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Toxicology Review of Zoster (non-live) Vaccine, supplement 398

From: Claudia Wrzesinski, DVM, PhD

Through: Martin Green, PhD

To: Ramachandra Naik, PhD; Laura Gottschalk, PhD

Division name: OVRR/DVRPA

File: BLA 125614/398

Submission date: September 24, 2020

Product: Shingrix, Zoster Vaccine Recombinant, Adjuvanted

Sponsor: GlaxoSmithKline Biologicals

Proposed Indication: Prevention of herpes zoster (shingles) in adults aged 50 years and older and adults aged 18 years and older who are at increased HZ risk due to immunodeficiency or immunosuppression caused by disease or therapy.

In this amendment the sponsor submitted:

- DART study "A Developmental Toxicity Study (including teratogenicity and postnatal investigations) of (b) (4) $AS01_B$ by Intramuscular Injection in Rabbits" (study number: 20152506) evaluating $AS01_B$ in rabbits
- Additional information for DART study "WIL AB14898: DQ –Developmental Toxicity Study (Including Teratogenicity and Postnatal Investigations) by the Intramuscular Route in the Rabbit" which was submitted and reviewed under the original BLA submission.

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Executive summary:

In this amendment the sponsor submitted: A) a DART study evaluating a full human dose of $AS01_B$ in (b) (4) rabbits "A Developmental Toxicity Study (including teratogenicity and postnatal investigations) of (b) (4) or $AS01_B$ by Intramuscular Injection in Rabbits" and B) additional information for DART study "WIL AB14898: DQ –Developmental Toxicity Study (Including Teratogenicity and Postnatal Investigations) by the Intramuscular Route in the Rabbit", the study was previously submitted and reviewed under the original BLA submission.

A) In the original BLA a rat DART study was submitted. Female rats were administered SHINGRIX or the $AS01_B$ Adjuvant System alone by intramuscular injection 4 and 2 weeks prior to mating, on Gestation Days (GD) 3, 8, 11, and 15, and on Lactation Day (LD) 7. The total dose was 0.2 mL at each occasion (a single human dose is 0.5 mL). No adverse effects on pre-weaning development up to post-natal day 25 were observed. There were no vaccine-related fetal malformations. In this current submission the sponsor submitted a rabbit DART study ((b) (4)-20152506) which was conducted to investigate the potential influence of a full human dose of $AS01_B$ on fertility parameters, embryo-fetal and pre- and post-natal survival, and development of the offspring. (b) (4) rabbits received saline, (b) (4), or $AS01_B$ formulation (0.5 mL/injection, full human dose) by intramuscular injection 28 and 14 days prior to mating, on GD 3, 11, 16, and 24, and after natural delivery on LD 7. Mated females and their litters were euthanized on GD 29 (cesarean section cohort) or on LD 35 (natural delivery cohort). Three rabbits in the $AS01_B$ group were found dead (GD 31, LD 28, and LD 30); the cause of death could not be determined. There were no $AS01_B$-related effects on clinical signs, dermal observations, or necropsy observations in the dams. There were no $AS01_B$-related effects on mating and female fertility, embryo-fetal pre- and post-natal survival, growth, or development. No adverse effects on pre-weaning development up to post-natal day 35 were observed.

B) The sponsor also provided additional background data for DART study ABI4898 which was submitted and reviewed under the original BLA submission. In study AB14898 fetal malformations were observed in fetuses of rabbits administered QS-21 at a dose of 200 µg/animal (high dose group). In this supplement the sponsor submitted new background data supporting that these findings are background findings and not evidence of QS-21-mediated fetal malformations.

A Developmental Toxicity Study (including teratogenicity and postnatal investigations) of (b) (4) or AS01$_B$ by Intramuscular Injection in Rabbits

In this review only group 1 (control) and 3 (AS01$_B$) will be evaluated, because only the administration of AS01$_B$ is of relevance for the Shingrix vaccine.

Introduction:

In the original BLA a rat DART study was submitted (study number: HEY0005); female rats were administered SHINGRIX or the AS01$_B$ Adjuvant System alone by intramuscular injection 4 and 2 weeks prior to mating, on Gestation Days (GD) 3, 8, 11, and 15, and on Lactation Day (LD) 7. The total dose was 0.2 mL at each occasion (a single human dose is 0.5 mL). No adverse effects on pre-weaning development up to post-natal day 25 were observed. There were no vaccine-related fetal malformations.

In this supplement the sponsor submitted a rabbit DART study (study number: (b) (4)-20152506) which was conducted to investigate the potential influence of a full human dose of AS01$_B$ on pregnant rabbits and fertility parameters, embryo-fetal and pre- and post-natal survival, growth and development of the offspring. Since Shingrix has been shown to induce significant systemic reactogenicity not only in rabbits (CRP increase, maternal toxicity) but also in humans (fatigue, headache, myalgia and shivering) which could adversely affect pregnancy outcome, the sponsor evaluated a full human dose of AS01$_B$ in a DART study. In this study the full human dose of AS01$_B$ is evaluated which is the component of the Shingrix vaccine inducing the main reactogenicity events. The submitted DART study provides new data in rabbits evaluating the potential effects of AS01$_B$ on parameters including numbers of corpora lutea, implantations, resorptions, live and dead fetuses per litter, sex ratio, fetal body weight, gravid uterine weight and placental morphology. This study was conducted to support the indication expansion of *Shingrix* to adults aged 18 years and older who are at increased risk of herpes zoster due to immunodeficiency or immunosuppression caused by disease or therapy and includes women of childbearing potential.

Summary:

(b) (4) rabbits received saline, (b) (4) or AS01$_B$ formulation (0.5 mL/injection, Group 3, full human dose) by intramuscular injection 28 days prior to mating, 14 days prior to mating, on Gestation Day (GD) 3, 11, 16, and 24, and after natural delivery on Lactation Day (LD) 7. (b) (4) is not relevant for the assessment of effects of gE/AS01$_B$ or AS01$_B$; however, the group can serve as control and results can be informative for the overall interpretation of the study. Mated females and their litters were euthanized on GD 29 (cesarean section cohort) or on LD 35 (natural delivery cohort). Three (b) (4) rabbits in the AS01$_B$ group were found dead; one animal died during the gestation period (GD 31) and 2 animals died during the lactation period (LD 28 and 30) showing reduced food consumption with body weight loss. The cause of death could not be determined, the mortality rate was above the submitted historical control data; mortality was only observed in the AS01$_B$ group. Since the sponsor will perform a

clinical study to monitor and evaluate pregnancy exposures and outcomes as a post-marketing commitment, which will address concerns of a potential negative effect of the vaccine on pregnant women; a follow up DART study was not deemed necessary. There were no $AS01_B$-related effects on clinical signs, dermal observations, body weights, or necropsy observations in the dams. There were no $AS01_B$-related effects on mating and female fertility, embryo-fetal pre- and post-natal survival, growth, or development. No adverse effects on pre-weaning development up to post-natal day 35 were observed.

