STN 125549

Toxicology Review of STN 125549 (*Neisseria meningitidis* Serogroup B Bivalent Recombinant Lipoprotein rLP2086 [subfamily A and B, E *coli*] Vaccine

BLA: 125549

Sponsor: Wyeth LLC, a subsidiary of Pfizer Inc., Worldwide Safety and Regulatory, Pearl River, NY 10965

Reviewer: Steven Kunder, Ph.D., DABT

Division: OVRR/DVRPA

Proposed use: Active immunization to prevent invasive meningococcal disease caused by *Neisseria meningitides* serogroup B in individuals aged 10 through 25 years. The vaccine is to be administered as a 3-dose series at months 0, 2, and 6.

TOXICOLOGY STUDIES:

WAY-263069 (rLP2086) and $AlPO_4$ (Cl136352): 5-cycle (1 dose/2 week cycle) Intramuscular Toxicity Study in Rabbits, (study report RPT-60511)

rLP2086 vaccine and $AlPO_4$ (cl136352) repeat 5 cycle (1 dose/2week cycle) intramuscular toxicity study in rabbits, (study no. RPT-74041)

The above repeat dose toxicology studies were reviewed under IND 13182 by Raymond Casteline.

REPRODUCTION TOXICOLOGY STUDIES
WAY-263069 (rLP2086) and $AlPO_4$ (CL-136352): A combined intramuscular fertility and developmental toxicity study in female rabbits (RPT-63113)

Meningococcal B vaccine (rlp2086): A combined intramuscular fertility and developmental toxicity study in female rabbits (RPT-75947)

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1 INTRODUCTION

The candidate bivalent rLP2086 vaccine (Trumenba), is a sterile liquid suspension composed of rLP2086 subfamily A and B proteins formulated at 120 mcg/mL/subfamily in 10 mM histidine buffer, pH 6.0, ----(b)(4)---- sodium chloride (NaCl) with 0.50 mg/mL aluminum as aluminum phosphate (AlPO4). Polysorbate 80 (PS-80) is added to the drug substance ----------(b)(4)-------. Therefore, PS-80 is not added during the drug product formulation but is present in the final drug product ------(b)(4)------. The drug product is filled into 1 mL syringes. A single dose of vaccine is 0.5 mL with no preservative.

Two repeat-dose toxicology studies and two reproductive toxicology studies were submitted to provide nonclinical support for this BLA.

Safety and immunogenicity/efficacy data from 7 Phase 1/2 clinical studies are presented by the sponsor BLA to support a positive benefit-risk profile of bivalent rLP2086 in individuals aged 10 through 25 years. The planned clinical dose is 120 µg in 500mL, administered intramuscularly at 0, 2 and 6 months.

2 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY STUDIES

2.1 Study title

WAY-263069 (rLP2086) and $AlPO_4$ (CL-136352): A combined intramuscular fertility and developmental toxicity study in female rabbits

2.1.1 Key study findings

The initial reproductive and developmental toxicology study was conducted to evaluate the effects of rLP2086 on fertility and development in (b)(4) rabbits. Three groups were used in this study: 2 control groups administered either 0.5 mL of saline or vehicle and a third group administered 0.5 mL of rLP2086 at a dose of 200µg. The vehicle in the control group and bivalent rLP2086 group was 0.25 mg/mL aluminum as $AlPO_4$, 5 mM succinate buffer, pH 6.0, 0.15 M NaCl, and 0.025% polysorbate 80. rLP2086 was administered IM to (b)(4) female rabbits (40/group) on Days 17 and 4 prior to mating (Days -17 and -4) and on GDs 10 and 24. Evaluations in all groups consisted of parental (F_0) generation mortality, clinical observations, injection site irritation, body weight, food consumption, gravid uterine weight, and hysterotomy findings on GD 29 (corpora lutea, litter size, embryo/fetal mortality); fetal sex, weight, and external, palatal, visceral, skeletal anomalies, and weights of the spleen and thymus; placental appearance; fecundity parameters (gestation index, gestation length), parturition, maternal care of offspring for females that delivered, postmortem observations, and first generation (F_1) litter size, mortality, clinical observations, sex distribution, body weight, and weights of the spleen and thymus. Serum samples for evaluating potential antibody titers to rLP2086 were also collected and evaluated from F_0 generation female rabbits and F_1 generation fetuses and pups.

IM administration of 200 µg vaccine to female rabbits 17 and 4 days prior to mating and on GDs 10 and 24 did not affect mating, fertility, or embryo/fetal viability, growth, or morphologic development. There was no adverse maternal toxicity in this study; findings in does included injection site irritation (transient, minimal severity) in the vehicle and 200 µg vaccine groups.

