Toxicology Review of Zoster (non-live) Vaccine

From: Nabil, Al Humadi, Claudia Wrzesinski

Through: Martin Green

To: Carmen Collazo, Michael Smith, Ramachandra Naik

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Product: Zoster vaccine, non-live, SHINGRIX™

Reviewer: Nabil Al-Humadi
BLA sections reviewed:
4.2.3.1 Single dose toxicity studies
4.2.3.2 Repeated dose toxicity studies
4.2.3.3 Genotoxicity (*in vivo* genotoxicity)
4.2.3.7 Other studies

Claudia Wrzesinski
BLA sections reviewed:
4.2.1.3 Safety pharmacology
4.2.3.3 Genotoxicity (*in vitro* genotoxicity)
4.2.3.5. Reproductive and Developmental Toxicity
4.2.3.6 Local Tolerance

Type and date of submission: Original, October 21st, 2016

Sponsor: GlaxoSmithKline Biologicals, S. A., Rue de l'Institute 89, Rixensart, Belgium

Proposed indication: Prevention of herpes zoster (shingles) in adults aged 50 years and older. By preventing herpes zoster, Shingrix reduces the overall incidence of postherpetic neuralgia.

Division name: OVRR/DVRPA

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EXECUTIVE SUMMARY:

The complete candidate vaccine, Shingrix, has been evaluated in 2 repeated dose toxicity studies in rabbits, one reproductive-developmental toxicity study in rats, one male fertility study in rats, two local tolerance studies in rabbits and one safety pharmacology study in rats. No significant toxicological findings were found which would prevent the use of this vaccine in adults 50 years of age at the recommended dose given as 2 intramuscular (IM) administrations 2-4 months apart.

Additionally, the sponsor submitted 3 safety pharmacology studies, 10 general toxicology studies, 10 genotoxciology studies, 5 reproductive toxicology studies and 3 local tolerance studies evaluating the AS01B adjuvant system or parts of it (MPL, QS-21).

Shingrix has been evaluated in repeated dose toxicity studies in the rabbits. Overall, the vaccine was well tolerated, but induced systemic as well as local reactogenicity. A transient but statistically significant increase in CRP levels was observed in rabbits receiving the Shingrix vaccine with levels up to 9 times (male animals) and 5 times (female animals) higher compared to control animals. These changes in CRP levels reflect an activation of the acute-phase response and indicate increasing levels of systemic inflammation, which potentially may be correlated with clinical adverse events like malaise and fatigue. Further, increases in bilirubin (up to 2x), popliteal lymph node weight (up to 50%), spleen weight (up to 17%), and thymus weight (up to 24%) were reported. Locally, mixed inflammatory cell infiltrate in the muscle and an enhanced activated appearance in the draining popliteal lymph nodes were observed.

The Shingrix vaccine was evaluated in a male fertility study in rats as well as in a reproductive developmental toxicity study in female rats. Treatment of male CD rats with the candidate vaccine at 20% of the full human dose did not affect male mating performance, fertility or early embryonic development. Treatment of female CD rats with the candidate vaccine at 40% of the full human dose per occasion was well tolerated and did not adversely affect embryo-fetal or pre- and post-natal survival, growth or development of the offspring. A reproductive toxicology study evaluating QS-21 in rabbits at doses up to 200 μg/dose (4 times the human dose) resulted in maternal toxicity (reduced food consumption, reduced body weight gain) as well as reduced fetal weight and malformations in the fetus (defect of the aortic arch). Doses of DQ adjuvant containing 100 or 20 μg/mL of QS-21 did not induce any adverse effects on maternal condition or embryo-fetal and post-natal development. Since Shingrix has been shown to induce significant systemic reactogenicity not only in rabbits (CRP increase, maternal toxicity) but also in humans (fatigue, headache, myalgia and shivering) which could adversely affect pregnancy outcomes, it might be warranted to evaluate a full human dose of the vaccine in a reproductive-developmental toxicity study in rabbits if this vaccine is recommended for females of child bearing age.

The sponsor submitted genotoxicity studies evaluating AS01B, MPL, and DQ/QS-21; no genotoxicity was observed in the submitted *in vitro* or *in vivo* studies. Safety pharmacology studies evaluating the candidate vaccine formulation, AS01B and MPL did not report clinically relevant adverse findings.

INTRODUCTION:

Herpes zoster (HZ; aka shingles) is a disease caused by reactivation after primary infection of latent varicella-zoster virus (VZV) that usually manifests as a localized rash, but can lead to postherpetic neuralgia (PHN). The incidence of HZ increases with age, with most cases seen in patients over age 50. Currently vaccination against HZ with Zostavax® is an approved preventative treatment; however, this vaccine show progressively decreased efficacy with increasing age and is not approved for use in immunocompromised individuals since it is a live virus vaccine. GSK Biologicals is developing a candidate HZ vaccine, which is an adjuvanted recombinant VZV-glycoprotein E (gE) subunit vaccine. Since the vaccine does not contain live virus, it is expected that this vaccine will be safe in all populations including highly immunocompromised persons. The adjuvant component is expected to increase immunogenicity and provide enhanced efficacy, especially in older adults for whom vaccination is most needed. Additionally, an adjuvanted gE vaccine that can induce a high and persistent immune responses may also provide enhanced efficacy in persons aged 50-59 years who are at increased risk for HZ and for whom no prevention option exists at this time. The purpose of this BLA is to evaluate the efficacy, safety and immunogenicity of an adjuvanted subunit HZ vaccine (gE/AS01B) in immunogenicity trial in subjects age 50 years and older. Additional immunogenicity trial in subjects, age 60 years and older in the US, will also be evaluated. A telecon took place between CBER and the sponsor on April 28, 2008, to discuss clarification of CBER's responses to the sponsor's questions and comments on the pre-read materials. No toxicology related issues were discussed.

The gE antigen is a purified recombinant protein produced in Chinese Hamster Ovary (CHO) cells. The gE protein is provided in a lyophilized form in monodose vials (50 µg/dose). The AS01B (liquid) Adjuvant System is provided in separate monodose vials (0.5 ml/dose). The AS01B Adjuvant System contains 50 µg of each of the immuno-enhancers *QS-21* (*Quillaja saponaria* Molina, fraction 21) and *MPL* (3-*O*-desacyl-4'-monophosphoryl lipid A) combined with liposomes. *QS-21* is a natural saponin molecule (triterpene glycoside) obtained from the (b) (4) of *Quillaja saponaria* Molina. *QS-21* is produced by (b) (4) . *MPL* (GSK Biologicals North America, USA) consists of a chemically detoxified form of the parent lipopolysaccharide (LPS) from the Gram-negative bacterium *Salmonella minnesota*. The liposomes consist of dioleoyl phosphatidylcholine (DOPC) and cholesterol.

The sponsor did not only evaluate the candidate vaccine but also submitted non-clinical studies evaluating the adjuvant alone or components of the adjuvant like MPL and QS-21. In the non-clinical studies the component QS-21 was evaluated alone as DQ. DQ stands for detoxified QS-21. In the vaccine formulation QS-21 is added post formation of the liposome and is associated with the liposomes through its interaction with cholesterol. The interaction with cholesterol reduces the known hemolytic activity of QS-21 which is seen when QS-21 is administered without cholesterol. DQ was considered to be the preferred candidate for primary pharmacodynamics and GLP toxicology testing.

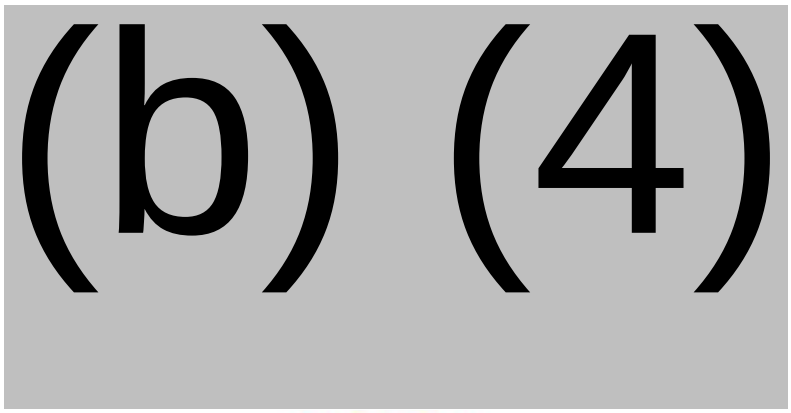


Figure 1: Schematic representation (as provided by the sponsor) of liposome containing QS-21 and MPL; note: DQ - the “detoxified” form of QS-21 - corresponds to the above structure without MPL. Figure was provided by the sponsor.

The candidate vaccine has been designed to induce robust cellular and humoral immune responses and to translate into robust vaccine efficacy in individuals with pre-existing immunity against VZV. The induction of both high cellular and humoral immune responses is desirable in the context of the development of a HZ vaccine. Specifically, a CD4+ T cell response is of particular relevance for protection against HZ.

Clinical studies submitted to support this BLA:

Study ID	Study Countries	Study Design Objectives*	Population (age) Schedule of Vaccination	Study Group	Number of Subjects	
					ATP Cohort of Immunogenicity	TVC
Pivotal studies in adults ≥ 50 YOA						
ZOSTER-006	Australia, Brazil, Canada, Czech Republic, Estonia, Finland, France, Germany, Hong Kong, Italy, Japan Mexico, South Korea, Spain, Sweden, Taiwan, UK, US	Phase III, randomized, observer-blind, pivotal efficacy study in adults ≥ 50 YOA. Follow-up driven by case accrual was to be at least 30 months after dose 2 (actual median safety follow-up time 4.4 years). **Primary objective:** VE in the prevention of HZ in adults ≥ 50 YOA. **Secondary objectives:** VE by age group in terms of: * Prevention of HZ. VE overall and by age group in terms of: * Prevention of overall PHN. * Reduction in duration of severe 'worst' HZ pain in subjects with confirmed HZ. * Reduction of HZ-related mortality and hospitalizations in subjects with confirmed HZ. * Reduction in incidence of HZ-associated complications in subjects with confirmed HZ. * Reduction in the use of pain medications in subjects with confirmed HZ. Safety and reactogenicity.	Adults ≥ 50 YOA stratified: 50-59 YOA, 60-69 YOA, 70-79 YOA and ≥ 80 YOA in an 8:5:3:1 ratio 2 doses at months 0 and 2	1) HZ/su 2) Placebo (saline)	1,070 (humoral immuno M3)[a] 212 (CMI immuno M3)[a] 1,067 (humoral immuno M3)[a] 218 (CMI immune M3)[a]	7,695 (EOS)[b] 7,344 (mTVC)[c] 7,710 (EOS)[b] 7,415 (mTVC)[c]

Study ID	Study Countries	Study Design Objectives*	Population (age) Schedule of Vaccination	Study Group	Number of Subjects	
					ATP Cohort of Immunogenicity	TVC
Pivotal studies in adults ≥ 50 YOA						
ZOSTER-022	Australia, Brazil, Canada, Czech Republic, Estonia, Finland, France, Germany, Hong Kong, Italy, Japan Mexico, South Korea, Spain, Sweden, Taiwan, UK, US	Phase III, randomized, observer-blind, pivotal efficacy study in adults ≥ 70 YOA. Follow-up driven by case accrual was to be at least 30 months after dose 2 (actual median safety follow-up time 4.2 years). **Primary objective of ZOSTER-022:** VE in the prevention of HZ in adults ≥ 70 YOA. **Secondary objectives of ZOSTER-022:** VE in terms of: * Prevention of overall PHN. * Reduction in duration of severe 'worst' HZ pain in subjects with confirmed HZ. * Reduction of HZ-related mortality and hospitalizations in subjects with confirmed HZ. * Reduction in incidence of HZ-associated complications in subjects with confirmed HZ. * Reduction in the use of pain medications in subjects with confirmed HZ. Safety and reactogenicity. **Co-primary objectives for pooled dataset of ZOSTER-006 and -022:** * VE in the prevention of overall PHN in subjects ≥70 YOA. * VE in the prevention of HZ in subjects ≥ 70 YOA. **Secondary objectives for pooled dataset of ZOSTER-006 and -022:** VE in terms of: * Prevention of overall PHN in subjects ≥ 50 YOA. Prevention of PHN in subjects ≥50 YOA with confirmed HZ. * Reduction in duration of severe 'worst' HZ pain in subjects ≥70 YOA with confirmed HZ. Safety and reactogenicity in subjects ≥70 YOA.	Adults ≥ 70 YOA Stratified: 70 79 YOA and ≥ 80 YOA in a 3:1 ratio. 2 doses at months 0 and 2	**ZOSTER-022:** 1) HZ/su 2) Placebo (saline) **Pooled dataset of ZOSTER-006 and -022:** 1) HZ/su 2) Placebo (saline)	387 (humoral immuno M3)[a] 412 (humoral immuno M3)[a] 1,457 (humoral immuno M3)[a] 1,479 (humoral immuno M3)[a]	6,950b 6,541 (mTVC)[d] 6,950b 6,622 (mTVC)[d] 14,645b 13,881 (mTVC)[e] 14,660b 14,035 (mTVC)[e]

Study ID	Study Countries	Study Design Objectives*	Population (age) Schedule of Vaccination	Study Group	Number of Subjects	
					ATP Cohort of Immunogenicity	TVC
Pivotal studies in adults ≥ 50 YOA						
ZOSTER-004	Canada, Germany, US	Phase III, randomized, open-label study; co-administration of HZ/su with unadjuvanted quadrivalent seasonal influenza vaccine (FLU-D-QIV). Duration of follow-up: 12 months post last vaccination. **Co-primary objectives:** * VRR to HZ/su (anti-gE Abs) in Co-Ad group at month 3. * NI in terms of humoral immune response (GMC ratio for anti-gE Abs) in Co-Ad group at month 3 vs. in control group at month 5. * NI in terms of HI antibody GMTs against the 4 influenza vaccine strains in Co-ad group vs. control group at day 21 post vaccination. **Secondary objectives:** * NI in terms of HI SCR against the 4 influenza vaccine strains in Co-ad group vs. control group at day 21 post vaccination. * GMTs, seroprotection rate, SCRs and mean geometric increase in response to FLU-D-QIV. * Safety and reactogenicity of both HZ/su and FLU-D-QIV.	Adults ≥ 50 YOA 1) Co-Ad group: 2 doses of HZ/su at months 0 and 2, and 1 dose of FLU-D-QIV at month 0 2) Control group: 1 dose of FLU-D-QIV at month 0, and 2 doses of HZ/su at months 2 and 4	1) Co-Ad 2) Control	386 395	413 415[f]
ZOSTER-007g	Belgium, Canada, US	Phase III, randomized, double-blind, lot-to-lot consistency study. Duration of follow-up: 12 months post last vaccination. **Primary objective:** Lot-to-lot consistency in terms of GMC ratio for anti-gE Abs at month 3 between 3 HZ/su production lots. **Secondary objectives:** * Lot-to-lot consistency of 3 manufacturing lots in terms of VRR to HZ/su (anti-gE Abs) at month 3. * Characterize humoral immune responses (anti-gE) at months 0 and 3. * Safety and reactogenicity.	Adults ≥ 50 YOA 2 doses at months 0 and 2	1) HZ/su Lot A 2) HZ/su Lot B 3) HZ/su Lot C	**M3** 210 210 202	**M3** 218 217 216