Study no.: 20152506, GSK Study ID: L7468
Conducting laboratory and location: (b) (4)
Date of study initiation: 08 November 2018
GLP compliance: Yes
QA reports: Yes
Drug, lot #: (b) (4) (Lot (b) (4)), $AS01_B$ (Lot AA1BA009A)

Doses:
(b) (4)
$AS01_B$: 50 µg of a saponin molecule (QS-21) and 50 µg of 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL)] was selected because it is the highest clinical dose used for the licensed Shingrix vaccine
.

Group	Test Material	Dose (µg/injection)	Number of Females	
			Assigned to Cesarean-Sectioning	Assigned to Natural Delivery
1	Control	0 (Control Article)	24	24
2	(b) (4)		24	24
3	$AS01_B$	50[b]	24	24

a. This dose was expected to give an approximate 20X dose multiple assuming a 3kg average rabbit weight and a 60 kg average human weight.
b. The dose of $AS01_B$ [50 µg of a saponin molecule (QS-21) and 50 µg of 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL)] was selected because it is the highest clinical dose used for the licensed Shingrix vaccine and other vaccine candidates in development.

Table 1: *Dosing in study L7468, (table provided by the sponsor).*

In this review only group 1 (control) and 3 ($AS01_B$) will be evaluated, because only the administration of $AS01_B$ is of relevance for the Shingrix vaccine.

Species/strain: (b) (4) rabbits
Number/sex/group: 24 females per dosage group
Route, formulation, volume, and infusion rate: Intramuscular, 0.5 mL per dose, injection sites were rotated to minimize any possible irritation (e.g., left and right hindlimb);

Study design:

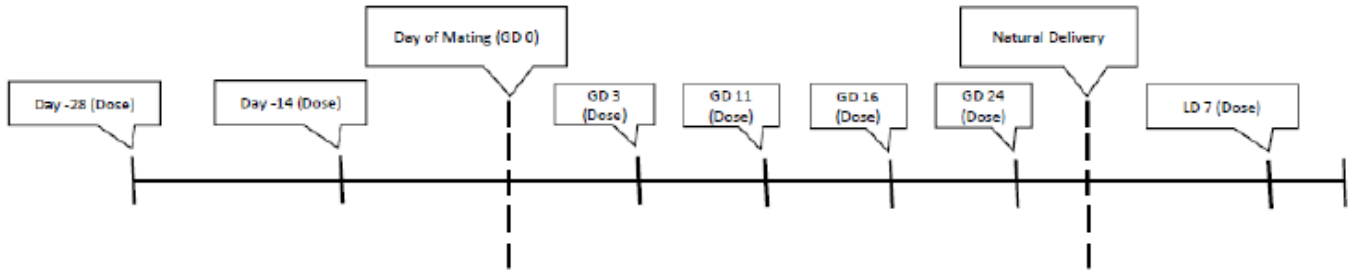

Figure 1: *Study design L7468, (figure provided by the sponsor).*

Parameters and endpoints evaluated:

Measurements and Observations – F0 Generation:

Clinical Observations:
Each rabbit was assessed for viability at least twice daily throughout the study. During LDs 1 to 3, viability was conducted for each doe and delivered litter by cage side observation twice daily. Clinical observations were recorded at least weekly during the dosing period, including on each day of dosing prior to dose administration, and once daily during the lactation period; however, during LDs 1 to 3, twice daily cage side observations were performed; so that does and litters were not disturbed.

Body Weight:
Body weight was measured once weekly during the premating phase, including on each day of dose administration (DS 1 and DS 15), and then on GD 0, 3, 8, 11, 16, 20, 24, 29, and 35 (where required for does that did not deliver), and on LD 4, 7, 10, 14, 17, 20, 24, 29 and 35.

Food Consumption:
Food consumption was measured daily, starting the day after arrival up to GD 29 (all rabbits) and continued daily for females assigned to natural delivery cohorts, including the day of scheduled euthanasia (LD 35). Acclimation values were not tabulated.

Mating Performance:
After 28 days of study (the first day of dose administration was considered DS 1), female rabbits were mated with untreated male rabbits (same source and strain), one male rabbit per female rabbit. Mating trial occurred over 5 consecutive days.
On the day of cohabitation, each female rabbit was placed in the cage of the assigned untreated male rabbit and monitored continuously until mating was confirmed by observation to have occurred at least twice or until the Study Supervisor, in consultation with the Study Director, determined that the pair would probably not mate. Each female was given up to 3 attempts to mate. The day that mating was confirmed was designated GD 0.

Delivery and Maternal Observations:
Female rabbits were evaluated for the following natural delivery observations: clinical signs observed, duration of gestation (GD 0 to the day the first kit was observed), litter size (defined as the number of kits present on day 4 and any removed kits), and kit viability on PND 4. The day that delivery occurred was designated as LD 1 for adult females and Postnatal Day (PND) 1 for F1 kits. Maternal behavior was recorded daily between LD 4 and 34. On PNDs 1 through 3, the litters were not disturbed to check for dead kits.

Embryo-Fetal Survival, Fetal Weight and Gravid Uterine Weight:
Surviving mated females were euthanized by an intravenous injection of a euthanasia solution (390 mg/mL pentobarbital sodium) on GD 29 (cesarean section cohort) or LD 35 (natural delivery cohort), and a gross necropsy of the thoracic, abdominal (stomachs rinsed with saline), pelvic viscera and injection sites was performed. The reproductive tract was dissected from the abdominal cavity.

For the natural delivery cohort, the number of implantation scars (former implantation sites before delivery) was recorded.

For the Cesarean-sectioning cohort, the gravid uterus (with evidence of at least apparently one live fetus) was weighed. The ovaries and uterus of pregnant females were examined for number and distribution of corpora lutea, implantation sites, placentae (size, color, or shape), early and late resorptions, and live and dead fetuses. Placentae were removed from the uterine wall so that all surfaces could be examined. Live fetuses were weighed individually and euthanized prior to performing visceral examinations or fresh eye and brain examinations.

Fetal Examinations – Rabbits Assigned to Cesarean-Sectioning:
To minimize bias, all fetal evaluations (including fetal weights, and external, visceral and skeletal examinations) were conducted without knowledge of dose group or maternal identification. Live fetuses were examined for external abnormalities with a dissecting microscope. Dead fetuses and any late resorptions were examined to the extent possible and then discarded.

