluations were conducted on F0 females. Fetuses on the embryo-fetal phase of the study were evaluated macroscopically at necropsy and subsequently by detailed internal visceral examination or skeletal examination. Clinical condition and survival, sex ratio, bodyweight, and pre-weaning reflex development were evaluated for the offspring on the littering phase of the study. Serum samples for antibody analysis were obtained from all F0 females on days -33 and -5 before pairing and from excess animals at day 6 after mating. Serum samples were obtained from embryo-fetal phase animals at day 20, from littering phase females at day 25 of lactation, from all fetuses of 11 litters per group at day 20 of gestation, and from up to 2 male and 2 female offspring in all litters at days 4 and 25 of age.

Results

No test article (AS03, split H5N1/AS03, or whole H5N1/AI) treatment-related effects on clinical observations, bodyweight, or food consumption were reported. A low incidence of tilted posture during lactation was observed in some females treated with whole H5N1/AI, and to a lesser extent among females treated with AS03 or split H5N1/AS03. In groups 4 and 6, following administration of the final dose on day 15 of gestation, mean food consumption was slightly but statistically

significantly lower than in controls. In group 6, low cumulative bodyweight gains during days 1-14 and 1-18 compared with controls were reported. Pale areas at the injection sites of animals in groups 2 and 4 (either post or pre and post mating) were reported. An increased incidence of pale and raised areas was reported at the injection sites of animals in group 6 (either post or pre and post mating).

No treatment related-effects on the mating performance or fertility of the F0 females. No treatment related-effects were observed on the length of gestation or their ability to give birth to a live litter. No treatment related-effects were reported on the embryo-fetal survival, growth, and development.

No treatment related-effects were reported on the clinical condition, survival, and growth of the F1 offspring between birth and day 25 of age.

Treatment with split H5N1/AS03 or whole H5N1/AI did not affect the reflex development of the F1 offspring prior to weaning. Thirteen offspring (7 litters) did not show the air righting reflex prior to day 21 of age and this finding may be related to treatment with AS03 (group 2). AS03 did not affect the attainment of the surface righting reflex or the ability of the offspring to show the startle response reflex and the pupil reflex.

No treatment related-effects were reported on the macropathology findings in the F1 offspring.

Serology data showed vaccine exposure of dams during pregnancy and exposure of fetuses and offspring to anti-H5N1 antibodies in all groups treated with either H5N1/AS03 or H5N1/AI.

Conclusion

No treatment (H5N1/AS03 vaccine or whole H5N1/AI vaccine) related-effects on the mating performance or fertility of the females were reported. No treatment (split H5N1/AS03 vaccine or whole H5N1/AI vaccine) related-effects on the embryo-fetal development or pre- and post-natal development of the offspring of naive or pre-immunized female rats on days 6, 8, 11 and 15 of gestation were reported.

Anti-H5N1 antibodies in all groups treated with either H5N1/AS03 or H5N1/AI were reported.

Study # 10 [GVB0009/064374]: Influenza vaccines (Fluarix and FluLaval) : Study of effects on embryo-fetal, pre- and post-natal development in CD rats by intramuscular administration (including pre-mating immunization phase).

Vaccine: Fluarix [(Trivalent split influenza [A/New Caledonia/20/99 (H1N1) 15µg, A/Wisconsin/67/2005 (H3N2) 15µg, and B/Malaysia/2506/2004 15µg] per 500 µl] and FluLaval [(Trivalent split Influenza [B/Malaysia/2506/2004 15 µg, A/Wisconsin/67/2005 15 µg, and A/New Caledonia/20/99 15 µg] per 500 µl].

Route of clinical administration: Intramuscular (IM) injection.

Indication: Active immunization against influenza disease.

Key study findings: No significant findings were reported.

Study no.: GVB0009/064374

Doses: Test article or control was dosed on day 28 pre-mating (PM) and gestation days (GD) 6, 8, 11, and 15. Animals were treated with 100 µl/occasion, which represents one fifth of full human dose.

Study design:

Group Number	Treatment	Dose Volume (µL)	Treatment Days	Number of Females
1	Saline	100	Day -28 before pairing, then days 6, 8, 11, 15 after mating	48
2	Fluarix	100	Day -28 before pairing, then days 6, 8, 11, 15 after mating	48
3	FluLaval	100	Day -28 before pairing, then days 6, 8, 11, 15 after mating	48

PM = Premating. Forty eight animals per group were allocated and treated in order to obtain 44 females with a positive indication of mating. Forty four females per group were treated during gestation.