Study ID	Study Countries	Study Design Objectives*	Population (age) Schedule of Vaccination	Study Group	Number of Subjects			
					ATP Cohort of Immunogenicity		TVC	
Pivotal studies in adults ≥ 50 YOA								
ZOSTER-026	Estonia, US	Phase III, randomized, open-label, schedule comparison study. Duration of follow-up: 12 months post last vaccination. **Co-primary objectives:** VRR to HZ/su (anti-gE Abs) at 1 month post dose 2 in the 0,6-month and 0,12-month schedule groups. **If the 0,6-month schedule VRR objective is met:** NI in terms of GMC ratio for anti-gE Abs (0,2-month over 0,6-month group) at 1 month post dose 2. **If the 0,12-month schedule VRR objective is met:** NI in terms of GMC ratio for anti-gE Abs (0,2-month over 0,12-month group) at 1 month post dose 2. **Secondary objectives:** * Immunogenicity with respect to humoral immune response to gE at Day 0, 1 month post dose 2 and one year post dose 2. Safety and reactogenicity.	Adults ≥ 50 YOA 1) Gr 0-2: 2 doses of HZ/su at months 0 and 2 2) Gr 0-6: 2 doses of HZ/su at months 0 and 6 3) Gr 0-12: 2 doses of HZ/su at months 0 and 12	1) Gr 0-2 2) Gr 0-6 3) Gr 0-12	**M3** 118 **M7** 114 **M13** 111	**M14**[h] 117 **M18**[h] 115 **M24**[h] 110	119 119 116	
EXPLO-CRD-004	Belgium	Phase I/II, exploratory, open-label, randomized study. Duration of follow-up: 10 months post last vaccination. **Co-primary objectives:** * Safety and reactogenicity of HZ/su with or without *Varilrix*. * Comparison of vaccine strategies to induce the optimum CD4+ and/or CD8+ T cell responses between vaccination groups at month 3. **Secondary objectives:** * Safety as measured by hematology and biochemistry parameters. * CMI responses elicited by various vaccine strategies. * Humoral immune responses elicited by various vaccine strategies.	Adults 18-30 YOA 1) gE/Y: 2 doses of HZ/su at months 0 and 2 2) gEVAR/Y: 2 doses of HZ/su and *Varilrix* at months 0 and 2	1) gE/Y 2) gEVAR/Y	10 10		10 10	
			Adults 50-70 YOA 1) VAR/E: 2 doses of *Varilrix* at months 0 and 2 2) gE/E: 2 doses of HZ/su at months 0 and 2 3) gEVAR/E: 2 doses of HZ/su and *Varilrix* at months 0 and 2	1) VAR/E 2) gE/E 3) gEVAR/E	45 45 44		45 45 45	
ZOSTER-018, ZOSTER-019 (EXT:EXPLO CRD-004 M30 and M42)	Belgium	Phase I/II, open-label extension follow-up at month 30 and month 42 of subjects vaccinated with HZ/su in study EXPLO-CRD-004 - persistence study. Duration of follow-up: 28 and 40 months post last vaccination. **Primary objective:** Descriptive assessment of persistence of the CMI responses to gE and VZV in HZ/su group at months 30 and 42. **Secondary objectives:** * Persistence of humoral immune responses to gE and VZV in HZ/su group at months 30 and 42. * Safety with respect to SAEs due to the study procedure during whole study (up to month 30 and month 42). * Clinically diagnosed HZ from month 12 in the EXPLO-CRD-004 study till end of ZOSTER-019.	Adults 18-30 YOA Adults 50-70 YOA No administration of HZ/su in this study	1) gE/Y 2) gE/E	**M30** 4 29	**M42** 3 20	**M30** 4 30	**M42** 3 20

Study ID	Study Countries	Study Design Objectives*	Population (age) Schedule of Vaccination	Study Group	Number of Subjects					
					ATP Cohort of Immunogenicity			TVC		
Pivotal studies in adults ≥ 50 YOA										
ZOSTER-003	Czech Republic, Germany, Netherlands, Sweden	Phase II, single-blind, randomized, antigen dose-selection study. Duration of follow-up: 1 month post last vaccination. **Primary objective:** Comparison of CD4+ T cell response to gE of gE/AS01B vaccines at month 3 in subjects ≥ 70 YOA. **Secondary objectives:** * Comparison of CD4+ T cell response to gE between gE1001B and gE100S groups at month 3 in subjects ≥ 70 YOA. * Comparison of humoral immune response to gE and VZV of gE/AS01B vaccines at month 3 in subjects ≥ 70 YOA. * Immunogenicity after 1 and 2 doses of study vaccine formulations with respect to CMI and humoral immune responses in subjects 60-69 YOA and ≥ 70 YOA. * Safety and reactogenicity (60-69 YOA and ≥70 YOA). * Clinically diagnosed HZ.	Adults ≥60 YOA Stratified: 60-69 YOA and ≥70 YOA in a 1:4 ratio 2 doses at months 0 and 2	1) gE251B: 25 µg gE/AS01$_B$ 2) gE501B: HZ/su 3) gE1001B: 100 µg gE/AS01$_B$ 4) gE100S: 100 µg gE/Saline 5) S gE1B: Saline, 1 dose, 100 µg gE/AS01$_B$, 1 dose	157 156 151 50 153			164 166 165 54 165		
ZOSTER-011, -012 and -013 (EXT 003 M12, M24, M36)	Czech Republic, Germany, Netherlands Sweden	A single-blind extension follow-up at months 12, 24 and 36 of ZOSTER-003 - persistence study. Duration of follow-up: 10, 22 and 34 months post last vaccination. **Primary objective:** None **Secondary objectives:** * Persistence of CMI (gE-specific CD4+ T cell response) and humoral immune responses (anti-gE and VZV Abs) at months 12, 24 and 36. * Safety with respect to SAEs during the whole study. * Clinically diagnosed HZ up to month 36.	ZOSTER-003 population No administration of HZ/su in these studies	1) gE251B: 25 µg gE/AS01$_B$ 2) gE501B: HZ/su 3) gE1001B: 100 µg gE/AS01$_B$ 4) gE100S: 100 µg gE/Saline 5) S gE1B: Saline, 1 dose, 100 µg gE/AS01$_B$, 1 dose	**M12** 146 144 147 48 145	**M24** 126 133 135 44 133	**M36** 117 123 127 40 128	**M12** 156 159 159 50 161	**M24** 150 155 154 49 157	**M36** 147 147 150 47 154
ZOSTER-024	Czech Republic, Germany, Netherlands Sweden	Phase II, open-label, single group, extension follow-up at months 48, 60 and 72 of HZ/su group of ZOSTER-003 - persistence study. Duration of follow-up: 70 months post last vaccination. **Primary objective:** Evaluation of CMI (gE- and VZV-specific CD4+ T cells) and humoral immune responses (anti-gE and VZV Abs) in HZ/su group at months 48, 60 and 72, overall and by age range. **Secondary objectives:** * Safety with respect to SAEs and pIMDs in each age range. * Suspected cases of HZ episodes.	ZOSTER-003 population No administration of HZ/su in this study	1) gE501B: HZ/su	**M48** 126	**M60** **NA**	**M72** **NA**	**M48** **129**	**M60** **124**	**M72** **119**

Study ID	Study Countries	Study Design Objectives*	Population (age) Schedule of Vaccination	Study Group	Number of Subjects	
					ATP Cohort of Immunogenicity	TVC
Pivotal studies in adults ≥ 50 YOA						
ZOSTER-010	Czech Republic, Spain, US	Phase II, randomized, observer-blind, adjuvant dose-selection study. Duration of follow-up: 12 months post last vaccination. **Primary objective:** Comparison of gE- and VZV-specific CD4+ T cell mediated and humoral immune responses between HZ/su, gE/AS01E and gE/Saline groups at month 3 in subjects ≥ 50 YOA. **Secondary objectives:** * Comparison of gE- and VZV-mediated CMI (including overall CD8+ T cell) and humoral immune responses between HZ/su, gE/AS01E, and gE/Saline groups at month 3 by age (50-59 YOA, 60-69 YOA and ≥70 YOA). * Safety and reactogenicity. * Suspected cases of HZ episodes.	Adults ≥ 50 YOA 2 doses at months 0 and 2	1) gE/AS01$_B$: HZ/su 2) gE/AS01$_E$: 50 µg gE/AS01$_E$ 3) gE/Saline: 50 µg gE/Saline 4) Saline	140 138 71 37	150 149 73 38
ZOSTER-023	Australia	Phase I, open-label, single country, safety and immunogenicity study. Duration of follow-up: 6 months post last vaccination. **Primary objective:** Safety and reactogenicity. **Secondary objective:** Immunogenicity with respect to humoral immune response to gE and VZV at months 0, 1 and 3.	Adults of Japanese ethnic origin 18-30 YOA and 50-69 YOA 2 doses at months 0 and 2	1) 18-30 YOA: HZ/su 2) 50-69 YOA: HZ/su	10 8	10 10
ZOSTER-032	Japan	Phase III, randomized, open-label study with SC vs. IM administration. Duration of follow-up: 12 months post last vaccination. **Co-primary objectives:** * Descriptive assessment of VRR and GMC to HZ/su (anti-gE Abs) when administered SC vs. IM at month 3. * Safety and reactogenicity up to month 3. **Secondary objectives:** * Immunogenicity with respect to humoral immune responses to gE (VRR and GMC) from month 0 to month 14. * Safety with respect to SAEs and pIMDs from month 3 to month 14.	Adults ≥ 50 YOA 2 doses at months 0 and 2	1) SC HZ/su 2) IM HZ/su	**M3** **M14** 29 30 29 28	**M3** **M14** 30 30 30 30

Study ID	Study Countries	Study Design Objectives*	Population (age) Schedule of Vaccination	Study Group	Number of Subjects	
					ATP Cohort of Immunogenicity	TVC
Pivotal studies in adults ≥ 50 YOA						
ZOSTER-033	Canada, Russian Federation	Phase III, non-randomized, open-label study in adults with a previous episode of HZ. Duration of follow-up: 12 months post last vaccination. **Co-primary objectives:** * VRR to HZ/su (anti-gE Abs) at month 3. * Safety and reactogenicity up to month 3. **Secondary objectives:** * Immunogenicity with respect to humoral immune responses to gE by age (50-59 YOA, 60-69 YOA and ≥70 YOA). * Safety with respect to SAEs and pIMDs from 30 days post last vaccination until month 14.	Adults ≥ 50 YOA 2 doses at months 0 and 2	1) HZ/su	82	96
ZOSTER-001	US	Phase I/IIa, randomized, observer-blind, placebo-controlled study. Duration of follow-up: 12 months post last vaccination. **Co-primary objectives:** * Safety and reactogenicity, including hematology and biochemistry parameters. * Immunogenicity with respect to CMI (CD4+ T cells) and humoral immune responses (antigE). **Secondary objectives**: * Immunogenicity with respect to CMI (CD4+ T cells) and humoral immune responses (antigE and VZV) at different timepoints. * Descriptive assessment of HZ and its complications.	Autologous hematopoietic stem cell transplant recipients ≥ 18 YOA 3 doses at months 0, 1 and 3	1) gE/AS01$_B$3: 3 doses of HZ/su 2) gE/AS01$_E$-3: 3 doses of gE/AS01$_E$ 3) gE/AS01$_B$2: 1 dose of placebo at month 0 + 2 doses of HZ/su at months 1 and 3 4) Placebo: 3 doses of saline	29 26 27 24	30 29 31[i] 30
ZOSTER-015	Germany, UK, US	Phase I/IIa, randomized, observer-blind, placebo-controlled study. Duration of follow-up: 12 months post last vaccination. **Co-primary objectives:** * Safety and reactogenicity, including hematology and biochemistry parameters and worsening of HIV condition. * Immunogenicity with respect to CMI (CD4+ T cells) and humoral immune responses (anti-gE). **Secondary objectives**: * Immunogenicity with respect to CMI (CD4+ T cells) and humoral immune responses (anti-gE and VZV) at different timepoints. * Descriptive assessment of HZ and its complications. * Effect of HZ/su on CD4+ T cell count and HIV viral load.	HIV-infected adults ≥ 18 YOA 3 doses at months 0, 2 and 6	1) HZ/su 2) Placebo	54 37	74 49

Ab = antibody; ATP = According to Protocol; CMI = Cell Mediated Immunity; EOS = end of study; FLU-D-QIV = GSK's unadjuvanted quadrivalent seasonal influenza vaccine; gE = glycoprotein E; GMC = Geometric Mean Concentration; GMT = Geometric Mean Titer; HI = Haemagglutinin Inhibition; HIV = Human Immunodeficiency Virus; HZ = Herpes Zoster; HZ/su = Herpes Zoster subunit candidate vaccine (50 µg gE/AS01B); IM = intramuscular; M = Month; mTVC = modified TVC; NA = not applicable; NI = non-inferiority; PHN = postherpetic neuralgia; pIMD = potential Immune-Mediated Disease; SAE = serious adverse event; SC = subcutaneous; SCR = Seroconversion Rate; TVC = Total Vaccinated Cohort; UK = United Kingdom; US = United States; VE = Vaccine Efficacy; VRR = Vaccine Response Rate; vs. = versus; VZV = Varicella Zoster Virus, YOA = years of age

* The detailed objectives as specified in the protocol and the statistical analysis plan are listed in the study narratives in m5.3.5.3 – Integrated summary of efficacy, Section 2
[a] For ATP cohort of humoral or CMI immunogenicity at other time points than month 3 (M3), refer to m5.3.5.1, ZOSTER-006 CSR, section 6.3.1.1.2 and m5.3.5.1 ZOSTER-022 CSR, section 6.4.1.1 and section 12.2
[b] TVC used for safety analysis
[c] mTVC used for final HZ efficacy analysis (Note: the mTVC for the EOS efficacy analysis included 7,340 subjects in the HZ/su group and 7,413 subjects in the placebo group)
[d] mTVC used for efficacy analysis
[e] mTVC used for EOS efficacy analysis in the pooled dataset of ZOSTER-006 and ZOSTER-022
[f] Although 415 subjects were part of the TVC and received FLU-D-QIV at dose 1, only 406 of them received at least 1 dose of HZ/su at the subsequent doses.
[g] Only the active phase of ZOSTER-007 has been completed (up to 1 month post last vaccination).
[h] The number of subjects in the adapted ATP cohort for immunogenicity are presented here. This cohort was used to denote that for each timepoint, the corresponding ATP cohort for immunogenicity/persistence has been used. More specifically, the analyses on the pre-vaccination and one month post dose 2 timepoints were based on the ATP cohort for immunogenicity, while the analysis on the 12 months post dose 2 timepoint was based on the ATP cohort for persistence.
[i] Although 31 subjects in group gE/AS01B2 were part of the TVC and received placebo at dose 1, only 29 of them received at least 1 dose of HZ/su at the subsequent doses.
Table 1: Clinical studies submitted to support this BLA

Stability Summary:

Shelf-life and storage conditions
Based on the available stability data for the phase III efficacy (60 months), phase III consistency (36 months) and commercial consistency lots (12 months) provided in dedicated 3.2.P.8.3 stability data sections of the gE drug product dossier, a shelf-life of 36 months at +2°C/+8°C is claimed for gE final container lots filled in 3 mL glass vials.