Abnormalities were classified as malformations, variations, or incidental findings. Malformations are defined as observations that are judged to potentially affect survival, growth, development, functional competence or external appearance. All other fetal observations (identified in the report as variations or incidental findings), represent retardations in development, transitory alterations or permanent alterations not believed to adversely affect survival, growth, development, function, longevity, or external appearance. In the study report, fetal malformations are discussed when they were found solely in the control group, when they occurred in a test article group at a higher incidence than the control group, or when considered related to the test article. Fetal variations are discussed when considered related to the test article.

Organ Weights:

The ovaries were weighed at necropsy for all female rabbits, including all nonpregnant rabbits, or rabbits euthanized before scheduled termination. Paired organs (ovaries) were weighed together. The gravid uterus (with at least one apparently live fetus) was weighed for all females assigned to cesarean sectioning at scheduled termination. Organ weights were not recorded for rabbits found dead.

Measurements and Observations – F_1 Generation – Natural Delivery Cohort

Clinical Observations:
On LD 1 through 3, viability was conducted for each litter by cageside observation twice daily (litters were not disturbed). From LD 4 through LD 35, kits were observed for general health/mortality and moribundity at least twice daily, but were not removed from cage during observation, unless necessary for identification or confirmation of possible findings. The kits in each litter were counted once daily beginning on PND 4.

Body Weight: Kits were individually weighed on PNDs 4, 7, 11, 14, 17, 21, 28, and 35.

Developmental Landmarks:

Parameters	Day Initiated
Hair Growth	PND 5
Eye Opening	PND 9
Air Righting Reflex	PND 10
Acoustic (Auditory) Startle[a]	PND 14
Pupil Constriction	Once on PND 22

a. The auditory startle stimulus was produced using a clipboard.

Table 2: *Evaluation of developmental endpoints in kits*

The number of kits meeting the criterion was recorded on each day of testing. Testing continued until the day the criterion was attained by all kits in the litter.

Unscheduled Deaths and Early Euthanasia:

One kit that died on Postnatal day 1 was evaluated for vital status at birth via lung floatation test (the lungs were removed and immersed in water). Kits with lungs that sank were identified as stillborn; kits with lungs that floated were identified as liveborn and to have died shortly after birth. The kit was examined for external and visceral abnormalities the extent possible; sexing and observation of milk in stomach were not possible due to cannibalization. A single cross-section was made between the parietal and frontal bones, and the brain was examined *in situ* for hydrocephaly; the remainder of the carcass was discarded. For kits that were found dead or were euthanized on or after postnatal day 4, a necropsy of the thoracic, abdominal, and pelvic viscera was performed (stomachs rinsed with saline and internal structures examined) to determine the cause of death or condition on the day the observation was made. The kits were sexed and evaluated for gross lesions; findings of absence of milk in stomach were recorded.

Kits were examined externally and for visceral defects. A single cross-section was made between the parietal and frontal bones, and the brain was examined *in situ* for hydrocephaly. External or visceral abnormalities were classified as malformations or variations. Following completion of visceral examinations, the entire kit was discarded, except that malformed viscera or gross lesions were preserved in 10% NBF, when possible.

Scheduled Euthanasia, Necropsy, and Organ Weights:
All fetuses/kits, including those selected for blood collection, and any unscheduled euthanasia, were euthanized by an intravenous or intraperitoneal injection (where appropriate) of 390 mg/mL pentobarbital sodium.
On PND 35, kits were euthanized, and blood was collected. Following blood collection, kits were sexed, and a necropsy of the thoracic, abdominal and pelvic viscera was performed, with gender determined. Stomachs were rinsed with saline and internal structures examined. The brain was weighed and retained for all kits at scheduled euthanasia, and kits that were found dead or euthanized before scheduled termination.

Immunogenicity Analysis:
Blood was collected for (b) (4) to measure the (b) (4) . This review will not discuss the (b) (4) analyses of the (b) (4) vaccine since in the review we only review the $AS01_B$ treatment group in comparison to the control group. No immunogenicity analysis was performed for animals receiving $AS01_B$.

Analysis of Data:
Descriptive statistics including number, mean, and/or standard deviation were reported as applicable. Clinical and necropsy observations data were summarized, but no inferential statistical analysis was performed. Female body weights and food consumption data recorded during the predose period were not tabulated, summarized, or analyzed statistically. All statistical tests were conducted at the 5% significance level. All pairwise comparisons were conducted using two sided tests and are reported at the 1% and 5% levels.

Variables for Inferential Analysis	Statistical Method		
	Parametric/Non-parametric	Non-parametric	Incidence
Body Weight	X	-	-
Body Weight Gains	X	-	-
Gravid Uterine Weight	X	-	-
Food Consumption	X	-	-
Fetal/Kit Body Weights[a,b]	X	-	-
Corpora Lutea Count	-	X	-
Number of Implants	-	X	-
Live Fetuses	-	X	-
Dead Fetuses	-	X	-
Number of Early Resorptions	-	X	-
Number of Late Resorptions	-	X	-
Sum of Resorptions	-	X	-
Fetal Percentage by Litter with Gross External/Visceral/Skeletal Abnormalities[c]	-	X	-
Mean Percent Affected Fetuses per Litter[d]	-	X	-
Incidences of Litters with Gross External/Visceral/Skeletal Abnormalities	-	-	X
Sex Ratio (% males per litter)[b]	-	X	-
Pre Implantation Losses[b]	-	X	-
Post Implantation Losses[b]	-	X	-

X = Statistical test performed; - = Not applicable

a Presented for males, females and sexes combined; live fetuses only.

b Calculated based on litter means/values.

c Calculated based on litter values by finding or classification, as appropriate.

d Calculated based on affected fetuses/litter.

Table 3: *Statistical analyses (table provided by the sponsor)*

The following pairwise comparisons were made:
Group 2 vs. Group 1
Group 3 vs. Group 1

Parametric/Non-parametric: Levene's test was used to assess the homogeneity of group variances. The groups were compared using an overall one-way ANOVA F test if Levene's test was not significant or the Kruskal-Wallis test if it was significant. If the overall F-test or Kruskal-Wallis test was found to be significant, then pairwise comparisons were conducted using Dunnett's or Dunn's test, respectively.
Non-parametric: The groups were compared using an overall Kruskal-Wallis test. If the overall Kruskal-Wallis test was found to be significant, then the above pairwise comparisons were conducted using Dunn's test. Datasets with two groups were compared using a two-sided t-test.
Incidence: A two-sided Fisher's exact test was used to conduct pairwise group comparisons of interest.