The F1 generation received no direct administration of vaccines. Any exposure to the test substance or metabolites was through the mother to the offspring *in utero* and/or through the milk.

Summary

The objective of this study was to evaluate the effect of 2 pandemic Influenza vaccines (Fluarix and FluLaval) on embryo-fetal and pre- and post-natal development in CrI:CD® (SD) IGS BR rats, following intramuscular administration. For each group, 22 animals (F0) were sacrificed on day 20 after mating (embryo-fetal phase) and the remaining 22 animals were allowed to rear their young to day 25 of age (littering phase). Animals were treated with 100µl/occasion of test article. A similarly constituted group acted as control (group 1) and received 100 µl of saline on the same occasions before pairing and after mating.

On day 20 after mating, embryo-fetal phase F0 animals were sacrificed for reproductive assessment and fetal examination. On day 25 of lactation, littering phase F0 animals were (allowed to litter and rear their offspring to weaning) sacrificed. The F1 offspring received no direct administration of the test article (any exposure was in utero or via the milk).

The following parameters were examined: clinical condition, bodyweight, food consumption, gestation length and parturition observations, and macroscopic

pathology evaluations were conducted on F0 females. Fetuses on the embryo-fetal phase of the study were evaluated macroscopically at necropsy and subsequently by detailed internal visceral examination or skeletal examination. Clinical condition and survival, sex ratio, bodyweight, pre-weaning reflex development, and macropathology were evaluated for the offspring on the littering phase of the study.

Serum samples for antibody analysis were obtained from all F0 females on days -33 and -5 before pairing and from excess animals at day 6 after mating. Serum samples were obtained from embryo-fetal phase animals at day 20, from littering phase females at day 25 of lactation, from all fetuses of 11 litters per group at day 20 of gestation, and from up to 2 male and 2 female offspring in all litters at days 4 and 25 of age.

Results

No test article (Fluarix and FluLaval) treatment-related effects on clinical observations, bodyweight, or food consumption were reported. Due to the failure of the litter to thrive, one female in the FluLaval treatment group of the littering phase was sacrificed prematurely. No treatment related-effect on the injection site, either in life or at necropsy, was reported.

No treatment related-effect were reported on mating performance or fertility of the F0 females or, the length of gestation or their ability to give birth to a live litter. No treatment related-effects were reported on numbers of corpora lutea, implantations, live young, sex ratio or pre and post implantation loss. Treatment with Fluarix caused slight loss (but statistically significantly higher than control) in the mean percentage of pre-implantation. This difference resulted in the mean number of implantations being slightly lower than in Controls.

Treatment with Fluarix or FluLaval caused slightly higher incidences of fetuses and litters with medially thickened/kinked ribs or complete cervical ribs when compared with the control group. Treatment with FluLaval caused slightly higher incidence of fetuses/litters with the abnormalities of incomplete ossification of cranial bones, cervical and caudal vertebrae and metacarpals/metatarsals, when compared to the control group.

No treatment related-effect were reported on embryo-fetal survival, growth, and development. No treatment related-effect were reported on the clinical condition, survival, and growth of the F1 offspring, between birth and day 25 of age. Treatment with Fluarix caused decrease in the mean sex ratio (% male offspring per litter) on day 1 of age and then to day 25 of age. This decrease attained statistical significance on day 4 of age before culling only.

No treatment related-effect were reported on the timing of the development of the surface or air righting reflexes in the F1 offspring prior to weaning. No treatment related-effect were reported on the ability of the offspring to show the startle

response reflex and the pupil reflex. No treatment related-effect were reported on macropathology findings in the F0 females or F1 offspring.

Conclusion

In conclusion, treatment of female rats with Fluarix vaccine or FluLaval vaccine did not cause maternal toxicity or adversely affect female mating performance or fertility, embryo-fetal survival, growth or development or the pre- and post-natal survival, growth or development of the offspring up to day 25 of age.

Summary of safety pharmacology studies:

Study # 11 [GVB 0016/072148]: Pan2 (Q-Flu/AS03B) cardiovascular and respiratory evaluation in the anaesthetized rat.

