Individuals using assistive technology may not be able to fully access the information contained in this file. For assistance, please send an e-mail to: ocod@fda.hhs.gov and include 508 Accommodation and the title of the document in the subject line of your e-mail.

Toxicology Review of Fluzone^{R} HD QIV

BLA: 103914/6290.0 &5001
Type and date of submission: Efficacy supplement, January 4, 2019; amendment, March 20, 2019

Sponsor: Sanofi Pasteur Inc., Swiftwater, PA
Product: Fluzone^{R} High-Dose Quadrivalent, 0.7 mL
Cross references: None
Proposed use: Active immunization for the prevention of influenza in persons 65 years and older

Reviewer: Ching-Long Joseph Sun, Ph. D., Division of Vaccines and Related Products Applications

Précis

The applicant conducted a single subcutaneous dose local tolerance study in rabbits and an intramuscular repeat-dose toxicity study in rabbits in support the HD QIV supplement. The single dose local tolerance study report is not reviewed as it was performed by single and not by intramuscular administration and not a dedicated toxicity study.

In respond to our information request (question 3) of February 28, 2019, the applicant provided in the amendment the composition of the Fluzone^{R} HD HV QIV tox Bulk drug product used in the toxicity studies. The only difference from the to-be-marked product is the specification of HA content. It was slightly higher at (b) (4) ug/mL/strain at the bulk used in the toxicity studies (vs (b) (4) ug/mL/strain).

In the repeated toxicity study, two groups of animals (10/sex/group) received intramuscular injections on days 1, 15 and 29 of either saline control or 0.8 mL Fluzone^{R} QIV-HD. After the treatment, half of animals/group was followed by a 2-week recovery period. Three biweekly intramuscular vaccine administrations at dose volume of 0.8 mL were well tolerated in animals. The findings were typical and/or expected local inflammatory (injection sites), immunogenic (spleen) and acute reaction responses to vaccine administrations and not considered adverse. After a 2-week recovery period, CRP had returned to baseline, and microscopic findings at the injection sites were either absent (necrosis, hemorrhage and edema) or observed (muscle fiber inflammation) at a lower frequency and/or severity, indicating on-going recovery. Increased germinal centers in the spleen as expected immunogenic response were still present.

Toxicology Study Review

Title and study number: Systemic and local tolerance toxicity study in (b) (4) rabbit following 3 intramuscular injections followed by a 2-week treatment-free period (Study # AB21745/SP178RD1703)
Performing laboratory: (b) (4)
Initiation date: February 6, 2017

Final report date: October11, 2018
Batch/lot number of test article: Q0580123
Animal species and strain: (b) (4) rabbit
Breeder/supplier: (b) (4)
Number of animals per sex per group: 10
Age: 13-14 weeks
Body weight range: 2.6-3.4 kg (males); 2.7-3.4 kg (females)
Route and site of administration: Intramuscular; the different dorsal lumbar muscle areas
Volume of administration: 0.8 ml (one human dose is 0.7 mL)
Frequency of administration and study duration: bi-weekly for 3 doses; 6 weeks
Dose: 0.8 mL with (b) (4) ug HA/mL/strain corresponding to human dose of 0.7 mL with (b) (4) ug HA/mL/strain
Stability: Documentation of the characterization and stability was provided in addendum 1
Means of administration: A syringe fitted with a needle
Report status: Final

Experimental design

Group	Test article	Dose* volume	HA content* (ug/mL/strain)	No. of animals/sex/group/sacrifice
1	Vehicle control	0.8 mL	0	5
2	Test item	0.8 mL	(b) (4)	5

*: to-be marked product is 0.7 ml with HA content of [redacted] ug/mL/strain (A/H1N1/California/07/2009, A/H3N2/Hong Kong/4801/2014, B/Phuket/3073/2013 and B/Brisbane/60/2008)

Methods:

Endpoint	
Hematology	(b) (4)
Clinical chemistry	(b) (4)
Coagulation	(b) (4)
C-reactive protein	(b) (4)
Immunogenicity	(b) (4)

Randomization procedure: The animals were allocated to dose group using a randomization procedure.