The shelf-life is calculated as from the manufacturing date (*i.e.* filling date).
The storage conditions are the following:
* Store in a refrigerator (+2°C/+8°C);
* Do not freeze;
* Store in the original package in order to protect from light. Given the fact that gE/AS01B vaccine is presented as two independent vials of gE Lyo and AS01B drug products, the expiry date of the dual presentation will be determined by whichever component expires earliest.

Toxicity studies submitted to support this BLA:

Single-Dose Toxicity:

- (b) (4) DT127 The acute intraperitoneal toxicity of Monophosphoryl Lipid A in rats- (b) (4)

Repeat-Dose Toxicity (including supportive toxicokinetics evaluations):

- (b) (4) 3262.1: 14-day intravenous toxicity study of MPL in dogs (b) (4)
- (b) (4) V20094: Repeated-dose toxicity study with Zoster candidate vaccine (gE/AS01B) administered subcutaneously (four times) or intramuscularly (four times) to male and female rabbits followed by a 4-week treatment free period
- (b) (4) 045/0022412 AS01B versus AS02V toxicity study by repeated (5 times) intramuscular administration to rabbits
- (b) (4) V20155: Repeated dose toxicity study with DQ administered intramuscularly to male and female rabbits
- (b) (4) V6721: Repeated-dose toxicity study with a VZV candidate vaccine (gE 100 µg/AS01B) administered intramuscularly (three times) to male and female rabbits
- (b) (4) V20165: Repeated-dose toxicity study with AS01B administered intramuscularly (seven times) to male and female rats followed by a 4-week treatment free period
- (b) (4) V20154: Repeated dose toxicity study with DQ administered intramuscularly to male and female rats
- (b) (4) 3262.2: 8-day intravenous toxicity study of MPL in rats- (b) (4)
- (b) (4) 3262.4: 7-day intravenous dose range-finding toxicity study in (b) (4) rats with MPL (b) (4)

Reproductive Studies:

Fertility and early embryonic development

- (b) (4) 0004: Zoster candidate vaccine: Study of effects on the fertility of male CD rats by intramuscular administration (including pre-mating immunization phase)

Embryo-fetal development

- (b) (4) 1729/7: MPL: Subcutaneous study of embryo-fetal development in the rat- in (b) (4)
- (b) (4) 1729/8: MPL: Subcutaneous study of embryo-fetal development in the rabbit- in (b) (4)

Prenatal and postnatal development, including maternal function

- (b) (4) 1729/17: MPL: Subcutaneous study of pre- and postnatal development in the rat- in (b) (4) - Marion
- (b) (4) 0005: Zoster candidate vaccine (gE/AS01B): Study of effects on embryo-fetal, pre- and post natal development in CD rats by intramuscular administration (including pre-mating immunization phase)
- (b) (4) 0020: DQ immunostimulant: Study for effects on female fertility, embryo-fetal and pre- and postnatal development in the CD rat by intramuscular administration (including pre-mating immunization phase)
- (b) (4) AB14898: DQ – Developmental toxicity study (including teratogenicity and postnatal investigations) by the intramuscular route in the rabbit

Local Tolerance:

- (b) (4) V 20212/01 Single dose toxicity and local tolerance study with DQ administered intramuscularly to male and female rats
- (b) (4) V 20212/02 Single dose toxicity and local tolerance study with DQ administered intramuscularly to male and female rabbits
- (b) (4) V6812/02 Single dose toxicity and local tolerance study with a VZV candidate vaccine (gE 100 µg / AS01B) administered
- (b) (4) V9912/05 Single dose toxicity and local tolerance study with Zoster candidate vaccine (gE/AS01B) administered subcutaneously to male and female rabbits

Other Toxicity Studies:

- (b) (4) V20041: Repeated dose toxicity and local tolerance study with QS-21 and with DQ administered intramuscularly to male and female rats-Reported in (b) (4)

Genotoxicology studies:

In vitro

- (b) (4)

In vivo

- (b) (4) 317/032657: Comparison of different test formulations in the rat micronucleus test
- (b) (4) 681/043748: AS01B assessment of effect on blood cells and bone marrow following intramuscular administration to CD rats
- (b) (4) 730/052198: MPL ((b) (4)) rat micronucleus test
- (b) (4) 0026/070209: An assessment of the effects of AS01B on red blood cells in peripheral blood and bone marrow
- (b) (4) V20204/4: Bone marrow micronucleus test with DQ in rats

Safety pharmacology studies:

- (b) (4) 1729/22: IMPL: Cardiovascular and respiratory effects in the anaesthetized dog following intravenous administration-Steve
- (b) (4) (b) (4) 0006: VZV candidate vaccine (gE 100 mcg/AS01B) cardiovascular and respiratory evaluation in the anaesthetized rat
- (b) (4) 0010: Cardiovascular and respiratory evaluation in the anaesthetized rat
- (b) (4) AA81874: AS01B Adjuvant - Effects on cardiovascular and respiratory functions following intramuscular administration in the conscious (b) (4)

Labeling: USE IN SPECIFIC POPULATIONS

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to Shingrix during pregnancy. Healthcare providers are encouraged to register women by calling XXXXXXXX.

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. There are no available human data to establish whether there is vaccine-associated risk with SHINGRIX in pregnant women.

A reproductive and developmental toxicity study was performed in female rats administered SHINGRIX or the $AS01_B$ Adjuvant System alone prior to mating, during gestation and lactation periods. The total dose was 0.2 mL at each occasion (a single human dose is 0.5 mL). This study revealed no adverse effects on fetal or pre-weaning development due to SHINGRIX *[see Data]*.

Data

Animal Data: In a reproductive and developmental toxicity study, female rats were administered SHINGRIX or the $AS01_B$ Adjuvant System alone by intramuscular injection 4 and 2 weeks prior to mating, on gestation Days 3, 8, 11, and 15, and on lactation Day 7. The total dose was 0.2 mL at each occasion (a single human dose is 0.5 mL). No adverse effects on pre-weaning development up to post-natal Day 25 were observed. There were no vaccine-related fetal malformations.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

SHINGRIX has not been evaluated for its carcinogenic or mutagenic potential. In a male fertility study, rats were vaccinated with 0.1 mL SHINGRIX (a single human dose is 0.5 mL) on 42, 28 and 14 days prior to mating. There were no effects on male fertility.

SAFETY PHARMACOLOGY STUDIES:

Reviewer: Dr. Claudia Wrzesinski

STUDY #1: (b) (4) 1729/22: MPL: Cardiovascular and Respiratory Effects in the Anaesthetized Dog Following Intravenous Administration

The (b) (4) dog was used for the evaluation of possible side effects of MPL on cardiovascular and respiratory parameters in a GLP study performed by (b) (4) , in 1999.

Two male and two female adult (b) (4) dogs were anesthetized using propofol anesthesia and received ascending doses of MPL at 1, 10 and 100 µg/kg at a dose volume of 1-2 ml/kg intravenously in the left jugular vein with at least 25 minutes between each dose. Two male and two female control animals received 3 volumes of vehicle (phosphate buffered saline), using the same regime as for test animals. Cardiovascular (blood pressure, heart rate, left ventricular pressure, mean femoral blood flow and ECG) and respiratory (peak inspiration and expiratory flow, respiration rate, tidal volume and minute volume) parameters were measured post-dose at 2, 10 and 20 minutes and were compared with pre-dose values. Cardiovascular and respiratory parameters were monitored continuously for a period of two hours post vaccination. There was little effect of treatment at any dose level of MPL. A small and gradual increase in mean heart rate over the MPL study period (from 83 at baseline to 93 beats per minute by the end of the experiment) and a decrease in the mean height of the T wave (due to 2 out of 4 animals) were noted although not being statistically different from controls. Also, a small increase in mean respiratory rate (from 15 at baseline to 18 breaths per minute), after administration of the top dose of MPL, was not considered to be physiologically relevant.

Study # 2: (b) (4) 0006: VZV Candidate Vaccine (gE 100 mcg/AS01B) Cardiovascular and Respiratory Evaluation in the Anaesthetized Rat

This GLP study was performed by (b) (4) , assessed the effects of gE 100 µg/AS01B candidate vaccine on the cardiovascular and respiratory systems in anaesthetized male (b) (4) rats.

Following the induction of surgical anesthesia, appropriate cannulations were performed in order to record cardiovascular (arterial blood pressure, heart rate and ECG (lead II)) and respiratory (tidal volume, respiration rate and minute volume) parameters. Cardiovascular and respiratory parameters were recorded continuously for at least a 30 minute stabilization period prior to the dose administration. Animals received either 1 ml/kg of gE 100 µg/AS01B or saline once intravenously. Following intravenous administration the parameters were recorded for 120 minutes post-dose. No treatment related effects were observed on any of the recorded cardiovascular or respiratory parameters. Values recorded for all cardiovascular and respiratory parameters during stabilization and post dose periods were within the normal range expected for this type of study.

Study # 3: (b) (4) 0010/063466: Cardiovascular and Respiratory Evaluation in the Anaesthetized Rat

In this study GSK Bio evaluated the effect of adjuvant materials (AS01B, (b) (4) on the cardiovascular and respiratory systems in anaesthetized male (b) (4) rats.

AS01B, (b) (4) adjuvants or saline were examined intravenously at a dose volume of 1 ml/kg equivalent. Following the induction of surgical anesthesia, appropriate cannulations were performed in order to record cardiovascular (arterial blood pressure, heart rate and ECG (lead II)) and respiratory (tidal volume, respiration rate and minute volume) parameters. Cardiovascular and respiratory parameters were recorded continuously for at least a 30 minute stabilization period prior to the dose administration. Following intravenous administration the parameters were recorded for 120 minutes post-dose. Intravenous administration of saline 0.9% w/v, AS01B, (b) (4) candidate vaccines did not produce any consistent treatment-related effects on any of the recorded cardiovascular or respiratory parameters. Values recorded for all cardiovascular and respiratory parameters during stabilization and post dose periods were within the normal range expected for this type of study.

Study # 4: (b) (4) AA81874: AS01B Adjuvant - Effects on Cardiovascular and Respiratory Functions Following Intramuscular Administration in the Conscious (b) (4)

The (b) (4) dog was used for the evaluation of possible side effects of AS01B on cardiovascular and respiratory parameters in a GLP study performed by (b) (4) in 2009.

In this study the effects of the test item AS01B Adjuvant on arterial blood pressure, heart rate, electrocardiogram, body temperature and respiratory parameters was evaluated following a single intramuscular administration in the conscious (b) (4) dog.

Four animals received saline (control) on day 0 and a full human dose of (50 µg MPL and 50 µg QS-21 in a liposome based formulation per 500 µL) AS01B adjuvant on day 7, each animal served as its own control. Body temperature, hemodynamic, cardiac and respiratory parameters were recorded in all animals on days 0 and 7, starting at least 1.5 hours before administration and for approximately 7 days following the administration. Data were analyses only over the first 3 days of recording (1, 3, 6, 24, 48 and 72 hours) after each administration.
AS01B Adjuvant, administered intramuscularly, induced a slight increase in body temperature 6 hours after treatment, compared with control (saline). AS01B Adjuvant did not relevantly affect the arterial blood pressure, the heart rate and the duration of the RR and PR intervals, of the QRS complex and of the QT and QTc intervals, irrespective of the formula used for QT interval correction, during the 72-hour period following administration. AS01B Adjuvant did not induce any disturbances in rhythm or waveform morphology of the ECG during the first 6-hour post-

treatment period. AS01B Adjuvant administered intramuscularly, did not relevantly affect the respiratory rate, the inspiratory and expiratory times, AUCITP (index of tidal volume) and AUCITP X Respiratory rate (index of minute volume).

In conclusion, a full human dose of AS01B adjuvant administered intramuscularly, did not affect the health status and the body weight gain of the animals throughout the study period. AS01B Adjuvant, administered intramuscularly, induced a slight increase in body temperature 6 hours after treatment, compared with control (saline). AS01B Adjuvant did not affect the cardiovascular function and the respiratory function.

GENERAL TOXICOLOGY STUDY REVIEWS:

Reviewer: Dr. Nabil Al-Humadi

Study # 1: Single Dose Toxicology and Local Tolerance Study with a VZV Candidate Vaccine (gE 100 µg/AS01B) Administered Intramuscularly to Male and Female Rabbits. Study number; 6812/02. (Reviewed by Dr. Al-Humadi in IND 13857)

Performing laboratory: (b) (4) .
Study initiation date: 23 January, 2006
Final Report date: 05 December, 2006
Test article batch/lot:
gE/100 µg/AS01B = (b) (4)
AS01B adjuvant = (b) (4)
Animal species and strain: (b) (4) rabbits
Breeder/supplier: (b) (4)
Number of animal per group and sex: 9 males and 9 females
Age: 12 week at start
Body weight range: 2221-2711 gram
Route and site of administration: Intramuscular
Volume of injection: 500 µL per animal per injection
Frequency of administration and study duration: One dose on day 0 and the study duration was 3 days.
Dose:
gE/100 µg/AS01B = monodose vial containing 62.5 µg of freeze-dried gE antigen per cake.
AS01B adjuvant = monodose vial containing 62.5 µg QS-21 and 62.5 µg MPL in a liposome-based formulation in a total volume of 625 µL.
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as a single-use vials (one vial per dose). Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study.

According to the testing laboratory, the shipment of gE antigen and of AS01B was received on 01/20/2006. The expiration date was 03/31/2009 and 10/31/2007 for gE antigen and AS01B, respectively.

Means of administration: The injection with the appropriate material was given to each animal in the central part of the right calf muscle.
Report status: Final

Experimental design:

Group	Treatment (Intramuscular)	Number of Animals (#/sex/group)
A	Saline	3M/3F
B	AS01B	3M/3F
C	gE 100 µg/AS01B	3M/3F

Table 2: *Experimental design (study # 6812/02)*

Methods:
The following parameters were evaluated: cage-side observation (twice daily), skin reactions at the intramuscular site of injection (approximately 3, 24, 48 and 72 hours after dosing), body weights (days 0 and 3), gross anatomy at termination, and histopathology on the right calf injection site. Liver, kidney, heart, and lungs were collected and preserved in formalin but not examined.

