

**REVIEW MEMORANDUM:
PRECLINICAL DEVELOPMENTAL TOXICITY STUDY REPORTS**

Date: June 9, 2009

From: Marion F. Gruber, Ph.D, OVR

To: File: STN 125259/0

Subject: **STN 125259/0** “Human Papillomavirus Type 16 and Type 18 Virus Like Particle (recombinant L1, ---b(4)----- cells and *Trichoplusia ni* cells) Vaccine with Alum and 3D-Monophosphoryl Lipid A Adjuvant; - b(4)-----, for the prevention of HPV-16 + HPV-18 infection”:

| | | |
|--------------------------------------|---|-------------------------|
| Preclinical studies reviewed: | Study report | -b(4)-249/033160 |
| | Study report | 1729/8 – D6154 |
| | Study report | 1729/7 – D6154 |
| | Study report | 1729/17 – D6154 |
| | Response to CR letter comment 12 | |

Background: In support of their proposed indication for Cervarix, GSK has conducted a pivotal developmental toxicity study -b(4)-249/033160 entitled “HPVPro/AS04D Prophylactic Human Papillomavirus Type 16 and Type 18 Candidate Vaccine Adjuvanted with AS04D: Study of effects on Pre and Post natal Development in b(4) rats by Intramuscular Administration (including pre-mating immunization phase)” and has submitted three additional reports from supportive developmental toxicity studies conducted with MPL adjuvant only, namely: **Study report 1729/8 – D6154:** MPL: Subcutaneous Study of Embryo-Fetal Development in the rabbit, **Study report 1729/7 – D6154** :MPL: Subcutaneous Study of Embryo-Fetal Development in the rat and **Study report 1729/17 – D6154:** MPL: Subcutaneous Study of Pre-and Postnatal Development in the rat.

The reviews of the individual study reports are attached below.

GSK has performed one pivotal developmental toxicity study -b(4)-249/033160 to evaluate the reproductive and developmental toxicity potential of CERVARIX (Human Papillomavirus Type 16 and Type 18 Candidate Vaccine Adjuvanted with AS04D). Four groups of female rats (--b(4)----- rat strain), 56 animals/group, were dosed either with saline only 30 days prior to mating and then on gestation days 6, 8, 11 and 15 of gestation; with saline 30 days prior to mating and then with HPVpro/AS04D vaccine on gestation days 6, 8, 11 and 15, with the HPVpro/AS04D 30 days prior to mating and on gestation days 6, 8, 11 and 15 and with AS04D adjuvant only 30 days prior to mating and on gestation days 6, 8, 11 and 15. Doses (100 ul HPVpro/AS04D representing one fifth the human dose [47 times the human dose relative to body weight]) were administered by IM injection into the anterior thigh muscle, control animals received saline vehicle

accordingly. Animals were subdivided into subgroups of 22 rats per group, and either underwent Caesarean sectioning on gestation day 20 or were allowed to rear their offspring.

There were no overt signs of treatment related maternal toxicity. Treatment did not affect body weights and body weight gains of the F0 generation neither did it affect body weight gain of the F1 generation born to treated dams. CERVERIX did not affect F0 female fertility, mating performance, embryo-fetal development and postnatal development. There were no observed treatment related effects on the incidence of major and minor abnormalities and skeletal variants in the offspring of dams treated with the test article except the 2 observations of small membranous intraventricular septal defect further discussed below. Also, postnatal growth and development did not appear to be affected by vaccine administration.

In pivotal study -b(4)- 249 fetal examinations showed two observations of small membranous intraventricular septal defect (IVSD), one (1) in group 3 (HPVpro/AS04D administered before and after mating), 1/166 fetuses evaluated (0.6 % fetuses affected, 4.5 % litters affected) and one (1) in group 4 (AS04D administered before and after mating), 1/169 fetuses evaluated (0.59 % fetuses affected, 4.5 % litters affected). These defects were listed as minor visceral fetal abnormalities. The sponsor stated that a review of recent studies conducted in the same laboratory revealed that this observation had not been reported in the control population in the last 15 studies. The sponsor then performed an extended review of the incidence of IVSD covering 98 studies performed between 1996 and 2005 --b(4)----- (included as Annex 3 in 4.2.3.5.2 study report -b(4)-249/033160). Four (4) cases of IVSD occurred in control animals (12259 fetuses examined, 0.033% by fetus and 0.235 % by litter) with slightly higher incidences in treated groups whereby treatment was not specified (e.g., “high dose” group had 9 cases of IVSD (9406 fetuses examined, 0.096% by fetus and 0.683% by litter).

In addition, among the malformations observed in supportive study 1729/8-D6154 conducted in rabbits treated with MPL s.c. daily from gestation days 7-19 there were two (2) cases of major ventricular septal defects, one (1) in group 3 (MPL intermediate dose group, 10 ug/kg/day) and one (1) in group 4 (MPL high dose group, 100 ug/kg/day) with an incidence of 0.6 % by fetus and 5.8% by litter in group 3 and 0.55% by fetus and 4.7 % by litter in group 4. In this study, this observation did not occur in the low dose MPL group and/or in the saline control group. Control group values from 6 embryo/fetal studies that preceded this study showed that of 1139 fetuses (118 litters) evaluated, there was 1 case of ventricular septal defect in study 4 (0.088% by fetus and 0.8% by litter). In addition the sponsor provided cumulative fetal defect data for ---b(4)----- rabbits, supplied by -----b(4)----- used in embryo-fetal studies at --b(4)----- since February 1994. The cumulative incidence of ventricular septal defect (major) in rabbits was 0.12%.

Since the finding of membranous ventricular septal defect observed in the pivotal study -b(4)- 249 and study 1729/8 – D6154 was isolated in nature, i.e., 1 fetus per litter/group and since it was also observed in the historical control data it was not clear whether this

observation was a treatment related effect. However, of concern was that the incidence of ventricular septal defect in both studies was higher than in the historical control and did not occur in concurrent control groups. Furthermore, in the rabbit study (1729/8-D6154) in which animals were treated with MPL, this finding occurred in the higher dose groups only and in the pivotal study (-b(4)- 249/033160) conducted in rats this finding occurred in groups 3 and 4, i.e. those groups that received HPV/AS04D or AS04D before and after mating.

Therefore, the sponsor was asked on August 31, 2007 to perform a post-hoc statistical analysis of the data from pivotal study -b(4)- 249 and study 1729/8 to further evaluate the statistical significance of this finding. In addition, the sponsor was asked to provide a reference supporting the statement that this finding represents a delay in fetal development and an explanation of their finding of the IVSD being “small” as used to describe the finding in study -b(4)- 249. The sponsor provided a response October 3, 2007 (sequence #17 to the Cervarix BLA). In that response, the sponsor states that a demonstration of statistical significant increase in incidence of IVSD observed in-b(4)-249 is not possible because the number of litters affected is below 5 and therefore, statistical tests are of minimal value in this analysis. Furthermore, the sponsor stated that the occurrence of IVSD in study 1729/8 is also not statistically significant. Sponsor concluded that the incidence of IVSD in rats and rabbits in studies -b(4)- 249 and -----b(4)- 1729/8 is of spontaneous nature. Sponsor attributed the occurrence of membranous intraventricular septal defects to a delay in normal development that will close with further normal development. CBER remained concerned because in the reported studies this event was not observed in concurrent controls and furthermore, the incidence was considered high relative to historical controls (in study -b(4)- 249: 0.6% by fetus and 4.5% by litter whereas historical background rates are 0.033% to 0.096% by fetus or 0.235% to 0.683% by litter).

The following comment (12 a and b) was communicated in the CR letter:

Regarding the developmental toxicity studies:

As stated in our communication of August 31, 2007, it is not clear whether the finding of membranous ventricular septal defect observed in studies -b(4)- 249 and -b(4)----- study 1729/8 – D6154 is a treatment related finding, since it is isolated (i.e., occurrence 1 fetus/litter/group and also observed in historical controls). However, we remain concerned because in the reported studies this event was not observed in concurrent controls and furthermore, the incidence is high compared to historical controls (in study -b(4)- 249: 0.6% by fetus and 4.5% by litter whereas historical background rates are 0.033% to 0.096% by fetus or 0.235% to 0.683% by litter). The Table entitled “Terminology of developmental abnormalities in common laboratory mammals” Version 2, 2006, (http://teratology.org/news_resources/DevToxTerms.htm), published by the Teratology Society suggests that a defect in the membranous ventricular septum may be associated with Tetralogy of Fallot, a congenital heart defect.

[censored text - clinical]

We acknowledge that VSD is reported to occur in 2-7% of human live births in

the US and is considered a very common congenital anomaly that can resolve spontaneously. Furthermore, we note that the VSDs observed in study -b(4)- 249 conducted in rats and -b(4)----- Study 1729/8-D6154 conducted in rabbits refer to the membranous septum and not to the muscular septum. *[censored text - clinical]* However, given the high incidence of ventricular septal defect in the reported developmental toxicity studies relative to concurrent and historical controls, and occurrence of this finding in more than one species, we request you:

- a. Please comment on the potential association of ventricular septal defect with HPV/AS04 vaccine.
- b. Please propose a risk assessment and risk management plan to address this observation.

GSK's response regarding question 12 a:

GSK Biologicals does not consider that there is any indication of a potential association between ventricular septal defect (VSD) and *Cervarix* administration as demonstrated in -b(4)- study -b(4)- 249, based on experience with current and historical data from both GSK and contract laboratories. The company states that the observation of a single affected fetus in any group of animals in -b(4)-study -b(4)- 249 and ---b(4)- study 1729/8-D6154 is typical for a spontaneous, background malformation such as VSD. The incidence of VSD is the minimal incidence that can be calculated for the group sizes used. Further support of this observation to be spontaneous is that VSD did not occur in multiple fetuses per litter, or in multiple litters per group. Sponsor states that the occurrence of a spontaneous background malformation such as VSD in the reported studies is typically variable across groups and across studies, due to animal strain variability. Therefore, it would be more appropriate to consider historical incidences across groups and across studies instead of the accumulated historical control incidence.

Sponsor states that in the historical control database (n=78 studies) of the laboratory (-b(4)-) that conducted rat study -b(4)- 249, a singly affected (VSD) fetus in a group occurred in control groups from 4 studies and in test-article treated groups from at least 6 Studies (event was deemed unrelated to test article). The incidence by fetus and litter for each of these 4 control groups ranged from 0.6% to 0.7% and 4.2% to 5.0%, respectively. Sponsor notes that the fetal and litter incidences of VSD in the vaccine treated groups of -b(4)-249 (0.6% and 4.5%, respectively) are of the same magnitude. Sponsor states that in both cases, it is the minimal incidence that can be counted for the group sizes used, and this is consistent with a spontaneous event. In addition, and as another point of reference, data among 67 rat developmental toxicity studies conducted within Safety Assessment at GlaxoSmithKline, showed 10 studies with VSD noted in the control group (incidence by fetus and by litter ranged from 0.6% to 2.4% and 4% to 10.5%, respectively) and 57 studies without VSD noted in the control group. Among 30 rabbit studies there were 3 studies with VSD noted in the control group (incidence by fetus and by litter ranged from 0.5% to 0.6% and 4.6% to 5.0%, respectively) and 27 studies without VSD noted in the

control group. Sponsor states that these data reflect the group incidence magnitude and variability that is typical of spontaneous malformation.

Sponsor states that a lack of an association between VSD and *Cervarix* administration is further substantiated by the observation that in study-b(4)- 249, a single fetus with VSD was noted in group 3 (vaccine administered before and during pregnancy) but not in group 2 (vaccine given during pregnancy only). Because the sensitive period for heart development is during pregnancy, the lack of a concordant result between groups 2 and 3 suggests the VSD was not associated with vaccine treatment, but rather is a spontaneous event.

Also, in the -b(4)-----rabbit study (1729/8-D6154) with MPL, sponsor states that there was no indication of a dose-responsive incidence of VSD. The incidence was 1 VSD at 10 ug MPL/kg/day and 1 VSD at 100 ug MPL/kg/day. Furthermore, the accompanying cardiac malformations for each of these fetuses were different: in the 10 ug MPL/kg/day fetus, the accompanying malformations were interrupted aortic arch, right subclavian artery arising from descending aorta and dilation of pulmonary trunk, and in the 100 ug MPL/kg/day fetus, the accompanying malformation was persistent truncus arteriosus.

Furthermore, GSK consulted with the authors of study -b(4)- 249 regarding a possible association between IVSD and 'Tetralogy of Fallot'. It was noted that 'Tetralogy of Fallot' is not a single defect, but rather it is a syndrome of four concurrent malformations, including: pulmonary stenosis, overriding aorta, hypertrophy of the right ventricle, and VSD. There were no instances of pulmonary stenosis, overriding aorta, or right ventricular hypertrophy in fetuses from the rat or the rabbit study. Authors state that the observation of VSD alone is not evidence of 'Tetralogy of Fallot,' because the syndrome only exists when all four defects occur simultaneously. Furthermore, cyanosis is expected to occur post nally with 'Tetralogy of Fallot,' and no cyanosis was reported in the pups in the postnatal portion of the -b(4)- 249 rat study.

GSK's response regarding question 12 b:

GSK and its consulted experts, based on the reasons cited in response to item 12a of the CR letter, do not conclude that there is any indication of a potential association of VSD with *Cervarix* vaccine, based on the animal toxicity studies. However, since a pregnancy registry is already planned for implementation upon approval of *Cervarix* in the U.S. and the outcomes of all registered pregnancies are to be assessed, the potential for VSD will be adequately covered.

Reviewer's comment: GSK has satisfactorily refuted a potential association of IVSD with HPV/AS04 vaccine. The reviewer notes that GSK is planning a pregnancy registry following licensure of *Cervarix* in the US which will capture outcomes of registered pregnancies. In this reviewer/s opinion no further risk management plan to assess the observation of VSD is necessary. Based on the results from reproduction toxicity studies performed, pregnancy category B may be considered.

Supervisor's concurrence: yes__X__ no_____

Review Memorandum

Date: April 4, 2007

From: Marion F. Gruber, Ph.D, OVRR

Subject: **STN 125259/0** “Human Papillomavirus Type 16 and Type 18 Virus Like Particle (recombinant L1, -----b(4)----- cells and *Trichoplusia ni* cells) Vaccine with Alum and 3D-Monophosphoryl Lipid A Adjuvant; -b(4)----, for the prevention of HPV-16 + HPV-18 infection”

- Module 4.2.3.5. Reproductive and Developmental Toxicity
 - 4.2.3.5.2. Embryo-fetal development
 - 4.2.3.5.2.1 **Study report -b(4)- 249/033160**
“HPVPro/AS04D Prophylactic Human Papillomavirus Type 16 and Type 18 Candidate Vaccine Adjuvanted with AS04D: Study of effects on Pre and Post natal Development in -b(4)- Rats by Intramuscular Administration (including pre-mating immunization phase),”
 - Annex 1: Serological report of reproduction toxicity study -b(4)- 249/033160
 - Annex 2: Serological report of the preliminary immunogenicity study with L1VLP16/L1VLP18 vaccine (HPVpro/AS04D) in female rats -b(4)- 20020475
 - 4.2.3.5.2. Fertility and early embryonic development
 - 4.2.3.5.2.1 **Study report -b(4)- 249/033160**
 - Addendum 1: Pre- and post-natal development study with VLP16/VLP18 vaccine (HPVpro/AS04D) administered IM in rats

Submission: March 29, 2007

Sponsor: GSK

Background: GSK has submitted a BLA for “Human Papillomavirus Type 16 and Type 18 Virus Like Particle Vaccine with Alum and Monophosphoryl Lipid A Adjuvant”

indicated for immunization of adolescents and adults for the prevention of Human Papillomavirus infections types HPV-16 and HPV-18.