Study no.: **RPT-63113**
Conducting laboratory and location: ----------(b)(4)----------
for Wyeth Research Drug Safety, ---(b)(4)----

Date of study initiation: 23 December 2005
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Meningococcal b vaccine (rlp2086), 7-5105-001A

2.1.2 Methods

Doses: 200 µg in 500mL
Species/strain: ----------(b)(4)---------- rabbits
Number/sex/group: F0 females: 40 (half intended for natural delivery, half intended for caesarian delivery
Route, formulation, volume: intramuscular; 0.25 mg/mL aluminum as $AlPO_4$, 5 mM succinate buffer, pH 6.0, 0.15 M NaCl, and 0.025% polysorbate 80; 500mL

Study design:
Dosing period: days 17 and 4 prior to mating and GDs 10 and 24

Dosage Group	N[a]	µg rLP2086[b] in the Vaccine Concentration in µg/mL)	Dose Volume (mL)[c]
Saline Control[d]	40	0	0.5
Vehicle Control[e]	40	0	0.5
Vaccine[f]	40	200 (400)	0.5

$AlPO_4$ = Aluminum phosphate; N = Number; NaCl = Sodium chloride.
a. 20 females were assigned to caesarean-sectioning and 20 females were assigned to natural delivery.
b. rLP2086 (A and B subfamilies) is the protein component of the vaccine.
c. All doses were administered into the hind legs.
d.The saline control was 0.9% isotonic saline.
e. The vehicle control was 0.25 mg/mL aluminum as $AlPO_4$, 5 mM succinate buffer, pH 6.0, 0.15 M NaCl, and 0.025% polysorbate 80 (initial formulation).
f. The subfamily A and B proteins are in a ratio of 1:1 in drug product matrix (vehicle control).

Hysterotomy on GD 29

From sponsor submission

Parameters and endpoints evaluated:

F_0:
generation mortality, clinical observations, injection site irritation, body weight, food consumption, gravid uterine weight, hysterotomy findings on GD 29 (corpora lutea, litter size, embryo/fetal mortality)

F1:
fetal sex, weight and external, palatal, visceral, skeletal anomalies and weights of the spleen and thymus; placental appearance; fecundity parameters (gestation index, gestation length), parturition, maternal care of offspring for females that delivered, postmortem observations, and F_1 generation litter size, mortality, clinical observations, sex distribution, body weight, and weights of the spleen and thymus.

Serum samples for potential antibodies to rLP2086 were collected from F0 dams and F1 fetuses and pups

Statistical methods:
Averages and percentages were calculated. Adult data were evaluated with the individual rabbit as the unit measured. Litter averages were used for the evaluation of F_1 generation fetal weights, fetal organ weights, fetal antibody titers, fetal ossification sites, pup weights, pup organ weights, and pup antibody titers. Litter ratios were analyzed for F_1 generation fetal sex ratio, pup sex ratio, percentage of resorbed conceptuses, percent normal placentae and percentage of fetuses with alterations. Litter counts were analyzed for corpora lutea, implantation sites, live and dead fetuses, early and late resorptions, fetal alterations and delivered, liveborn and stillborn pups.

Proportional data (F_0 and F_1 generation clinical and necropsy observations; injection site irritation observations; numbers of does pregnant, with resorptions, with all conceptuses resorbed, and with viable fetuses; percent normal placentae; litters and fetuses with any alterations; litter and fetuses with each gross external, soft tissue and skeletal alteration; number of does that delivered a litter, with stillborn pups, with no liveborn pups, and with all pups dying; liveborn and stillborn pups; pups found dead or missing; mating, fertility, abortion, gestation, viability and lactation indices) were analyzed using the variance test for homogeneity of the binomial distribution.

Continuous data (F_0 generation body weights, body weight changes, food consumption, gravid uterine weight, F_0 generation antibody titers, F_1 generation fetal weights, fetal sex ratio, fetal organ weights, fetal antibody titers, percent resorbed conceptuses per litter, percent fetuses with any alteration per litter, ossification site averages, pup weights, pup sex ratio, pup organ weights, pup antibody titers) were analyzed using Bartlett's test of homogeneity of variances and the analysis of variance, when appropriate [ie, Bartlett's test was not significant ($p \leq 0.001$)]. If the analysis of variance was significant ($p \leq 0.05$), Dunnett's test was used to identify the statistical significance of the individual

groups. If the analysis of variance was not appropriate [ie, Bartlett's test was significant ($p \leq 0.001$)], the Kruskal-Wallis test was used, when less than or equal to 75% ties were present. In cases where the Kruskal-Wallis test was statistically significant ($p \leq 0.05$), Dunn's method of multiple comparisons[7] was used to identify the statistical significance of the individual groups.

Litter averages for fetal and pup body weights were also analyzed using an analysis of covariance with litter size as the covariable. If the analysis of covariance was significant ($p \leq 0.05$), t-tests comparing the adjusted means were used to identify the statistical significance of each individual group.
Count data (corpora lutea, implantation sites, live and dead fetuses, early and late resorptions, duration of gestation, number of delivered, liveborn and stillborn pups, surviving pups per litter live litter size at weighing, number of implantation sites) were evaluated using the procedures described above for the Kruskal-Wallis test.