Key study findings: No significant findings were reported.

Study no.: GVB 0016/072148

Summary and conclusion

The purpose of this study was to assess the effects of Pan2 (Q-Flu/AS03B), an inactivated influenza vaccine antigen (Type A/H3N2) plus AS03B adjuvant, candidate vaccine on the cardiovascular and respiratory systems in anaesthetized male Han Wistar rats.

Test article (Pan2 (Q-Flu/AS03B) candidate vaccine) was injected intravenously (once) at a dose volume of 1 ml/kg equivalent to an over dosage relative to the bodyweight based on a 70 kg human (receiving 0.5 ml) and 250 g rat (receiving 1 ml/kg) equivalent to approximately 140-fold higher than the intended human dose. Control group was dosed intravenously (once) with saline 0.9% w/v with a dose volume of 1 ml/kg.

Cardiovascular (arterial blood pressure, heart rate and ECG (lead II)) and respiratory (tidal volume, respiration rate and minute volume) parameters were evaluated continuously for at least a 30 minute stabilization period prior to the dose administration. Following intravenous administration, the parameters were recorded for 120 minutes post-dose.

No test article related effects on any of the recorded cardiovascular or respiratory parameters were reported. Values recorded for all cardiovascular and respiratory parameters during stabilization and post-dose periods were within the normal range.

In conclusion, Pan2 (Q-Flu/AS03B) vaccine did not cause any adverse effects on the cardio-respiratory systems.

Study # 12 [AS03A]: Adjuvant-effects on cardiovascular and respiratory functions, following intramuscular administration in the conscious Beagle dog monitored by telemetry.

Key study findings: Decrease in food consumption, decrease in body weight, and increase in body temperature were reported.

Study no.: AA80120

Summary and conclusion

The purpose of this study was to assess the effects of AS03A adjuvant on the arterial blood pressure, heart rate, electrocardiogram, body temperature, and respiratory parameters in conscious male Beagle dogs.

Test article was injected intramuscularly (once) at a dose volume of 0.5 mL per animal which is equivalent to 1 full human dose. Control group was dosed intramuscularly (once) with saline on study day 0 and with AS03A adjuvant on study day 7. Each animal served as its own control and treated with the control and the test article with a wash-out period of 7 days in between each treatment.

Body temperature, haemodynamic, cardiac (RR intervals, PR intervals, QRS complex, and QT intervals) and respiratory (respiration rate, inspiratory time, expiratory time, AUCITP deflection, and AUCITP X respiratory rate) parameters were evaluated in all animals on study days 0 and 7 starting at least 1.5 hours before administration and for approximately 7 days following the administration. Data were analyzed over the first 3 days of recording only (1, 3, 6, 24, 48, and 72 hours) after each dose administration. Morbidity/mortality, clinical observations, and body weights were also recorded.

Decrease in food consumption and in body weight loss was reported in 2 out of the 4 animals in group 2 (adjuvant treated). Slight increase in body temperature were reported in group two 6 hours after dosing. No test article-related effects on the arterial blood pressure, the heart rate, and the duration of the RR and PR intervals of the QRS complex and of the QT and QTc intervals, irrespective of the formula used for QT interval correction, during the 72 hours period following dosing were reported. Thus, AS03A adjuvant did not cause any potential deleterious effect on the atrio-ventricular and intra-ventricular conduction velocity, and on ventricular re-polarization. No test article-related effects on the any disturbances in rhythm or wave for morphology of the ECG during the first 6-hour post-treatment period. No test article-related effects were reported in the respiratory rate, the inspiratory and expiratory time, AUCITP (index of tidal volume) and AUCITP X respiratory rate (index of minute volume).

In conclusion, slight decrease in food consumption and body weight loss was reported in group 2. Because of the low number of animals used in this study, the treatment relation of these findings cannot be excluded. Six hours post treatment,

slight increase in body temperature was also reported. AS03A treatment did not affect the cardiovascular and the respiratory functions.

Summary of gene toxicology studies:

Study # 13: SB62 Batches SB621011 and SB621017 –b(4)---- testing for mutagenic activity with Salmonella typhimurium TA 1535, TA 1537, TA 98, and TA 100 and Escherichia coli WP2UVRA.