Product information

Cervarix is composed of recombinant C-terminally truncated HPV-16 L1 and HPV-18 L1 proteins, assembled into VLPs and adjuvanted with GSK's proprietary adjuvant AS04. The HPV-16 L1 and HPV-18 L1 proteins are the active ingredients of the vaccine produced with a Baculovirus expression system. The AS04 adjuvants is composed of an aluminum salt, $Al(OH)_3$ and 3-O- desacyl-4'-monophosphoryl lipid A, MPL.

One dose of Cervarix contains 20 ug of HPV-16 and 20 ug of HPV-18 proteins adjuvanted with AS04 composed, per human dose, of 500 ug aluminum hydroxide and 50 ug MPL.

Preliminary studies:

A proposal for a reproductive toxicity study to assess the effects of this vaccine on pre- and postnatal development in ---b(4)----- rats had been submitted to FDA on December 9, 2002 to IND -b(4)-. At that time, the sponsor had requested a teleconference with CBER to agree on the study design. A teleconference took place on January 16, 2003, between representatives of GSK and CBER, during which CBER communicated comments to the sponsor (see IND -b(4)- file).

In order to select a relevant animal model, the sponsor has conducted a preliminary immunogenicity study with the objective of determining antibody production in non-pregnant ---b(4)----- female rats (5/group) in response to immunization with HPV-16 and HPV-18 L1 VLPs formulated either on AS04D or $Al(OH)_3$ (Annex 2: "Serological report of the preliminary immunogenicity study -b(4)- 20020475"). Groups of 4 or 5 OFA (-b(4)-) rats (females, 5 weeks old) were immunized IM 4 or 5 times (day 0, 30, 32, 35, and 39) with 1/5 or 1/10 HD of VLPs 16 and 18 formulated either on AS04D or $Al(OH)_3$. NaCl served as a control. Animals were bled at day 0 and at day 5 post their last injection. In this preliminary experiment, the female rats used were not pregnant. Quantitation of anti-VLPs 16/18 antibody was performed by ELISA using VLP-16 or VLP-18 as coating antigen. Results showed high antibody titers to both, HPV-16 and HPV-18 L1 VLPs in groups receiving priming injections at day 0. Based on the results from this experiment, the sponsor selected 1/5 of the human dose for the reproductive toxicity study.

Review of Module 4.2.3.5.2.1

Study title: "HPVPro/AS04D Prophylactic Human Papillomavirus Type 16 and Type 18 Candidate Vaccine Adjuvanted with AS04D: Study of Effects on Pre- and Post-Natal Development in -b(4)- Rats by Intramuscular Administration (Including Pre-Mating Immunization Phase)" audited final report -b(4)- 249/033160

Study director: M.P. Dent, B. Sc.

Conducted by: -----b(4)-----

-b(4)-

---b(4)--

---b(4)--

---b(4)--

Objective: to assess the effects of intermittent IM administration of HPVpro/AS04D, a prophylactic human papilloma virus type 16 and 18 candidate vaccine adjuvanted with AS04D, on embryo-fetal, pre-and post-natal development in the rat.

Study was conducted in compliance with GLP as set forth in UK GLP regulations 1999, OECD principles of GLP (rev. 1997) and EC commission directive 1999/11/EC of March 8, 1999. The study was designed taking into account the principles of ICHS5a.

Vaccine composition: Lot -----b(4)-----: Mono-dose vial containing 20 ug of HPV-16 L1 VLP, 20 ug HPV-18 L1 VLP, 50 ug MPL and 500 ug Al(OH)₃ (aluminum hydroxide salt) in a total volume of 500 ul. Page 13 of the study report indicates that the vaccine lot used is composed of HPV-18 L1 VLP only and contains 20 ug instead of 50 ug MPL. However, GSK clarified in a teleconference that this is a typographical error. Note that the submission contained a COA indicating the correct vaccine formulation.

Adjuvant (AS04D): Lot -----b(4)-----: Mono-dose vial containing 50 ug MPL and 500 ug Al(OH)₃ (aluminum hydroxide salt) in a total volume of 500 ul. A COA was contained in the submission.

Animal model: As stated by the sponsor, the rat was chosen “because of its use as a predictor of reproductive toxic change in man and the requirement for a rodent species by regulatory agencies. The -b(4)- strain was used because of the historical control data available in the laboratory.”

Treatment regimen: The IM route is the intended clinical ROA. The dose volume of 100 ul of HPVpro/AS04D candidate vaccine per animal per treatment represents one fifth the human dose, 47 times the human dosage relative to body weight (based on a 70 kg human and 300 g rat). Animals received the test article by bolus IM administration at a volume of 100 ul/rat in the anterior thigh, with alternate hind limbs used for subsequent injections.

Sponsor states that the analyses of blood serum obtained from dams and fetuses for vaccine induced antibody was performed by the sponsor as a separate study (refer to review of Module 4.2.3.5.2 Annex 1: Serological report of reprotoxicity study BVR/249).

Study timing:

| | | |
|------------------------|---------|----------|
| Animals arrived | January | 8, 2003 |
| Pre-Immunization bleed | January | 14, 2003 |
| Treatment (pre-mating) | January | 19, 2003 |

| | |
|-------------------------|-------------------|
| Animals paired | February 18, 2003 |
| Treatment commenced | February 25, 2003 |
| Necropsy completed | April 10, 2003 |
| Experimental completion | July 18, 2003 |

Animals: A total of 240 virgin female ---b(4)----- rats (of ---b(4)----- origin) weight range (30 days prior to pairing) 127-179 g (32-36 days of age) were obtained from ---b(4)-----, allowed 6 days acclimatization prior to pre-immunization bleed, and housed in stainless steel cages, 1 rat/cage after mating. Animals were allocated to treatment groups by selecting animals from each body weight range (± 5 g ranges).

Fifty-six (56) female -----b(4)----- rats were assigned to 4 different treatment groups:

| Group | Treatment | Dosage (vol/inj.) | Treatment days | No. females | Animal number |
|-------|---|-------------------|---|-------------|---------------|
| 1 | Saline | 100 ul | Day -30 prior to mating, and days 6, 8, 11, 15 after mating | 56 | 1001-1056 |
| 2 | Saline premating, then HPVpro/AS04D vaccine | 100 ul | Saline: Day -30 prior to mating Then vaccine: days 6, 8, 11, 15 after mating | 56 | 1057-1112 |
| 3 | HPVpro/AS04D vaccine | 100 ul | Day -30 prior to mating, and days 6, 8, 11, 15 after mating | 56 | 1113-1168 |
| 4 | AS04D | 100 ul | Day -30 prior to mating, and days 6, 8, 11, 15 after mating | 56 | 1169-1224 |

- 56 animals were allocated to each group so that 44 females could be selected with positive indication of mating, GD0: when positive evidence of mating was detected
- excess animals were killed on GD 6

Of the 44 animals continued for each group, half were sacrificed on day 20 after mating, and 22 animals were allowed to rear their young to day 25 of age.

Clinical observations (F0 females):

Animals were observed 2x daily for evidence of their reaction to treatment or ill health; a more detailed examination was performed on each animal once each week; the injection sites were examined daily for local swelling and reddening from day -30 prior to pairing to day 25 of lactation.

Detailed observations were made in association with dosing according to the following frequency: pre-dose observations including injection sites, as the animals are returned to

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their cages, at the end of dosing each group, between 1 and 2 hours after completion of dosing all groups, as late as possible in the work day.

Body weight of the F₀ females was obtained on day -30, treatment day and weekly until mating; after mating on days 0, 3, 6, 8, 11, 15, 17 and 20; during lactation on days 1, 4, 7, 11, 14, 18, 21 and 25.

Food consumption was monitored weekly during the premating phase; on GDs 0-2, 3-5, 6-7, 8-10, 11-14, 15-16 and 17-19 and on LD 1-3, 4-6, 7-10, 11-13, 14-17, 18-20 and 21-24.

Mating procedure females were paired on a one-to-one basis with stock males of the same strain. The day on which evidence of mating was found (copulation plugs, presence of spermatozoa in vaginal smears) was designated GD0.

Duration of gestation was defined as the time elapsing between detection of mating and commencement of parturition. From day 20 post-mating, all littering females were checked 3x daily for evidence of parturition. Individual gestation lengths were calculated from the day of mating to day of parturition.

Offspring observations-littering phase

All offspring were examined 24 hours after birth and each live offspring assessed for surface righting reflex, also recorded for each litter were:

Number born (live and dead)

Individual bodyweights of live offspring (days 1, 4, 7, 11, 14, 18, 21 and 25 of age)

Individual sexes

Observations on individual offspring

Litters were observed daily for evidence of ill health or reaction to maternal treatment.

Mortality and litter size

Daily records were maintained for mortality and litter size, dead offspring were examined externally and internally.

Litters were culled to 10 (5M + 5F, where possible) on day 4 of age (culling was used to standardize the rate of growth of the offspring).

Sex ratio was recorded on days 1 and 4 (before and after culling) and 25 days of age.

Body weight: Offspring were weighed individually on days 1 and 4 and then on days 7, 11, 14, 18, 21 and 25.

Pre-weaning examination on the F₁ generation include surface righting (from day 1 of age until achieved), air righting reflex (from day 14 of age until achieved), auditory function (day 20 of age, startle response to sudden noise), visual function (pupil reflex on day 20 of age).

Blood sampling-antibody assays

From all excess animals at day 6 after mating.

All F₀ females were blood sampled (0.8 ml from retro-orbital sinus) on days -35 and -5 prior to pairing, at termination (GD 20 or LD 25).

All fetuses from 11 litters/group had fetal cord blood samples obtained on GD 20.

Three male and 3 female offspring in all litters had blood samples obtained (from retro-orbital sinus) on day 25; culled offspring had blood samples obtained by decapitation on day 4.

Macroscopic pathology

All adult females:

Macroscopic pathology entailed a macroscopic examination for evidence of disease or adverse reactions to treatment (abnormal tissue retained). Necropsy included a detailed examination of external features and orifices, neck and associated tissues and the thoracic, abdominal and pelvic cavities and their viscera.

Animals allocated to embryo-fetal phase:

Necropsy was done at day 20 after mating; recorded were:

Number of corpora lutea in each ovary, number of implantation sites, number of resorption sites (early/late), number and distribution of fetuses (live and dead), sex of fetuses, weight of fetuses, weight of placentae, external abnormalities of individual fetuses and placentae.

All fetuses examined externally, half of each litter allocated to fresh visceral examination at necropsy and subsequent skeletal examination with the other half allocated to visceral examination after free-hand serial sectioning.

Blood samples for antibody assay taken from all parent females and cord blood sampled from all fetuses from 11 litters per group.

Animals allocated to post-natal phase:

F₀ females failing to produce a viable litter were necropsied at day 25 after mating. F₀ females that produced a litter were examined for macroscopic abnormalities and number of implantation sites recorded on day 25 of lactation.

F₁ males and females were necropsied on day 25 of age.

Data processing

Embryo-fetal phase: data were presented for body weight, food consumption, reproductive tract, pre-implantation loss, post-implantation loss, external, visceral and skeletal findings from fetuses, fetal weights and placental weights.

Peri/post-natal phase: data were presented for body weights, food consumption, gestation index, post-implantation survival index, live birth index, viability index, lactation index, sex ratio, audio/visual function test.

Data were expressed as group means with standard deviations. Findings from external, visceral and skeletal examination of fetuses were tabulated on an individual basis and as group incidences of fetuses and litters affected. Findings were classified as major abnormalities (considered detrimental and are usually rare) and minor abnormalities/variants (little detrimental effect on the animal, transient changes and frequently occurring in the control population).

RESULTS

Summary of adult performance

| Group | 1 | 2 | 3 | 4 |
|--|----|----|----|----|
| Mated | 44 | 44 | 44 | 44 |
| Allocated to embryo-fetal phase | 22 | 22 | 22 | 22 |
| Not pregnant | 1 | 0 | 0 | 0 |
| With live young at day 20 after mating | 21 | 22 | 22 | 22 |
| Allocated to littering phase | 22 | 22 | 22 | 22 |
| Not pregnant | 0 | 0 | 0 | 1 |
| With live young on day 25 of lactation | 22 | 22 | 22 | 21 |

Mortality/Clinical signs

There were no treatment related reactions and animals showed good health. Injection site observations were restricted to two animals in group 3 (1121: red discharge at injection site after –30 day immunization; 1148: swelling of the site on days 19 and 20 of gestation).

There were no effects of treatment on bodyweight or body weight change prior to pairing or throughout gestation and lactation.

Gestation Body Weight (gram)

| Group | | Day | | | | | | | |
|----------|------|-----|-----|-----|-----|-----|-----|-----|-----|
| | | 0 | 3 | 6 | 8 | 11 | 15 | 17 | 20 |
| 1 | Mean | 263 | 282 | 299 | 309 | 327 | 353 | 379 | 427 |
| | SD | 22 | 24 | 26 | 27 | 29 | 31 | 34 | 39 |
| | N | 43 | 43 | 43 | 43 | 43 | 43 | 43 | 43 |
| 2 | Mean | 259 | 278 | 293 | 304 | 321 | 348 | 376 | 424 |
| | SD | 19 | 21 | 21 | 21 | 22 | 23 | 25 | 28 |
| | N | 44 | 44 | 44 | 44 | 44 | 44 | 44 | 44 |
| 3 | Mean | 256 | 275 | 290 | 300 | 318 | 343 | 370 | 418 |
| | SD | 19 | 19 | 20 | 21 | 22 | 22 | 23 | 27 |
| | N | 44 | 44 | 44 | 44 | 44 | 44 | 44 | 44 |
| 4 | Mean | 259 | 278 | 293 | 304 | 323 | 349 | 375 | 423 |
| | SD | 21 | 24 | 25 | 26 | 28 | 29 | 31 | 35 |
| | N | 43 | 43 | 44 | 43 | 43 | 43 | 43 | 43 |

| Group | | Post-partum Body Weight (gram) | | | | | |
|----------|------|--------------------------------|-----|-----|-----|-----|-----|
| | | Day | | | | | |
| | | 1 | 4 | 11 | 14 | 18 | 25 |
| 1 | Mean | 321 | 335 | 362 | 365 | 362 | 335 |
| | SD | 25 | 24 | 27 | 28 | 24 | 27 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 |
| 2 | Mean | 325 | 339 | 336 | 370 | 366 | 335 |
| | SD | 22 | 22 | 22 | 24 | 24 | 20 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 |
| 3 | Mean | 322 | 337 | 367 | 371 | 363 | 332 |
| | SD | 19 | 19 | 22 | 24 | 25 | 26 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 |
| 4 | Mean | 321 | 335 | 361 | 361 | 360 | 331 |
| | SD | 34 | 33 | 33 | 34 | 26 | 33 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 |

Food consumption

Raw data contained in Appendices 5 - 7 showed that maternal food consumption was not adversely influenced by vaccination throughout gestation.

Necropsy findings of adult females

Necropsy findings were unremarkable for each treatment regime.

Caesarean data (Table 12, App. 8, Table 13, App. 9 of module 4.2.3.5.2.1)

With the exception of one control female, all animals allocated to the embryo-fetal assessment on day 20 after mating were pregnant and had live litters at day 20 of gestation.