Results:

F0 Generation Dams:

Mortality:

Three rabbits in the AS01B group were found dead on GD 31, LD 28, and LD 30. In-life and postmortem observations for these rabbits are detailed below. All other rabbits survived to scheduled euthanasia.

Rabbit 9797 in the AS01B group was found dead during the morning viability checks on GD 31 (7 days after the 6th injection). This rabbit had no adverse clinical signs. Body weight and food consumption data were unremarkable. No abnormalities were detected at necropsy examination. This rabbit was pregnant. The average duration of gestation for this group was 32.2 days.

Rabbit 9837 in the AS01B group was found dead on LD 28 (21 days after the 7th and last injection). This rabbit had no adverse clinical signs. This rabbit lost 0.14 kg (4.0%) of body weight from LD 17 to 24 (vs. controls, which gained a mean of 0.02 kg). Individual cage food consumption (maternal animal plus offspring) on the 3 days prior to death (GD 24-25, 25-26 and 26-27) was 229, 126 and 145g, respectively, while the mean cage food consumption for the controls ranged from 303 to 505g for the same time periods. No abnormalities were detected in this rabbit at necropsy. The litter for this rabbit consisted of 7 kits.

Rabbit 9839 in the AS01B group was found dead on LD 30 (23 days after the 7th and last injection). This rabbit had no clinical signs. This rabbit lost 0.35 kg (10.0%) of body weight from LD 20 to 29. This rabbit lost more body weight than any individual control rabbit during the same time period. The individual cage food consumption (maternal animal plus offspring) on GD 27-28, 28-29 and 29-30 was 126, 143 and 124g, respectively while the individual cage values for control animals ranged from 384 to 505g for the same time periods. No abnormalities were detected in this rabbit at necropsy examination. The litter for this rabbit consisted of 7 kits.

The cause of death was not apparent for the 3 found dead animals. The submitted historical control data (HCD) list that 11 out of 1376 pregnant (b) (4) rabbits were found dead (this corresponds to around 1 out of 125 animals) while in the AS01B group 3 out of 48 animals were found dead during this study; 1 out of 48 animals died during the gestation period (GD 31) and 2 animals died during the lactation period (LD 28 and 30). The sponsor did not include HCD for mortality of pregnant animals during the lactation phase at the time of the submission. An IR was sent asking for HCD regarding mortality of pregnant animals during the lactation phase. The test facility ((b) (4)) compiled HCD in the post-partum lactation period including data on (b) (4) rabbits from 7 different DART studies during the post-partum phase (the duration of the lactation phases from these 7 studies varied between 21, 29, and 35 days). In these studies, 1 out of 174 female animals was found dead during the lactation phase. Overall, the mortality rate of dams receiving AS01B is higher in this submitted DART study than reported in the HCD. Additionally, no animals were found dead in either the control group or the (b) (4) vaccinated group.

Clinical Observations: There were no treatment-related clinical observations noted.

Body Weight and Body Weight Changes: There were no treatment-related effects on mean body weights or mean body weight gains during the premating, gestation, or lactation periods**.**

Food Consumption: There were no treatment-related effects on mean food consumption during the premating, gestation, or lactation periods. In the 2 rabbits that were found dead at the end of the lactation period, there were body weight losses and food consumption decreases within the week of being found dead. HCD collected during the prenatal and embryofetal development stages (HCD for the post-partum lactation period is not available) show that incidental maternal toxicity has occurred at a range of 0- 2 does/group.

Reproductive Performance: Natural delivery and Cesarean birth group

Parameter		F0 generation	
		Control	$AS01_B$
Female rabbits paired	N	48	48
Rabbits that mated	N (%)	44 (91.7)	46 (95.8)
Female Fertility Index (both groups)	N/N (%)	39/44 (88.6)	45/46 (97.8)
Rabbits pregnant/ rabbits paired	N/N (%)	39/48 (81.2)	45/48 (93.8)

Table 4: *Summary of mating and fertility: F0 generation female rabbits, N/N (Number of pregnancies/number of rabbits that mated)*

Reproductive Performance: There were no treatment-related effects on the number of rabbits mated (91.7% to 95.8%), fertility index (88.6% to 97.8%), or number of rabbits pregnant/number of rabbits paired (81.2% to 93.8%), see table 4.

Natural Delivery and Litter Observations:

Parameter		F0 generation	
		Control	$AS01_B$
Rabbits tested	N	24	24
Pregnant	N (%)	17 (70.8)	22 (91.7)
Delivered litters	N (%)	17 (100.0)	21 (95.4)[a]
Delivered litters with one or more liveborn kits	N	17	20
Duration of gestation	MEAN±S.D.	32.7±0.7	32.2±0.5
Implantation sites	N	144	174 (
Implantation sites per delivered litter	MEAN±S.D	8.5±2.1	8.3±2.8
Does with stillborn kits	N (%)	0 (0.0)	1 (4.8)
Does with no liveborn kids	N (%)	0.0±0	1 (4.8)
Gestation index	(%)	100.0	95.4
Gestation index	N/N	17/17	21/22
Does with all kits dying da 1-4 postpartum	N (%)	0 (0.0)	0 (0.0)
Does with all kits dying da 5-35postpartum	N (%)	1 (5.9)	0 (0.0)
Kits delivered	N	138	150
Kits delivered	MEAN±S.D	8.1±2.0	7.5±1.7
Live born	N (%)	126 (91.3)	144 (96.0)