2.1.3 Results

F0 Generation

Mortality/Clinical signs: There were no adjuvant or vaccine-related deaths. One F_0 generation doe given saline was found dead after delivery of a litter. No cause of death was apparent at necropsy. All other rabbits survived to scheduled sacrifice.

Body weight: Body weights and body weight gains were not affected by vaccine treatment. Gravid uterine weight was not affected by treatment.

Food consumption: Food consumption was unaffected by adjuvant or vaccine. A statistically significant ($p \leq 0.05$) reduction in the absolute feed consumption value on GDs 16 to 20 in does given the vaccine did not appear treatment related. All other food consumption was comparable between groups, and no statistically significant differences occurred.

Mating and fertility:
The number of rabbits that mated (80%) was reduced in the group given the vaccine compared to either control (93% each), but the decrease was considered unrelated to treatment because it was not statistically significant and was within the range of historical controls for the testing facility. Poor male mating performance was proposed by the sponsor as contributing to the reduced number of mated rabbits in the vaccine group, as there were fewer mounting attempts by males (significantly reduced at $p \leq 0.05$ in the vaccine group), and all females were administered chorionic gonadotropin on the day of mating to ensure ovulation. Although there were fewer mated rabbits in the vaccine group, there was no affect on fertility. Fertility rates in rabbits receiving saline, adjuvant, and vaccine were 92%, 100% and 92%, respectively.

Hysterotomy Findings

In the caesarean-section subgroups, pregnancy occurred in 15 (75.0%), 17 (85.0%) and 9 (45.0%) of the 20 does per group given saline, adjuvant, or vaccine respectively. These reductions in the pregnancy rate (statistically significant in the vaccine-treated group at $p \leq 0.01$ as compared to the adjuvant group) reflected that fact that all does with a confirmed mating were assigned to natural delivery and all does without a confirmed mating were assigned to caesarean-sectioning. The sponsor allocated does this way to maximize the number of pregnancies in the caesarian delivery groups delivering offspring. None of the caesarian-section or littering parameters was affected by treatment. Litter averages for corpora lutea, implantations, litter size, live/dead fetuses, early and late resorptions, fetal body weights and percentage resorbed conceptuses were not affected by treatment.

Fetal examinations
No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by the vaccine. There were no significant differences between groups in the litter or fetal incidences of any gross external, soft tissue or skeletal alterations. Fetal ossification averages were comparable among the groups.

In does given saline, adjuvant alone and vaccine with adjuvant, litters with fetuses had 9 (60.0%), 8 (47.0%) and 4 (44.4%) alterations, respectively. The numbers of fetuses with any alteration observed were 15 (14.4%), 11 (8.6%) and 6 (8.3%), and the percentages of fetuses with any alteration per litter were 16.5, 11.7 and 9.2 in these same respective dosage groups.

Fetal external and visceral examination
There were no vaccine-related gross external or visceral alterations.

Fetal skeletal examination
There were no treatment-related skeletal malformations. Variations occurred in all groups and are not of toxicological concern.

Fetal organ weights
Spleen and thymus weights were unaffected by vaccine treatment.

Natural Delivery Observations
Pregnancy occurred in 19 (95.0%), 20 (100%) and 20 (100%) of the groups (20/group) that were given saline, adjuvant or vaccine, respectively. One doe (animal 5105) given the vaccine did not deliver a litter, was sacrificed on GD 35 and had one fetus and one early resorption *in utero*. This single event appeared unrelated to the vaccine. One or more liveborn pups were delivered by every other pregnant doe assigned to natural delivery. There were 19, 20 and 19 litters delivered in each dosage group.

Natural delivery and litter observations were unaffected by administration of the vaccine with adjuvant. Values for the numbers of does delivering litters, the duration of gestation, averages for implantation sites per delivered litter, the gestation index (number of does with one or more liveborn pups/number of pregnant rabbits), the numbers of does with all pups dying, litter sizes, viability and lactation indices, surviving pups per litter, percent male pups per number of pups sexed per litter, live litter size at weighing and pup weight per litter were comparable between groups. One litter from a doe (animal 5092) receiving vaccine had all pups in the litter die by LD 4. No other adverse findings were associated with this doe, thus the cause of the litter loss does not appear to be vaccine related.

There was a small but non-statistically significant increase in stillborn pups in both vehicle and vaccine treated groups.

Dose group	**Stillborn**	**Total pups**	**%**
Saline control	0		0
Vehicle/adjuvant control	3	141	2.1
vaccine	5	143	3.5

The incidence of vehicle and adjuvant stillborn births is within the testing facility historical control range.

The number of pups surviving per litter at PPD 21 was 6.7, 6.0 and 6.0 following maternal administration of saline, adjuvant or vaccine, respectively. Overall pup survival does not appear affected by vaccine treatment.

Clinical observations
Natural delivery subgroups had no consistent clinical observation at delivery related to vaccine treatment.

Natural delivery pup organ weights
Spleen and thymus weights were unaffected by vaccine treatment.