Litter data on GD 20 did not indicate treatment related effects as assessed by the mean numbers of corpora lutea, implantations, sex ratio or pre- or post-implantation losses. Furthermore, there appeared to be no treatment related effect on mean placental, litter and fetal weights.

Caesarean Data Summary

| Group | | 1 | 2 | 3 | 4 |
|-------------------------|------|-------|-------|-------|-------|
| Corpora lutea | Mean | 18.8 | 17.0 | 17.1 | 17.1 |
| | SD | 3.6 | 2.3 | 1.9 | 2.4 |
| | N | 21 | 22 | 22 | 22 |
| Implan- tations | Mean | 17.2 | 16.0 | 16.1 | 16.2 |
| | SD | 2.3 | 1.6 | 2.3 | 1.5 |
| | N | 21 | 22 | 22 | 22 |
| Early re- sorptions | Mean | 1.0 | 0.8 | 1.0 | 1.0 |
| | SD | | | | |
| | N | 21 | 22 | 22 | 22 |
| Late re- sorptions | Mean | 0.0 | 0.0 | 0.0 | 0.0 |
| | SD | | | | |
| | N | 21 | 22 | 22 | 22 |
| Total re- sorptions | Mean | 1.0 | 0.8 | 1.0 | 1.0 |
| | SD | | | | |
| | N | 21 | 22 | 22 | 22 |
| Live males | Mean | 7.2 | 7.2 | 7.4 | 7.0 |
| | SD | 2.1 | 2.4 | 2.8 | 2.1 |
| | N | 21 | 22 | 22 | 22 |
| Live females | Mean | 9.0 | 8.0 | 7.7 | 8.2 |
| | SD | 2.1 | 2.4 | 2.7 | 1.7 |
| | N | 21 | 22 | 22 | 22 |
| Total live | Mean | 16.2 | 15.2 | 15.1 | 15.2 |
| | SD | 1.9 | 1.9 | 2.6 | 2.1 |
| | N | 21 | 22 | 22 | 22 |
| % pre-impl. loss | Mean | 8.0 | 6.8 | 6.6 | 6.5 |
| | SD | | | | |
| | N | 21 | 22 | 22 | 22 |
| % post-impl. loss | Mean | 5.9 | 4.9 | 6.5 | 6.4 |
| | SD | | | | |
| | N | 21 | 22 | 22 | 22 |
| Sex ratio % male | Mean | 44.4 | 47.3 | 48.5 | 45.9 |
| | SD | | | | |
| | N | 21 | 22 | 22 | 22 |
| Fetal body weight | Mean | 3.70 | 3.76 | 3.79 | 3.73 |
| | SD | 0.24 | 0.24 | 0.25 | 0.23 |
| | N | 21 | 22 | 22 | 22 |
| Placental Weight | Mean | 0.51 | 0.53 | 0.54 | 0.53 |
| | SD | 0.04 | 0.05 | 0.05 | 0.07 |
| | N | 21 | 22 | 22 | 22 |
| Litter Weight | Mean | 59.83 | 56.97 | 57.10 | 56.89 |
| | SD | 6.88 | 7.34 | 10.36 | 8.9 |
| | N | | | | |

TABLE 16

Fetal examinations - minor skeletal abnormalities/variants - group incidences

| Group | Fetuses | | | | Litters | | | |
|---|----------|------------------|------------------|----------|----------|------------------|------------------|----------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Treatment pre-mating | Saline | Saline | HPV pro/ASO4D | ASO4D | Saline | Saline | HPV pro/ASO4D | ASO4D |
| Treatment post-mating | Saline | HPV pro/ASO4D | HPV pro/ASO4D | ASO4D | Saline | HPV pro/ASO4D | HPV pro/ASO4D | ASO4D |
| Number examined | 169 | 164 | 166 | 164 | 21 | 22 | 22 | 22 |
| Cranial sutural bone | - | - | - | 1 | - | - | - | 1 |
| Vertebral element abnormality | | | | | | | | |
| thoracic | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 2 |
| lumbar | 1 | - | - | - | 1 | - | - | - |
| Ribs thickened/kinked | 3 | 2 | - | - | 2 | 2 | - | - |
| Irregularly ossified | - | 1 | - | - | - | 1 | - | - |
| Total affected by one or more of the above | 6 | 3 | 1 | 3 | 4 | 3 | 1 | 3 |
| Rib and vertebral configuration | | | | | | | | |
| Cervical rib | 4 | - | - | 4 | 3 | - | - | 3 |
| Short/absent 13 th rib(s) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Number with 13/14 or 14/14 ribs | 20 | 15 | 20 | 13 | 10 | 9 | 12 | 9 |
| Complete 14 th rib(s) | - | 1 | - | - | - | 1 | - | - |
| 20 thoracolumbar vertebrae | - | 1 | - | - | - | 1 | - | - |
| Offset alignment pelvic girdle | - | 1 | - | - | - | 1 | - | - |
| Incomplete ossification | | | | | | | | |
| Cranial centres | 24 | 24 | 25 | 16 | 9 | 15 | 11 | 7 |
| Hyoid | 9 | 9 | 11 | 3 | 7 | 6 | 9 | 3 |
| Vertebrae | | | | | | | | |
| cervical | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 1 |
| thoracic | 10 | 9 | 3 | 4 | 8 | 9 | 2 | 4 |
| lumbar | 1 | 1 | - | - | 1 | 1 | - | - |
| sacrocaudal | 9 | 13 | 12 | 8 | 7 | 11 | 8 | 4 |
| Sternebrae | | | | | | | | |
| 5 th and/or 6 th | 95 | 98 | 85 | 79 | 21 | 21 | 21 | 21 |
| other | 6 | 3 | 5 | 6 | 5 | 3 | 2 | 5 |
| total | 95 | 98 | 86 | 81 | 21 | 21 | 21 | 21 |
| Pelvic bones | 6 | 9 | 9 | 6 | 4 | 6 | 5 | 4 |
| Metacarpals/metatarsals | 1 | 2 | 5 | 3 | 1 | 2 | 2 | 3 |
| Precocious ossification | | | | | | | | |
| Cervical vertebral centra (>3 ossified) | 12 | 11 | 4 | 12 | 6 | 5 | 3 | 9 |
| Additional observations at necropsy | | | | | | | | |
| Renal cavitation | 3 | 4 | 4 | 3 | 3 | 4 | 3 | 2 |
| Hydroureter | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 2 |
| Shiny skin | - | - | 1 | 1 | - | - | 1 | 1 |
| Left umbilical artery | - | 1 | 2 | 1 | - | 1 | 2 | 1 |

Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

TABLE 15

Fetal examinations - minor visceral abnormalities - group incidences

| Group | | Fetuses | | | | Litters | | | |
|---------------------------|----------------------------|---------|------------------|------------------|-------|---------|------------------|------------------|-------|
| | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Treatment pre-mating | | Saline | Saline | HPV pro/ASO4D | ASO4D | Saline | Saline | HPV pro/ASO4D | ASO4D |
| Treatment post-mating | | Saline | HPV pro/ASO4D | HPV pro/ASO4D | ASO4D | Saline | HPV pro/ASO4D | HPV pro/ASO4D | ASO4D |
| Number examined | | 171 | 169 | 166 | 169 | 21 | 22 | 22 | 22 |
| Number affected | | 28 | 32 | 41 | 30 | 15 | 17 | 18 | 16 |
| Eye(s) | variation in size | 1 | - | 2 | - | 1 | - | 2 | - |
| | dilated orbital sinus | - | - | 1 | - | - | - | 1 | - |
| Thyroid | rudimentary/small | 2 | 2 | - | - | 2 | 2 | - | - |
| Thymus | partially undescended | 1 | - | 3 | - | 1 | - | 3 | - |
| Innominate artery | absent/rudimentary | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 1 |
| Ventricular septal defect | small | - | - | 1 | 1 | - | - | 1 | 1 |
| Azygos vein | narrow | - | - | 1 | - | - | - | 1 | - |
| Lungs | partially/fused lobes | - | 1 | 1 | 1 | - | 1 | 1 | 1 |
| Inferior vena cava | premature branching | - | - | 1 | - | - | - | 1 | - |
| Diaphragm | thin with protruding liver | 8 | 10 | 7 | 7 | 8 | 7 | 5 | 4 |
| Liver | thinning | 1 | - | 2 | - | 1 | - | 2 | - |
| | additional lobe | - | 1 | 1 | 1 | - | 1 | 1 | 1 |
| Kidney(s) | rudimentary/absent papilla | - | 2 | - | 1 | - | 2 | - | 1 |
| Ureter(s) | displaced | - | - | - | 1 | - | - | - | 1 |
| | dilated | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Uterine horn wall | dark area | - | - | - | 1 | - | - | - | 1 |
| Testis(es) | displaced | 4 | 3 | 2 | 3 | 4 | 3 | 2 | 3 |
| Umbilical artery | left sided | - | 1 | 2 | 1 | - | 1 | 2 | 1 |
| Haemorrhages | | | | | | | | | |
| Brain/spinal cord | | 4 | 5 | 5 | 4 | 3 | 5 | 4 | 4 |
| Eye/surrounding tissue | | - | 1 | - | - | - | 1 | - | - |
| Dorsal fat pad | | - | - | 1 | - | - | - | 1 | - |
| Intra-thoracic | | - | - | 1 | - | - | - | 1 | - |
| Intra-abdominal | | 1 | 1 | 3 | 4 | 1 | 1 | 3 | 4 |
| Hepatic | | 1 | 2 | 1 | 3 | 1 | 2 | 1 | 3 |
| Subcutaneous | | 7 | 5 | 10 | 7 | 5 | 3 | 8 | 6 |

Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

The type and distribution of visceral and skeletal findings in the treated groups were comparable to those in control animals. However, in group 3 treated with HPVpro/ASO4D one fetus out of 166 evaluated was observed to have a small ventricular septal defect. In group 4 treated with ASO4D before and after mating, one fetus out of 169 treated was observed to have a small ventricular septal defect. The sponsor states that a review of recent historical control data from studies conducted in the same

laboratory revealed that this observation had not been reported in the control populations in the last 15 studies. The sponsor attributes the occurrence of this isolated finding in the present study to a spontaneous etiology unrelated to treatment with candidate vaccine. In addition, the sponsor performed an extended review of the incidence of small intraventricular septal defect (IVSD) covering 98 studies performed between 1996 and 2005 presumably at ----b(4)----- (included as Annex 3 in 4.2.3.5.2 study report -b(4)-249/033160). Four (4) cases of IVSD occurred in control animals (12259 fetuses examined, 0.033%) with slightly higher incidences in treated groups whereby treatment was not specified (e.g., “high dose” group had 9 cases of IVSD [9406 fetuses examined, 0.096%]). The sponsor attributes this finding to delayed fetal development and considers the low incidence in the HPV study of no biological significance.

Skeletal findings that were considered major abnormalities, were a) absent central aspect cervical vertebral arches and were observed in 1 fetus (total eval. 334) in group 2 receiving HPVpro/AS04D vaccine during gestation, b) fused cervical vertebral arches observed in 1 fetus (total eval. 335) in group 4 receiving adjuvant only and c) split sternum observed in 1 fetus (total eval. 335) in group 4 receiving adjuvant only. Note that these findings did not occur in group 3 receiving HPV/AS04D vaccine prior to and during gestation. The sponsor has submitted historical control data from 36 embryo-fetal studies with similar necropsy/fetal examinations performed between 2001 and 2005 (included as Annex 3). Data show that absent/misshapen cervical vertebra were recorded in the historical control data base. However, fused cervical vertebral arches, split sternum and azygos vein narrow, were not detected in the historical control data. Since these findings occurred sporadically in the HPV study, they are likely not treatment related.

Parturition and post-partum litter data (App. 12, 14-15, 16-17, 18, 19)

All animals allocated to the littering phase were pregnant and subsequently gave birth to a litter, the duration of gestation was within the expected range of 21.5 - 23 days and unaffected by treatment. Clinical signs did not indicate any effects of treatment. The number of offspring born, sex ratio and subsequent survival to day 25 of age were similar in all groups. There was no effect of maternal treatment on bodyweight of either male or female offspring. There was no effect of maternal treatment on the mean age of attainment for surface righting or air reflex righting. All offspring assessed for auditory and visual responses on day 20 of age were successful with the exception of one group 3 offspring that could not be assessed for pupil reflex. Macropathological examinations of offspring killed at day 25 of age revealed no findings considered to be related to maternal treatment.

| | | Post-partum litter data | | | | | Total litter size Day 1 |
|-------|----|-------------------------|------|------|------|------|-------------------------------|
| Group | | Total impl. | | | | | |
| 1 | X | 15.6 | 14.1 | 13.6 | 9.6 | 9.6 | 14.4 |
| | SD | 2.9 | 3.0 | 3.4 | 1.3 | 1.3 | 3.0 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 |
| 2 | X | 16.6 | 15.4 | 15.2 | 9.9 | 9.9 | 15.6 |
| | SD | 1.6 | 1.4 | 1.4 | 0.4 | 0.4 | 1.5 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 |
| 3 | X | 16.0 | 15.0 | 14.6 | 10.0 | 10.0 | 15.1 |
| | SD | 1.6 | 1.8 | 1.7 | 0.2 | 0.2 | 1.8 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 |
| 4 | X | 15.6 | 15.0 | 14.6 | 9.6 | 9.6 | 15.1 |
| | SD | 2.6 | 2.7 | 2.7 | 0.9 | 0.9 | 2.7 |
| | N | 21 | 21 | 21 | 21 | 21 | 21 |

| Post-partum litter data (cont.) | | | | |
|---------------------------------|---|------------------------------|------------------|---------------------------|
| Group | | Post implant. Surv. index | Live birth index | Lactation index Day 25 |
| 1 | X | 91.7 | 98.1 | 100.0 |
| | N | 20 | 22 | 22 |
| 2 | X | 94.2 | 98.4 | 98.6 |
| | N | 22 | 22 | 22 |
| 3 | X | 94.5 | 99.1 | 99.5 |
| | N | 22 | 22 | 22 |
| 4 | X | 96.0 | 99.1 | 97.6 |
| | N | 21 | 21 | 21 |

Study report BVR249/033160 Addendum 1: Pre- and post-natal development study with VLP16/VLP18 vaccine (HPVpro/AS04D) administered IM in rats

The purpose of this addendum is to present the fertility data for the female rats vaccinated with HPVpro/AS04D. The data presented include mating/pregnancy status for females used on study as well as data from females not selected for the continuation through pregnancy/lactation. Pregnancy information for these females had been retained as transcribed data within the records for the stock males. Results from the non-selected females (12/group) were presented for completeness. Mating and fertility data were collected as part of the original protocol and retained with the raw data but findings had not been presented in the original report as the study focused on the effects of vaccination during pregnancy and lactation. The study included a group (3) that consisted of females treated with the HPVpro/AS04D prior to mating (-30 day) as well as during gestation (e.g., GDs 6, 8, 11 and 15). Significant anti-VLP16 and anti-VLP18 antibody responses were observed in these females prior to pairing. The addendum provides the individual animal and summary data on the mating performance and fertility of the females used in the study and assesses the effect of pre-mating vaccination and antibody levels on fertility. As stated above 56 females/group were treated before pairing, 22 females/group were each allocated to the subgroup scheduled for Caesarean sectioning and natural delivery. The remaining 12 females per group were not allocated to the study but were terminated before projected day of littering and uterine contents were assessed for evidence of pregnancy.