Live born	MEAN±S.D	7.4±2.4	7.2±1.7
Still born	N (%)	0.0±0	0.0±0
Still born	MEAN±S.D	0.0±0	1 (0.7)
Kits found dead or euthanized due to adverse clinical observations			
Days 4-7	N/N (%)	6/126 (4.8)	7/144 (4.9)
Days 8-11	N/N (%)	7/120 (5.8)	4/137 (2.9)
Days 12-14	N/N (%)	0/113 (0.0)	0/133 (0.0)
Days 15-17	N/N (%)	0/113 (0.0)	1/133 (0.8)
Days 18-21	N/N (%)	1/113 (0.9)	0/132 (0.0)
Days 22-28	N/N (%)	1/112 (0.9)	0/132 (0.0)[b]
Days 29-35	N/N (%)	1/111 (0.0)	3/119 (2.5)[c]
Delivered litters with one of more liveborn kits	N	17	20
Day 4[d]	MEAN±S.D	7.4 ± 2.4	7.2 ± 1.7
Day 7	MEAN±S.D	7.0 ± 2.4	6.8 ± 1.6
Day 11	MEAN±S.D	6.6 ± 2.1	6.6 ± 1.6
Day 14	MEAN±S.D	6.6 ± 2.1	6.6 ± 1.6
Day 17	MEAN±S.D	6.6 ± 2.1	6.6 ± 1.6
Day 21	MEAN±S.D	6.6 ± 2.1	6.6 ± 1.6
Day 28	MEAN±S.D	6.5 ± 2.2	6.6 ± 1.6
Day 35	MEAN±S.D	6.5 ± 2.2	6.4 ± 1.6
Percent of male kits (day 4)	MEAN±S.D	52.6 ± 23.3	61.7 ± 17.7
Kit weight/litter			
Day 4[d]	g	80.3 ± 18.8	86.7 ± 14.7
Day 7	g	113.8 ± 22.6	119.2 ± 20.8
Day 11	g	160.6 ± 30.2	166.9 ± 27.8
Day 14	g	210.1 ± 35.4	212.4 ± 38.4
Day 17	g	246.5 ± 42.1	255.4 ± 48.1
Day 21	g	295.5 ± 51.1	308.9 ± 62.1
Day 28	g	502.3 ± 74.2	500.2 ± 97.1
Day 35	g	743.0 ±100.6	749.4 ±143.0

Table 5: *Summary of natural delivery observation: F0 generation female rabbits;*p<0.05; **p<0.01; N/N (Number of pregnancies/number of rabbits that mated);* [a)] *excluding rabbit 9797 which was found dead on GD 31;* [b)] *excludes 7 kits in litter 9837 that were euthanized on Day 28 postpartum due to death of doe;* [c)] *excludes 6 kits in litter 9839 that were euthanized on Day 30 postpartum due to death of doe.* [d)] *includes liveborn kits and kits that died before weighing on Day 4 postpartum*

There were no treatment-related effects on natural delivery or litter observations. Values for the numbers of does delivering litters, the duration of gestation, averages for implantation sites per delivered litter, does with stillborn and liveborn kits, the gestation index (number of does with one or more liveborn kits/number of pregnant rabbits), dams with all kits dying, kits found dead or presumed cannibalized, viability and lactation indices, surviving kits per litter, percentage of male kits per litter, live litter size at weighing, and kit weights per litter were all comparable among the three groups. All differences in these parameters, including those of statistical significance, were considered unrelated to the test article or adjuvant because the magnitude of the difference was minor.

There was a slight statistically significant decrease in gestation length for the AS01B group which was not considered treatment related as the difference was minor (32.2 days vs 32.7 days in controls). There was a slight increase in the total no. of liveborn kits that was statistically significant in the (b) (4) group (145, 98.6% vs 126, 91.3% in controls) that was not

considered test article-related because the magnitude of the difference is minor and shows an increase in liveborn.

Parameter: clinical observations F1		F0 generation	
		Control	$AS01_B$
Litters examined	N	17	20
Dehydration, total	N/N	51/6	31/6
mild	N/N	22/5	21/4
moderate	N/N	30/4	6/2
severe	N/N	1/1	4/2
Think body condition	N/N	57/7	22/6
Cold to touch	N/N	4/3	7/4
Decreased motor activity	N/N	2/2	2/2
Head and/or back scab	N/N	6/1	13/1
No use of both hindlimbs	N/N	0/0	1/1
No righting reflex	N/N	0/0	1/1
Head tilt to the right and/or left	N/N	1/1	0/0
Dyspnea	N/N	1/1	0/0
Low carriage	N/N	1/1	0/0

Table 6: *Summary of natural delivery clinical observation of kits;*p<0.05; **p<0.01; N/N (total frequency (days kits) / litter with observations*

There were no treatment-related clinical signs noted in the F1 generation

Parameter: developmental landmarks F1		F0 generation	
		Control	$AS01_B$
Litters examined	N	17	20
Hair growth: day 5	MEAN±S.D	100.0 ± 0.0	100.0 ± 0.0
Eye opening: day 9	MEAN±S.D	21.7 ± 31.2	5.8 ± 12.1
Eye opening: day 10	MEAN±S.D	67.2 ± 33.3	41.7 ± 36.3*
Eye opening: day 11	MEAN±S.D	92.7 ± 13.0	87.8 ± 20.2
Eye opening: day 12	MEAN±S.D	100.0 ± 0.0	100.0 ± 0.0
Air righting day 10	MEAN±S.D	24.8 ± 25.0	19.5 ± 23.4
Air righting day 11	MEAN±S.D	16.8 ± 22.4	38.5 ± 33.2
Air righting day 12	MEAN±S.D	42.4 ± 37.5	46.2 ± 40.3
Air righting day 13	MEAN±S.D	52.4 ± 37.3	58.4 ± 36.4
Air righting day 14	MEAN±S.D	67.8 ± 26.3	72.1 ± 31.8
Air righting day 15	MEAN±S.D	82.2 ± 24.4	87.7 ± 25.7
Air righting day 16	MEAN±S.D	93.3 ± 16.2	98.2 ± 5.8
Air righting day 17	MEAN±S.D	98.6 ± 5.6	98.9 ± 5.0
Air righting day 18	MEAN±S.D	99.3 ± 2.8	99.4 ± 2.5
Air righting day 19	MEAN±S.D	100.0 ± 0.0	100.0 ± 0.0
Acoustic startle day 14:	MEAN±S.D	83.3 ± 21.0	94.2 ± 17.6
Acoustic startle day 15:	MEAN±S.D	100.0 ± 0.0	100.0 ± 0.0
Pupil constriction day 22:	MEAN±S.D	100.0 ± 0.0	100.0 ± 0.0

Table 7: *Summary of natural delivery clinical observation of kits; *p<0.05; **p<0.01;*

There were no significant differences in the mean number of litters achieving criterion for hair

growth, air righting, acoustic (auditory) startle, or pupil constriction among between the control group and the animals receiving AS01B.

There was a statistical difference in the eye-opening don day 10 as well a difference on day 9; by day 12 all animals in both dose groups have their eyes opened.