Statistical analysis: Statistical analyses were performed for males and females separately by the (b) (4) data acquisition system as follows: the best transformation for the data (none, log or rank) was determined depending upon the kurtosis of the data, the probability of the Bartlett's test for homogeneity of the variances and an assessment of whether the size of the groups is approximately equal or not. Non- or log-transformed data was analyzed by parametric methods. Rank transformed data was analyzed using non-parametric methods. Homogeneity of means was assessed by analysis of variances (ANOVA). Data were then analyzed to compare the treated group with the control group

using the two-sample t-test for parametric data or the Mann-Whitney test for non-parametric data.

The following parameters were evaluated.

Parameters	Frequency of Testing
Morbidity/mortality	Twice daily
Clinical signs	Daily and weekly physical examination
Local tolerance	Prior to and daily
Body weight	Twice weekly
Food consumption	Daily
Body Temperatures	4 occasions during pre-treatment, prior to each dose, 3, 6 and 24 hours after each dose
Ophthalmologic examination	Pre-treatment, days 3, 13 and 43
Clinical chemistry and coagulation	Pretest, days 3, 31 and 43 thru the ear artery
Hematology	Same as above
C-reactive protein	Same as above
Immune response	Pretest, days 15, 31 and 43 thru the ear artery
Necropsy	Days 31 and 43
Tissues for histopathology	Same as above

Postmortem procedures: The following tissues were collected at necropsy and microscopically examined. Those tissues marked with an asterisk were weighed.

SYSTEM	ORGAN COLLECTED	ORGAN NOT COLLECTED
DIGESTIVE	Large intestine (cecum, colon, rectum); small intestine (duodenum, jejunum, ileum), liver*, stomach, gall bladder, pancreas, esophagus, salivary gland (parotid, mandibular, sublingual), Peyer's patch	
RESPIRATORY	Lung with bronchi, trachea	Nasal cavity
CARDIOVASCULAR	Heart*, aorta	
IMMUNOLOGIC/ HEMATOPOIETIC	Bone with bone marrow (femur, sternum), lymph node (mesenteric, mandibular, Iliac, sacral), spleen*, thymus*	

SYSTEM	ORGAN COLLECTED	ORGAN NOT COLLECTED
UROGENITAL	Epididymes*, kidneys*, urinary bladder, ovaries*, prostate*, testes*, seminal vesicle, uterus*, cervix, vagina, oviduct	Fallopian tubes
NEUROLOGIC	Brain*, spinal cord	
HORMONAL	Adrenals, mammary glands, thyroid glands*, parathyroid glands, pituitary gland*	
OTHER	Skeletal muscle, skin, sciatic nerve (left), tongue, eyes, optic nerve, Harderian glands, injection sites	
GROSS LESIONS	None	

Results:

Mortality: All animals survived to scheduled euthanasia.

Clinical signs: There were no signs of toxicity that were considered adverse.

Local tolerance: A few local observations (edema, erythema, induration and/or hematoma) were noted with a slightly higher frequency in the vaccine-treated animals. However, these observations except hematoma were also seen in the control. Therefore, these local reactions were not considered as toxicologically significant.

Body weight and food consumption: They were not affected by the treatment.

Body temperature: There was no effect of the vaccine on the body temperature.

Ophthalmology: No vaccine-related ocular changes were noted.

Immune response: Significant higher antibody responses (IgG titers) against all 4 strains (A/H1N1/California/07/2009, A/H3N2/Hong Kong/4801/2014, B/Phuket/3073/2013 and B/Brisbane/60/2008) achieving 3-3.3 $\log_{10}$ EU on day 15 and 4-4.8 $\log_{10}$ EU on days 31 and 43. In the control group as well as in pretest samples, no or very low background was detected.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Glutamate dehydrogenase (not determined) Gamma-glutamyl transferase Aspartate aminotransferase Alanine aminotransferase Sorbitol dehydrogenase (not determined) Total bile acids (not determined)
B) HEPATOBILIARY		Alkaline phosphatase Total bilirubin
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	C-reactive protein Day 31 4-5 X↑	Albumin Globulin (calculated) A/G Ratio Cholesterol Creatine kinase Cholinesterase (not determined) Total protein Triglyceride