Mating and fertility

Female fertility did not appear to be affected by immunization with HPVpro/AS04D prior to mating as indicated by numbers of pregnant females and numbers of implantation counts (see tables 1 and 2 reproduced below).

TABLE 1

Mating, allocation and fertility data

| | | | | | |
|-----------------------|---|--------|--------------|--------------|-------|
| Group | : | 1 | 2 | 3 | 4 |
| Treatment pre-mating | : | Saline | Saline | HPVpro/AS04D | AS04D |
| Treatment post-mating | : | Saline | HPVpro/AS04D | HPVpro/AS04D | AS04D |

| Group | 1 | 2 | 3 | 4 |
|-----------------------------------|----|-----|-----|-----|
| Mating data: | | | | |
| Females paired | 56 | 56 | 56 | 56 |
| Females mated | 55 | 55 | 56 | 56 |
| Females pregnant | 54 | 55 | 56 | 55 |
| Animal distribution to subgroups: | | | | |
| Allocated to Day 20 pc kill | 22 | 22 | 22 | 22 |
| Allocated to give birth | 22 | 22 | 22 | 22 |
| Not used # | 12 | 12 | 12 | 12 |
| Fertility indices (%): | | | | |
| Mating index: Mated/paired | 98 | 98 | 100 | 100 |
| Conception index: Pregnant/mated | 98 | 100 | 100 | 98 |
| Fertility index: Pregnant/paired | 96 | 98 | 100 | 98 |

TABLE 2

Implantation counts

| | | | | | |
|-----------------------|---|--------|--------------|--------------|-------|
| Group | : | 1 | 2 | 3 | 4 |
| Treatment pre-mating | : | Saline | Saline | HPVpro/AS04D | AS04D |
| Treatment post-mating | : | Saline | HPVpro/AS04D | HPVpro/AS04D | AS04D |

| Sacrifice day: | | Day 20 pc | Day 21 pp | Spare females# | Overall |
|----------------|------|-----------|-----------|----------------|---------|
| Group 1 | Mean | 17.2 | 15.6 | 15.7 | 16.3 |
| | SD | 2.3 | 2.9 | 3.6 | 2.9 |
| | n | 21 | 22 | 11 | 54 |
| Group 2 | Mean | 16.0 | 16.6 | 15.7 | 16.2 |
| | SD | 1.6 | 1.6 | 2.1 | 1.7 |
| | n | 22 | 22 | 11 | 55 |
| Group 3 | Mean | 16.1 | 16.0 | 16.8 | 16.2 |
| | SD | 2.3 | 1.6 | 2.4 | 2.1 |
| | n | 22 | 22 | 12 | 56 |
| Group 4 | Mean | 16.2 | 15.6 | 16.6 | 16.1 |
| | SD | 1.5 | 2.6 | 1.7 | 2.0 |
| | n | 22 | 21 | 12 | 55 |

pc = post coitum

pp= post partum

Implantation counts based on the recorded number of pups.

Pre-coital interval and mating evidence

Within each group, the incidence of females with pre-coital intervals of 1, 2, 3, or 4 days closely approximated 25 % of the population which is consistent with the expected values for rats exhibiting a normal 4 day estrous cycle. The numbers of copulation plugs and the estimates of sperm numbers in the vaginal washings were similar in all groups

Conclusion: Based on the parameters evaluated vaccination with HPVpro/ASO4D does not appear to affect female fertility.

Serology data: Annex 1: Serological report of study -b(4)-249/033160

Dams: Serological evaluations of study -b(4)-/249 were performed on serum samples collected from dams on days -35 and -5 prior to pairing, on GD day 6 after mating (on excess animals of groups 2, 3 and 4 that were bled and killed), on GD 20 (on 22 pregnant females from groups 2, 3 and 4 that were bled and sacrificed) and on LD 25 (on the remaining females of each group).

Fetuses: On GD20, 11 pregnant females from each group underwent C-sectioning. Blood was collected from their fetuses and sera from fetuses of the same litter were pooled.

Pups: On day 4 of age, pups in excess per litter were bled; sera from these pups of the same litter were pooled. On day 25 of lactation, 3 males and 3 females were sacrificed in all litters, sera were pooled (1 pool of 3 males and 1 pool of 3 females offspring for each litter) for serological analysis.

Seroconversion rates, anti-VLP16 and anti-VLP 18 geometric mean titers were determined for all groups. Quantitation of antibodies was performed by ELISA using VLP16 and VLP18 as coating antigens.

Anti-VLP16 and anti-VLP 18 antibody responses

| Groups | Schedule | Animals | Timing | Anti-VLP 16 | | Anti-VLP 18 | |
|--------|---|---------|---------------|--------------|-----------|--------------|-----------|
| | | | | S.C | GMT | S.C | GMT |
| 1 | Saline (D-30, 5, 8, 11, 15) | Dams | D-35 (n=56) | 0 % | < 15 | 0 % | < 5 |
| | | | D25 (n = 22) | 0 % | < 15 | 0 % | < 5 |
| | | Fetuses | GD20 (n = 11) | 0 % | < 15 | 0 % | < 5 |
| 2 | Saline (D-30) vaccine (D6, 8, 11, 15) | Dams | D-5 (n =56) | 0 % | < 15 | 0 % | < 5 |
| | | | GD6 (n = 11) | 0 % | < 15 | 0 % | < 5 |
| | | | GD20 (n = 22) | 100 % | 1567 | 100 % | 1800 |
| | | | D25 (n = 22) | 100 % | 1082 | 100 % | 2889 |
| | | Fetuses | GD20 (n = 11) | 100 % | 220 | 100 % | 254 |
| | | Pups | D4 (n = 22) | 100 % | 713 | 100 % | 855 |
| | | (M/F) | D25 (n = 22) | 100 % | 1161/1192 | 100 % | 2755/2851 |
| 3 | Vaccine (D-30, 6, 8, 11, 15) | Dams | D-5 (n =56) | 100 % | 2114 | 100 % | 2305 |
| | | | GD6 (n = 12) | 100 % | 1778 | 100 % | 1943 |
| | | | GD20 (n = 22) | 100 % | 3428 | 100 % | 5180 |
| | | | D25 (n = 22) | 100 % | 2102 | 100 % | 3617 |
| | | Fetuses | GD20 (n = 11) | 100 % | 827 | 100 % | 1026 |
| | | Pups | D4 (n = 21) | 100 % | 1325 | 100 % | 2230 |
| | | (M/F) | D25 (n = 22) | 100 % | 1726/1779 | 100 % | 3272/3379 |
| 4 | AS04D (D-30, 6, 8, 11, 15) | Dams | D-5 (n =56) | 0 % | < 15 | 0 % | < 5 |
| | | | GD6 (n = 12) | 0 % | < 15 | 0 % | < 5 |
| | | | GD20 (n = 22) | 100 % | 291 | 100 % | 335 |
| | | | D25 (n = 21) | 71 % (15/21) | 87 | 86 % (18/21) | 63 |
| | | Fetuses | GD20 (n = 11) | 82 % (9/11) | 53 | 91 % (10/11) | 28 |
| | | Pups | D4 (n = 19) | 37 % (7/19) | 46 | 53 % (10/19) | 18 |
| | | M | D25 (n = 21) | 76 % (16/21) | 62 | 76 % (16/21) | 51 |
| | | F | D25 (n = 21) | 71 % (15/21) | 91 | 71 % (15/21) | 87 |

n = number of animals tested

M: pool of male offspring F: pool of female offspring

GMTs calculated on responders

Overall the results suggest that the vaccine antigens are immunogenic in pregnant animals and also suggest a transfer of antibodies from vaccinated dams to fetuses and offspring during development *in utero* and lactation. No anti-VLP16 and VLP18 antibodies were detected in the sera collected from dams or fetuses in saline-treated control animals. Anti-VLP16 and VLP18 antibody responses were observed in 100% of dams after 4 and 5 IM administrations of the HPVpro/AS04D candidate vaccine given during pregnancy or 1 month prior to and during pregnancy, respectively. Antibody responses were observed in 100% of fetuses, as well as in 100% of the pups from dams receiving 4 or 5 vaccine administrations. However, there were also weak anti-VLP16 and anti-VLP18 antibody responses observed in 100% of dams receiving AS04D adjuvant. Although weak, these responses were well above the cut-off of the ELISA (15 EU/ml and 5 EU/ml respectively for anti-VLP16 and anti-VLP18 serologies). In addition, some seroconversion was also observed in the related fetuses and pups. The sponsor initiated an investigation in order to explain this finding. Mix-up between vaccine adjuvant vials as well as formulation flow sheets were considered unlikely, remaining traces of antigen when preparing/formulating the adjuvant was checked in the

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adjuvant batch used in -b(4)-249 (e.g., ----b(4)----) by ELISA and ----b(4)----- and was found negative, inadvertent vial labeling was considered unlikely as well as inadvertent labeling of serum samples. Retesting of all serum samples from the adjuvant group animals confirmed the positive results. Testing of sera from rats that received another adjuvant system (AS04C) alone with the specific anti-VLP 16 & 18 ELISA's did not show a positive response nor did sera from mice injected with AS04A adjuvant. An immunological study was conducted in non-pregnant rats to investigate potential contamination of the AS04D adjuvant lot. No anti-VLPs 16/18 antibodies were observed with the AS04D lot used in -b(4)-249 or a AS04D control lot.

Study audits focused on dosing and bleeding records and included raw data review and detailed discussions with staff regarding SOPs in place. No deviations or deficiencies were identified. In summary, an explanation as to why adjuvant treated animals displayed low levels of circulating anti-VLP16 and anti-VLP18 L1 antibody titers have not been found.

Comments:

1. In this particular animal model and under the test conditions studied, Caesarean and post-partum data, necropsy findings from adult females as well as fetal examinations, suggest that, overall, the HPVpro/AS04D vaccine does not affect pre- and postnatal/pre-weaning development.

However, fetal examinations revealed to observations of ventricular septal defect, one in group 3, treated before and after mating with HPVpro/AS04D, in one fetus out of 166 evaluated (0.6 % fetuses affected). In group 4, treated with AS04D alone before and after mating, one fetus out of 169 (0.69 % fetuses affected) was observed to have a ventricular septal defect. The sponsor stated that a review of recent studies conducted in the same laboratory revealed that this observation had not been reported in the control population in the last 15 studies. The sponsor then performed an extended review of the incidence of intraventricular septal defect (IVSD) covering 98 studies performed between 1996 and 2005 at ----b(4)----- (included as Annex 3 in 4.2.3.5.2 study report -b(4)-249/033160). Four (4) cases of IVSD occurred in control animals (12259 fetuses examined, 0.033%) with slightly higher incidences in treated groups whereby treatment was not specified (e.g., "high dose" group had 9 cases of IVSD [9406 fetuses examined, 0.096%]).

In addition, among the malformations observed in supportive study 1729/8-D6154 conducted in rabbits treated with MPL there were 2 cases of major ventricular septal defects, one in groups 3 (MPL intermediate dose group, 10 ug/kg/day) and one in group 4 (MPL high dose group, 100 ug/kg/day). The incidence (mean % fetuses) was 0.6 in group 3 and 0.5 in group 4. This malformation did not occur in the low dose MPL group and/or in the saline control group. Sponsor provided laboratory standard data for ----b(4)----- rabbits, supplied by ----b(4)----- used in embryo-fetal studies at -b(4)----- since February 1994.

The cumulative incidence of ventricular septal defect (major) in rabbits was 0.12%.

It is not clear whether the finding of ventricular septal defect observed in the pivotal study -b(4)- 249 and study 1729/8 – D6154 is a treatment related finding, since it is isolated in nature, i.e., 1 fetus per litter/group and since it was also observed in the historical control data. However, the incidence of ventricular septal defect in both studies is higher than in the historical control. Furthermore, in the rabbit study treated with MPL, this finding occurred in the higher dose groups only and in the pivotal study conducted in rats treated with HPV/AS04D groups 3 and 4 received additional doses of adjuvant prior to mating.

Therefore, the sponsor was asked to perform a post-hoc statistical analysis of the data from pivotal study -b(4)- 249 and study 1729/8 to further evaluate the statistical significance of this finding. In addition, the sponsor was asked to provide a justification supporting the statement that this finding represents a delay in fetal development and an explanation of their finding of the IVSD being “small” as used to describe the finding in study -b(4)- 249.

2. Skeletal findings that were considered major abnormalities, were a) absent central aspect cervical vertebral arches and were observed in 1 fetus (total eval. 334) in group 2 receiving HPVpro/AS04D vaccine during gestation, b) fused cervical vertebral arches observed in 1 fetus (total eval. 335) in group 4 receiving adjuvant only and c) split sternum observed in 1 fetus (total eval. 335) in group 4 receiving adjuvant only. Note that these findings did not occur in group 3 receiving HPV/AS04D vaccine prior to and during gestation. The sponsor has submitted historical control data from 36 embryo-fetal studies with similar necropsy/fetal examinations performed between 2001 and 2005 (included as Annex 3). Data show that absent/misshapen cervical vertebra were recorded in the historical control data base. However, fused cervical vertebral arches, split sternum and azygos vein narrow, were not detected in the historical control data. However, since these findings occurred sporadically in the HPV study, they are likely not treatment related.
3. HPVpro/AS04D vaccine did not affect fertility of the female rats.
4. Weak anti-VLP16 and anti-VLP18 antibody responses were observed in 100% of dams receiving AS04D adjuvant. The sponsor's investigation as to the cause of this finding revealed no deviations or deficiencies were identified. Although an explanation as to why adjuvant treated animals displayed low levels of circulating anti-VLP16 and anti-VLP18 L1 antibody titers has not been found, this would not jeopardize the conclusion of the study results regarding effects of the vaccine on embryo/fetal pre- and postnatal development.

Review Memorandum

Date: November 16, 2007

From: Marion F. Gruber, Ph.D, OVR

Subject: **STN 125259/0** “Human Papillomavirus Type 16 and Type 18 Virus Like Particle (recombinant L1, ---b(4)----- cells and *Trichoplusia ni* cells) Vaccine with Alum and 3D-Monophosphoryl Lipid A Adjuvant; ---b(4)---, for the prevention of HPV-16 + HPV-18 infection”:

- Module 4.2.3.5. Reproductive and Developmental Toxicity
 - 4.2.3.5.2. Embryo-fetal development
 - 4.2.3.5.2.1 **Study report 1729/7 – D6154**
MPL: Subcutaneous Study of embryo-fetal development in the rat

Submission: March 29, 2007

Sponsor: GSK

Background: GSK has submitted a BLA for “Human Papillomavirus Type 16 and Type 18 Virus Like Particle Vaccine with Alum and Monophosphoryl Lipid A Adjuvant” indicated for immunization of adolescents and adults for the prevention of Human Papillomavirus infections types HPV-16 and HPV-18.

To support the proposed indication the sponsor has conducted a pivotal developmental toxicity study in the rat entitled “HPVPro/AS04D Prophylactic Human Papillomavirus Type 16 and Type 18 Candidate Vaccine Adjuvanted with AS04D: Study of effects on Pre and Post natal Development in -b(4)-Rats by Intramuscular Administration (including pre mating immunization phase).” The audited final report -b(4)- 249/033160 is contained in Module 4.2.3.5.2.1 of this submission.

Data from study 1729/7 – D6154 was requested by CBER to obtain additional information on the MPL adjuvant and its potential effects on embryo-fetal development.