Antibody Analysis
Anti-test article antibodies were not detectable in any predose samples from does from the saline control, vehicle control, or vaccine groups or in any GD 29 specimens from does or fetuses from the saline control or vehicle control groups. Anti-vaccine antibodies (anti-LP2086A and anti-LP2086B) were detected in all GD 29 specimens from does and fetuses from the vaccine group. These results confirm that the saline control and vehicle control group animals were not exposed to vaccine while does administered the rLP2086 vaccine with adjuvant had a serum antibody response to rLP2086 indicating vaccine exposure.

Summary: WAY-263069 (rLP2086) and $AlPO_4$(CL-136352): A Combined Intramuscular Fertility and Developmental Toxicity Study in Female Rabbits, Study Number: RPT-63113

Dose[a]	**0 (Saline Control)**	**Vehicle Control**[b]	**200 µg rLP2086 in PF-05212366**[c]
Sex	**F**	**F**	**F**
Number of Animals[d]	40	40	40
Treated F_0 Females			
Number Died or Euthanized Moribund	1[e]	0	0
Number of Females Mated (Confirmed)	37	37	32
Number of Pregnant Females	34	37	29
Fertility Rate (%)[f]	91.9	100	90.6
Noteworthy Findings			
Clinical Observations	-	-	-
Injection Site Reaction	-	-	-
Necropsy Observations	-	-	-
Body Weight	-	-	-
Body-Weight Gain	-	-	-
Food Consumption (Premating, Gestation, Lactation)	-	-	-
Gravid Uterine Weight	-	-	-
Cesarean Section Observations (GD 29)			
Number Evaluated	20	20	20
Number Pregnant Rabbits	15	17	9
Mean Number Corpora Lutea[g]	7.9	8.0	8.0
Mean Number Implantations[g]	7.6	7.8	8.0
Mean Number Early Resorptions[g]	0.2	0.0	0.0
Mean Number Late Resorptions[g]	0.5	0.1	0.0
Mean Litter Size[g]	6.9	7.6	8.0
Number Live Fetuses[g]	104	128	72
Embryo-Fetal Mortality	0	1	0
Mean Fetal Sex Ratio (% Males)	37.4	55.1*	51.2
Noteworthy Findings			
Fetal Weight	-	-	-
Fetal Morphology (External, Palatal, Visceral, or Skeletal)	-	-	-
Spleen Weights	-	-	-
Thymus Weights	-	-	-
Placental Appearance	-	-	-
Additional Examinations			
Antibody Analysis[j]			
F_0 Female Rabbits	Negative	Negative	Positive
F_1 Fetuses and Pups (M/F)	Negative	Negative	Positive

Table based upon sponsor submission

* p ≤0.05, significantly different from control based on appropriate trend or pairwise comparison. A full description of the statistical decision tree can be found in the final report for this study.

- = No noteworthy finding; $AlPO_4$ = Aluminum phosphate; F_0 = Maternal/paternal generation; F_1 = First generation; F = Female; GD = Gestation day; GLP = Good Laboratory Practice; IM = Intramuscular; M = Male; NaCl = Sodium chloride; (b)(4) = ----(b)(4)-------; PPD = Postpartum day; RPT = Report.

a. Dose volume was 0.5 mL/injection.
b. The vehicle control was 0.25 mg/mL aluminum as $AlPO_4$, 5 mM succinate buffer, pH 6.0, 0.15 M NaCl, and 0.025% polysorbate 80.
c. The dosing formulation contained 400 µg/mL rLP2086 (200 µg/mL of each subfamily), 0.25 mg/mL aluminum as $AlPO_4$, 5 mM succinate buffer,
0.15 M NaCl pH 6.0, and 0.025% polysorbate 80.
d. 20 females were assigned to caesarean-sectioning and 20 females were assigned to natural delivery.
e. 1 female was found dead at the morning viability check on lactation Day 1. There was no discernable cause of death.
f. Calculated as follows: the number of pregnant rabbits divided by the number mated rabbits and multiplied by a 100 to obtain a percentage. According to historical control data, in 5 studies of similar design conducted at the contract testing facility from July 2001 to December 2005, the average number of rabbits that mated in each study was 80.0%, 83.3%, 92.5%, 100%, or 100%.
g. Data reported as group mean values.
h. Calculated (in days) at the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the time the first pup was delivered.
i. Number of rabbits with live offspring/number of pregnant rabbits.
j. Results denoted as either negative for no antibodies detected or positive for an increase in antibodies.

2.1.4 Discussion and Conclusions

There were no vaccine-related deaths in the does. One female in the saline control group was found dead at the morning viability check on lactation Day 1; there was no apparent cause of death.
No clinical or necropsy observations were attributed to rLP2086 vaccine. No irritation was observed at the injection sites. Body weights, body-weight gains, and food consumption values during the premating, gestation, and lactation periods and gravid uterine weights were unaffected by either the vehicle or the vaccine.