Necropsy Observations
There were no treatment-related necropsy observations in the rabbits at the end of the gestation or lactation periods

Embryo-Fetal Survival, Fetal Weight, and Gravid Uterine Weight in the Cesarean section group

Maternal performance – Cesarean section		F0 generation	
		Control	AS01B
Number of females	N	24	24
Number of female pregnant	N	22	23
	%	91.7	95.8
Females with live fetuses	N	22	23
	%	100.0	100.0
Female with all nonviable	N	0	0
	%	0.0	0.0
Female with resorption	N	4	7
	%	18.2	30.4
Females euthanized preterminal	N	0	0
	%	0.0	0.0
Found dead	N	0	0
	%	0.0	0.0
Unscheduled euthanasia	N	0	0
	%	0.0	0.0
Number of corpora lutea	Mean	10.2	9.2
	SD	2.4	2.5
Number of implantations	Mean	8.5	8.1
	SD	1.4	1.4
Pre-implantation loss	Mean	14.54	9.73
	SD	13.76	12.44
Post-implantation loss	Mean	2.02	4.23
	SD	4.47	6.71
Total number or resorptions	Mean	0.2	0.3
	SD	0.4	0.6
Number of early resorptions	Mean	0.2	0.2
	SD	0.4	0.4
Number of late resorptions	Mean	0.2	0.1
	SD	0.0	0.3
Total number of fetuses	Mean	8.3	7.7
	SD	1.3	1.7
Number of live fetuses	Mean	8.3	7.7
	SD	1.3	1.7
Live male fetus/litter (%)	Mean	46.44	48.52

	SD	15.62	18.47
Number of dead fetuses	Mean	0.0	0.0
	SD	0.0	0.0
Mean fetal weight (g)	Mean	42.98	43.72
	SD	3.36	3.99
Gravid uterus weight (g)	Mean	520.46	497.02
	SD	3.71	4.27

Table 8: *Summary of maternal performance and mortality – Cesarean section; *p<0.05; **p<0.01;*

There was no treatment-related effect on numbers of corpora lutea, implantations, resorptions, live and dead fetuses per litter, sex ratio, fetal body weight, gravid uterine weight or placental morphology.
The mean number of live fetuses/litter was slightly lower in the AS01B group (7.7) vs controls (8.3) but was not considered treatment-related as it was not statistically significant and within historical control (mean 8.8, range 7.0-10.1).

The post-implantation loss was slightly higher in the AS01B group (4.23) vs controls (2.02) but was not considered treatment-related as neither were statistically significant, nor did this higher post-implantation loss translate to lower numbers of live fetuses/litter and both were within historical control ranges for this laboratory, mean of 3.5, range of 0.8-22.9.

Thirty percent of females treated with AS01B showed resorptions while only 18% of females in the control group showed resorption. However, this value is within the reported historical control data (5.0% to 55.0%).

Parameter: developmental landmarks F1		**F0 generation**	
		Control	**AS01B**
Number of fetuses examined	N	183	178
Number of litters examined	N	22	23
Summary of fetal abnormalities by classification: gestation – Cesarean section: external			
Incidental	N	0	1
Malformation	N	0	1
Summary of fetal abnormalities by classification: gestation – Cesarean section: fresh visceral			
Variation	N	3	4
Malformation	N	3	2
Summary of fetal abnormalities by classification: gestation – Cesarean section: skeletal			
Variation	N	135	123
Malformation	N	4	1
Summary of fetal abnormalities by classification: gestation – Cesarean section: external			
Head, Domed - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Limb: Hindlimb, Malrotated - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Mouth: Tongue, Protruding - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Trunk: Abdomen, Distended - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Adrenal gland, Large - Malformation	Fetuses N(%)	3(1.52)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)

Brain: Misshapen - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Eye, Small - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Gallbladder, Small - Variation	Fetuses N(%)	1(0.45)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)
Abdomen, Fluid filled - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Liver: Lobe, Misshapen - Variation	Fetuses N(%)	0(0.00)	1(0.48)
	Litters N(%)	0(0.0)	1(4.3)
Lung: Lobe, Absent - Variation	Fetuses N(%)	2(1.22)	2(1.27)
	Litters N(%)	2(9.1)	2(8.7)
Lung: Lung, Small - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Clavicle, Misshapen - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Forelimb: Forepaw phalanges, Unossified - Variation	Fetuses N(%)	12(6.57)	6(3.29)
	Litters N(%)	6)27.3)	6(26.1)
Forelimb: Humerus, Bent - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Forelimb: Radius, Bent - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Forelimb: Ulna, Bent - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Hindlimb: Femur, Bent - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Hindlimb: Fibula, Bent - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Hindlimb: Fibula, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Hindlimb: Hindpaw phalanges, Unossified - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Hindlimb: Metatarsal, Bent - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Hindlimb: Tibia, Bent - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Pelvic girdle: Ilium, Misshapen - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Pelvic girdle: Pubis, Incomplete ossification - Variation	Fetuses N(%)	2(1.07)	0(0.00)
	Litters N(%)	2(9.1)	0(0.0)
Rib: Rib, Branched - Malformation	Fetuses N(%)	1(0.45)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)
Rib: Rib, Nodule - Variation	Fetuses N(%)	1(0.45)	2(1.35)
	Litters N(%)	1(4.5)	2(8.7)
Rib: Rib, Wavy rib - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Rib: Rib, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Scapula: Misshapen - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Skull: Frontal, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Skull: Frontal, Misshapen - Malformation	Fetuses N(%)	0(0.00)	1(0.72)

	Litters N(%)	0(0.0)	1(4.3)
Skull: Hyoid ala, Bent - Variation	Fetuses N(%)	5(2.60)	4(2.37)
	Litters N(%)	5(22.7)	4(17.4)
Skull: Hyoid ala, Malpositioned - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Fetuses N(%)	0(0.0)	1(4.3)
Skull: Hyoid body, Incomplete ossification - Variation	Litters N(%)	1(0.45)	0(0.00)
	Fetuses N(%)	1(4.5)	0(0.0)
Skull: Interparietal, Incomplete ossification - Variation	Litters N(%)	0(0.00)	1(0.72)
	Fetuses N(%)	0(0.0)	1(4.3)
Skull: Interparietal, Unossified - Variation	Litters N(%)	0(0.00)	1(0.72)
	Fetuses N(%)	0(0.0)	1(4.3)
Skull: Mandible, Incomplete ossification - Variation	Litters N(%)	0(0.00)	1(0.72)
	Fetuses N(%)	0(0.0)	1(4.3)
Skull: Mandible, Misshapen - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Skull: Maxilla, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	2(1.27)
	Litters N(%)	0(0.0)	2(8.7)
Skull: Nasal, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Skull: Parietal, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Skull: Premaxilla, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Skull: Squamosal, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Skull: Supraoccipital, Misshapen - Malformation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Skull: Suture bone, Supernumerary site - Variation	Fetuses N(%)	1(0.45)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)
Skull: Tympanic annulus, Unossified - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Skull: Zygomatic arch, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	2(1.45)
	Litters N(%)	0(0.0)	1(4.3)
Sternebra, Fused - Variation	Fetuses N(%)	4(2.12)	4(1.93)
	Litters N(%)	3(13.6)	2(8.7)
Sternebra, Misshapen - Variation	Fetuses N(%)	1(0.45)	2(1.45)
	Litters N(%)	1(4.5)	1(4.3)
Sternebra, Unossified - Variation	Fetuses N(%)	11(5.60)	2(1.10)
	Litters N(%)	6(27.3)	2(8.7)
Sternebra, Incomplete ossification - Variation	Fetuses N(%)	7(3.82)	3(2.03)
	Litters N(%)	6(27.3)	3(13.0)
Sternebra, Isolated ossification site - Variation	Fetuses N(%)	1(0.45)	2(1.10)
	Litters N(%)	1(4.5)	2(8.7)
Supernumerary rib: Cervical, Full - Variation	Fetuses N(%)	0(0.00)	0(0.00)
	Litters N(%)	0(0.0)	0(0.0)
Supernumerary rib: Cervical, Short - Variation	Fetuses N(%)	1(0.45)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)
Supernumerary rib: Thoracolumbar, Full - Variation	Fetuses N(%)	85(46.86)	81(44.64)
	Litters N(%)	19(86.4)	21(91.3)
Supernumerary rib: Thoracolumbar, Short - Variation	Fetuses N(%)	49(27.74)	50(28.50)
	Litters N(%)	19(86.4)	21(91.3)
Vertebra: Caudal vertebra, Misaligned - Variation	Fetuses N(%)	2(0.96)	1(0.72)
	Litters N(%)	2(9.1)	1(4.3)