Parameters	Frequency of Testing
Cageside observation[1]	Twice daily
Clinical observations[2]	NC
Body weight	Days 0 and 3
Food consumption	NC
Body temperature	NC
Ophthalmologic exam	NC
Clinical chemistry	NC
Hematology	NC
Coagulation	NC
Immunological response	NC
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Approximately 3, 24, 48 and 72 hours after dosing
Necropsy	Day 3
Histopathology	Day 3

(NC = Not collected)
Table 3: *Parameters evaluated (study # 6812/02)*

[1] Cageside observations include mortality, morbidity, general health and signs of toxicity.
[2] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

SYSTEM	ORGAN COLLECTED	ORGAN NOT COLLECTED
DIGESTIVE	Liver	Large intestine (cecum, colon, rectum); small intestine (duodenum, jejnum, ileum), esophagus, gall bladder, , salivary gland (mandibular), pancreas, stomach
RESPIRATORY	Lung (with main-stem bronchi), trachea	Nasal turbinates
CARDIOVASCULAR	Heart	Aorta
IMMUNOLOGIC/ HEMATOPOIETIC		Lymph nodes(1 related to route of administration, and 1 from a distant location), thymus, bone with marrow(sternum), bone with marrow (femur), lymph node (mandibular, mesenteric), spleen
UROGENITAL	Kidneys	Fallopian tubes, cervix, uterus, epididymis, ovaries, prostate, seminal vesicle, testes, urinary bladder, uterus (with cervix), vagina
NEUROLOGIC		Brain (cerebrum, cerebellum, medulla/pons), optic nerve, sciatic nerve, spinal cord (cervical, lumbar, mid-thoracic)
HORMONAL		Zymbal's Gland (if present), adrenals, mammary glands, thyroid (with parathyroid glands), pituitary glands
OTHER		Harderian gland (if present), eyes, skeletal muscle, skin, tongue
GROSS LESIONS		
INJECTION SITE OR SITE OF APPLICATION		

Table 4: *Table of histopathology (study # 6812/02)*

Histopathology was not performed on the liver, kidney, heart, and lungs because no gross macroscopic changes were observed.

Results:
Morbidity and mortality: All animals survived to their scheduled termination.

Systemic toxicity:
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs or body weight (gain) were found.

SEX	MALES			FEMALES		
GROUPS	1 (CONTROL)	2	3	1 (CONTROL)	2	3
NUMBER OF ANIMALS	3	3	3	3	3	3
BODY WEIGHT Day 0	2477.3	2540.7	2340.0	2521.7	2465.0	2486.0
BODY WEIGHT Day 3	2529.3	2564.0	2367.7	2538.3	2514.7	2505.3

Table 5: *Table of body weights in grams (study # 6812/02)*
Gross Pathology:

Group	Findings
1M	NF
2M	Yellow nodule in the liver (1/3)*
3M	NF
1F	NF
2F	NF
3F	NF

* Number of animals with this finding/total number of animals per group. NF = no findings.
Table 6: *Gross pathology (study # 6812/02)*
Microscopic finding are listed below:

Groups	Findings
1M	Mononuclear cell infiltrate (2/3)*, fiber(s) degeneration
2M	Very slight to slight widespread mononuclear cell infiltrate (3/3) in the calf muscle
3M	Very slight to slight widespread mononuclear cell infiltrate (3/3) in the calf muscle
1F	Mononuclear cell infiltrate (3/3)
2F	Very slight to slight widespread mononuclear cell infiltrate (3/3) in the calf muscle
3F	Very slight to slight widespread mononuclear cell infiltrate (3/3) in the calf muscle

* Number of animals with this finding/total number of animals per group.
Table 7: *Microscopic findings (study # 6812/02)*

Local toxicity: Non treatment-related yellow nodule attached to the liver of one male in group 2 was reported. All animals in groups 2 and 3 showed treatment-related widespread, predominantly extra-muscular, mononuclear cell infiltrate. The inflammation was very slight except for two females in group 3, in which the inflammation was scored as slight. Non treatment-related very slight mononuclear cell infiltrate was reported in two males and all females of group 1. One male in group 1 showed slight fiber degeneration also.

Test article related effects and assessments:

Test article related effects are listed in the table below:

Test article related effects	Effects considered incidental
Widespread, predominantly extra-muscular, mononuclear cell infiltrate	

Table 8: *Test article related effects (study # 6812/02)*

Adverse gross or microscopic alteration that could be indicative of systemic or local toxicity was not reported.

Other than chronic inflammation at the site of injection "which was attributable to recovery of trauma due to injection" and the widespread, predominantly extra-muscular, mononuclear cell infiltrate, there were no other treatment-related effects on histopathology, and any histopathology findings were considered as incidental to the study and not related to the test article.

There were no treatment-related effects on body weights or gross pathology. Based on the overall findings in this study, it can be concluded that in (b) (4) rabbits single intramuscular administration of Herpers Zoster VZV-gE vaccine had no adverse effects in terms of systemic toxicity and local tolerance at the dose level of 62.5 µg antigen with 62.5 µg QS-21 and 62.5 µg MPL in a liposome-based formulation in a total volume of 625 µL as adjuvant.

However, several endpoints such as opthalmologic examination, clinical chemistry, hematology, coagulation, histopathological examination, and immunological responses were not evaluated in this study.

GLP study deviations or amendments: No GLP study deviations or amendments were reported in this study.

Study # 2: AS01B Versus AS02V Toxicity Study by Repeated (Five Times) Intramuscular Administration to Rabbits. Study number; (b) (4) 045. (Reviewed by Dr. Al-Humadi in IND 13857)

Performing laboratory: (b) (4)

Study initiation date: 27 and 28 August, 2001 for males and females respectively.
Final Report date: 15 December, 2006

Test article batch/lot:
SB AS01B adjuvant = (b) (4)
SB AS02V adjuvant = (b) (4)
Animal species and strain: (b) (4)
Breeder/supplier: (b) (4) .
Number of animals per group and sex: 35 males and 35 females
Age: Approximately 11½ to 14½ week at start
Body weight range: 1.72-2.60 kg
Route and site of administration: Intramuscular, in the gastrocnemius muscle/alternately on both hindlimbs, commencing with the left hindlimb.
Volume of injection: 500 µL per animal per injection
Frequency of administration and study duration: Five doses were given on days 0, 14, 28, 42, and 56.
Dose:
SB AS01B adjuvant = Composition per vial:
KH_2PO_4: 41 mM
Na_2HPO_4: 9 mM
NaCl: 100 mM
SB AS 1
SB AS02V adjuvant = Composition per vial:
KH_2PO_4: 1.47 mM
Na_2HPO_4: 8.1 mM
NaCl: 137 mM
SB AS 2
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use vials (one vial per dose). Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study.

According to the testing laboratory, the shipment of AS01B and of AS02V was received on 07/19/2001 and an additional shipment was received on 09/20/2001. The expiration date was 01/30/2004 and 01/04/2004 for AS01B and AS02V, respectively. As stated in the study protocol, GMP material supplied by the sponsor was used to dose animals and quality control of dosage form was not assessed by (b) (4) . Quality control of the vaccine (antigen and adjuvant) is the responsibility of the sponsor.

Means of administration: The injection with the appropriate material was given to each animal in the gastrocnemius muscle/alternately on both hindlimbs, commencing with the left hindlimb.

Report status: Final
Experimental design:

Group	Treatment (Intramuscular)	Number of Animals (#/sex/group)
1	Saline	10M/10F
2	AS01B	10M/10F
3	AS02V	10M/10F

Table 9: *Experimental design (study #* (b) (4) *045)*

Methods:
The following parameters were evaluated: clinical signs (twice daily), skin reactions at the intramuscular site of injection (approximately at 1, 3, and 24 hours after dosing), body weights (on days -7, 0, 3, and 7, at weekly intervals thereafter and before necropsy), food consumption (daily and reported weekly), ophthalmoscopy (once during pretest and on days 59 and 84), rectal body temperature (prior to and 4 and 24 hours after injection on days 0 and 56), hematology and clinical chemistry (pretreatment and on days 1, 3, 54, 57, 59, and 84), blood for cytokines determination (prior to, 4, 8, 24, and 48 hours after injection on days 0 and 42), gross anatomy at termination, organ weights, and histopathology on a selection of tissues.

Parameters	Frequency of Testing
Cageside observation[3]	Twice daily
Clinical observations[4]	Twice daily
Body weight	On days -7, 0, 3, and 7, at weekly intervals thereafter and before necropsy
Food consumption	Daily and reported weekly
Body temperature	Prior to and 4 and 24 hours after injection on days 0 and 56
Ophthalmologic exam	Once during pretest and on days 59 and 84
Clinical chemistry*	Pretreatment and on days 1, 3, 54, 57, 59, and 84
Hematology*	Pretreatment and on days 1, 3, 54, 57, 59, and 84
Coagulation	NC
Cytokines determination*	Prior to, 4, 8, 24, and 48 hours after injection on days 0 and 42
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Approximately at 1, 3, and 24 hours after dosing
Necropsy	Days 59 and 84
Histopathology	Days 59 and 84

* Blood collected from central auricular artery. NC = Not collected
Table 10: *Parameters evaluated (study #* (b) (4) *045)*

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Organ/Tissue	Collected	Not collected
Adrenal glands	X!*	
Aorta	X!	
Bone (sternum & femur)		
Bone marrow (sternum & femur)		
Brain (cerebrum,	X!*	

[3] Cageside observations include mortality, morbidity, general health and signs of toxicity.
[4] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Organ/Tissue	Collected	Not collected
cerebellum, medulla/ pons, and olfactory bulb)		
Colon	X!	
Cecum	X	
Duodenum	X	
Epididymides	X!*	
Esophagus	X	
Eyes (optic nerve)	X!	
Fallopian tubes (oviduct)		
Femur (head and knee joint)	X!	
Gall bladder	X!	
Gross lesions (if any)		X
Harderian gland (if applicable)	X	
Heart	X!*	
Ileum	X	
Injection sites	X!	
Jejunum	X	
Kidneys	X!*	
Lacrimal glands		X
Larynx		X
Liver	X!*	
Lung (main-stem; bonchi)	X!*	
Lymph nodes (cervical)	X!*	
Lymph nodes-popliteal (left and right)	X*	
Lymph nodes (iliac and mesenteric)	X!	
Mammary glands	X	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		X
Ovaries	X!*	
Pancreas	X!	
Peyer's patch (if applicable)		X
Pituitary gland	X!*	
Prostate	X!*	
Rectum	X	
Salivary glands (mandibular)	X	
Sciatic nerve	X	
Seminal vesicles	X!*	

Organ/Tissue	Collected	Not collected
Skeletal muscle (thigh only)	X	
Sternum	X	
Skin (hind limb)	X	
Spinal cord (cervical, lumbar, thoracic)	X!	
Spleen	X!*	
Stomach	X!	
Testes	X!*	
Thymus	X!*	
Thyroid (w/ parathyroid glands)	X!*	
Tongue	X	
Trachea	X	
Ureters		X
Uterus (w/ cervix)	X!*	
Urinary bladder	X!	
Vagina	X!	
Zymbal's gland (if applicable)		X

Table of Histology – Tissues collected and marked with an X only were not processed histologically, but are held in fixative against any future requirement for microscopic examination.
Table 11: *Tissues collected (study #* (b) (4) *045)*

Results:
Morbidity and mortality: All animals survived to their scheduled termination.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium
CARBOHYDRATE METABOLISM		Glucose

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
LIVER FUNCTION: A) HEPATOCELLULAR	LDH: SD1 M ↓2.35 G2, SD1 M ↑5.90 G3 Aspartate aminotransferase (AST or SGOT): SD57 M ↓1.67 G2, SD57 M ↓1.82 G3, SD59 F ↓1.71 G2	Alanine aminotransferase (ALT or SGPT) Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids
B) HEPATOBILIARY	Total bilirubin: SD1 M ↑2.00 G3, SD59 F ↑2.00 G3, SD84 F ↑2.00 G3 Alkaline phosphatase (ALP): SD57 M ↓1.52 G2 Gamma-glutamyl transferase (GGT): SD57 M ↓2.00 G2	Total bile acids
ACUTE PHASE REACTANTS		C-reactive protein, fibrinogen (also under coagulation),
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Fasting Triglycerides: SD1 M ↓2.59 G2, SD1 M ↓3.20 G3, SD3 M ↓2.10 G2, SD3 M ↓2.53 G3, SD54 M ↓1.90 G2, SD54 M ↓2.49 G3, SD57 M ↓1.62 G2, SD57 M ↓3.10 G3, SD59 M ↓3.11 G2, SD59 M ↓4.37 G3, SD84 M ↓1.95 G2, SD84 M ↓2.87 G3 Creatine kinase: SD3F ↑1.69 G3, SD59M ↓1.96 G2, SD59F ↓1.58 G2, SD59F ↑5.55 G3, SD84M ↓1.62 G3	Albumin (A) Globulin (G, calculated) or A/G Ratio Total Cholesterol Cholinesterase Total protein

Table 12: *Clinical chemistry results (study #* (b) (4) *045)*

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	Total Leukocytes (WBC): SD1 M ↑1.8 G2, SD1 F ↑1.75 G2, SD1 F ↑1.69 G3, SD57 M ↑1.68 G2, SD57 M ↑1.57 G3, SD57 F ↑1.83 G3 Neutrophil count: SD1 M ↑3.8 G2, SD1 M ↑2.8 G3, SD1 F ↑3.36 G2, SD1 F ↑3.60 G3, SD57 M ↑4.90 G2, SD57 M ↑5.00 G3, SD57 F ↑3.91 G2, SD57 F ↑4.58 G3 Macrophage count: SD1 M ↑2.25 G2, SD1 M ↑1.83 G3, SD1 F ↑1.67 G2, SD1 F ↑2.27 G3, SD3 M ↑1.54 G3, SD3 F ↑1.73 G2, SD3 F ↑1.91 G3, SD54 M ↑1.73 G3, SD54 F ↑1.80 G2, SD57 F ↑2.28 G2, SD57 F ↑2.11 G3, SD59 M ↑2.13 G3, SD59 F ↑1.57 G3	lymphocyte count Total Leukocytes (WBC) Large Unstained Cells (LUC)
CLOTTING POTENTIAL	Fibrinogen: SD1 M ↑2.00 G2, SD1 M ↑1.60 G3, SD1 F ↑1.67 G2, SD1 F ↑1.80 G3, SD3 M ↑1.66 G2,	Activated partial-thromboplastin time clotting time Prothrombin time Mean platelet volume

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)	NOT OF NOTE
	SD3 F ↑1.95 G2, SD3 F ↑1.72 G3, SD57 M ↑2.31 G2, SD57 M ↑2.21 G3, SD59 M ↑1.92 G2, SD59 M ↑2.17 G3, SD59 F ↑1.57 G2, SD59 F ↑1.79 G3 Platelet count: SD59 M ↑1.62 G3	
OTHERS		Bone marrow cytology

Table 13: *Hematology results (study #* (b) (4) *045)*

Systemic toxicity:
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, rectal temperature (on days 0 or 56 of study), or organ weight were found. Number of animals exhibited signs at the injection sites shortly after dosing on days 14, 28, 42, and 56 of study. These signs were generally limited to very slight to well-defined erythema and blue/black coloration of the skin. The erythema was seen for a maximum of 2 days after dosing and the blue/black coloration persisted for up to 7 days after dosing. The incidence of these observations was generally slightly higher for the groups receiving the adjuvants than the controls, but there were no clear differences between the two adjuvants. One male (group 2) was unwilling to use the injected hindlimb at approximately 3 hours after the second dose. This observation were also seen in one female (groups 2) and two females (group 3) following and at approximately 3 hours after the fifth injection. This sign was no longer apparent at 24 hours after dosing.