Key study findings: No significant findings were reported.

Study no.: -b(4)--inv-21354/768632

Summary and conclusion

The mutagenic activity of SB62, batches SB621011 and SB621017 –b(4)---- was tested in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2uvrA at exposure levels ranging from –b(4)----- of the original oil in water solution per plate. The tests were –b(4)----- presence and -----b(4)-----

The sensitivity of the assay and the metabolizing activity of the –b(4)---- were demonstrated using concurrent positive controls. The results obtained in both assays were similar. In any of the 5 bacterial strains, in either activation condition, with either batch of the test item, no mutagenic activity was observed.

In both mutation assays at –b(4)-----, precipitation of both test items was observed. At the lower dose ranges, no toxicity to the bacteria was observed. However, excessive precipitation at the higher dose levels obscured the background lawns of microcolonies and toxicity could not be assessed accurately.

In conclusion, SB62 batches SB621 011 and SB621 017 –b(4)- were not mutagenic to *Salmonella typhimurium* or *Escherichia coli* when tested in –b(4)----- up to and above their limit of solubility in the test system.

Study # 14: SB62 new SB62 old SB62/Alpha-tocopheryl –b(4)---- in vitro mutation test using mouse lymphoma L5178Y cell.

Key study findings: No significant findings were reported.

Study no.: BVR 785/052587

Summary and conclusion

To test the mutagenic potential of SB62 new, SB62 old, and SB62/alpha-tocopheryl –b(4)----, an in vitro mammalian cell mutation assay were used. –b(4)-

Summary and conclusion

The purpose of this study is to determine the potential of SB62 new, SB62 old, and SB62 alpha-tocopheryl –b(4)--, to induce an increase in micronuclei in bone marrow cells of Sprague-Dawley (CD) rats. This study compared three batches of SB62 emulsions with increasing concentrations of alpha-tocopheryl –b(4)---, a ---b(4)-----

SB62 new, SB62 old, and SB62 alpha-tocopheryl –b(4)---- were supplied at a concentration of less than 0.05 mg/mL (tocopheryl –b(4)-----), 0.25, and 4.94 mg/mL, respectively. At a dose volume of 2 mL/kg/day, the test substances were tolerated with acceptable levels of bone marrow toxicity. Higher dose volumes of 5 and 10 mL/kg/day caused substantial bone marrow toxicity. Thus, the 2 mL/kg/day dose volume was defined as the maximum for the micronucleus test. The main test was performed using male animals only.

At a dose volume of 2 mL/kg/day on two consecutive occasions, approximately 24 hours apart, animals were dosed intravenously by bolus injection in the lateral tail vein. The positive control group received a single oral administration of cyclophosphamide at 20 mg/kg approximately 24 hours prior to termination. The negative control group received the vehicle, phosphate buffered saline (PBS).

Twenty-four hours after administration of the second dose, bone marrow smears were obtained from seven males in the negative control and in each of the test substance groups. Twenty four hours after a single dose, bone marrow smears were also obtained from five males in the positive control group. The presence of micronuclei in 2000 immature erythrocytes was examined. The proportion of immature erythrocytes was assessed by examination of at least 1000 erythrocytes from each animal. The incidence of micronucleated mature erythrocytes was also recorded.

In rats treated with SB62 new, SB62 old, or SB62 alpha-tocopheryl –b(4)--- (at any treatment level) compared to vehicle control values, no statistically significant increases in the frequency of micronucleated immature erythrocytes and no substantial decreases were reported in the proportion of immature erythrocytes.

Significant increases in the frequency of micronucleated immature erythrocytes were reported in the positive control group.

In conclusion, the test substances (SB62 new, SB62 old and SB62 alpha tocopheryl –b(4)-----), when administered intravenously by bolus injection at a dose volume of 2 mL/kg/day on two consecutive days, did not cause an increase in the induction of micronucleated immature erythrocytes. The test substances did not cause bone marrow cell toxicity.