Objective: to assess the effects of MPL on the embryonic and fetal development of the rat when administered subcutaneously

Test article composition: **MPL** -----b(4)----- (April 1999) manufactured by RIBI ImmunoChem Research

Animal model: As stated by the sponsor, the rat was chosen “because of its acceptance by regulatory authorities and availability of background data

Treatment regimen: Test and control articles administered s.c. to mated female rats daily from day 6 to 17 of gestation, inclusive, animals were injected at one of 4 areas, rotating daily, (left shoulder, right shoulder, left hip, right hip), animals were necropsied on day 20, fetuses were removed, killed and examined

Study timing:

| | |
|-----------------------|--------------------|
| Animals arrived | July 16 & 23, 1999 |
| Treatment (initiated) | July 19, 1999 |
| Necropsy completed | August 11, 1999 |

Animals: A total of 96 healthy time-mated female ----b(4)----- rats, 9 wks of age, weight range 175.7-267.9 g were obtained from ----b(4)----- . Mating was confirmed by the presence of a vaginal plug or sperm in a vaginal smear. The day on which mating was observed was designated day 0 of gestation, females delivered to -b(4)--- by day 3 of gestation. On day 4 of gestation the animals were assigned to treatment groups using a randomization procedure based on day of gestation and body weight. Ninety-six (96) female rats were assigned to 4 different treatment groups: saline control, 1, 10 and 100 ug/kg/day of MPL (group assignment shown in table below)

Dose levels

The following dose levels were selected:

| Group number | Group description | Dose level (µg/kg/day) | Dose concentration (µg/mL) | Dose volume (mL/kg) | Number of females |
|--------------|-------------------|---------------------------|-------------------------------|------------------------|-------------------|
| 1 | Control | 0 | 0 | 5 | 24 |
| 2 | Low | 1 | 0.2 | 5 | 24 |
| 3 | Intermediate | 10 | 2 | 5 | 24 |
| 4 | High | 100 | 20 | 5 | 24 |

The dose levels were selected in conjunction with the Sponsor. The high dose level was 100 x the human therapeutic dose. Individual dose volumes were adjusted according to the latest recorded body weight.

Clinical observations:

Animals were observed at least once daily for evidence of ill health or overt toxicity; animals were also observed at intervals up to 4 hours after dosing for signs of reaction to treatment.

Body weight of the F₀ females were recorded on days 4, 6, 7, 8, 9, 12, 15, 17 and 20 of gestation.

Food consumption was recorded for days 4 - 6, 6 - 7, 7 - 8, 8 - 9, 9 - 12, 12 - 15, 15 - 17 and 17 - 20 of gestation

Macroscopic pathology

All adult females: pregnancy status, gravid uterus weight, number of corpora lutea, number and intrauterine position of implantation sites, number of resorption (early/late), number and distribution of fetuses (live and dead)

All fetuses were examined externally and sexed; half of the fetuses of each litter were dissected and the viscera examined. They were then eviscerated and stained for skeletal examinations.

RESULTS

Mortality/Clinical signs

There were no treatment related reactions and all animals survived to scheduled kill (Table 1 reproduced below). Clinical observations were unremarkable. There were no effects of treatment on bodyweight or body weight change throughout gestation.

TABLE 1
Summary of female performance

| Test article Group Level (µg/kg/day) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 |
|--|-------------------|-----------|----------------|-----------|
| Number of animals: | Group 1 | Group 2 | Group 3 | Group 4 |
| In group | 24 | 24 | 24 | 24 |
| Not pregnant | 2 | 4 | 1 | 1 |
| Pregnant (%) | 22 (91.7) | 20 (83.3) | 23 (95.8) | 23 (95.8) |
| Died/killed | 0 | 0 | 0 | 0 |
| With total embryo/foetal loss | 0 | 0 | 0 | 1 |
| With live fetuses on Day 20 | 22 | 20 | 23 | 22 |

Food consumption

Raw data contained in Appendices 4 showed that maternal food consumption was not adversely influenced by treatment throughout gestation.

Caesarean data (Table 1 App. 5.1 of module 4.2.3.5.2.1)

There were no test article related effects on Caesarean parameters as shown in Table 6 reproduced below. One female in the high dose group (no 83) showed total embryo-fetal loss and had only 2 implantations, both were early intrauterine deaths. However, implantation occurred prior to initiating of treatment (day 7) so that this event is unlikely to be due to treatment. The pregnancy rate was not affected and there were no adverse effects of treatment on the uterine/ implantation data.

TABLE 6
Group mean caesarian data

| Test article Group Level (µg/kg/day) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 | |
|---|-------------------|---------|----------------|----------|------------|
| 6.1 Uterine/implantation data | | | | | |
| | Group 1 | Group 2 | Group 3 | Group 4 | Statistics |
| Number of females with live fetuses at Day 20 gestation | 22 | 20 | 23 | 22 | |
| Mean number of corpora lutea per female | 15.6 | 16.7 | 15.7 | 16.1 | J |
| Mean number of implantations per female | 13.2 | 13.5 | 13.7 | 13.9 | J |
| Pre-implantation loss: | | | | | |
| Mean % | 13.4 | 15.8 | 12.1 | 12.7 | |
| Number of dams affected | 12 | 18* | 14 | 16 | F+ |
| Early intrauterine deaths: | | | | | |
| Mean number | 1.2 | 1.1 | 0.6 | 0.6 | |
| Number of dams affected | 14 | 11 | 10 | 11 | F+ |
| Late intrauterine deaths: | | | | | |
| Mean number | 0.0 | 0.1 | 0.0 | 0.1 | |
| Number of dams affected | 0 | 1 | 0 | 3 | DR* F+ |
| Dead fetuses: | | | | | |
| Mean number | 0.0 | 0.0 | 0.0 | 0.0 | |
| Number of dams affected | 0 | 0 | 0 | 0 | X |
| Post-implantation loss: | | | | | |
| Mean % | 8.5 | 7.8 | 4.2 | 5.7 | |
| Number of dams affected | 14 | 11 | 10 | 13 | F+ |
| Mean number of fetuses per female | 12.0 | 12.4 | 13.2 | 13.1 | J |

F+ = Cochran-Armitage and Fisher's exact tests, one-sided for increasing incidence
J = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon Rank Sum tests
X = not analysed

* P<0.05
** P<0.01
*** P<0.001
DR = significant dose response

TABLE 6
Group mean caesarian data

| Test article Group Level (µg/kg/day) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 | |
|---|-------------------|---------|----------------|----------|------------|
| 6.2 Foetal data - females with live foetuses | | | | | |
| | Group 1 | Group 2 | Group 3 | Group 4 | Statistics |
| Number of male foetuses | 120 | 132 | 145 | 143 | |
| Number of female foetuses | 144 | 115 | 150 | 145 | |
| % male foetuses | 46.5 | 53.4 | 47.7 | 49.0 | J |
| Mean litter weight (g) | 44.57 | 45.27 | 49.18 | 48.84 | DR* J |
| Mean placental weight (g) | 0.54 | 0.56 | 0.54 | 0.57 | J |
| Mean foetal weight (g) | 3.73 | 3.66 | 3.74 | 3.75 | J |
| Mean foetal weight (g) - males only | 3.85 | 3.71 | 3.86 | 3.86 | J |
| Mean foetal weight (g) - females only | 3.63 | 3.60 | 3.62 | 3.65 | J |
| J = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon Rank Sum tests * P<0.05 ** P<0.01 *** P<0.001 DR = significant dose response | | | | | |

TABLE 6
Group mean caesarian data

| Test article Group Level (µg/kg/day) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 | |
|--|-------------------|---------|----------------|----------|------------|
| 6.3 Foetal defect data | | | | | |
| | Group 1 | Group 2 | Group 3 | Group 4 | Statistics |
| <u>EXTERNAL AND VISCERAL DEFECTS</u> | | | | | |
| Number of foetuses examined | 264 | 247 | 303 | 288 | |
| Number of litters examined | 22 | 20 | 23 | 22 | |
| Number showing malformations | 3 | 1 | 1 | 1 | |
| Mean % of foetuses examined | 1.3 | 0.5 | 0.3 | 0.3 | |
| Number of litters affected | 3 | 1 | 1 | 1 | F+ |
| Number showing variations | 37 | 22 | 27 | 37 | |
| Mean % of foetuses examined | 15.3 | 9.1 | 9.6 | 12.5 | |
| Number of litters affected | 15 | 11 | 13 | 15 | F+ |
| <u>SKELETAL DEFECTS</u> | | | | | |
| Number of foetuses examined | 131 | 122 | 151 | 143 | |
| Number of litters examined | 22 | 20 | 23 | 22 | |
| Number showing malformations | 0 | 0 | 0 | 0 | |
| Mean % of foetuses examined | 0.0 | 0.0 | 0.0 | 0.0 | |
| Number of litters affected | 0 | 0 | 0 | 0 | X |
| Number showing variations | 115 | 114 | 131 | 127 | |
| Mean % of foetuses examined | 87.0 | 93.7 | 88.7 | 89.0 | |
| Number of litters affected | 22 | 20 | 23 | 22 | X |
| Total number of foetuses showing malformations | 3 | 1 | 1 | 1 | |
| % of foetuses examined | 1.1 | 0.4 | 0.3 | 0.3 | |
| Number of litters affected | 3 | 1 | 1 | 1 | F+ |
| F+ = Cochran-Armitage and Fisher's exact tests, one-sided for increasing incidence X = not analysed | | | | | |

Visceral malformations occurred in 3 fetuses in the control group and one fetus in each of the low, intermediate and high dose groups. Sponsor attributes these to spontaneous occurrence in the rat strain.

| Group No | Dam No | Fetus No | Malformation |
|----------|--------|----------|---|
| 1 | 6 | L1 | Interrupted aortic arch, right subclavian artery arising from descending aorta (may be retro-oesophageal) |
| | 12 | R8 | Retro-oesophageal subclavian artery, subclavian artery arising from descending aorta |
| | 17 | R5 | Hydronephrosis |
| 2 | 47 | R2 | Internal hydrocephaly |
| 3 | 68 | L8 | Hydronephrosis |
| 4 | 83 | L10 | Abnormal lung lobe formation (major); situs inversus (thorax); right-sided pulmonary trunk |

External and visceral malformation and variations observed in this study were unaffected by treatment. They were in the range of what is observed in the historical control database and occurred in the untreated (control) as well as in the treated groups. There were no skeletal malformations. The number of fetuses showing skeletal variations was in the range of that observed in the historical control database. In general, incidences of skeletal variations were unaffected by treatment. There were slightly more fetuses with incomplete or non-ossification of the hyoid arch in the treated groups compared to control group (i.e., mean % fetuses with hyoid arch not ossified in control: 16.6 %, in dose group 1: 18%, in dose group 2: 19.8% and in dose group 3: 21 % (historical control data: 11.3%). However, the mean % fetuses affected in the control group was also above that observed on the historical control data base (i.e., 16.6 % in the control group, 11.3 % in the historical control data base). In addition, there were more fetuses with extra thoraco-lumbar ribs in the treated groups compared to the control group, but there was no dose response effect (i.e., mean % fetuses affected in control : 7.2 %, in dose group 1: 17.8%, in dose group 2: 9.8% and in dose group 3: 24.5 % (historical control data: 12.4%).

Summary: The current preclinical study no. 1729/7 – D6154 was a preliminary reproductive toxicology study to assess the potential of MPL adjuvant on embryo-fetal development in the rat. The study did not identify any reproductive or developmental risks. In addition, formulation, dosing schedule and route of administration used in current study 1729/7 – D 6154 differed from those proposed in the clinical study in that MPL adjuvant was used at doses of 1, 10 and 100 ug/kg/day in the absence of vaccine antigen, using daily dosing and given via the sc route. Together study 1729/7 – D6154 serves as a supportive study to pivotal study -b(4)-249 demonstrating absence of teratogenic effects of MPL immunostimulant under the conditions of the study using the rat model.

Review Memorandum

Date: April 25, 2007

From: Marion F. Gruber, Ph.D, OVR

Subject: **STN 125259/0** “Human Papillomavirus Type 16 and Type 18 Virus Like Particle (recombinant L1, -----b(4)----- cells and *Trichoplusia ni* cells) Vaccine with Alum and 3D-Monophosphoryl Lipid A Adjuvant; - b(4)-----, for the prevention of HPV-16 + HPV-18 infection”:

- Module 4.2.3.5. Reproductive and Developmental Toxicity
 - 4.2.3.5.2. Embryo-fetal development
 - 4.2.3.5.2.1 **Study report 1729/8 – D6154**
MPL: Subcutaneous Study of embryo-fetal development in the rabbit

Submission: March 29, 2007

Sponsor: GSK

Background: GSK has submitted a BLA for “Human Papillomavirus Type 16 and Type 18 Virus Like Particle Vaccine with Alum and Monophosphoryl Lipid A Adjuvant” indicated for immunization of adolescents and adults for the prevention of Human Papillomavirus infections types HPV-16 and HPV-18.

To support the proposed indication the sponsor has conducted a pivotal developmental toxicity study in the rat entitled “HPVPro/AS04D Prophylactic Human Papillomavirus Type 16 and Type 18 Candidate Vaccine Adjuvanted with AS04D: Study of effects on Pre and Post natal Development in -b(4)-Rats by Intramuscular Administration (including pre mating immunization phase).” The audited final report -b(4)- 249/033160 is also contained in Module 4.2.3.5.2.1 of this submission.

Data from study 1729/8 – D6154 was requested by CBER to obtain additional information on the MPL adjuvant and its potential effects on embryo-fetal development.

Objective: to assess the effects of MPL on the embryonic and fetal development of the rabbit when administered subcutaneously

Test article composition: **MPL** Lot ----b(4)----- (April 1999) manufactured by RIBI ImmunoChem Research, now Corixa, (COI included in the final study report as Appendix 7).

Animal model: As stated by the sponsor, the rabbit was chosen “because of its acceptance by regulatory authorities and quantity of published background data”

Treatment regimen: Test and control articles administered s.c. to mated female rabbits daily from day 7 to 19 of gestation, inclusive, animals were injected at one of 4 areas, rotating daily, (left shoulder, right shoulder, left hip, right hip), animals were necropsied on day 29, fetuses were removed, killed and examined

Study timing:

| | | |
|-----------------------|---------------------|------|
| Animals arrived | August 11, 13 & 16, | 1999 |
| Treatment (initiated) | August 15, | 1999 |
| Necropsy completed | September 11, | 1999 |

Animals: A total of 96 healthy time-mated female (----b(4)----- strain) rabbits, 4-5 months of age, weight 2.5 kg, were obtained from ----b(4)----- . After coitus, each female received an IV injection of 25 IU chorionic gonadotropin to ensure ovulation. The day on which mating was observed was designated day 0 of gestation, females delivered to ---b(4)----- by days 1, 2, 3 of gestation. Animals were assigned to treatment groups using a randomization procedure based on day of gestation and body weight.

Ninety-six (96) female rabbits were assigned to 4 different treatment groups as shown in the table below: saline, 1, 10 and 100 ug/kg/day of MPL.

Dose levels

The following dose levels were selected:

| Group number | Group description | Dose level (ug/kg/day) | Dose concentration (ug/mL) | Dose volume (mL/kg) | Number of females |
|--------------|-------------------|------------------------|----------------------------|---------------------|-------------------|
| 1 | Control | 0 | 0 | 1.5 | 24 |
| 2 | Low | 1 | 0.67 | 1.5 | 24 |
| 3 | Intermediate | 10 | 6.67 | 1.5 | 24 |
| 4 | High | 100 | 66.7 | 1.5 | 24 |

The dose levels were selected in conjunction with the Sponsor. The high dose level was 100x the proposed human therapeutic dose. Individual dose volumes were adjusted according to the latest recorded body weight.