During week 7 of study, males in group 3 showed decrease in their food consumption when compared to the control group. This effect did not persist, was not seen in the females and was attributed to slight low values for 3 animals. The different was therefore considered to be incidental and not related to treatment.

On study day 1, lactate dehydrogenase (LDH) levels were decreased and increased significantly in groups 2 and 3, respectively. Aspartate aminotransferase (AST) were decreased in groups 2 and 3 on study day 57. This decrease was also seen in group 2 females on study day 59. Total bilirubin increased significantly in group 3 males (study day 1) and females (study days 59 and 84). Alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) was decreased in group 2 males on study day 57. Triglyceride levels were decreased significantly in groups 2 and 3 males on days 1, 3, 54, 57, 59, and 84. Triglyceride levels were decreased in groups 2 and 3 females on study day 57 only. Creatine kinase levels were increased significantly in group 3

females on study days 3 and 59. The levels of creatine kinase were decreased significantly in group 2 (males and females) on study day 59. This decrease was also seen in group 3 males on study day 84.

Total leukocytes were increased significantly on study days 1 (groups 2M and 2F and group 3F) and 57 (groups 2 and 3, M & F). Neutrophil counts were increased significantly in groups 2 and 3 (males and females) on study days 1 and 57. Macrophages were increased significantly in groups 2 and 3 of males and females on study day 1. Macrophages were also increased in group 3 males and groups 2 and 3 females on study day 3. This increase were seen on study days 54 (group 3 males and group 2 females), 57 (groups 2 and 3 females), and 59 (group 3 males and females).
Fibrinogen levels were increased significantly in groups 2 and 3 of both males and females on study days 1 and 59. These levels were also increased on study day 3 in group 2 males and in groups 2 and 3 females. On study day 57, only males in both groups 2 and 3 showed this increase. An increase in platelet count was reported on study day 59 in group 3 males.

Organ Weight (Days 59/84):

SEX	MALES			FEMALES		
GROUPS	1 (CONTROL)	2	3	1 (CONTROL)	2	3
NUMBER OF ANIMALS	5	5	5	5	5	5
BODY WEIGHT (terminal)	3148/3042	2980/2914	2936/3082	3304/3038	2922/3200	2960/3042
PITUITARY	0.027/0.025	0.026/0.027	0.025/0.025	0.034/0.025	0.029/0.028	0.030/0.030
BRAIN	9.88/9.65	9.24*/9.68	9.78/9.67	9.26/9.38	9.55/9.30	9.34/9.54
ADRENALS	0.194/0.191	0.180/0.207	0.205/0.190	0.217/0.183	0.189/0.251	0.201/0.220
EPIDIDYMIDES	2.18/1.99	1.67*/1.94	1.69*/1.82			
HEART	6.69/5.85	6.84/6.31	6.30/6.59	5.81/5.43	6.07/6.18	5.99/5.60
KIDNEYS	18.10/13.63	15.03*/14.12	15.12*/15.53	15.52/13.33	15.23/15.72	14.97/14.07
LIVER	87/68	71/70	72/65	78/60	71/69	71/59
LUNGS and BRONCHI	19.38/13.91	16.04/20.68	18.70/18.25	13.21/12.51	16.35/16.35	16.46/12.30
SPLEEN	0.699/0.694	0.809/0.721	0.762/0.672	0.994/0.610	0.894/0.927	0.782/0.895
TESTES	5.25/5.32	5.12/5.51	4.44/4.77			
THYROID and PARATHYROID	0.158/0.159	0.170/0.156	0.166/0.165	0.148/0.122	0.137/0.149	0.146/0.154
THYMUS	2.90/3.26	2.36/2.26	2.86/2.82	2.90/2.38	1.85/2.13	2.02/1.78
LT. POPLITEAL	0.061/0.070	0.086/0.067	0.087/0.064	0.114/0.078	0.146/0.089	0.127/0.075
RT. POPLITEAL	0.080/0.079	0.075/0.065	0.098/0.061	0.104/0.075	0.072*/0.078	0.088/0.082
OVARIES				0.331/0.320	0.302/0.269	0.318/0.313
UTERUS & CERVIX				6.70/4.47	5.73/5.31	4.80/4.71

Absolute weights are expressed as mean (grams). *Different from controls at P≤0.05.
Table 14: Organ weights *(study #* (b) (4) *045)*

Brain weights were significantly decreased in males group 2 on study day 59. Epididymides and kidneys weights were decreased significantly in males groups 2 and 3 on day 59. No significant changes in body or organ weights were reported on study day 84 (recovery).

Gross Pathology:

Group	Findings (Day 59)
1M	Dark area at injection site 5 (1/5), masses in the liver, enlarged cervical lymph node (1/5), congested iliac lymph node (1/5), regional to mass mesenteric lymph node (1/5), congested lungs and bronchi (1/5)
2M	Congested iliac lymph node (1/5), enlarged salivary gland (1/5), pale area at the salivary gland
3M	Dark area at injection site 5 (2/5)
1F	Abnormal contents in the gall bladder (1/5), misshapen spleen (1/5)
2F	Dark area at injection site 5 (2/5), congested lungs and bronchi (1/5), cyst in the uterus (1/5), dark area in the adipose tissue (1/5), dark area at injection site 3 (2/5), thickened injection site 1 (1/5), dark area at injection site 1 (1/5), dark area in the muscle (1/5)
3F	Congested lungs and bronchi (1/5), fluid distention in the uterus (1/5), dark area at injection site 3 (1/5), dark area at injection site 1 (1/5), pale area at salivary glands (1/5), unilaterally enlarged salivary glands(1/5), pale salivary gland (1/5)

(NF = No findings)
Table 15: *Gross pathology results at day 59 (study #* (b) (4) *045)*

Group	Findings (Day 84)
1M	Congested iliac lymph node (1/5), dark area in skeletal muscle (1/5), misshapen spleen (1/5), splenulus adipose tissue (1/5), congested thymic lymph nodes (1/5), enlarged thymic lymph nodes (1/5), abnormal contents in the GI tract (1/5), pale area in the salivary gland (1/5)
2M	Dark area in the kidneys (1/5), congested lungs and bronchi (3/5)
3M	Congested iliac lymph node (1/5), abnormal contents in the GI tract (1/5), pale area in the salivary gland (3/5)
1F	Congested iliac lymph node (1/5), incomplete collapse of the lungs and bronchi (1/5), unilaterally small ovaries (1/5), misshapen spleen (1/5), misshapen uterus (1/5), splenulus adipose tissue (1/5), abnormal contents in the GI tract (1/5)
2F	Incomplete collapse of the lungs and bronchi (1/5), misshapen spleen (1/5), small thymus (1/5)
3F	Cyst in the kidneys (1/5), dark area in the ovaries (1/5), swollen spleen (1/5), splenulus adipose tissue (1/5), dark area in the skin/subutis

(NF = No findings)
Table 16: *Gross pathology results at day 84 (study #* (b) (4) *045)*

Microscopic finding are listed below:

Groups	Findings
1M	Cortical fatty vacuolation in the adrenals (1/5), inflammation with fibrosis at the injection site 5 (1/5), fatty infiltration at the injection site 5 (2/5), basophilic cortical tubules in the kidneys (4/5), dilated cortical tubules in the kidney (4/5), accumulation of cellular debris in collecting ducts in the kidneys (2/5), interstitial inflammatory infiltration in the kidney (2/5), cortical mineralization in the kidney (3/5), medullary mineralization in the kidney (1/5), cortical cast (1/5), chronic inflammatory cells within the portal area (3/5), focal inflammation with associated hepatocytic degeneration in the liver (1/5), follicular hyperplasia of the cervical LN (1/5), alveolar proteinosis in the lungs and bronchi (4/5), acinar cell vacuolation and apoptosis in the pancreas (1/5), accessory splenic tissue in the pancreas (1/5), cyst in the parathyroids (1/5), epithelial erosion in the stomach (3/5), spermatid giant cells in the testes (3/5), fatty infiltration at the injection site 2 (1/5)
2M	Hemorrhage in the brain (1/5), degenerate spermatogenic cells in ducts of the epididymides (1/5), inflammation with fibrosis at the injection site 5 (4/5), fatty infiltration at the injection site 5 (3/5), basophilic cortical tubules in the kidneys (5/5), dilated cortical tubules in the kidney (5/5), accumulation of cellular debris in collecting ducts in the kidneys (4/5), interstitial inflammatory infiltration in the kidney (2/5), cortical mineralization in the kidney (5/5), medullary mineralization in the kidney (2/5), erythrocytes and erythrophagocytosis in sinuses in the iliac LN (1/5), hemorrhage in the iliac LN (1/5), alveolar proteinosis in the lungs and bronchi (3/5), acinar cell vacuolation and apoptosis in the pancreas (2/5), epithelial erosion in the stomach (3/5), spermatid giant cells in the testes (4/5), inflammation with fibrosis at injection site 1 (2/5), fatty infiltration at injection site 2 (3/5), inflammation with fibrosis at injection site 3 (5/5), inflammation with fibrosis at injection site 4 (1/5), acute inflammation in the salivary glands (1/5), necrosis in the salivary glands (1/5)
3M	Hemorrhage in the brain (1/5), inflammation with fibrosis at the injection site 5 (4/5), fatty infiltration at the injection site 5 (2/5), subcutaneous hemorrhage at injection site 5 (1/5), basophilic cortical tubules in the kidneys (5/5), dilated cortical tubules in the kidneys (4/5), accumulation of cellular debris in collecting ducts in the kidneys (4/5), interstitial inflammatory infiltration in the kidney (1/5), cortical mineralization in the kidney (4/5), medullary mineralization in the kidney (3/5), alveolar proteinosis in the lungs and bronchi (2/5), acinar cell vacuolation and apoptosis in the pancreas (3/5), accessory splenic tissue in the pancreas (1/5), epithelial erosion in the stomach (2/5), spermatid giant cells in the testes (4/5), involution/atrophy in the thymus, follicular dilatation in the thyroids (1/5), focal parafollicular cell hyperplasia in the thyroids (1/5), inflammation with fibrosis at injection site 1 (1/5), inflammation with fibrosis at injection site 3 (4/5), inflammation with fibrosis at injection site 4 (2/5), fatty infiltration at injection site 4 (1/5)
1F	Cortical fatty vacuolation in the adrenals (2/5), fatty infiltration at injection

Groups	Findings
	site 4 (1/5), accumulation of cellular debris in collecting ducts in the kidneys (5/5), basophilic cortical tubules in the kidneys (5/5), dilated cortical tubules in the kidneys (5/5), interstitial inflammatory infiltration in the kidney (2/5), cortical mineralization in the kidney (5/5), medullary mineralization in the kidney (5/5), chronic inflammatory cells within the portal area (1/5), erythrocytes and erythrophagocytosis in sinuses in the iliac LN (1/5), alveolar proteinosis in the lungs and bronchi (3/5), prominent presence of corpora lutea in the ovaries (1/5), follicular cyst in the ovaries (5/5), mineralization in the ovaries (1/5), follicular hemorrhage in the ovaries (1/5), acinar cell vacuolation and apoptosis in the pancreas (2/5), acinar atrophy with chronic inflammation in the pancreas (1/5), cyst in the parathyroids (1/5), growth anomaly in the spleen (1/5), epithelial erosion in the stomach (2/5), mucosal lymphoid infiltration in the stomach (1/5), involution/atrophy in the thymus (2/5), cyst in the thyroids (1/5), inflammation with fibrosis at injection site 3 (1/5), inflammation with fibrosis at injection site 1 (1/5)
2F	Periostial inflammation in the femur inc. joint (1/5), inflammation with fibrosis at injection site 5 (5/5), fatty infiltration at injection site 5 (2/5), accumulation of cellular debris in collecting ducts in the kidneys (5/5), basophilic cortical tubules in the kidneys (5/5), dilated cortical tubules in the kidneys (5/5), interstitial inflammatory infiltration in the kidneys (2/5), cortical mineralization in the kidney (5/5), medullary mineralization in the kidney (4/5), chronic inflammatory cells within the portal area (2/5), erythrocytes and erythrophagocytosis in sinuses in the iliac LN (1/5), hemorrhage in the iliac LN (1/5), alveolar proteinosis in the lungs and bronchi (3/5), follicular cyst in the ovaries (5/5), acinar cell vacuolation and apoptosis in the pancreas (3/5), accessory splenic tissue in the pancreas (1/5), epithelial erosion in the stomach (3/5), dilated uterus (1/5), inflammatory cell infiltrate in the adipose tissue (1/5), inflammation with fibrosis at injection site 3 (2/5), hemorrhage at injection site 3 (1/5), subcutaneous inflammation at injection site 3 (1/5), inflammation with fibrosis at injection site 1 (1/5), inflammation with fibrosis at injection site 2 (1/5), fatty infiltration at injection site 2 (2/5)
3F	Cortical fatty vacuolation in the adrenals (1/5), inflammation with fibrosis at injection site 5 (3/5), fatty infiltration at injection site 5 (3/5), accumulation of cellular debris in collecting ducts in the kidneys (4/5), basophilic cortical tubules in the kidneys (5/5), dilated cortical tubules in the kidneys (5/5), interstitial inflammatory infiltration in the kidneys (3/5), cortical mineralization in the kidney (5/5), medullary mineralization in the kidney (5/5), chronic inflammatory cells within the portal area (2/5), focal necrosis with inflammatory infiltrate (1/5), erythrocytes and erythrophagocytosis in sinuses in the iliac LN (2/5), alveolar proteinosis in the lungs and bronchi (1/5), follicular cyst in the ovaries (3/5), mineralization in the ovaries (1/5), acinar cell vacuolation and apoptosis in the pancreas (4/5), epithelial erosion in the stomach (2/5), involution/atrophy in the thymus (1/5), lymphocytic thyroiditis in the thyroids (1/5), cyst in the thyroids (2/5), inflammation with

Groups	Findings
	fibrosis at injection site 3 (2/5), inflammation with fibrosis at injection site 4 (1/5), inflammation with fibrosis at injection site 1 (2/5), fatty infiltration at injection site 2 (1/5), acute inflammation in the salivary gland, fibrosis in the salivary gland

(NF = No findings)
Table 17: *Microscopic results (study #* (b) (4) *045)*

Body temperature:

Group	SD 0/1 of study			SD 56/57 of study		
	Pre-dose	4 hours after dose	24 hours after dose	Pre-dose	4 hours after dose	24 hours after dose
1 M/F	0	0	0	0	0	0
2 M/F	0	0	0	0	0	0
3 M/F	0	0	0	0	0	0

Table of occurrences for body temperature >40º
Table 18: Body temperature results *(study #* (b) (4) *045)*

Local toxicity: Dark area at injection site 5 was reported in males (groups 1 and 3) and females (group 2) treated animals. Dark areas were also reported at injection sites 1 and 3 in female groups 2 and 3.