OVERALL SUMMARY:

General Toxicology:

Treatment with the adjuvant AS03 caused minimal or mild subacute inflammation of the subcutaneous and/or epimysial tissues. Minor inflammation was reported at the injection site. Fasciitis and cellulitis was reported in animals treated with the adjuvanted vaccines. The incidence and severity of granulomatous myositis in animals treated with the adjuvanted vaccines was higher than the control groups. The perineural connective tissue surrounding the sciatic nerves findings (inflammatory cell infiltrates, hemorrhage, necrosis, and fibrosis) were considered to be an inflammatory response to the vaccine adjuvant and to the dosing procedure. Perivascular cuffing was reported at the injection site of test article treated rabbits. This was characterized by the presence of a predominantly lymphocytic cellular infiltrate centered around blood vessels. Injection site reactions were attributable to recovery of trauma due to injection.

Monocytosis, neutrophil or heterophil, and eosinophil counts increases could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair. Treatment related-increases were reported in fibrinogen and white blood cell count. This suggests an acute and transient inflammatory response and might correlates with the erythema and/or edema and the inflammation reported microscopically at the injection site. The increases in fibrinogen levels were not considered frank toxicity but rather an anticipated effect associated with an immunological response.

The increase in mean and individual animal total protein and globulin values and the lower mean and individual animal albumin to globulin ratio (A/G) values for the adjuvant and vaccine treated groups might be the result of an increase in immunoglobulin synthesis due to polyclonal activation of B lymphocytes by the adjuvant. An increase was reported in males' total bilirubin. This increase was not reported after the subsequent dose administrations in males and considered non-adjuvant related. However, an increase in females' total bilirubin were reported which might indicate test article-related effect in the liver. The increase in bilirubin levels in females at recovery might indicate a delayed type effect by the test article on the liver. The increase in mean and individual creatine kinase activity values for the adjuvant and vaccine treated groups observed might be a reflection of minimal muscle degeneration subsequent to the inflammatory response to intramuscular injection of the adjuvant and the vaccine antigen/adjuvant.

There were an increase in mean and individual animal platelet count for the adjuvant and vaccine treated groups. The minimal to slight differences in coagulation (except for fibrinogen) parameters subsequent to adjuvant and

vaccine administration were considered representative of release of acute inflammatory proteins from the liver.

Microscopic findings of lymphoid hyperplasia were consistent with the increases reported in the adjusted spleen weight of all treated groups. Stimulation of the immune system by the test article-treatment might be the cause of these findings.

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

Reproductive toxicology:

No test article-related effects were reported on mortality, clinical observations, body weight, body weight changes, or food consumption during pre-mating, gestation, or postnatal periods.

No treatment-related effects were reported on gross pathology. Increased incidences of swollen area(s) at the injection site were reported in the treated groups. Limited movement of the hindlimbs (this was considered to be associated with the presence of swelling) was reported. This sign was recoverable. Pale areas were reported at the injection sites.

Recoverable, test article-related (adjuvant or adjuvant + antigen) significant decrease was reported in body weight gain and food consumption.

No treatment related-effects were reported on the mating performance or fertility of the F0 females. No treatment related-effects were reported on the length of gestation or their ability to give birth to a live litter. No treatment related-effects were reported on the embryo-fetal survival, growth, and development.

Treatment with Fluarix or FluLaval caused slightly higher incidences of fetuses and litters with medially thickened/kinked ribs or complete cervical ribs when compared with the control group. Treatment with FluLaval caused slightly higher incidence of fetuses/litters with the abnormalities of incomplete ossification of cranial bones, cervical and caudal vertebrae and metacarpals/metatarsals, when compared to the control group.

No treatment-related effects on air righting, surface righting, auditory response, and pupil reflex were reported in pups. No treatment-related effects on gross pathology were reported in the litter and the pups.

Vaccine exposure of dams during pregnancy to anti-H1N1 or anti-H5N1 antibodies was indicated by the serological analysis data. Fetal samples analysis, showed trans-placental transfer of anti-H5N1 antibodies from dams to fetuses during gestation. Pup samples analysis, collected on day 25 of lactation,

showed increased antibody responses compared to fetuses suggesting a transfer of anti-H5N1 antibodies via the milk during the lactation period.