Clinical observations:

Animals were observed once daily for evidence of ill health or overt toxicity; animals were also observed at intervals up to 1 hours after dosing for signs of reaction to treatment.

Morbidity and mortality

All animals were examined 2 daily to detect any which were dead or moribund. One animal that aborted on day 28 was sacrificed and examined macroscopically.

Body weight of the F₀ females were recorded on days 4, 7, 8, 9, 12, 15, 19, 24 and 29 of gestation.

Food consumption was recorded daily from day 4 to 29 of gestation and reported on the body weight intervals.

Macroscopic pathology

All adult females: pregnancy status, gravid uterus weight, number of corpora lutea, number and intrauterine position of implantation sites subdivided into:

Live fetuses

Early intrauterine deaths

Late intrauterine deaths

Dead fetuses

Uteri of any apparently non-pregnant females was immersed in a 10% ammonium sulphide solution to reveal any evidence of implantation

Individual fetal and placental weights were recorded and foetuses were examined externally.

One half of the fetuses/litter were decapitated, heads placed in Bouin's solution for fixation and decalcification and transferred to alcohol, serial sections were examined. All fetuses were dissected, sexed and the viscera examined. They were then eviscerated and stained for skeletal examinations. Fetal abnormalities were classified as malformations (rare and/or potentially lethal) and variations (commonly occurring non-lethal abnormalities).

RESULTS

Mortality/Clinical signs

One low dose female aborted on day 28 of gestation and one intermediate dose female aborted on day 29 of gestation. All fetuses found, dead or alive, were of apparent normal development for the stage of gestation. In addition, there was one female in the low and 5 females in the intermediate MPL dosing group with total embryo/fetal loss. No total embryo/fetal loss occurred in the placebo or in the high dose MPL group. (Table 1 reproduced below)

TABLE 1
Summary of female performance

| Test article Group Level ($\mu\text{g/kg/day}$) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 |
|---|-------------------|-----------|----------------|-----------|
| Number of animals: | Group 1 | Group 2 | Group 3 | Group 4 |
| In group | 24 | 24 | 24 | 24 |
| Not pregnant | 2 | 2 | 1 | 3 |
| Pregnant (%) | 22 (91.7) | 22 (91.7) | 23 (95.8) | 21 (87.5) |
| Died/killed | 0 | 0 | 0 | 0 |
| Aborted and killed | 0 | 1 | 1 | 0 |
| With total embryo/foetal loss | 0 | 1 | 5 | 0 |
| With live foetuses on Day 29 | 22 | 20 | 17 | 21 |

Clinical observations of F0 rabbits were unremarkable (Table 2 reproduced below)

TABLE 2
Summary of clinical observations and necropsy findings

| Test article Group Level ($\mu\text{g/kg/day}$) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 |
|---|-------------------|-----------|----------------|-----------|
| 2.2 Necropsy findings | Group 1 | Group 2 | Group 3 | Group 4 |
| Number of animals examined* | 22 | 20 | 17 | 21 |
| Number of animals with finding: | | | | |
| Not remarkable (%) | 17 (77.3) | 19 (95.0) | 16 (94.1) | 17 (81.0) |
| Injection site - red | 2 | 1 | 1 | 4 |
| Placenta - raised area | 1 | | | |
| Skin subcutis - sore | 2 | | | |

* animals with live foetuses on Day 29

There were no effects of treatment on bodyweight or body weight change throughout gestation (Table 3 reproduced below).

TABLE 3
Group mean body weight data

| | | | | |
|-------------------|---------|---|-----|-----|
| Test article | Control | | MPL | |
| Group | 1 | 2 | 3 | 4 |
| Level (µg/kg/day) | 0 | 1 | 10 | 100 |

3.1 Body weight (kg)

| Day of gestation | Group 1 | Group 2 | Group 3 | Group 4 | Statistics |
|---------------------------------|---------|---------|---------|---------|------------|
| 4 | 3.38 | 3.51 | 3.45 | 3.48 | A |
| 7 | 3.47 | 3.60 | 3.55 | 3.57 | |
| 8 | 3.48 | 3.62 | 3.54 | 3.57 | |
| 9 | 3.48 | 3.62 | 3.53 | 3.58 | |
| 12 | 3.49 | 3.59 | 3.51 | 3.60 | |
| 15 | 3.44 | 3.59 | 3.53 | 3.58 | |
| 19 | 3.44 | 3.60 | 3.51 | 3.58 | |
| 24 | 3.62 | 3.68 | 3.64 | 3.71 | |
| 29 | 3.72 | 3.82 | 3.76 | 3.79 | |
| % body weight change Days 4-29 | 10.5 | 9.2 | 9.4 | 9.1 | |
| % body weight change Days 7-19 | -0.6 | 0.1 | -1.1 | 0.3 | |
| % body weight change Days 19-29 | 8.4 | 6.5 | 7.3 | 5.9 | |

A = ANOVA, regression and Dunnett's tests

Food consumption

Raw data contained in Appendix 4 showed that maternal food consumption was not adversely affected by treatment throughout gestation.

Caesarean Data (Table 6 reproduced below and App. 5.1 of module 4.2.3.5.2.1)

One animal in the low dose group and five animals in the intermediate dose group had total embryo-fetal loss. Of these 5 animals in the intermediate dose group, 3 animals had only 1 implantation, one female had 2 and one female had 4 implantations. In all animals, all implantations were early uterine deaths. Sponsor states that it is not unusual for litters to be resorbed in rabbits when the implantation rate is low. Of note, the first treatment started on day 7, thus, low number of implantations on these animals is unlikely due to treatment with MPL. In addition, no total embryo/fetal loss occurred in the high dose MPL group.

In females with live fetuses at caesarean sectioning, the mean numbers of corpora lutea were marginally lower in the MPL treated groups, compared to controls which resulted in a statistically significant dose-response, resulting in a small, dose related decrease in mean number of implantations. However, when compared to historical control data, all values were within the range of the historical control data (Appendix 8 of submission). Since treatment started on day 7 of gestation it is unlikely that the lower number of corpora lutea observed in MPL treated groups is due to MPL.

In the low dose group the mean number of late intrauterine deaths was slightly higher than expected from the current background data (0.2 to 0.7). However, there was no dose relationship, as this observation was not made in the intermediate and high dose MPL group.

TABLE 6
Group mean caesarian data

| Test article Group Level (µg/kg/day) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 | |
|---|-------------------|------------|----------------|------------|--|
| 6.1 Uterine/implantation data | | | | | |
| | Group 1 | Group 2 | Group 3 | Group 4 | Statistics |
| Number of females with live foetuses at Day 29 gestation | 22 | 20 | 17 | 21 | |
| Mean number of corpora lutea per female | 12.8 | 11.8 | 11.6 | 11.4 | DR* J |
| Mean number of implantations per female | 10.7 | 9.9 | 9.7 | 9.6 | J |
| Pre-implantation loss: mean % number of dams affected | 16.5 16 | 16.9 15 | 16.7 10 | 15.1 15 | F+ |
| Early intrauterine deaths: mean number number of dams affected | 0.7 8 | 0.4 6 | 0.2 2 | 0.5 7 | F+ |
| Late intrauterine deaths: mean number number of dams affected | 0.1 3 | 0.9 9 | 0.3 4 | 0.5 7 | F+ |
| Dead foetuses: mean number number of dams affected | 0.0 0 | 0.1 1 | 0.0 0 | 0.05 1 | X |
| Post-implantation loss: mean % number of dams affected | 9.1 11 | 13.9 13 | 5.2 6 | 10.3 13 | F+ |
| Mean number of foetuses per female | 9.9 | 8.5 | 9.2 | 8.5 | DR* J |
| F* = Cochran-Armitage and Fisher's exact tests, one-sided for increasing incidence J = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon Rank Sum tests X = not analysed | | | | | * P<0.05 ** P<0.01 *** P<0.001 DR = significant dose response |

Fetal data

Mean sex ratio was unaffected by treatment. In the low and high dose groups, mean placental and fetal weights were significantly higher than in the control group due to fewer fetuses in these groups.

Malformations were listed on pages 28-30 of the final report (table 6 reproduced below). The abnormalities observed are known to occur spontaneously in this strain of rabbit in the testing laboratories and their nature and intergroup- distribution do not indicate a

treatment related effect.

TABLE 6
Group mean caesarian data

| Test article Group Level (µg/kg/day) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 | |
|--|-------------------|---------|----------------|----------|------------|
| 6.3 Foetal defect data | | | | | |
| | Group 1 | Group 2 | Group 3 | Group 4 | Statistics |
| Number of foetuses examined | 217 | 170 | 156 | 179 | |
| Number of litters examined | 22 | 20 | 17 | 21 | |
| <u>EXTERNAL AND VISCERAL DEFECTS</u> | | | | | |
| Number showing malformations | 5 | 3 | 8 | 3 | |
| Mean % of foetuses examined | 3.4 | 1.5 | 4.8 | 1.5 | |
| Number of litters affected | 5 | 3 | 4 | 3 | F+ |
| Number showing variations | 44 | 28 | 20 | 44 | |
| Mean % of foetuses examined | 21.0 | 15.9 | 12.5 | 24.4 | |
| Number of litters affected | 21 | 13 | 10 | 17 | F+ |
| <u>SKELLETAL DEFECTS</u> | | | | | |
| Number showing malformations | 7 | 6 | 8 | 2 | |
| Mean % of foetuses examined | 3.1 | 3.2 | 5.7 | 1.2 | |
| Number of litters affected | 6 | 5 | 5 | 2 | F+ |
| Number showing variations | 183 | 162 | 151 | 153 | |
| Mean % of foetuses examined | 84.9 | 95.8 | 97.0 | 85.3 | |
| Number of litters affected | 22 | 20 | 17 | 21 | X |
| Total number of foetuses showing malformations | 11 | 9 | 16 | 5 | |
| % of foetuses examined | 5.1 | 5.3 | 10.3 | 2.8 | |
| Number of litters affected | 10 | 7 | 6 | 5 | F+ |

F+ = Cochran-Armitage and Fisher's exact tests, one-sided for increasing incidence
X = not analysed

Comment:

The current preclinical study no. 1729/8 – D6154 was a preliminary reproductive toxicology study to assess the potential of MPL adjuvant on embryo-fetal development in the rabbit. Formulation, dosing schedule and route of administration used in current study 1729/8 – D 6154 differed from those proposed in the clinical study in that MPL adjuvant was used at doses of 1, 10 and 100 ug/kg/day in the absence of vaccine antigen and alum and used daily dosing and the sc route.

In this study 1729/8-D6154 there were 2 cases of major ventricular septal defects, one (1) in groups 3 (MPL intermediate dose group, 10 ug/kg/day) and one in group 4 (MPL high dose group, 100 ug/kg/day) with an incidence of 0.6 % by fetus and 5.8% by litter in group 3 and 0.55% by fetus and 4.7 % by litter in group 4. This observation did not occur in the low dose MPL group and/or in the saline control group. Control group values from 6 embryo/fetal studies that preceded this study showed that of 1139 fetuses (118 litters) evaluated, there was 1 case of ventricular septal defect in study 4 (0.088% by fetus and 0.8% by litter). In addition the sponsor provided cumulative fetal defect data for ----b(4)----- rabbits, supplied by -----b(4)----- used in embryo-fetal studies at ---b(4)---since February 1994. The cumulative incidence of ventricular septal defect (major) in rabbits was 0.12%.

It is not clear whether the finding of intraventricular septal defect observed in this study and in pivotal study -b(4)- 249 is a treatment related finding, since it is isolated in nature, i.e., 1 fetus per litter/group and since it was also observed in the historical control data. However, concerning is that the incidence of ventricular septal defect in both studies is higher than in the historical control and did not occur in concurrent control groups. Furthermore, in this study (1729/8-D6154) in which animals were treated with MPL, this finding occurred in the higher dose groups only and in the pivotal study (-b(4)-249/033160) conducted in rats this finding occurred in groups 3 and 4, i.e. those groups that received HPV/AS04D or AS04D before and after mating.

The sponsor was asked to perform a post-hoc statistical analysis of the data from pivotal study -b(4)-249 and study 1729/8 to further evaluate the statistical significance of this finding. In addition, the sponsor was asked to provide a reference supporting the statement that this finding represents a delay in fetal development and an explanation of their finding of the IVSD being "small" as used to describe the finding in study -b(4)-249. The sponsor received this request on August 31, 2007 and provided a response October 3, 2007 (sequence #17 to the Cervarix BLA). In this response, the sponsor states that a demonstration of statistical significant increase in incidence of IVSD observed in -b(4)- 249 is not possible because the number of litters affected is below 5 and therefore, statistical tests are of minimal value in this analysis. Furthermore, sponsor states that the occurrence of IVSD in study 1729/8 is also not statistically significant. Sponsor concludes that the incidence of IVSD in rats and rabbits in studies -b(4)-249 and --b(4)--1729/8 is of spontaneous nature. Sponsor attributes the occurrence of membranous intraventricular septal defects to a delay in normal development that will close with further normal development.

Review Memorandum

Date: June 12, 2007

From: Marion F. Gruber, Ph.D., OVR

Subject: **STN 125259/0** “Human Papillomavirus Type 16 and Type 18 Virus Like Particle (recombinant L1, ---b(4)----- cells and *Trichoplusia ni* cells) Vaccine with Alum and 3D-Monophosphoryl Lipid A Adjuvant; -b(4)-----, for the prevention of HPV-16 + HPV-18 infection”:

- Module 4.2.3.5. Reproductive and Developmental Toxicity
 - 4.2.3.5.2. Embryo-fetal development
 - 4.2.3.5.2.1 **Study report 1729/17 – D6154**
MPL: Subcutaneous Study of pre-and postnatal development in the rat

Submission: March 29, 2007

Sponsor: GSK

Background: GSK has submitted a BLA for “Human Papillomavirus Type 16 and Type 18 Virus Like Particle Vaccine with Alum and Monophosphoryl Lipid A Adjuvant” indicated for immunization of adolescents and adults for the prevention of Human Papillomavirus infections types HPV-16 and HPV-18.

To support the proposed indication the sponsor has conducted a pivotal developmental toxicity study in the rat entitled “HPVPro/AS04D Prophylactic Human Papillomavirus Type 16 and Type 18 Candidate Vaccine Adjuvanted with AS04D: Study of effects on Pre and Post natal Development in -b(4)-Rats by Intramuscular Administration (including pre mating immunization phase).” The audited final report -b(4)-249/033160 is also contained in Module 4.2.3.5.2.1 of this submission.

Study 1729/17 – D6154 was requested by CBER to obtain additional data on the MPL adjuvant and its potential effects on pre- and postnatal development.

Objective: to assess the effects of MPL on the pre-and postnatal development, including maternal function, in the rat when administered subcutaneously

Test article composition: MPL Lot ---b(4)----- (received by ---b(4)---- in September 1999) COI **not** included in the final study report. Testing laboratory indicates that the expiry date of the test article was not stated by the sponsor.