Histologically, minimal to moderate, sporadic, inflammation with fibrosis was reported (at terminal kill) at the five injection sites in the treated animals. An increased incidence and degree of severity, when compared to control, of this inflammation were reported in both male and female rabbits given AS01B or AS02V adjuvants. This inflammation was accompanied by fatty infiltration in some of the injection sites.

An extensive number of tissues were examined for histology. Findings relative to the kidney, liver and lung appeared evenly distributed between treated and control groups of animals. No increased incidences of histological findings indicative of potential adverse events were observed in the treated groups relative to the controls.

Cytokines determination:
Blood (for cytokine determination) serum samples were collected and stored frozen for possible future analysis.

Test article related effects and assessments:

Test article related effects	Effects considered incidental
↓Aspartate aminotransferase (AST or SGOT), ↑Total bilirubin, ↓Triglyceride, ↓Creatine kinase (group 2 males and females), ↑Leukocytes count, ↑Neutrophil count, ↑Macrophages, ↑Fibrinogen	↓G2 and ↑G3 LDH, ↓G2 Alkaline phosphatase, ↓G2 GGT, ↑G3 platelet count.

Test article related effects	Effects considered incidental
Dark areas at the injection site Inflammation with fibrosis at the injection site	

Table 19: Test article related effects *(study #* (b) (4) *045)*

Treatment related effects on clinical pathology parameters such as the increases in fibrinogen levels, macrophage, neutrophil, and leukocyte counts were reported. The increases in these parameters are not considered signs of frank toxicity but more of anticipated effects associated with an immunological response.

Since control animals showed similar responses, adverse microscopic alteration, related to test article treatment, that could be indicative of systemic or local toxicity were not observed.

Other than chronic inflammation (which was attributable to recovery of trauma due to injection), very slight to well-defined erythema at the site of injection, and blue/black coloration of the skin, there were no treatment-related effects on histopathology, and any histopathology findings were considered as incidental to the study and not related to the test article.

Unwillingness to use the injected hindlimb in one male and one female (group 2), and two females (group 3) were reported. These observations were seen at approximately 3 hours after the second dose in the male and after the fifth dose in females. This sign was no longer apparent at 24 hours after dosing.

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, rectal temperature (on days 0 or 56 of study), or organ weight were found.

Blood samples for cytokine determinations were collected but not analyzed.

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

Study # 3: Repeated-dose Toxicity Study with a VZV Candidate Vaccine (gE 100 µg/AS01B) Administered Intramuscularly (Three Times) to Male and Female Rabbits. Study number; (b) (4) V6721. (Reviewed by Dr. Al-Humadi in IND 13857)

Performing laboratory: (b) (4)

Study initiation date: 23 and 24 January, 2006 for males and females, respectively.
Final Report date: 20 December, 2006
Test article batch/lot:

gE/100 µg/AS01B = (b) (4)

AS01B adjuvant = (b) (4)
Animal species and strain: (b) (4) rabbits
Breeder/supplier: (b) (4)
Number of animal per group and sex: 32 males and 32 females
Age: 12 week at start
Body weight range: 2200-2700 gram
Route and site of administration: Intramuscular
Volume of injection: 500 µL per animal per injection
Frequency of administration and study duration: Three doses on days 0, 14, and 28.
Dose:
VZV (gE-CHO rDNA) vaccine = 50 µg lyophilized.
AS01B adjuvant = MPL 50 µg and QS-21 50 µg.

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use vials (one vial per dose). Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study.

According to the testing laboratory, the shipment of gE antigen and of AS01B was received on 01/20/2006. The expiration date was 03/31/2009 and 10/31/2007 for gE antigen and AS01B, respectively.

Means of administration: The injection with the appropriate material was given to each animal in:
Day 0, right hind leg (right hamstring muscle)
Day 14, left hind leg (left calf muscle)
Day 28, right hind leg (right calf muscle)
Report status: Final

Experimental design:

Group	Treatment (Intramuscular)	Volume (µL)	Number of Animals (#/sex/group)	
			S1 (n=5)	S2 (n=5)
6721/01A	Saline	500	10M/10F	10M/10F
6721/01B	AS01B	500	10M/10F	10M/10F
6721/01C	gE 100 µg/AS01B	500	10M/10F	10M/10F

S1 = subgroup 1 animals were sacrificed on day 31 (i.e. 3 days post 3rd inoculation).
S2 = subgroup 2 animals were sacrificed on day 56 (i.e. 28 days post 3rd inoculation).
Table 20: *Experimental design (study #* (b) (4) *V6721)*

Methods:
The following parameters were evaluated: cage side observation and clinical observations (twice daily), skin reactions at the intramuscular site of injection (approximately at 3, 24 and 48 hours after each injection), body weights (in subgroup 1; days -7, -4, 0, 3, 7, 14, 21, 28, and 31, and in subgroup 2; days -7, -4, 0, 3, 7, and then weekly until day 56), food consumption (in subgroup 1; days -4, 0, 3, 7, 14, 21, and 28; and in subgroup 2; days -4, 0, 3, 7, and then weekly until day 56), ophthalmoscopy (in subgroups 2 animals, on days -4, 31 and 52), rectal body temperature (in

subgroup 1 only, prior and circa 4 and 24 hours after the first and the third inoculation), hematology and clinical chemistry (subgroups 2 only, pretest and on days 1, 3, 24, 29, and 56), gross anatomy at termination, organ weights and histopathology on a selection of tissues (day 31 for subgroup 1 and day 56 for subgroup 2). Blood samples for antibody-determination (day 31 for subgroup 1 and day 56 for subgroup 2) were taken and analyzed (non-GLP) under the responsibility of the sponsor.

Parameters	Frequency of Testing
Cageside observation[5]	Twice daily
Clinical observations[6]	Twice daily
Body weight	In subgroup 1; days -7, -4, 0, 3, 7, 14, 21, 28, and 31, and in subgroup 2; days -7, -4, 0, 3, 7, and then weekly until day 56
Food consumption	In subgroup 1; days -4, 0, 3, 7, 14, 21, and 28; and in subgroup 2; days -4, 0, 3, 7, and then weekly until day 56
Body temperature	In subgroup 1 only; prior and circa 4 and 24 hours after the first and the third inoculation
Ophthalmologic exam	In subgroups 2; animals on days -4, 31 and 52
Clinical chemistry*	Subgroups 2 only; pretest and on days 1, 3, 24, 29, and 56
Hematology*	Subgroups 2 only; pretest and on days 1, 3, 24, 29, and 56
Coagulation	NC
Immunological response*	Day 31 for subgroup 1 and day 56 for subgroup 2
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	NC
Necropsy	Day 31 for subgroup 1 and day 56 for subgroup 2
histopathology	Day 31 for subgroup 1 and day 56 for subgroup 2

* Ear artery. (NC = Not collected)
Table 21: *Parameters evaluated (study # (b) (4) V6721)*

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Organ/Tissue	Collected	Not collected
Adrenal glands	X!*	

[5] Cageside observations include mortality, morbidity, general health and signs of toxicity.
[6] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Organ/Tissue	Collected	Not collected
Aorta	X!	
Bone (sternum & femur)		X
Bone marrow (sternum & femur)		X
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	X!*	
Cervix		X
Colon	X!	
Duodenum		X
Epididymides	X!*	
Esophagus		X
Eyes (optic nerve)	X!	
Fallopian tubes (oviduct)		X
Gall bladder		X
Gross lesions (if any)	X!	
Harderian gland (if applicable)		X
Heart	X!*	
Ileum		X
Jejunum		X
Kidneys	X!*	
Lacrimal glands		X
Larynx		X
Liver	X!*	
Lung (main-stem; bonchi)	X!*	
Lymph nodes (cervical)		X
Lymph nodes (mandibular)	X!	
Lymph nodes (mesenteric)	X!	
Mammary glands		X
Muscle at injection sites	X!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		X
Ovaries	X!*	
Pancreas	X!	
Peyer's patch (if applicable)		X
Pituitary gland	X!*	
Prostate	X!*	
Rectum		X

Organ/Tissue	Collected	Not collected
Salivary glands (mandibular)		X
Sciatic nerve	X!	
Skeletal muscle	X!	
Skin		X
Spinal cord (cervical, lumbar, thoracic)	X!	
Spleen	X!*	
Stomach	X!	
Testes	X!*	
Thymus	X!*	
Thyroid (w/ parathyroid glands)	X!*	
Tongue		X
Ureters		X
Uterus (w/ cervix)	X!*	
Urinary bladder	X!	
Vagina		X
Zymbal's gland (if applicable)		X

Table 22: *Histopathology tissues (study #* (b) (4) *V6721)*

Histopathological examination was performed on the tissues and organs in the table above as indicated for all animals. All injection sites were sampled and preserved, but only the site injected on day 28 (right calf muscle) was examined microscopically in order to better assess the evolvement of the local reaction over time. The tissues selected were (b) (4), sectioned at 5 μm and (b) (4). With the preserved right calf muscle three areas were processed, i.e. central and adjacent left and right area. These areas were also examined macroscopically for gross findings. Tissues/organs that were not submitted to histopathological examination were kept in (b) (4). Bone marrow smears (fixated in (b) (4) were prepared at scheduled necropsies of the animals and kept in storage for possible examination.

Results:
Morbidity and mortality: All animals survived to their scheduled termination.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Lactate dehydrogenase (LDH): SD3 1st inoculation F ↓1.60 G3 SD-4 prior 3rd inoculation F ↓1.60 G2	Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids
B) HEPATOBILIARY		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein, fibrinogen (also under coagulation),
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Creatine kinase: SD3 1st inoculation M ↑1.56 G3 SD3 1st inoculation F ↓2.25 G2 SD3 1st inoculation F ↓2.78 G3 SD1 3rd inoculation F ↓1.84 G3 Fasting Triglycerides: SD1 1st inoculation M ↑1.54 G3 SD28 3rd inoculation M ↑1.60 G2	Albumin (A) Globulin (G, calculated) or A/G Ratio Total Cholesterol Cholinesterase Total protein

ND = not determined

Table 23: *Clinical chemistry results (study #* (b) (4) *V6721)*

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	Neutrophil count: SD1 1st inoculation M ↑2.00 G2 Monocyte count: SD3 1st inoculation M ↑2.00 G2 SD3 1st inoculation M ↑1.70 G2 SD3 1st inoculation M ↑1.61 G3 SD1 3rd inoculation F ↓1.60 G3 Basophils: SD28 3rd inoculation M ↑1.63 G2 SD28 3rd inoculation F ↓2.00 G2 Eosinophils count: SD1 3rd inoculation F ↓2.00 G3 SD3 3rd inoculation F ↑1.75 G3	lymphocyte count Neutrophil count Total Leukocytes (WBC) Large Unstained Cells (LUC)
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

ND = Not determined

Table 24: *Hematology results (study # (b) (4) V6721)*

Systemic toxicity:
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, body temperature, or organ weight were found.

There were no treatment-related effects on organ weights or weight rations for males receiving the adjuvanted vaccine. Males receiving the adjuvanted vaccine had statistically significantly increased adrenals (0.242 vs 0.169 grams) and epididymides (2.25 vs 1.82 grams) absolute weights on study day 31. These increases were not reported at the recovery period (study day 56).

There was no treatment-related clinical pathology in treated males; however, treated females demonstrated a statistically significant decrease in LDH (SD3 1st inoculation and SD -4 prior to 3rd inoculation) levels. Creatine kinase levels were significantly increased in group 3 males on study day 3 after 1st inoculation and decreased in groups 2 and 3 females on study day 3 after first inoculation. It was also decreased in group 3 females on study day 1 after 3rd inoculation. Triglyceride levels were significantly increased in group 3 males on study day 1 after the 1st inoculation. It was also increased in groups 2 males on study day 28 after the 3rd inoculation. The values of the above changes were not considered to be biologically significant because they were inconsistent and did not correlate with any histopathology findings.

Among the hematology parameters, neutrophil count was increased significantly in group 2 males on study day 1 after the 1st inoculation. Monocyte counts were increased significantly in groups 2 and 3 males on study day 3 after the 1st inoculation. Monocyte counts were decreased significantly in groups 2 and 3 females on study day 13 after the 3rd inoculation. There was significant increase in monocyte count in group 3 females on study day 3 after the 3rd inoculation. Basophils were decreased significantly in groups 2 and 3 males on study day 1 after the 3rd inoculation. Basophils were increased significantly in groups 2 and 3 males on study day 28 after the 3rd inoculation. In group 2 females, basophils were decreased significantly on study day 28 after the 3rd inoculation. Eosinophil count was increased significantly in group 2 males on study day 3 after the 1st inoculation. Eosinophil counts were also increased significantly in group 2 females on study day 2 after the 1st inoculation. Eosinophil count was decreased significantly in group 3 females on study day 1 after the 3rd inoculation but increased significantly in group 3 females on study day 3 after the 3rd inoculation.

Organ Weight (SD31/SD56):

SEX	MALES			FEMALES		
GROUPS	1 (SD31/SD56) (CONTROL)	2 (SD31/SD56)	3 (SD31/SD56)	1 (SD31/SD56) (CONTROL)	2 (SD31/SD56)	3 (SD31/SD56)
NUMBER OF ANIMALS	5/5	5/5	5/5	5/5	5/5	5/5
BODY WEIGHT (terminal)	3137/3435	3315/3730	3385/3723	3391/3893	3440/3937	3353/4091
BRAIN	9.49/9.97	9.44/9.89	9.13/9.99	9.25/9.47	9.68/9.55	9.70/9.78
ADRENALS	0.169/0.223	0.242**/0.270	0.186/0.278	0.187/0.208	0.200/0.211	0.169/0.213
EPIDIDYMIDES	1.82/2.39	2.25*/2.62	2.01/2.15			
HEART	7.22/7.36	8.94/7.93	7.65/8.16	7.86/8.69	7.81/8.51	7.63/8.54
KIDNEYS	15.29/15.78	15.44/16.78	17.18/16.50	15.85/16.25	16.87/16.56	16.47/17.43
LIVER	105.44/99.40	105.24/118.40	123.67/114.24	124.96/116.12	118.74/130.16	107.59/128.41
PITUITARY	0.027/0.030	0.029/0.037	0.030/0.030	0.035/0.032	0.043/0.035	0.035/0.034
POP-LN-L	0.222/0.229	0.232/0.269	0.238/0.269	0.284/0.297	0.370/0.316	0.317/0.329
POP-LN-R	0.221/0.255	0.272/0.288	0.327/0.254	0.247/0.299	0.352/0.296	0.361/0.323
PROSTATE	2.60/3.78	3.12/4.03	3.21/3.72			
SPLEEN	1.058/0.935	1.159/1.146	1.231/1.183	1.291/1.349	1.453/1.403	1.713/1.267
TESTES	3.54/5.18	4.41/4.45	3.96/5.07			
THYROID and PARATHYROID	0.204/0.210	0.181/0.204	0.205/0.218	0.208/0.253	0.232/0.242	0.226/0.242
THYMUS	4.66/4.00	4.65/5.38	4.69/4.62	4.32/4.56	5.34/4.99	5.02/4.93
OVARIES				0.247/0.250	0.336/0.294	0.365/0.294
UTERUS				4.088/4.586	5.139/4.966	4.709/5.854

Absolute weights are expressed as mean (grams). *Different from controls at P≤0.05; **Different from controls at P≤0.01.