The number of rabbits that mated was reduced in the group given 200 µg vaccine (80.0%) compared with either control (93% each), but the decrease was apparently unrelated to treatment as it lacked statistically significance and was within the range of historical controls (80.0% to 100%) for the testing facility (----------(b)(4)----------Preclinical Services).
Although there were fewer mated rabbits in the 200 µg vaccine group, there was no effect on fertility. Fertility rates in rabbits given saline, vehicle, and rLP2086 were 91.9%, 100%, and 90.6%, respectively.
In the subgroups of rabbits that were caesarean-sectioned, there were no effects of rLP2086 on numbers of corpora lutea (F_0), litter size (F_1), embryo-fetal mortality, fetal sex, weight, external, palatal, visceral, or skeletal morphology, weights of the spleen and thymus, or placental appearance. In the subgroups of rabbits that naturally delivered offspring, there were no effects of the vaccine on the duration of gestation, the gestation index, parturition, litter size, postpartum maternal care of offspring, offspring mortality and growth, or weights of the spleen and thymus. The numbers of pups surviving to postpartum day (PPD) 21, mean numbers of surviving pups/litter on PPD 21, and mean weights of pups on PPD 21 were similar between groups.

IM administration of rLP2086 to maternal does (F_0) prior to mating (17 and 4 days before mating) and on GDs 10 and 24 did not affect fertility or growth and development of F_1 fetuses and pups. There were no effects of bivalent rLP2086 on the external, palatal, visceral, or skeletal morphology of F_1 fetuses based on examination of a limited number of litters. The low mating rate among vaccine treated rabbits resulted in the sponsor deciding to conduct a repeat reproductive and developmental toxicity study to achieve higher mating success. The sponsor performed the repeat study at a different laboratory (Wyeth Drug Safety) as well.

2.2 Study title

Meningococcal B vaccine (rLP2086): A combined intramuscular fertility and developmental toxicity study in female rabbits (RPT-75947)

2.2.1 Key study findings

This repeat study was conducted to evaluate an adequate number of litters by cesarean section arm as recommended by the ICH guidelines. The previously conducted fertility and developmental toxicity study in (b)(4) rabbits had a low pregnancy rate in animals assigned to the caesarian section component resulting in a minimal number of litters available for the assessment of embryo/fetal development.

Evaluations for vaccine-related effects consisted of maternal mortality, clinical observations, abortion rate, body weight, food consumption, injection site irritation, fecundity parameters (mating and fertility indices), gravid uterine weight, hysterectomy findings on GD 29 (corpora lutea, litter size, embryo/fetal mortality), postmortem observations, and macroscopic observations of the injection sites; fetal sex, weight, and external, palatal, visceral, and skeletal anomalies; and placental appearance.

IM administration of 200 µg vaccine to female rabbits 17 and 4 days prior to mating and on GDs 10 and 24 did not affect mating, fertility, or embryo/fetal viability, growth, or morphologic development. There was no adverse maternal toxicity in this study; findings in does included injection site irritation (transient, minimal severity) in the vehicle and 200 µg vaccine groups.

Study no.: RPT-75947
Conducting laboratory and location: Wyeth Drug Safety, ----(b)(4)----
Date of study initiation: 21 November 2008
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

2.2.2 Methods

Doses: 200µg vaccine
Species/strain: -------(b)(4)-------- rabbits

Number/sex/group: F0 females: 25
Route, formulation, and volume: intramuscular; 0.5 mg/mL aluminum as $AlPO_4$, 10 mM histidine buffer, pH 6.0, 0.15 M NaCl, and 0.005% polysorbate 80; 0.5 mL

Study design:

Dosing period: days 17 and 4 prior to mating and GDs 10 and 24

Dosage Group	N	µg rLP2086[a] in the Vaccine (Concentration in µg/mL)	Dose Volume (mL)[b]
Saline Control[c]	25	0	0.5
Vehicle Control[d]	25	0	0.5
Vaccine[e]	25	200 (400)	0.5

$AlPO_4$ = Aluminum phosphate; N = Number; NaCl = Sodium chloride.
a. rLP2086 (A and B subfamilies) is the protein component of the vaccine.
b. All doses administered into the hind legs
c. The saline control was 0.9% isotonic saline.
d. The vehicle control was 0.5 mg/mL aluminum as $AlPO_4$, 10 mM histidine buffer, pH 6.0, 0.15 M NaCl, and 0.005% polysorbate 80 (final formulation).
e. The subfamily A and B proteins are in a ratio of 1:1 in drug product matrix (vehicle control).