Table of Clinical Chemistry Results

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT	NOT OF NOTE
RED BLOOD CELLS		Hematocrit Hemoglobin conc. Mean corp. Hb. Mean corp. Volume Mean corp. Hb. conc. Total erythrocyte count Reticulocyte
WHITE BLOOD CELLS		Total leukocytes Differential white blood cell counts Large unstained cells (not determined)
CLOTTING POTENTIAL		Activated partial-thromboplastin time Prothrombin time Platelet count Fibrinogen

Table of Hematology Results

Organ weights: Higher spleen weights were seen on day 31 (22 % in males and 82 % in females) and still present on day 43 (37 % in males and 16% in females).

Gross pathology: Dark foci and firm area at the injection sites in the treatment group were observed. These findings were no longer present after the recovery period.

Microscopic findings: An increase in the number of germinal centers in the spleen was seen in all except one vaccine-treated animal. Inflammation, necrosis, hemorrhage, edema, degeneration/regeneration and/or mineralization were seen at the injection sites. The incidences (n=5) of these findings are summarized below.

Day 31	1M	2M	1F	2F
Day 1 injection site				
Inflammation	0	4	2	4
Necrosis	0	0	1	1
Hemorrhage/edema	0	4	0	4
Day 15 injection site				
Inflammation	1	5	1	3
Necrosis	0	1	0	2
Degeneration/regeneration	0	4	0	2
Mineralization	0	1	0	0
Day 29 injection site				
Inflammation	3	5	3	5
Necrosis	2	5	2	5
Hemorrhage/edema	1	5	1	5

Spleen Increased in number of germinal centers	0	5	0	5

At the end of recovery period, finding in the spleen noted at day 31 was still present. No hemorrhage/edema at the injection sites was noted on day 43. Other findings at the injection sites were observed at reduced incidences/severity. The incidences are shown below:

Day 43	1M	2M	1F	2F
Day 1 Injection site				
Inflammation	0	0	0	3
Degeneration/regeneration	0	0	0	1
Mineralization	0	0	0	1
Day 15 Injection site				
Inflammation	0	2	1	3
Degeneration/regeneration	0	0	0	1
Day 29 Injection site				
Inflammation	0	5	1	5
Degeneration/regeneration	0	4	0	5
Mineralization	0	3	0	4
Spleen				
Increased germinal canters	0	4	0	5

Test article related effects
Local inflammation at the injection sites. Immunogenic response in the spleen. Elevation of C reactive protein. Typical and expected local inflammatory/immunogenic and acute reaction to vaccine administration. Completely or partially reversible after a 2-week treatment-free period except the spleen.

Assessment

There were no biologically significant Fluzone^{R} HD QIV-related effects on clinical observations, mortality, body weights, body temperature, ophthalmology, coagulation, hematology and macroscopic examination. Slight CRP elevation (up to 4-5 folds) was observed, indicative some acute reactions mostly attributable to the vaccine. Microscopic findings at the injection sites (inflammation, necrosis, hemorrhage, necrosis and

degeneration/regeneration) and the spleen (increase germinal centers) were typical local and immunogenic reactions to the vaccine administration.

Robust antibody titers against all four strains at days 15, 31 and 43 were indicative of an active delivery of the test articles in the treated animals.

Based on overall evaluation of the toxicity study report, it can be concluded that three bi-weekly intramuscular injections of 0.8 ml of Fluzone^{R} QIV HD were well tolerated in rabbits.

GLP study deviations or amendments: Minor protocol amendments were recorded in the draft report. None of them influenced the quality, integrity or interpretation of the results.

Recommendation
The supplement has adequate nonclinical toxicology data in support Fluzone^{R} QIV HD for the indication from a toxicological standpoint. No animal developmental toxicity study of the product was performed and is so indicted in section 8.1 of the labeling. Thus, the proposed section 8.1 regarding animal data/study and section 13.1 are adequate from a toxicological standpoint.

Concurrence: Martin D. Green, Ph. D., Division of Vaccines and Related Products Applications