Table 25: *Organ weight results (study # (b) (4) V6721)*

Gross Pathology:

Group	Findings (Day 31)
1M	Red area in the calf muscle (left, 1/5), red area in the calf muscle (right, 1/5), hemorrhage, hamstring (left, 1/5), encrustations in the skin (1/5), red appearance in the thyroid (1/5)
2M	Petechia (e) in the calf muscle (left, 1/5), red area in the calf muscle (right, 1/5), hemorrhage, hamstring (left, 1/5), uni-lateral pitted area in the kidneys (1/5), uni-lateral red area in the mandibular LN(1/5), red appearance in the popliteal LN (left, 1/5), red appearance in the thyroid (2/5)
3M	Uni-lateral red area in the adrenals (1/5), uni-lateral cyst in the kidneys (1/5), Hyalin tissue in the mandibular LN(1/5), swollen seminal vesicles (1/5), yellow content in the seminal vesicles (1/5)
1F	Uni-lateral cyst in the adrenals (1/5), hemorrhage in the calf muscle (right, 1/5), two bladders (1/5), hemorrhage "hamstring" (left, 1/5), cyst in the kidneys (2/5), firm rough surface in the liver (1/5), encrustations in the skin (2/5), shaving wound in skin (1/5)
2F	Encrustations in the skin (1/5)
3F	Petechia (e) in the calf muscle (right, 1/5), focal atelectasis in the lungs (1/5), encrustations in the skin (1/5), uni-lateral cyst in the thyroid (1/5), red appearance in the uterus (1/5)

(NF = No findings)
Table 26: *Gross pathology results at day 31 (study #* (b) (4) *V6721)*

Group	Findings (Day 56)
1M	Cyst in the kidneys (1/5), nodule in the thymus (1/5)
2M	Red enlarged in the mandibular LN (1/5), small prostate (1/5)
3M	Uni-lateral cyst in the adrenals (1/5), cyst in the kidneys (1/5)
1F	Cyst parametrial adipose tissue in the abdominal cavity (1/5), discharge in the eye (1/5), agenesis in the gall bladder (1/5), cyst in the kidneys (1/5), didelphys in the uterus (1/5)
2F	Cyst parametrial adipose tissue in the abdominal cavity (1/5), discharge in the eye (1/5), uni-lateral constrictions in the kidneys (1/5), uni-lateral cyst in the thyroid (1/5)
3F	Cyst parametrial adipose tissue in the abdominal cavity (1/5)

(NF = No findings)
Table 27: *Gross pathology results at day 56 (study #* (b) (4) *V6721)*

Microscopic finding are listed below:

Groups	Findings (Day 31)
1M	Very slight mononuclear cell infiltrate (4/5), very slight fiber (s) degeneration (1/5), very slight macrophage aggregate (s) in the right inguinal LN (1/5), sinusoidal blood in the left popliteal LN (1/5), slight focal

Groups	Findings (Day 31)
	macrophage accumulation (1/5), focal basophilic tubules in the kidneys (4/5), multi focal mononuclear cell infiltrate in the kidneys (1/5), mineralization in the kidneys (3/5), multi focal mononuclear cell infiltrate in the larynx (2/5), BALT hyperplasia in the lungs (3/5), cystically dilated sinus in the mandibular LN (1/5), small macrophage aggregates in the mesenteric LN (1/5), multi focal epithelial hyperplasia in the prostate (1/5), focal hyperkeratosis in the skin (1/5), multi focal mononuclear cell infiltrate in the stomach (1/5), focal ulceration (1/5), increased multinucleated giant cells in the testes (2/5), microhemorrhage in the thymus (1/5)
2M	Hemorrhage (focal) in the calf muscle (TR, right) (3/5), very slight widespread mononuclear cell infiltrate (3/5), very slight multifocal mononuclear cell infiltrate (1/5), very slight mononuclear cell infiltrate (1/5), very slight fiber (s) degeneration(1/5), very slight macrophage aggregate (s) in the right inguinal LN (1/5), very slight macrophage aggregate (s) in the left inguinal LN (1/5), increased polymorphonuclear leukocytic infiltration in the right popliteal LN (4/5), enhanced activate appearance in the right popliteal LN (1/5), microhemorrhage in the left popliteal LN (1/5), multi focal cortical vacuolation in the adrenals (2/5), focal basophilic tubules in the kidneys (4/5), multi focal mononuclear cell infiltrate in the kidneys (2/5), tubular dilatation in the kidneys (1/5), mineralization in the kidneys (4/5), scar tissue in the kidneys (1/5), multi focal mononuclear cell infiltrate in the larynx (3/5), multi focal alveolitis in the lungs (1/5), multi focal polymorphonuclear leukocytic infiltration in the lungs (2/5), BALT hyperplasia in the lungs (2/5), cystically dilated sinus in the mandibular LN (1/5), uni-lateral vascular hyperemia (1/5), multi focal epithelial hyperplasia in the prostate (1/5), multi focal mononuclear cell infiltrate in the stomach (3/5), focal ulceration (2/5), increased multinucleated giant cells in the testes (2/5), increased number of cortical macrophage in the thymus (1/5), microhemorrhage in the thymus (1/5), uni-lateral hemorrhage in the thyroid (1/5), multi focal mononuclear cell infiltrate in the trachea/bronchi (2/5), urolithiasis in the urinary bladder (3/5),
3M	Hemorrhage (focal) in the calf muscle (TR, right) (2/5), very slight to slight widespread mixed inflammatory-cell infiltration in right calf muscle (5/5), increased polymorphonuclear leukocytic infiltration in the right popliteal LN (4/5), enhanced activated appearance in the right popliteal LN (5/5), increased polymorphonuclear leukocytic infiltration in the left popliteal LN (4/5), enhanced activated appearance in the left popliteal LN (3/5), multi focal cortical vacuolation in the adrenals (1/5), multi focal mononuclear cell infiltrate in the adrenals (1/5), focal basophilic tubules in the kidneys (4/5), multi focal mononuclear cell infiltrate in the kidneys (2/5), mineralization in the kidneys (2/5), cyst in the kidneys (1/5), multi focal mononuclear cell infiltrate in the larynx (1/5), laryngitis in the larynx (1/5), cystically dilated sinus in the mandibular LN (4/5), hemorrhage in the mandibular LN (2/5), multi focal epithelial hyperplasia in the prostate (2/5), mixed inflammatory cell infiltrate in surrounding tissue in the sciatic nerve (2/5), increased

Groups	Findings (Day 31)
	germinal center development in the spleen (1/5), multi focal mononuclear cell infiltrate in the stomach (3/5), increased number of cortical macrophage in the thymus (1/5), focal interstitial mononuclear cell infiltrate in the thyroid (1/5), focal c-cell hyperplasia in the thyroid (1/5), multi focal mononuclear cell infiltrate in the trachea/bronchi (2/5)
1F	Hemorrhage (focal) in the calf muscle (TR, right) (1/5), very slight macrophage aggregate (s) in the right inguinal LN (1/5), very slight macrophage aggregate (s) in the right popliteal LN (2/5), very slight macrophage aggregate (s) in the left popliteal LN (1/5), enhanced activated appearance in the left popliteal LN (2/5), multi focal cortical vacuolation in the adrenals (1/5), diffuse lamina propria plasma cell accumulation in the colon (1/5), two gall bladders (1/5), focal basophilic tubules in the kidneys (3/5), multi focal mononuclear cell infiltrate in the kidneys (1/5), tubular dilatation in the kidneys (1/5), mineralization in the kidneys (4/5), cyst in the kidneys (2/5), multi focal mononuclear cell infiltrate in the larynx (2/5), focal hepatocellular necrosis in the liver (1/5), multi focal alveolitis in the lungs (2/5), focal alveolar microhemorrhage in the lungs (2/5), cystically dilated sinus in the mandibular LN (2/5), hemorrhage in the mandibular LN (1/5), tunica media vacuolation in the mesenteric artery (1/5), mineralization in the ovaries (4/5), focal polymorphonuclear leukocytic infiltration in the parathyroids (1/5), multi focal acanthosis in the skin (2/5), crust formation in the skin (1/5), focal dermatitis in the skin (1/5), multi focal mononuclear cell infiltrate in the stomach (1/5), focal ulceration (2/5), microhemorrhage in the thymus (1/5), cyst in the thyroid (1/5), focal c-cell hyperplasia in the thyroid (1/5), multi focal mononuclear cell infiltrate in the trachea/bronchi (1/5), urolithiasis in the urinary bladder (2/5)
2F	Hemorrhage (focal) in the calf muscle (TR, right) (1/5), very slight widespread mononuclear cell infiltrate (5/5), very slight macrophage aggregate (s) in the right inguinal LN (1/5), very slight macrophage aggregate (s) in the right popliteal LN (1/5), increased polymorphonuclear leukocytic infiltration in the right popliteal LN (3/5), enhanced activated appearance in the right popliteal LN (2/5), very slight macrophage aggregate (s) in the left popliteal LN (1/5), increased polymorphonuclear leukocytic infiltration in the left popliteal LN (2/5), enhanced activated appearance in the left popliteal LN (2/5), multi focal cortical vacuolation in the adrenals (3/5), multi focal mononuclear cell infiltrate in the adrenals (1/5), focal myocardial mononuclear cell infiltrate in the heart (2/5), focal basophilic tubules in the kidneys (4/5), tubular dilatation in the kidneys (2/5), mineralization in the kidneys (5/5), multi focal mononuclear cell infiltrate in the larynx (4/5), portal bile duct/ductular cell hyperplasia in the liver (1/5), alveolar microhemorrhage in the lungs (1/5), cystically dilated sinus in the mandibular LN (1/5), mineralization in the ovaries (5/5), corpora lutea in the ovaries (1/5), focal hemorrhage in the pancreas (1/5), focal mononuclear cell infiltrate in the pancreas (1/5), multi focal acanthosis in the skin (1/5), crust formation in the skin (1/5), multi focal mononuclear cell infiltrate in the

Groups	Findings (Day 31)
	stomach (3/5), focal ulceration (1/5), epithelial cyst in the stomach (2/5), focal interstitial mononuclear cell infiltrate in the thyroid (1/5), multi focal mononuclear cell infiltrate in the trachea/bronchi (1/5), endometrial hyperplasia in the uterus (1/5)
3F	Very slight to slight widespread mixed inflammatory-cell infiltration in right calf muscle (5/5), mineralization (1/5), very slight macrophage aggregate (s) in the right inguinal LN (1/5), very slight macrophage aggregate (s) in the left inguinal LN (1/5), increased polymorphonuclear leukocytic infiltration in the right popliteal LN (4/5), enhanced activated appearance in the right popliteal LN (4/5), increased polymorphonuclear leukocytic infiltration in the left popliteal LN (3/5), enhanced activated appearance in the left popliteal LN (2/5), focal keratitis in the eyes (1/5), myocardial mononuclear cell infiltrate in the heart (1/5), focal basophilic tubules in the kidneys (2/5), multi focal mononuclear cell infiltrate in the kidneys (2/5), tubular dilatation in the kidneys (1/5), mineralization in the kidneys (2/5), luminal polymorphonuclear leukocytic infiltration in the larynx (1/5), multi focal mononuclear cell infiltrate in the larynx (3/5), multi focal alveolitis in the lungs (5/5), multi focal polymorphonuclear leukocytic infiltration in the lungs (1/5), lobar emphysema in the lungs (1/5), mineralization in the ovaries (5/5), mixed inflammatory cell infiltrate in surrounding tissue in the sciatic nerve (3/5), multi focal acanthosis in the skin (1/5), crust formation in the skin (1/5), hemorrhage in the skin (1/5), multi focal mononuclear cell infiltrate in the stomach (2/5), focal interstitial mononuclear cell infiltrate in the thyroid (1/5), cyst in the thyroid (1/5), vascular hyperemia in the uterus (1/5)

(NF = no findings)
Table 28: *Microscopic results (study #* (b) (4) *V6721)*

Day 31
Right treated calf muscle (injected on day 28, sampled 3 days after inoculation): An inflammatory response was observed in all treated animals. The inflammation extended throughout the muscle and/or along the epimysium ('widespread') in most animals, but consisted of limited numbers of inflammatory cells, hence the grading of very slight to slight. In the AS01B group, the inflammation was of mononuclear cell type (predominantly small- to medium-sized macrophages). In the gE 100 μg/AS01B group, the inflammation was of a mixed cell type (a mixture of mononuclear, i.e. small- to medium-sized macrophages and lymphocytes; and granulocytic inflammatory cells) and associated with mineralization in 2 males and 1 female. Injection with saline induced a very slight to slight mononuclear cell infiltrate, which was in most cases a typical, elongated trail of predominantly small-sized macrophages, which is considered to be a response against the inoculation procedure.
Draining inguinal and popliteal lymph nodes: Almost all gE 100 μg/AS01B, a few AS01B animals and 2 saline controls exhibited enhanced activated appearance (increased cellularity of medullary cords) of popliteal lymph nodes. Increased polymorphonuclear leukocytic infiltration was observed in the right popliteal lymph nodes of most AS01B and gE 100 μg/AS01B animals and in the left popliteal lymph nodes of a few AS01B animals and several gE 100 μg/AS01B

animals. Very slight macrophage aggregates were observed in the draining lymph nodes of some animals including saline controls. The cytology of the macrophages in the group resembled those in the saline controls and was, therefore, considered to be unrelated to injection with the vaccine formulations.
Sciatic nerve: A mixed inflammatory-cell infiltrate was observed in the adipose tissue closely around the sciatic nerve of 2 males and 3 females of the gE 100 μg/AS01B group. This infiltrate was considered to be a reaction to the test article, which had leaked from the injection site into the surrounding adipose tissue including the nerve.