Hysterotomy on GD 29

Parameters and endpoints evaluated:
F0:
mortality, clinical observations, abortion rate, body weight, food consumption, injection site irritation, fecundity parameters (mating and fertility indices), gravid uterine weight, hysterectomy findings on GD 29 (corpora lutea, litter size, embryo/fetal mortality), postmortem observations, and macroscopic observations of the injection sites

F1 caesarian litter:
fetal sex, weight, and external, palatal, visceral, and skeletal anomalies; and placental appearance

Statistical methods:
Maternal body weight, body weight gain, food consumption, injection site irritation, fecundity parameters (mating index, fertility index), abortion rate, gravid uterine weight, hysterotomy parameters, fetal weight, fetal sex, and fetal examination findings were analyzed for a difference among groups using a nonparametric one way analysis of variance, with follow up tests to compare all groups in a pairwise manner. Analyses of injection site irritation were performed on each day of dosing with respect to the average post-dose irritation score. Analyses of hysterotomy findings were done with respect to number per litter or proportion per litter, depending on the parameter being analyzed. Analyses of fetal examination findings were done with respect to proportion of affected fetuses per litter and proportion of litters with at least one affected fetus. Fetal

weight was also analyzed with a nonparametric one-way analysis of variance after adjusting for a covariate to account for litter size.

2.2.3 Results

F0 Females

Mortality, Clinical Observations, Postmortem Observations and Injection Site Irritation

There were no deaths, abortions, postmortem observations, or vaccine related clinical observations in does in the vaccine group. There were no adverse injection site irritation in does administered the vaccine. One female in the vehicle control group aborted on GD 28; no abortions occurred in the saline or vaccine groups. Clinical observations were limited to red pigment on drop pan and loose feces, which occurred at similar incidences in the saline, vehicle, and vaccine groups, were generally sporadic and/or transient, and did not affect the overall health of the animals. Postmortem observations consisted of discolored (tan) kidneys in the one vehicle control animal that aborted. Early interruption of pregnancy (total resorptions) occurred in one animal in the vehicle control group. Edema and/or erythema were observed at the injection site in both vehicle and vaccine groups. These observations occurred at low incidence, were of minimal severity (grade 1), were generally transient (present at 2 and/or 24 hours postdose but no longer present at the subsequent evaluation 6 days postdose).

Body Weights and Body Weight Gains

There were no effects of the vaccine on body weights or body weight gains. Premating and gestation body weights and body weight gains, and adjusted pregnancy weight gain (overall gestation weight gain minus the gravid uterus) were comparable in the groups administered saline, vehicle, and vaccine. The vehicle did not affect body weights or body weight gains.

Gravid Uterine Weights

Gravid uterine weights were similar in the groups administered saline, vehicle or vaccine.

Food Consumption

There was one period of reduced food consumption during gestation, otherwise food consumption was comparable across groups. Food consumption was significantly lower in the vaccine group compared to the vehicle control group on GDs 24 through 28. The difference was small (20% lower than vehicle controls) and did not affect body weight gain on GDs 24 through 28 or final body weight on GD 29.

Mating Performance and Fertility

Mating or fertility was comparable across groups; the mating and fertility indices in the study ranged from 88% to 92% and 91% to 100%, respectively.

Hysterotomy Findings

There was no effect of the vaccine on the number of corpora lutea, implantations, or embryo-fetal viability (pre- and post-implantation loss, number of resorptions and live fetuses). The mean number of implantations was slightly higher in the vaccine group (9.09) compared to the vehicle and saline control groups (7.75 and 7.14, respectively), as a result of lower pre-implantation loss in this group. The mean number of live fetuses was also higher in the vaccine group (8.55) compared to the saline and vehicle control (7.20 and 6.62, respectively) as a result of the higher number of implantations in the vaccine group. The mean number of implantations and live fetuses for all groups were within the historical range (7.32 to 9.50 for implantations; 6.89 to 9.00 for live fetuses) indicating that the higher values in the vaccine group reflect the normal variability for these parameters in rabbits. One female in the vehicle control group had early interruption of pregnancy. The vehicle did not affect hysterotomy parameters.

F1 Offspring
Fetal Sex Distribution
Fetal sex distribution was not affected by the vaccine or the vehicle. Fetal sex distribution was significantly higher in the vaccine group compared to the vehicle control (0.55 compared to 0.37); however, it was similar to the saline control (0.49). This difference appears unrelated to vaccine administration.

Fetal Body Weight
There were no effects of the vaccine on fetal body weight. Absolute fetal body weights were lower in the vaccine group compared to the saline and vehicle groups as a result of larger litters in this group. When adjusted to account for litter size (using the number of uterine implantations as a covariate), fetal body weights were comparable across groups. The vehicle did not affect fetal body weights.

Fetal Morphological Examinations
Fetal External Examination
There were no fetal external anomalies related to vaccine administration; findings occurred at single incidences in the saline, vehicle, and vaccine groups. The vehicle did not affect fetal external morphology.

Fetal Visceral Examination
There were no fetal visceral anomalies related to vehicle or vaccine administration; findings occurred at single or comparable incidences in the saline, vehicle, and vaccine groups. The vehicle did not affect fetal visceral morphology.