Animal model: As stated by the sponsor, the rat was chosen “because of its acceptance by regulatory authorities and availability of background data”

Treatment regimen: Test and control articles administered s.c. to time-mated female rats daily from day 6 of gestation to day 21 post-partum, inclusive, females were allowed to litter and rear their offspring. Twenty animals of each sex were randomly selected from each group to form the F1 generation, animals were maintained untreated for up to 12 weeks post-weaning before being paired for up to 15 days. Mated F1 females were killed on day 13 of gestation and uterine contents were examined. The F1 males were killed in week 17.

Study timing:

| | | |
|-----------------------|--------------------|-------------------|
| Animals arrived | September 1 and 3, | 1999 |
| Treatment (initiated) | September 5, | 1999 |
| Necropsy completed | October 16, | 1999 (F0 females) |
| | February 15 | 2000 (F1 animals) |

Animals: A total of 96 healthy time-mated female (---b(4)----- strain) rats, 9 weeks of age, weighing at least 160 g, were obtained from -----b(4)----- . Mating was confirmed by the presence of a vaginal plug or sperm in a vaginal smear. The day on which mating was observed was designated day 0 of gestation, females delivered to --b(4)--- by day 2 of gestation. Animals were assigned to treatment groups using a randomization procedure based on day of gestation and body weight. Allocation of the F1 generation was by random selection of 20 males and 20 females from the available litters.

Ninety-six (96) female rabbits were assigned to 4 different treatment groups: 0, 1, 10 and 100 ug MPI/kg/day as shown on the table below.

The following dose levels were selected:

| Group number | Group description | Dose level (µg/kg/day) | Dose concentration (µg/mL) | Dose volume (mL/kg) | Number of P females |
|--------------|-------------------|---------------------------|-------------------------------|------------------------|------------------------|
| 1 | Control | 0 | 0 | 5 | 24 |
| 2 | Low | 1 | 0.2 | 5 | 24 |
| 3 | Intermediate | 10 | 2 | 5 | 24 |
| 4 | High | 100 | 20 | 5 | 24 |

The dose levels were selected in conjunction with the Sponsor. The high dose level was 100x the human therapeutic dose. Individual dose volumes were adjusted according to the latest recorded body weight.

Clinical observations:

Animals were observed once daily for evidence of ill health or overt toxicity; animals were also observed at intervals up to 1 hours after dosing for signs of reaction to treatment.

Morbidity and mortality

All animals were examined 2 daily to detect any which were dead or moribund.

Body weight of the F₀ females were recorded on days 4, 6, 7, 8, 9, 12, 15, 17 and 20 of gestation and on days 1, 4, 7, 14 and 21 *post-partum*.

F1 male body weights were recorded weekly. F1 female body weights were recorded weekly prior to pairing and until confirmation of mating and on days 0, 3, 6, 10 and 13 of gestation.

Food consumption was recorded for the F₀ females over days 4-6, 6-7, 7-8, 8-9, 9-12, 12-15, 15-17 and 17-20 of gestation and over days 1-4, 4-7, 7-14 and 14-21 *post-partum*. For F1 animals food intake was recorded weekly prior to pairing and for F1 females over days 0-3, 3-6, 6-10, and 10-13 of gestation.

Litter data:

F₀ females were allowed to litter and date of parturition and the duration of gestation were recorded.

For each litter to day 21 post-partum

- Number of pups born (live and dead)
- Daily live litter size and sex (reported days 1, 4, 7, 14 and 21)
- Daily clinical observations
- Individual pup weights on days 1, 4, 7, 14 and 21 post-partum
- Necropsy findings of dead and culled pups where conditions permitted

Litters were culled on day 4 to a maximum of 8 pups with an equal sex distribution, where possible; animals considered unlikely to survive to weaning were pre-selected for cull and a random selection procedure was used for additional pups

Development parameters

Pinna unfolding, incisor eruption, eye opening, surface righting reflex day 1 *post-partum*, air righting reflex day 17 *post-partum*, grip strength day 21 *post-partum*, pupillary reflex day 21 *post-partum*, auditory response day 21 *post-partum* and visual placing response day 21 *post-partum*. Responses were scored as 0 = no response, 1 = poor response and 2 = normal response

F1 physical development

Vaginal opening and balano-preputial separation were assessed daily from 30-40 days of age, respectively, until development was complete

F1 learning ability

During weeks 4 and 5 of the maturation phase all control and high dose group animals were assessed for their learning ability and behavior in the swimming maze

F1 motor activity

During week 4 the motor activity of control and high dose group animals was assessed by automated photocell activity records for 30 minutes, animals in low and intermediate groups were assessed at week 6, activity counts were recorded at 2 minute intervals.

F1 generation mating

After 12 week of maturation, one male was paired with one female of the same parental group, avoiding sibling pairings. Mating was confirmed by the presence of a vaginal plug in situ or sperm in a vaginal washing

F1 caesarean data

Mated females were killed on day 13 of gestation, ovaries and uteri removed and data recorded for

- Pregnancy status
- Number of corpora lutea
- Number and position of implantations subdivided into live embryos, early and late intrauterine deaths

Necropsy

All animals (except the F1 females) were killed and given a macroscopic examination, Uteri of females was immersed in a 10% ammonium sulphide solution and number of implantations was recorded

Tissue retention (from adult animals)

Ovaries, uterus, cervix, vagina, pituitary, gross lesions, testes, epididymides, seminal vesicles, prostate, coagulating gland

RESULTS

Mortality and P generation female performance

All animals survived to scheduled kill, 1 control F0 female, 2 low dose, 0 intermediate dose and 2 high dose F0 females had total litter loss (reproduced in Table 1 below).

TABLE 1
Summary of female performance - P generation

| | | | | |
|---|-----------|-----------|-----------|-----------|
| Test article | Control | | MPL | |
| Group | 1 | 2 | 3 | 4 |
| Level (µg/kg/day) | 0 | 1 | 10 | 100 |
| Number of animals: | Group 1 | Group 2 | Group 3 | Group 4 |
| In group | 24 | 24 | 24 | 24 |
| Not pregnant | 1 | 2 | 1 | 2 |
| Pregnant (%) | 23 (95.8) | 22 (91.7) | 23 (95.8) | 22 (91.7) |
| Died/killed | 0 | 0 | 0 | 0 |
| With total litter loss | 1 | 2 | 0 | 2 |
| With live pups at Day 21 <i>post-partum</i> | 22 | 20 | 23 | 20 |

Clinical observations

Clinical observations were recorded in Table 2 and Appendix 1 and were unremarkable.

Body weight and food intake

Group mean body weight gain (Figure, 1 Table 3, Appendix 2) was similar in all groups during gestation and lactation. There were no effects of treatment on bodyweight or body weight change throughout gestation. During gestation and lactation, group mean food intake of the treated females was similar to, or greater than, that of the controls.

Litter data F0 generation

Pup numbers and survival: One (1) control F0 female, 2 low dose, 0 intermediate dose and 2 high dose F0 females had total litter loss. The mean duration of gestation, mean number of implantations, number of pups born and pup sex ratio was similar in all groups (reproduced in Table 5 below).

TABLE 5
Group mean litter data - P generation

| Test article Group Level (µg/kg/day) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 | |
|---|-------------------|---------|----------------|----------|------------|
| 5.1 Pup numbers - females rearing young to weaning | | | | | |
| | Group 1 | Group 2 | Group 3 | Group 4 | Statistics |
| Number of females with live pups at Day 21 <i>post-partum</i> | 22 | 20 | 23 | 20 | X |
| Mean duration of gestation (days) | 22.3 | 22.4 | 22.1 | 22.2 | X |
| Mean number of implantation sites | 12.7 | 13.3 | 12.8 | 13.2 | J |
| Mean number of pups born | 11.3 | 12.0 | 11.8 | 12.2 | J |
| Mean number of pups alive Day 1 | 10.8 | 11.8 | 11.6 | 11.7 | X |
| Mean % male pups Day 1 | 45.1 | 52.1 | 52.6 | 51.7 | J |
| Mean number of pups alive Day 4 before culling | 10.1 | 11.3 | 10.9 | 10.8 | X |
| Mean number of pups culled Day 4 | 2.9 | 3.4 | 3.3 | 3.0 | X |
| Mean number of pups alive Day 4 after culling | 7.3 | 7.9 | 7.5 | 7.8 | X |
| Mean number of pups alive Day 7 | 7.3 | 7.9 | 7.5 | 7.8 | X |
| Mean number of pups alive Day 14 | 6.5 | 6.7 | 6.9 | 7.6 | X |
| Mean number of pups alive Day 21 | 6.4 | 6.7 | 6.9 | 7.6 | X |

J = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon Rank sum tests
X = not analysed

TABLE 5
Group mean litter data - P generation

| Test article Group Level (µg/kg/day) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 | |
|---|-------------------|---------|----------------|----------|------------|
| 5.2 Gestation and neonatal survival indices | | | | | |
| | Group 1 | Group 2 | Group 3 | Group 4 | Statistics |
| Gestation index % | 100.0 | 100.0 | 100.0 | 100.0 | X |
| Post-implantation survival index % | 88.9 | 90.0 | 92.8 | 91.5 | F- |
| Live birth index % | 96.5 | 98.2 | 97.9 | 96.3 | F- |
| Viability index 1 % | 93.8 | 96.1 | 93.7 | 92.4 | F- |
| Viability index 2 % | 100.0 | 100.0 | 100.0 | 100.0 | X |
| Viability index 3 % | 89.8 | 84.7 | 92.1 | 97.5 | F- |
| Viability index 4 % | 99.4 | 100.0 | 99.1 | 100.0 | F- |

F- = Cochran-Armitage and Fisher's exact tests, one-sided for decreasing incidence
X = not analysed

Clinical observations and necropsy findings of dead and culled F1 pups where conditions permitted

Appendix 4.3 (individual line listings) lists clinical observations and necropsy findings observed in the pre-weaning F1 progeny. Data were not summarized by sponsor, however, review of the line listings in appendix 4.3 revealed that clinical observations and necropsy findings were comparable among treatment groups and there were no apparent treatment-related observations.

Pup weights

Pup weights were summarized in Table 5.3 and appendices 4.4 and 4.7 and were comparable in all groups throughout the lactation period.

Physical development

Physical development was assessed by pinna unfolding, incisor eruption and eye opening. Data were comparable among treatment groups (Table 5.4 and Appendix 4.5).

Functional development

Functional tests included surface righting, air righting, auditory response, pupillary reflex, visual placing and grip strength. With the exception of grip strength, there were no apparent differences in the scoring between control and treated groups assessed at days 1, 17 and 21. Sponsor states that there was a statistically significant decreased dose response in the intermediate and high group for mean scores for the grip strength, assessed as the ability to grip and hang from a suspended wire. The biological significance of this observation is not clear. Furthermore, this test is not routinely applied to assess functional development and its validation is unclear. Since all other developmental tests reveal no differences between treatment and controls, this particular finding is not considered treatment related. (Table 5.5, Appendix 4.6)

Necropsy data (reproduced in Table 6 below)

Sponsor did perform a necropsy of females, including females that showed total litter loss. Findings were unremarkable. Necropsy examinations were also performed on F1 generations pups, i.e., dead and culled pups and revealed no remarkable findings. Of note is that this study did not include a pre-specified visceral and skeletal examination of the F1 pups as the study design did not contain a Caesarean subgroup.

TABLE 6
Summary necropsy data - P generation

| | | | | |
|-------------------|---------|---|-----|-----|
| Test article | Control | | MPL | |
| Group | 1 | 2 | 3 | 4 |
| Level (µg/kg/day) | 0 | 1 | 10 | 100 |

6.1 Females

| | Group 1 | Group 2 | Group 3 | Group 4 |
|---------------------------------|-----------|-----------|-----------|-----------|
| Number of animals examined* | 22 | 20 | 23 | 20 |
| Number of animals with finding: | | | | |
| Not remarkable (%) | 18 (81.8) | 16 (80.0) | 18 (78.3) | 15 (75.0) |
| Eye - protruding | | | | 1 |
| Injection site - red | 1 | 1 | | 1 |
| Kidney - pelvic dilatation | 1 | 1 | 1 | |
| Skin subcutis - hairloss | 2 | 3 | 2 | 3 |
| Urinary bladder - distension | 1 | | | |
| Uterus - distension | | | 2 | |

+ females with live pups at Day 21 *post-partum*

TABLE 6
Summary necropsy data - P generation

| | | | | |
|-------------------|---------|---|-----|-----|
| Test article | Control | | MPL | |
| Group | 1 | 2 | 3 | 4 |
| Level (µg/kg/day) | 0 | 1 | 10 | 100 |

6.2 F_{1a} weanlings

| | Group 1 | Group 2 | Group 3 | Group 4 |
|---------------------------------|-----------|-----------|------------|------------|
| Number of animals examined | 101 | 93 | 118 | 111 |
| Number of animals with finding: | | | | |
| Not remarkable (%) | 90 (89.1) | 83 (89.2) | 106 (89.8) | 101 (91.0) |
| Kidney - large | | 1 | | 1 |
| - pelvic dilatation | 10 | 10 | 9 | 10 |
| Skin subcutis - hairloss | 1 | | 1 | |
| - sore | | | 2 | |
| Tail - kinked | | | 1 | |
| Ureter - distension | | 1 | 1 | 1 |

F1 generation

Morbidity and mortality data were shown in Table 7 reproduced below. One female animal was sacrificed due to poor conditions, this animal was an offspring of F0 females treated with low does of MPL.

TABLE 7
Summary of adult performance - F₁ generation

| Test article Group | Control 1 | 2 | MPL 3 | 4 |
|--|--------------|-----------|-----------|------------|
| Level (µg/kg/day) | 0 | 1 | 10 | 100 |
| Number of animals: | Group 1 | Group 2 | Group 3 | Group 4 |
| Males | | | | |
| In group | 20 | 20+ | 20 | 20 |
| Died/killed | 0 | 0 | 0 | 0 |
| Inducing pregnancy | 19 | 15 | 18 | 20 |
| Females | | | | |
| In group | 20 | 20 | 20 | 20 |
| Died/killed before start of maturation phase | 0 | 1 | 0 | 0 |
| Not pregnant | 1 | 4 | 2 | 0 |
| Pregnant (%) | 19 (95.0) | 15 (75.0) | 18 (90.0) | 20 (100.0) |
| Died/killed | 0 | 0 | 0 | 0 |
| With total embryo loss | 0 | 0 | 0 | 0 |
| With live embryos at Day 13 of gestation | 19 | 15 | 18 | 20 |
| * one male not paired due to death of allocated female during maturation phase | | | | |

Clinical observations (pale teeth, damaged/missing paws, swollen paw, swollen tail, lachrymation, chromodacryorrhea, eyes half closed, hair loss, thinning fur, rough hair coat, sores/lesions of the F1 generation (20 males/group examined and 20 females/group examined except group 2 where 19 females were examined) during maturation and females during gestation were commonly occurring lesions and considered unrelated to treatment.

There were no differences in groups in F1 generation male of female body weight during the pre-pairing period or in female body weight gain during gestation (Appendix 7 and Table 9) and food intake was similar in all groups (Table 10, Appendix 8).

Learning ability was assessed by escape times and numbers of correct escapes in the swimming maze trail. Female and male results were separately recorded. Only animals whose parents where treated with control (group 1) or the highest MPL dose (group 4) were assessed. There were minor statistical differences observed between groups that are not likely considered treatment related (Table 11). Motor activity was also assessed and differences between groups were minor and likely contributable to differences in age. (Table 13).