Safety pharmacology toxicology:

Decrease in food consumption and in body weight was reported in adjuvant treated group. Slight increase in body temperature were reported in test article-treated group. No test article-related effects were reported on the arterial blood pressure, the heart rate, and the duration of the RR and PR intervals of the QRS complex and of the QT and QTc intervals, irrespective of the formula used for QT interval correction, during the 72 hours period following dosing. Thus, AS03A adjuvant did not cause any potential deleterious effect on the atrio-ventricular and intra-ventricular conduction velocity, and on ventricular re-polarization. No test article-related effects on the disturbances in rhythm or wave for morphology of the ECG during the first 6-hour post-treatment period. No test article-related effects were reported in the respiratory rate, the inspiratory and expiratory time, AUCITP (index of tidal volume) and AUCITP X respiratory rate (index of minute volume).

Gene toxicology:

SB62 batches SB621 011 and SB621 017 –b(4)-- were not mutagenic to *Salmonella typhimurium* or *Escherichia coli* when tested in ----b(4)----- up to and above their limit of solubility in the test system.

SB62 new, SB62 old, and SB62/alpha-tocopheryl –b(4)---- did not demonstrate mutagenic potential in the in vitro cell mutation assay.

The test substances (SB62 new, SB62 old and SB62 alpha-tocopheryl –b(4)----), when administered intravenously by bolus injection at a dose volume of 2 mL/kg/day on two consecutive days, did not cause an increase in the induction of micronucleated immature erythrocytes. The test substances did not cause bone marrow cell toxicity.

Pregnancy category: B

Justification: No data are available from adequate and well-controlled studies for Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted in pregnant women. Reproductive studies included in this BLA showed no adverse effects on mating, female fertility, pregnancy, parturition, lactation parameters, and embryo-fetal or pre-weaning development were observed. There were no vaccine-related fetal malformations or other evidence of teratogenesis.

Internal communication:

As reported in the reproductive toxicology studies above, the highest dose used to treat the animals was 200 µl/occasion, which is less than the full human dose (500 µl/occasion). Ideally, the human vaccine dose should be administered to animals in toxicity studies, where feasible. This is to best evaluate the potential for clinical key relevant toxicities. Adequate justification is needed for an alternative dosing strategy. Since 500 µl/occasion dose is not feasible in rats¹, the administration of a dose that exceeds the human dose on a mg/kg basis while still capable of inducing an immune response in the animal² is consistent with our practice.

Note: The sponsor has submitted amendment 21 on December 7, 2012, which included additional information in regards to the dosing calculations:

In study Bridge GPS 1536-08129, the female rats allocated to the study group receiving H5N1/AS03 vaccine were administered 1.5 µg HA, as compared to the full human dose of 3.75 µg HA.

- 1.5 µg HA per 250 g rat is equivalent to 6 µg HA per kg (0.006 mg HA per kg)
- 3.75 µg HA per 50 kg female human is equivalent to 0.075 µg HA per kg (0.000075 mg HA per kg)
- 6 µg HA per kg / 0.075 µg HA per kg = 80 fold difference.

The dose of vaccine administered to the female rats corresponds to an 80-fold overdose on a body weight basis (µg/kg).

Thus, the repro/tox information submitted are acceptable as per the FDA practice.

Proposed label wording:

Pregnancy Category B

A reproductive and developmental toxicity study has been performed in female rats at a dose approximately 80 times the human dose (**on a mg/kg basis**) and has shown no evidence of impaired fertility or harm to the fetus due to Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted. The effect of Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted on embryo-fetal and pre-weaning development was evaluated in rats. Animals were administered Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted by intramuscular injection once prior to gestation, during the period of organogenesis (gestation days 7, 9, and 12), later in pregnancy (gestation day 16) and during lactation (day 7), 0.2

¹ <http://research.utsa.edu/files/larc/Ratbiomethodologyhandouts.pdf>
<http://www.bu.edu/orccommittees/iacuc/policies-and-guidelines/administration-of-drugs-and-experimental-compounds-in-mice-and-rats/>

² Guidance for Industry: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications

mL/dose/rat (approximately 80-fold excess relative to the projected human dose on a body weight basis). No adverse effects on mating, female fertility, pregnancy, parturition, lactation parameters, and embryo-fetal or pre-weaning development were observed. There were no vaccine-related fetal malformations or other evidence of teratogenesis.

No data are available from adequate and well-controlled studies for Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted in pregnant women.

Because animal reproduction studies are not always predictive of human response, Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted should be given to a pregnant woman only if clearly needed.

OVERALL CONCLUSION:

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

Concurrence: Martin D. Green