Fetal Skeletal Examination
There were no fetal skeletal anomalies related to vaccine administration; findings generally occurred at comparable incidences in the saline, vehicle, or vaccine groups. A higher incidence of fetuses with a decreased number of caudal vertebrae occurred in the vaccine group (7% affected fetuses per litter) compared to 3% and 1% in the saline and vehicle controls, respectively. The incidence of

this variation was within the historical control range (0% to 7%). The incidence of fetuses with greater than 26 presacral vertebrae was higher in the vaccine group compared to the vehicle control group (23% compared to 3%); however, the incidence in the vaccine group was similar to the saline control group (23% and 20%, respectively) and was within the historical control range (7% to 30%). The increased incidence of these two vertebral anomalies was interpreted to be unrelated to vaccine administration. The vehicle did not affect fetal skeletal morphology.

Placental Morphology
There were no effects of the vehicle or vaccine on placental morphology. Discolored placenta (brown) occurred in the vaccine group but was not vaccine-related because the finding was limited to fetuses from one litter.

Anatomic Pathology
A necropsy was done on each female rabbit, including the animal that was electively euthanized. The necropsy included macroscopic examination of the injection site (dosing site only). Injection site findings of rabbits given saline, vehicle or the vaccine were observed. These findings consisted of focal red or white discoloration at the injection sites. There were no significant differences in incidence or size (diameter) of these findings between the saline, vehicle, or vaccine groups.

Antibody Analysis
Anti-vaccine antibodies were not detectable in any predose specimens from does from the saline control, vehicle control, or vaccine groups or in any GD 29 specimens from does or fetuses from the saline control or vehicle control groups. Anti-vaccine antibodies (anti-LP2086A and anti-LP2086B) were detected in all GD 29 specimens from does and fetuses from the vaccine group. These results confirm that the saline control and vehicle control group animals were not exposed to vaccine while does administered the rLP2086 vaccine with adjuvant had a serum antibody response to rLP2086 indicating vaccine exposure.

Summary table: Meningococcal B Vaccine (rLP2086): A Combined Intramuscular Fertility and Developmental Toxicity Study in Female Rabbits

Female Fertility and Developmental Toxicity: 200 µg/dose

Dose[a]	**0 (Saline Control)**	**Vehicle Control**[b]	**200 µg rLP2086 in PF-05212366**[c]
Sex	**F**	**F**	**F**
Number of Animals[d]	25	25	25
Treated F_0 Females			
Number Died or Euthanized Moribund	0	0	0
Number of Females Mated (Confirmed)	22	23	22
Number of Pregnant Females	20	22	22
Fertility Rate (%)[e]	91.0	95.7	100
Number of Abortions	0	1 (GD 28)	0
Noteworthy Findings			

Dose[a]	**0 (Saline Control)**	**Vehicle Control[b]**	**200 µg rLP2086 in PF-05212366[c]**
Clinical Observations			
Red pigment on drop pan	1	1	2
Loose feces	8	10	5
Injection Site Reaction			
Edema			
Day 1	+ (N=1)	+ (N=3)	+ (N=2)
Day 14	-	-	+ (N=1)
Day 28	+ (N=1)	+ (N=1)	+ (N=3)
Day 42	-	+ (N=2)	+ (N=2)
Erythema			
Day 1	+ (N=4)	+ (N=3)	+ (N=4)
Day 14	+ (N=3)	+ (N=3)	+ (N=3)
Injection Site Reaction (continued)			
Day 28	+ (N=1)	+ (N=6)	+ (N=7)
Day 42	-	+ (N=2)	+ (N=1)
Necropsy Observations			
Discolored (tan) kidneys	0	1	0
Gross Pathology			
Injection site			
Focal red or white discoloration	9	14	11
Body Weight	-	-	-
Body-Weight Gain	-	-	-
Food Consumption[f]			
GDs 24 through 28	102.44	109.91	-14.5
Gravid Uterine Weight	-	-	-
Cesarean Section Observations (GD 29)			
Number Evaluated	25	25	25
Number Pregnant Rabbits	20	21	22
Mean Number Corpora Lutea[g]	10.15	9.90	10.18
Mean Number Implantations[g]	7.75	7.14	9.09
Mean Number Early Resorptions[g]	0.50	0.29	0.45
Mean Number Late Resorptions[g]	0.05	0.05	0.09
Mean Litter Size[g]	7.20	6.62	8.55
Number Live Fetuses[g]	144	139	188
Embryo-Fetal Mortality	0	0	0
Mean Fetal Sex Ratio (%Males)	48.6	41.0	55.3
Noteworthy Findings			
Fetal Weight	-	-	-
Fetal Morphology (External, Visceral, or Skeletal)	-	-	-
Spleen Weights	-	-	-
Thymus Weights	-	-	-
Placental Appearance	-	-	-
Additional Examinations			
Antibody Analysis[h]			
F_0 Female Rabbits	Negative	Negative	Positive
F_1 Fetuses and Pups (M/F)	Negative	Negative	Positive

Table based upon sponsor submission

- = No noteworthy finding; + = Very slight; $AlPO_4$ = Aluminum phosphate; F_0 = Maternal/paternal generation; F_1 = First generation; F = Females;