Vertebra: Caudal vertebra, Unossified - Variation	Fetuses N(%)	1(0.45)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)
Vertebra: Caudal vertebra, Incomplete ossification - Variation	Fetuses N(%)	3(1.41)	2(1.45)
	Litters N(%)	3(13.6)	2(8.7)
Vertebra: Cervical arch, Misshapen - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Vertebra: Cervical arch, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Vertebra: Cervical centrum, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)
	Litters N(%)	0(0.0)	0(0.0)
Vertebra: Cervical centrum, Isolated ossification site - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Vertebra: Lumbar arch, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Vertebra: Lumbar vertebra, Absent - Malformation	Fetuses N(%)	0(0.00)	0(0.00)
	Litters N(%)	0(0.0)	0(0.0)
Vertebra: Lumbar vertebra, Supernumerary - Malformation	Fetuses N(%)	2(1.01)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)
Vertebra: Sacral arch, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	1(4.3)
Vertebra: Thoracic arch, Fused - Malformation	Fetuses N(%)	1(0.45)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)
Vertebra: Thoracic arch, Incomplete ossification - Variation	Fetuses N(%)	1(0.45)	1(0.72)
	Litters N(%)	1(4.5)	1(4.3)
Vertebra: Thoracic centrum, Fused - Malformation	Fetuses N(%)	2(1.01)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)
Vertebra: Thoracic centrum, Incomplete ossification - Variation	Fetuses N(%)	1(0.45)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)

Table 9: *Summary of fetal abnormalities by finding: gestation – Cesarean section; *p<0.05; **p<0.01*

In the AS01B group, one fetus had a small eye and one multiply malformed fetus (domed head, hindlimbs malrotated, protruding tongue, distended abdomen, brain misshapen, small lungs, bent long bones (humerus/radius/ulna/femur/ tibia/fibula/metatarsal, and frontal/mandible/supraoccipital misshapen) was noted in a different litter.

In controls, there were 3 fetuses in 1 litter with large adrenal glands (also 2 other fetuses in this same litter had supernumerary lumbar vertebrae), 2 fetuses in 1 litter with fused thoracic centrum (one of these fetuses also had a branched rib, the other fetus had a fused thoracic arch).

All malformations and variations that were observed were considered unrelated to (b) (4) and AS01B because: 1) the abnormality was limited to a single fetus; 2) the abnormality occurred at a similar incidence in the control group; and/or 3) the litter and/or fetal incidence was within the range of the historical control data for the Testing Facility

Test Material	Dam Number	Fetus Number	Malformation(s)
Control article	9726	3	Branched Ribs; Fused Thoracic Centra
		8	Fused Thoracic Arches; Fused Thoracic Centra
	9747	1	Large Adrenal Glands
		3	Supernumerary Lumbar Vertebrae

		4	Large Adrenal Glands
		7	Large Adrenal Glands
		8	Supernumerary Lumbar Vertebrae
AS01$_B$	9822	6	Small Eye
	9841	5	Domed Head; Hindlimbs Malrotated; Protruding Tongue; Distended Abdomen; Misshapen Brain, Frontals, Mandibles, Supraoccipital; Small Lung; Bent Long Bones (Humeri, Radii, Ulnae, Femurs; Fibulas, Tibias); Bent Metatarsal

Table 10: *Summary of fetal malformation (detailed description) – Cesarean section; *p<0.05; **p<0.01;*

Serology:

Animals receiving the AS01$_B$ adjuvant did not receive a specific antigen during the test article administration. Therefore, no antigen specific immune response could be evaluated in the dams or kits. The immune response to the (b) (4) was evaluated but will not be discussed in this review.

Assessment:

(b) (4) rabbits received saline, (b) (4), or AS01$_B$ formulation (0.5 mL/injection, full human dose) by intramuscular injection 28 days prior to mating, 14 days prior to mating as well as on GD 3, 11, 16, and 24, and after natural delivery on LD 7. (b) (4) is not relevant for the assessment of effects of gE/AS01$_B$ or AS01$_B$; however, the group can serve as control and results can be informative for the overall interpretation of the study. Mated females and their litters were euthanized on GD 29 (cesarean section cohort) or on LD 35 (natural delivery cohort).

Three (b) (4) rabbits in the AS01$_B$ group were found dead; one animal died during the gestation period (GD 31) and 2 animals died during the lactation period (LD 28 and 30) and showed reduced food consumption with body weight loss. The cause of death could not be determined. The submitted HCD (from 68 full DART studies) list that 11 out of 1376 pregnant (b) (4) rabbits were found dead (this corresponds to around 1 out of 125 animals) while in this current study 3 out of 48 animals were found dead; 1 out of 48 animals died during the gestation period (GD 31) and 2 animals died during the lactation period (LD 28 and 30). The sponsor did not include HCD for mortality of pregnant animals during the lactation phase at the time of the submission. An IR was sent asking for HCD regarding mortality of pregnant animals during the lactation phase. The test facility ((b) (4)) compiled HCD in the post-partum lactation period including data on (b) (4) rabbits from 7 different DART studies during the post-partum phase (the duration of the lactation phases from these 7 studies varied between 21, 29, and 35 days). In these studies, 1 out of 174 female animals was found dead during the lactation phase. Overall, the mortality rate of dams receiving AS01$_B$ is higher in this submitted DART study than reported in the HCD. Additionally, no animals were found dead in either the control group or the (b) (4) vaccinated group.