Physical development assessed by balano-preputial separation and vaginal opening were unaffected by treatment (reproduced in Table 12 below)

TABLE 12
Summary of physical development data - F₁ generation

| Test article | | Control | | MPL | | | | | | | | | | | |
|---|---|---------|----|-----|-----|----|----|----|----|----|----|----|----|----|---|
| Group | | 1 | 2 | 3 | 4 | | | | | | | | | | |
| Level (µg/kg/day) | | 0 | 1 | 10 | 100 | | | | | | | | | | |
| 12.2 Vaginal opening | | | | | | | | | | | | | | | |
| Group and sex | Number of animals with complete development on Day post-partum: | | | | | | | | | | | | | | Mean day post-partum for complete development |
| | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | |
| 1F | 0 | 0 | 1 | 3 | 4 | 3 | 1 | 6 | 1 | 0 | 0 | 1 | 0 | 0 | 35.5 |
| 2F | 0 | 1 | 1 | 1 | 3 | 2 | 4 | 3 | 0 | 1 | 0 | 1 | 1 | 1 | 36.2 |
| 3F | 0 | 0 | 0 | 4 | 3 | 3 | 7 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 35.1 |
| 4F | 0 | 0 | 2 | 1 | 2 | 5 | 3 | 4 | 2 | 1 | 0 | 0 | 0 | 0 | 35.6 |
| Statistics | | | | | | | | | | | | | | | J |
| J = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon Rank sum tests | | | | | | | | | | | | | | | |

J = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon Rank sum tests

TABLE 12
Summary of physical development data - F₁ generation

| Test article | | Control | | MPL | | | | | | | | | | | |
|----------------------------------|---|---------|----|-----|-----|----|----|----|----|----|----|----|-------|----|---|
| Group | | 1 | 2 | 3 | 4 | | | | | | | | | | |
| Level (µg/kg/day) | | 0 | 1 | 10 | 100 | | | | | | | | | | |
| 12.1 Balano-preputial separation | | | | | | | | | | | | | | | |
| Group and sex | Number of animals with complete development on Day post-partum: | | | | | | | | | | | | | | Mean day post-partum for complete development |
| | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52-54 | 55 | |
| 1M | 0 | 0 | 2 | 1 | 3 | 5 | 2 | 3 | 2 | 0 | 0 | 2 | 0 | 0 | 45.8 |
| 2M | 0 | 0 | 1 | 3 | 4 | 0 | 5 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 45.4 |
| 3M | 0 | 0 | 0 | 6 | 3 | 5 | 0 | 0 | 5 | 0 | 0 | 1 | 0 | 0 | 45.3 |
| 4M | 0 | 0 | 1 | 1 | 5 | 2 | 3 | 3 | 2 | 2 | 0 | 0 | 0 | 1 | 46.2 |
| Statistics | | | | | | | | | | | | | | | J |

J = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon Rank sum tests

Mating data of F1 generation

The pre-coital time was 2-3 days in all groups and mating, fertility and fecundity indices were similar in all groups (see Table 14 reproduced below). Note that the F1 generation was not directly treated with test article. Sponsor states that one control, 4 low dose and 2 intermediate dose males failed to sire pregnancies after positive evidence of mating. At necropsy, one of the low dose males had only one epididymis and both testes were small and 1 of the intermediate dose males had small testes. The other of those males had normal reproductive organs.

TABLE 14
Group mating data – F₁ generation

| Test article Group Level (µg/kg/day) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 | |
|--|-------------------|---------|----------------|----------|------------|
| 14.3 Mating indices | | | | | |
| | Group 1 | Group 2 | Group 3 | Group 4 | Statistics |
| Number of paired males | 20 | 19+ | 20 | 20 | |
| Number of paired females | 20 | 19 | 20 | 20 | |
| Number of mated males | 20 | 19 | 20 | 20 | |
| Number of mated females | 20 | 19 | 20 | 20 | |
| Number of pairings inducing pregnancy | 19 | 15 | 18 | 20 | |
| Mating index % | 100.0 | 100.0 | 100.0 | 100.0 | X |
| Fertility index % | 95.0 | 78.9 | 90.0 | 100.0 | F- |
| Pecundity index % | 95.0 | 78.9 | 90.0 | 100.0 | F- |

+ one additional male not paired due to death of allocated females

F- = Cochran-Armitage and Fisher's exact tests, one sided for decreasing incidence
X = not analysed

Mean numbers of corpora lutea, implantations or incidence of pre-implantation loss was not adversely affected by maternal (F0) treatment (Table 15 reproduced below).

TABLE 15
Group mean uterine/implantation data - F₁ generation

| Test article Group Level (µg/kg/day) | Control 1 0 | 2 1 | MPL 3 10 | 4 100 | |
|--|-------------------|------------|----------------|------------|------------|
| | Group 1 | Group 2 | Group 3 | Group 4 | Statistics |
| Number of females with live embryos at Day 13 gestation | 19 | 15 | 18 | 20 | X |
| Mean number of corpora lutea per female | 17.6 | 17.5 | 17.6 | 18.2 | J |
| Mean number of implantations per female | 15.2 | 15.4 | 15.8 | 15.7 | J |
| Pre-implantation loss: mean % number of dams affected | 11.6 15 | 11.0 11 | 9.5 13 | 12.0 10 | F+ |
| Early intrauterine deaths: mean number number of dams affected | 0.5 7 | 0.9 8 | 0.5 7 | 0.6 8 | F+ |
| Late intrauterine deaths: mean number number of dams affected | 0.2 3 | 0.0 0 | 0.2 4 | 0.7 8 | DR* F+ |
| Post-implantation loss: mean % number of dams affected | 4.1 9 | 6.0 8 | 4.6 11 | 9.2 14 | F+ |
| Mean number of live embryos per female | 14.6 | 14.5 | 15.2 | 14.4 | J |

F+ = Cochran-Armitage and Fisher's exact tests, one sided for increasing incidence
 J = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon Rank Sum tests
 X = not analysed

* P<0.05
 ** P<0.01
 *** P<0.001
 DR = significant dose response

Necropsy examinations of the F1 generation and females did not indicate maternal treatment related effects.

Laboratory historical control data (Appendix 15) with the test species derived from 6 rat embryo-fetal studies that preceded the present study (1995- 1999) indicated that the Caesarean results observed in the present study was in the range of the historical control data.

Comments:

Administration of 1, 10 and 100 ug/kg/day MPL to the F0 generation from day 6 of gestation to day 21 of lactation had no affect on the F1 generation under the conditions of the study and parameters assessed.

The experimental design of this study does not follow current recommendations outlined in CBER's guideline regarding developmental toxicity studies for vaccines, ("Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications , 2006") however, this study was conducted prior to the availability of the guidance document. Of note is that this study did not include visceral and skeletal examinations of the F1 generation. Thus, the study is limited in terms of providing information on potential teratogenic effects due to MPL. However, other parameters assessed, such as uterine parameters, body weight, viability, and development of offspring suggest that MPL does not adversely affect pre-and postnatal development in the test species under the conditions of the study. A certificate of analysis for the test article, i.e., MPL was not provided nor was the expiry date for the test article indicated.

Despite these limitations results of this study can be taken as supplemental information supporting findings from the pivotal developmental toxicity study -b(4)-249 conducted with Cervarix.

REVIEW MEMORANDUM

From: Marion F. Gruber, Ph.D., OVRR

Date: June 09, 2009

Subject: Review Memorandum

STN 125259/028 Section 1.11.2

Safety Information Amendment; Partial Response to FDA Complete
Response letter (dated December 1, 2007),

Response to toxicology Question 12

Background: In support of their proposed indication for Cervarix, GSK has conducted a pivotal developmental toxicity study -b(4)-**249/033160** entitled “HPVPro/AS04D Prophylactic Human Papillomavirus Type 16 and Type 18 Candidate Vaccine Adjuvanted with AS04D: Study of effects on Pre and Post natal Development in -b(4)-rats by Intramuscular Administration (including pre-mating immunization phase)” and has submitted three additional reports from supportive developmental toxicity studies conducted with MPL adjuvant only, namely: **Study report 1729/8 – D6154: MPL: Subcutaneous Study of Embryo-Fetal Development in the rabbit**, **Study report 1729/7 – D6154 MPL: Subcutaneous Study of Embryo-Fetal Development in the rat** and **Study report 1729/17 – D6154: MPL: Subcutaneous Study of Pre-and Postnatal Development in the rat**.

In the pivotal study -b(4)-**249** in rats and in supportive study 1729/8-D6154 conducted in rabbits fetal examinations revealed observations of small membranous intraventricular septal defect (IVSD) (for details, refer to review memo of November 29, 2007). CBER stated in its communication to the sponsor of August 31, 2007, that it is not clear whether the finding of membranous ventricular septal defect observed in studies -b(4)-249 and --b(4)----- study 1729/8 –D6154 is a treatment related finding, since it is isolated (i.e., occurrence 1 fetus/litter/group and also observed in historical controls.) However, CBER was concerned because in the reported studies this event was not observed in concurrent controls and furthermore, the incidence is high compared to historical controls (in study -b(4)-249: 0.6% by fetus and 4.5% by litter whereas historical background rates are 0.033% to 0.096% by fetus or 0.235% to 0.683% by litter). Thus, the following comment was communicated in the CR letter:

Regarding the developmental toxicity studies:

As stated in our communication of August 31, 2007, it is not clear whether the finding of membranous ventricular septal defect observed in studies -b(4)-249 and -b(4)-----study 1729/8 – D6154 is a treatment related finding, since it is isolated (i.e., occurrence 1 fetus/litter/group and also observed in historical controls). However, we remain concerned because in the reported studies this event was not observed in concurrent controls and furthermore, the incidence is

high compared to historical controls (in study -b(4)-249: 0.6% by fetus and 4.5% by litter whereas historical background rates are 0.033% to 0.096% by fetus or 0.235% to 0.683% by litter). The Table entitled "Terminology of developmental abnormalities in common laboratory mammals" Version 2, 2006, (http://teratology.org/news_resources/DevToxTerms.htm), published by the Teratology Society suggests that a defect in the membranous ventricular septum may be associated with Tetralogy of Fallot, a congenital heart defect.

[censored text - clinical]

We acknowledge that VSD is reported to occur in 2-7% of human live births in the US and is considered a very common congenital anomaly that can resolve spontaneously. Furthermore, we note that the VSDs observed in study -b(4)- 249 conducted in rats and ---b(4)---- Study 1729/8-D6154 conducted in rabbits refer to the membranous septum and not to the muscular septum. *[censored text - clinical]* However, given the high incidence of ventricular septal defect in the reported developmental toxicity studies relative to concurrent and historical controls, and occurrence of this finding in more than one species, we request you:

- a. Please comment on the potential association of ventricular septal defect with HPV/AS04 vaccine.
- b. Please propose a risk assessment and risk management plan to address this observation.

GSK's response regarding question 12 a:

GSK Biologicals does not consider that there is any indication of a potential association between ventricular septal defect (VSD) and *Cervarix* administration as demonstrated in -b(4)- study -b(4)-249, based on experience with current and historical data from both GSK and contract laboratories. The company states that the observation of a single affected fetus in any group of animals in -b(4)- study -b(4)- 249 and ---b(4)---- study 1729/8-D6154 is typical for a spontaneous, background malformation such as VSD. The incidence of VSD is the minimal incidence that can be calculated for the group sizes used. Further support of this observation to be spontaneous is that VSD did not occur in multiple fetuses per litter, or in multiple litters per group. Sponsor states that the occurrence of a spontaneous background malformation such as VSD in the reported studies is typically variable across groups and across studies, due to animal strain variability. Therefore, it would be more appropriate to consider historical incidences across groups and across studies instead of the accumulated historical control incidence.

Sponsor states that in the historical control database (n=78 studies) of the laboratory (-b(4)-) that conducted rat study -b(4)- 249, a singly affected (VSD) fetus in a group occurred in control groups from 4 studies and in test-article treated groups from at least 6 Studies (event was deemed unrelated to test article). The incidence by fetus and litter for each of these 4 control groups ranged from 0.6% to 0.7% and 4.2% to 5.0%, respectively. Sponsor notes that the fetal and litter incidences of VSD in the vaccine treated groups of

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-b(4)-249 (0.6% and 4.5%, respectively) are of the same magnitude. Sponsor states that in both cases, it is the minimal incidence that can be counted for the group sizes used, and this is consistent with a spontaneous event. In addition, and as another point of reference, data among 67 rat developmental toxicity studies conducted within Safety Assessment at GlaxoSmithKline, showed 10 studies with VSD noted in the control group (incidence by fetus and by litter ranged from 0.6% to 2.4% and 4% to 10.5%, respectively) and 57 studies without VSD noted in the control group. Among 30 rabbit studies there were 3 studies with VSD noted in the control group (incidence by fetus and by litter ranged from 0.5% to 0.6% and 4.6% to 5.0%, respectively) and 27 studies without VSD noted in the control group. Sponsor states that these data reflect the group incidence magnitude and variability that is typical of spontaneous malformation.

Sponsor states that a lack of an association between VSD and *Cervarix* Administration is further substantiated by the observation that in study -b(4)-249, a single fetus with VSD was noted in group 3 (vaccine administered before and during pregnancy) but not in group 2 (vaccine given during pregnancy only). Because the sensitive period for heart development is during pregnancy, the lack of a concordant result between groups 2 and 3 suggests the VSD was not associated with vaccine treatment, but rather is a spontaneous event.

Also, in the ---b(4)--- rabbit study (1729/8-D6154) with MPL, sponsor states that there was no indication of a dose-responsive incidence of VSD. The incidence was 1 VSD at 10 ug MPL/kg/day and 1 VSD at 100 ug MPL/kg/day. Furthermore, the accompanying cardiac malformations for each of these fetuses were different: in the 10 ug MPL/kg/day fetus, the accompanying malformations were interrupted aortic arch, right subclavian artery arising from descending aorta and dilation of pulmonary trunk, and in the 100 ug MPL/kg/day fetus, the accompanying malformation was persistent truncus arteriosus.

Furthermore, GSK consulted with the authors of study -b(4)-249 concerning FDA's comments regarding a possible association between IVSD and 'Tetralogy of Fallot'. It was noted that 'Tetralogy of Fallot' is not a single defect, but rather it is a syndrome of four concurrent malformations, including: pulmonary stenosis, overriding aorta, hypertrophy of the right ventricle, and VSD. There were no instances of pulmonary stenosis, overriding aorta, or right ventricular hypertrophy in fetuses from the rat or the rabbit study. Authors state that the observation of VSD alone is not evidence of 'Tetralogy of Fallot,' because the syndrome only exists when all four defects occur simultaneously. Furthermore, cyanosis is expected to occur post natally with 'Tetralogy of Fallot,' and no cyanosis was reported in the pups in the postnatal portion of the BVR 249 rat study.

GSK's response regarding question 12 b:

GSK and its consulted experts, based on the reasons cited in response to item 12a of the CR letter, do not conclude that there is any indication of a potential association of VSD with *Cervarix* vaccine, based on the animal toxicity studies. However, since a pregnancy registry is already planned for implementation upon approval of *Cervarix* in the U.S. and

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the outcomes of all registered pregnancies are to be assessed, the potential for VSD will be adequately covered.

Reviewer's comment: GSK has satisfactorily refuted a potential association of IVSD with HPV/AS04 vaccine. The reviewer notes that GSK is planning a pregnancy registry following licensure of Cervarix in the US which will capture outcomes of registered pregnancies. In this reviewer/s opinion no further risk management plan to assess the observation of VSD is necessary.