GD = Gestation day; GLP = Good Laboratory Practice; ICH = International Conference on Harmonization; IM = Intramuscular; M = Males; NaCl = Sodium chloride; (b)(4) = ----(b)(4)-------; PPD = Postpartum day; RPT = Report.
a. Dose volume was 0.5 mL/injection.
b. The vehicle control was 0.5 mg/mL aluminum as $AlPO_4$, 10 mM histidine buffer, pH 6.0, 0.15 M NaCl, and 0.005% polysorbate 80.
c. The dosing formulation contained 400 µg/mL rLP2086 (200 µg/mL of each protein subfamily), 0.25 mg/mL aluminum as $AlPO_4$, 5 mM succinate buffer, 0.15 M NaCl pH 6.0, and 0.025% polysorbate 80.
d. All animals were assigned to caesarean-sectioning.
e. Calculated as follows: the number of pregnant rabbits divided by the number mated rabbits and multiplied by a 100 to obtain a percentage. According to historical control data, in 5 studies of similar design conducted at the contract testing facility from July 2001 to December 2005, the average number of rabbits that mated in each study was 80.0%, 83.3%, 92.5%, 100%, or 100%.
f. Group means are shown for controls. For treated group, percent differences from saline controls are shown.
g. Data reported as group mean values.
h. Results denoted as either negative for no antibodies detected or positive for an increase in antibodies.

2.2.4 Discussion and Conclusions

Treatment of F0 females from Day 6 of gestation to Day 20 of lactation had no effect upon the number of implantation sites, total litter size on Day 1 in F1 dams, live litter size, sex ratio, body weights, behavioral functions, offspring survival assessed through to weaning or macroscopic pathology. There were no test article-related clinical signs or effects on body weight, sexual maturation or sensory examinations in F1 animals selected for subsequent mating. Likewise, no test article-related effects on mating performance, fertility, reproductive parameters or macroscopic pathology were observed in F1 animals.

The absolute dose administered to rabbits in this study, 200 µg, represents an exposure of approximately 66.6 µg/kg (200µg/3 kg rabbit). This compares to the clinical dose of 200 µg, or 4µg/kg (200µg/50 kg human). The respective rabbit dose is approximately 16.67x the human dose, rounded to 17x by the sponsor. The dose multiple relative to the clinical dose also presents a margin for safety.

3 CONCLUSION

The lack of rLP2086-related adverse findings in the two 5-cycle repeat-dose toxicity studies in which the vaccine was administered to rabbits at twice the highest clinical dose administered supports the safety of bivalent rLP2086 administration to humans at a dose of 200 µg for up to 4 doses. Additionally, the lack of vaccine-related findings in the 2 fertility and developmental toxicity studies where bivalent rLP2086 was administered to rabbits at a multiple of the highest clinical dose supports the safety of rLP2086 administration to women of childbearing potential.

In the 2 repeat-dose toxicity studies, there were increases in mean body temperature in animals administered the vehicle or bivalent rLP2086 (100 and/or 400 µg) and increases in fibrinogen and total globulins observed during the dosing phase in animals after each dose (100 and/or 400 µg). Changes in

mean body temperature were not considered adverse based on the small magnitude of the increases. Increases in fibrinogen and total globulins were attributed to an acute phase inflammatory reaction, an anticipated response after administration of bivalent rLP2086, and were not considered adverse based on their magnitude. These findings reversed prior to the next dose or during the 4-week observation period. Transient increases in fibrinogen were observed in the Phase 1 clinical study and are often observed in clinical studies with vaccines.

In conclusion, IM administration of rLP2086 to rabbits at doses that resulted in a substantial anti-rLP2086 antibody response and equaled or exceeded the highest clinical dose on a per dose basis, did not produce any adverse effect in rabbits, and did not affect fertility or growth and development of F_1 fetuses and pups. In addition, the similar results obtained in the two 5-cycle repeat-dose rabbit toxicity studies indicate that the nonclinical toxicity and safety profile of rLP2086 observed using the final formulation of the vaccine is comparable to the initial process/formulation that was used in the early clinical studies with rLP2086.

4 PROPOSED PACKAGE INSERT WORDING

Pregnancy Category B.

Reproduction studies have been performed in rabbits at doses up to 17 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to Trumenba. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, Trumenba should only be continued during pregnancy if clearly needed.

Justification: The reproductive toxicology studies conducted by the sponsor support the pregnancy labeling claim.

Carcinogenesis, mutagenesis, and impairment of fertility

No studies have been performed in animals to evaluate the carcinogenic potential and the genotoxic risk of Trumenba vaccine.

Reproduction studies have been performed in rabbits at doses up to 17 times the human dose on mg/kg basis and revealed no evidence of impaired fertility or harm to the fetus due to Trumenba vaccine.

5 OVERALL CONCLUSION

Based on nonclinical toxicity assessments, there are no significant safety issues to preclude the BLA from being approved.

Concurrence: Martin D. Green