At the moment the sponsor does not have data available with a full human dose of AS01B in rabbits for the Shingrix vaccine or any other AS01B vaccine under development. In order to clarify if the decreased food consumption together with body weight loss and death were incidental or were connected to the AS01B administration in (b) (4) rabbits, a follow up DART study using the full human dose of Shingrix could be helpful. However, since the sponsor will perform a clinical study to monitor and evaluate pregnancy exposures and outcomes as a post-marketing commitment, which will address concerns of a potential negative effect of the vaccine on pregnant women; a follow up DART study was not deemed necessary.

In the literature, it is reported that female rabbits in the last week of pregnancy and the first week of lactation can have higher mortality rates than nonpregnant females [Rosell, 2016]; higher mortality during the later postpartum phase has not been described. Mortality in conjunction with reduced food intake and indigestion has been reported for rabbits [Harcourt-Brown, 2002; McInnes, 2012].

There were no (b) (4) or AS01B related clinical signs, dermal scoring of injectionnsites, or effects on body weights, body weight changes, or food consumption in the F0 generation.

There were no (b) (4) or AS01B-related effects on maternal necropsy observations, maternal organ weights, embryo-fetal survival, or fetal weight. There were no (b) (4) or AS01B-related fetal external, visceral, or skeletal malformations or variations.

There were no (b) (4) or AS01B-related effects on natural delivery and litter observations, kit weights, kit clinical observations, reflex and physical development, necropsy observations, or brain weights.

There were no significant differences in the mean number of litters achieving criterion for hair growth, air righting, acoustic (auditory) startle, or pupil constriction among between the control group and the animals receiving AS01B. There was a statistical difference in the eye-opening don day 10 as well a difference on day 9; by day 12 all animals in both dose groups have their eyes opened.

Conclusions:

In conclusion, saline control, or AS01B adjuvant at 0.5 mL/injection was given by intramuscular injection to rabbits 28 days prior to mating, 14 days prior to mating as well as on GD 3, 11, 16, and 24, and after natural delivery on LD 7. There were no AS01B-related effects on clinical signs, dermal observations, or necropsy observations in the dams. There were no AS01B-related effects on mating and female fertility, embryo-fetal pre- and post-natal survival, growth, or development. No adverse effects on pre-weaning development up to post-natal day 35 were observed.

Three (b) (4) rabbits in the AS01B group were found dead; one animal died during the gestation period (GD 31) and 2 animals died during the lactation period (LD 28 and 30) and showed

reduced food consumption with body weight loss. The cause of death could not be determined. Since the sponsor will perform a clinical study to monitor and evaluate pregnancy exposures and outcomes as a post-marketing commitment, which will address concerns of a potential negative effect of the vaccine on pregnant women, a follow up DART study was not deemed necessary.

Additional information regarding DART study "WIL AB14898: DQ – Developmental Toxicity Study (Including Teratogenicity and Postnatal Investigations) by the Intramuscular Route in the Rabbit" which was submitted and reviewed under the original BLA submission.

Summary:

Study WIL AB14898 was submitted and reviewed with the original BLA submission. In study WIL AB14898, three groups of female (b) (4) rabbits were administered intramuscular injections of DQ adjuvant containing 20, 100 or 200 µg/mL of QS21, 28 and 14 days before the start of mating and on GD 3, 8, 11, 15 and 24, then on LD 7. A control group of female (b) (4) rabbits was administered sterile physiological saline (0.9 % NaCl). Animals were either assigned to a caesarean sub-group (all females were necropsied on day 29 *post-coitum)* or littering sub-group.

Intramuscular administrations of DQ adjuvant containing 200 µg/mL of QS-21 to (b) (4) rabbits starting 28 and 14 days before the start of mating and on gestation days 3, 8, 11, 15 and 24 and on day 7 of lactation induced a significant maternal mean body weight loss associated with reduced mean food consumption at the end of the gestation period. In addition, lower mean fetal weight was noted at this dose level. Defects of the aortic arch (retro or high arched) were observed in three fetuses from separate litters suggestive of a possible association with treatment at this dose. Doses of DQ adjuvant containing 100 or 20 µg/mL of QS-21 did not induce any adverse effects on maternal condition or embryo-fetal and post-natal development.

Examination of the live fetuses revealed 7 malformed fetuses from 6 different litters in the 200 µg/mL QS-21 group, 0 malformed fetuses in the 100 µg/mL QS-21 group, 2 malformed fetuses from separate litters in the 20 µg/mL QS-21 group, compared with 1 malformed fetus in the control group. In the 20 or 100 µg/mL QS-21 dose groups, neither the incidence nor type of the malformations suggested any association with treatment due to their diverse nature or since they are part of the background of morphological changes in the (b) (4) rabbit strain used in this study. In the 200 µg/mL QS-21 group, three fetuses from separate litters had defects of the aortic arch (retro or high arched) suggestive of a possible association with treatment at the highest dose.

In this supplement submission the sponsor submitted new background data supporting that the findings observed in fetuses of female rabbits administered QS-21 at a high dose (200 µg/animal) in DART study (AB14898) are background findings and not evidence of QS-21-mediated fetal malformations.

In order to consider a developmental toxicity signal as test-article related, a distinct embryologic abnormality needs to occur at a higher incidence than the background incidence and the abnormality needs to be consistent between fetuses. The malformations that occurred more than once in the high dose group were (i) malpositioned and/ or malformed kidneys, and (ii) “great vessels malformations”. The former findings were genetically linked to one male and the incidence of both findings fall within the reported historical control data as evidenced information from the Contract Research Organization which was submitted in this submission.

The three fetuses from separate litters showed major vessels defects (two with “aortic arch retroesophageal” and one with “narrowed and high arched aortic arch”) which were originally reported as suggestive of a possible association with treatment at the highest dose. Further review revealed that 2 of these 3 fetuses (from 2 independent litters) had malformations with the same description (“malformed great vessels: aortic arch retroesophageal etc”), whereas the 3rd case was associated with a primary kidney malformation and considered to be different from the other 2 cases. These 2 cases and other malformations were found to be a known part of the background malformations of the strain of rabbits. Defects of the aortic arch (retro or high arched) were observed in 2 fetuses from separate litters and were found to be within the background of this strain of rabbit based on historical background date of the Contract Research Organization and not suggestive of a possible association with treatment.

Concurrence: Martin D. Green