Other organs: No vaccine-related histopathological changes were observed, other than a prominent germinal center development in the spleen of 1 gE 100 μg/AS01B male, which was considered to be a physiological reaction.

Day 56
Right treated calf muscle (injected on day 42, sampled 28 days after inoculation): An inflammatory response was observed in 1 AS01B male, 1 gE 100 μg/AS01B male and 4 gE 100 μg/AS01B females. The inflammation consisted of slight mixed inflammatory cell infiltration in 1 gE 100 μg/AS01B male (multifocal), and 2 gE 100 μg/AS01B females (1 with fibrosis and the other widespread with multinucleated giant cells) and was considered vaccine-related, because mixed inflammation of this degree has not been observed in saline controls of the present and previous studies. This relationship was less clear for the very slight mixed inflammatory cell infiltration in 1 gE 100 μg/AS01B female and the very slight mononuclear cell infiltrate in 1 AS01B male. Very slight mixed or mononuclear inflammation has been observed in saline controls but without the few lymphocytes as observed in these two animals. The very slight mononuclear cell infiltrate in 1 gE 100 μg/AS01B female was similar to that observed regularly in saline controls and was therefore considered to be unrelated to inoculation with the vaccine formulation.
Untreated triceps: Very slight mononuclear cell infiltrates were observed in 1 gE 100 μg/AS01B male and 1 gE 100 μg/AS01B female.
Draining inguinal and popliteal lymph nodes: One AS01B female exhibited enhanced activated appearance (increased cellularity of medullary cords) of the right popliteal lymph node. Increased polymorphonuclear leukocytic infiltration was observed in the right popliteal lymph nodes of 1 AS01B and 1 gE 100 μg/AS01B female. Very slight macrophage aggregates were observed in the draining lymph nodes of animals of all groups including the saline control. The cytology of the macrophages in the vaccine groups resembled those in the saline controls and was, therefore, considered to be unrelated to injection with the vaccine.
Other organs: A decreased incidence of mononuclear cell infiltrate in the trachea/bronchi of AS01B females was the only statistically significant difference between the test groups and saline controls. The decrease was considered to be fortuitous, illustrating the variability in incidence of this histopathological change. All but few of the histopathological findings observed were considered to be common for rabbits of this strain and age. The exceptions were a) a very slight, focal glia cell proliferation in the cerebral cortex of the brain of 1 gE 100 μg/AS01B male; b) slight chronic epicarditis in the heart of another gE 100 μg/AS01B male; and c) an embryonal remnant in the heart of 1 saline control female. The focal glia cell proliferation consisted of two small accumulations of glia cells and neurons with a slight preponderance of glia cells. The finding might be a congenital anomaly. Examination of the additionally prepared sections of the

brains of all saline control and gE 100 μg/AS01B animals, did not show such a proliferation. Because the slight epicarditis was observed only in one animal, this incidental finding was considered not treatment-related.

Body temperature:
Not test article-related effect on body temperature was reported.

Group	SD 0/1 of study			SD 56/57 of study		
	Pre-dose	4 hours after dose	24 hours after dose	Pre-dose	4 hours after dose	24 hours after dose
1 M/F	0	0	0	0	0	0
2 M/F	0	0	0	0	0	0
3 M/F	0	0	0	0	0	0

Table of occurrences for body temperature >40º
Table 29: *Body temperature results (study # (b) (4) V6721)*

Local toxicity: Hematoma at the injection sites were reported in the males' control (3/10), adjuvant (1/10) and the candidate vaccine (3/10) groups. This observation were also reported in females' control (2/10) and adjuvant (1/10) groups. Since this observation was reported in the control groups, it is considered non test article-related.

At study day 31 sacrifices, macroscopic examination at necropsy revealed red areas and/or hemorrhages at the inoculation site of the calf muscles and hamstring in some animals including saline controls. In addition, petechiae were observed in the calf muscles of one AS01B male and one gE 100 μg/AS01B female. Encrustations were found on the skin of some animals including saline controls. One saline control female exhibited a shaving wound. The gross changes in the other organs were considered to be unremarkable because they are part of common background pathology and they occurred in one or two animals or the incidences were distributed about equally amongst the controls.

At study day 56 sacrifices, macroscopic examination at necropsy did not reveal gross changes at the inoculation sites. The observed gross changes, including the congenital changes in a saline control (the absence of a gall bladder or 'agenesis' and the presence of a double uterus or 'didelphys'), were considered to be unremarkable.

An extensive number of tissues were examined for histology. Findings relative to the kidney, liver, and lung appeared evenly distributed between treated and control groups of animals. No increased incidence of histological findings indicative of potential adverse events was observed in the treated groups relative to the controls.

Serology:
The serological analysis showed that anti-gE antibody responses were observed in 100% of male and female rabbits after 3 administrations of VZV candidate vaccine adjuvanted with AS01B. No anti-gE antibodies were detected in the sera collected from animals in saline-treated control group and AS01B adjuvant group. Similar level of anti-gE responses observed between male and

females rabbits at days 3 and 28. It was concluded that vaccine take was observed in 100% of rabbits immunized with VZV candidate vaccine.

Test article related effects and assessments:

Test article related effects are listed in the table below:

Test article related effects	Effects considered incidental*
↓F Creatine kinase, ↑M monocytes, ↓F monocytes, Mixed inflammatory-cell infiltrate in the adipose tissue closely around the sciatic nerve. Popliteal lymph nodes; enhanced activated appearance and increased polymorphonuclear leukocytic infiltration.	↑ Triglycerides, ↑ neutrophils, ↑↓ eosinophils, ↑↓ basophils, Hematoma at the injection site,

Table 30: *Test article related effects (study # (b) (4) V6721)*

There were no clear treatment-related effects on clinical pathology parameters, although there were a number of statistically significant differences. Many of these differences were of a magnitude or nature that was not clinically significant or that remained within the normal range of values established for gender, laboratory or species.

Adverse gross or microscopic alteration that could be indicative of systemic or local toxicity was not observed.

There were no treatment-related effects on histopathology, and any histopathology findings were considered as incidental to the study and not related to the test article.

There were no treatment-related effects on clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, body temperature, or organ weights.

Draize scoring (for edema, erythema and eschar formation) were mentioned in appendix 1 but no results were reported.

Based on the overall findings in this study, it can be concluded that in (b) (4) rabbits repeated intramuscular administration of VZV candidate vaccine had no adverse effects in terms of systemic toxicity and local tolerance at the dose level of 50 µg antigen with AS01B (50 µg MPL and 50 µg QS-21) as adjuvant.

Immunology performed in this study verified that an active dose was administered.

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

Conclusion:
Based on nonclinical toxicity assessments of this study and the assessment of the supportive studies (micronucleus, and bone marrow and blood cells tests, above), there are no significant safety issues to report in this study.

Study # 4: Repeated-dose Toxicity Study with Zoster Candidate Vaccine (gE/AS01B) Administered Subcutaneously (Four Times) or Intramuscularly (Four Times) to Male and Female Rabbits Followed by a 4-Week Treatment Free Period. Study number; (b) (4) V 20094.

Performing laboratory: (b) (4).
Study initiation date: 12 and 13 December, 2011 for males and females, respectively.
Final Report date: 13 June, 2012
Test article batch/lot:

Test article	Lot number	Expiration date
gE/AS01B	(b) (4)	(b) (4)
AS01B adjuvant	(b) (4)	
Saline	(b) (4)	

Animal species and strain: (b) (4) rabbits
Breeder/supplier: (b) (4)
Number of animal per group and sex: 52 males and 52 females
Age: 12 week at start
Body weight range: 2000-2300 gram
Route and site of administration: Subcutaneous (SC) or intramuscular (IM)
Volume of injection: 500 µL per animal per injection
Frequency of administration and study duration:

On days 0, 15/14 (males/females), 28, and 42, the SC injections with the appropriate material were given in the subcutis overlaying the central part of the left anterior thigh muscle of the animals of treatment groups 1, 2, and 3.

On days 0, 15/14 (males/females), 28, and 42, the IM injections were made in the central part of the right anterior thigh muscle of the animals of treatment groups 1, 4, and 5.

Study duration was 70 and 71 for males and females, respectively.

Dose:
For gE: Monodose vial containing 62.5 µg of freeze-dried gE antigen per cake.
For AS01B adjuvant: 700 µl monodose vial containing 50 µg QS-21 and 50 µg MPL in a liposome-based formulation per 500 µl.

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study.

Please see expiration dates above.

Means of administration: Subcutaneous (SC) or intramuscular (IM)
Report status: Final

Experimental design:

Males:

Group	Color Code	Treatment	Volume	Animal Numbers of Males (even nos.)	
				S1 (n=5)	S2 (n=5)
20094/1	White	Saline	0.5 mL	2-10	12-20
20094/2	Blue	gE/AS01B (SC)	0.5 mL	22-30	32-40
20094/3	Green	AS01B (SC)	0.5 mL	42-50	52-60
20094/4	Red	gE/AS01B (IM)	0.5 mL	62-70	72-80
20094/5	Yellow	AS01B (IM)	0.5 mL	82-90	92-100

Females:

Group	Color Code	Treatment	Volume	Animal Numbers of Females (odd nos.)	
				S1 (n=5)	S2 (n=5)
20094/1	White	Saline	0.5 mL	1-9	11-19
20094/2	Blue	gE/AS01B (SC)	0.5 mL	21-29	31-39
20094/3	Green	AS01B (SC)	0.5 mL	41-49	51-59
20094/4	Red	gE/AS01B (IM)	0.5 mL	61-69	71-79
20094/5	Yellow	AS01B (IM)	0.5 mL	81-89	91-99

S1 = subgroup 1 were sacrificed on day 45 (i.e. 3 days post 4th injection)
S2 = subgroup 2 were sacrificed on day 70/71 (i.e. 28/29 days post 4th injection)

Table 31: *Experimental design (study # (b) (4) V 20094)*

Methods:
The following parameters were evaluated: cage side observation and clinical observations (twice daily), skin reactions at the intramuscular site of injection (approximately at 3, 24 and 48 hours after each injection), body weights (in subgroup 1; days -4, 0, 3, 7, 15, 21, 28, 35, 42 and 45; and in subgroup 2; days -4, 0, 3, 7, and weekly until day 70/71), food consumption (in subgroup 1; days -4, 0, 3, 7, 15, 21, 28, 35 and 42; and in subgroup 2; days -4, 0, 3, 7, and weekly until day 70/71), ophthalmoscopy (pre-dose and on days 45 and 66 in subgroup 2 animals only), body temperature (prior to and circa 4, 24 and 48 hours after each injection [subgroup 1 only]), hematology, clinical chemistry, and coagulation (subgroups 2 only, predose, 1, 7, 38, 43, 49 and 70/71 [males/females]), gross anatomy at termination, organ weights and histopathology on a selection of tissues (day 45 for subgroup 1 and day 70/71 for subgroup 2). Blood samples for antibody-determination (day 45 for subgroup 1 and day 70/71 for subgroup 2) were taken and analyzed (non-GLP) under the responsibility of the sponsor.

Parameters	Frequency of Testing
Cageside observation[7]	Twice daily

[7] Cageside observations include mortality, morbidity, general health and signs of toxicity.

Parameters	Frequency of Testing
Clinical observations[8]	Twice daily
Body weight	In subgroup 1; days -4, 0, 3, 7, 15, 21, 28, 35, 42 and 45; and in subgroup 2; days -4, 0, 3, 7, and weekly until day 70/71
Food consumption	In subgroup 1; days -4, 0, 3, 7, 15, 21, 28, 35 and 42; and in subgroup 2; days -4, 0, 3, 7, and weekly until day 70/71
Body temperature	Prior to and circa 4, 24 and 48 hours after each injection [subgroup 1 only]
Ophthalmologic exam	Pre-dose and on days 45 and 66 in subgroup 2 animals only
Clinical chemistry*	Subgroups 2 only, predose, 1, 7, 38, 43, 49 and 70/71 [males/females]
Hematology*	Subgroups 2 only, predose, 1, 7, 38, 43, 49 and 70/71 [males/females]
Coagulation*	Subgroups 2 only, predose, 1, 7, 38, 43, 49 and 70/71 [males/females]
Immunological response*	Day 45 for subgroup 1 and day 70/71 for subgroup 2
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Approximately at 3, 24 and 48 hours after each injection
Necropsy	Day 45 for subgroup 1 and day 70/71 for subgroup 2
Histopathology	Day 45 for subgroup 1 and day 70/71 for subgroup 2

* Blood collected from the ear artery.

Table 32: *Parameters evaluated (study # (b) (4) V 20094)*

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	!	
Bone (sternum & femur)	!	
Bone marrow (sternum & femur)	!	
Brain	!*	
Cecum		X
Cervix		X
Colon	!	
Duodenum	!	

[8] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Organ/Tissue	Collected	Not collected
Epididymides	!*	
Esophagus		X
Eyes (optic nerve)	!	
Gall bladder	!	
Gross lesions (if any)	!	
Harderian gland (if applicable)		X
Heart	!*	
Ileum		X
Jejunum		X
Kidneys	!*	
Knee joint	!	
Lacrimal glands		X
Larynx		X
Liver	!*	
Lung	!*	
Lymph nodes (Inguinal)	!	
Lymph nodes (Iliac)	!	
Lymph nodes (popliteal)	!*	
Lymph nodes (mandibular and mesenteric)	!	
Mammary glands		X
Mesenteric artery	!	
Muscle at injection sites	!	
Muscle (skeletal)	!	
Ovaries	!*	
Pancreas	!	
Peyer's patch (if applicable)		X
Pituitary gland	!*	
Prostate	!*	
Rectum		X
Salivary glands (submaxilary)		X
Sciatic nerve	!	
Skeletal muscle		X
Skin		X
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	!*	
Stomach	!	

Organ/Tissue	Collected	Not collected
Testes	!*	
Thymus	!*	
Thyroid (w/ parathyroid glands)	!*	
Tongue		X
Ureters		X
Uterus	!*	
Urinary bladder	!	
Vagina		X
Zymbal's gland (if applicable)		X

Table of Histology – Histopathological examination was performed on the tissues and organs in the table above as indicated for all animals.
Table 33: *Tissues collected (study # (b) (4) V 20094)*

Results:
Morbidity and mortality: No test article-related morbidity or mortality was reported.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	**Aspartate aminotransferase** (AST or SGOT) SD7 M ↓0.6 G2 SD7 M ↓0.6 G3	Alanine aminotransferase (ALT or SGPT) Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids Lactate dehydrogenase (LDH)
B) HEPATOBILIARY	**Total bilirubin** SD1 M ↑ 1.74 G2 SD1 M ↑ 1.93 G3 SD1 M ↑ 2.06 G4 SD1 M ↑ 1.90 G5 SD43 M ↑ 2.45 G3 SD43 M ↑ 3.50 G5 SD49 M ↑ 1.72 G5 SD7 F ↑ 1.83 G2 SD7 F ↑ 1.67 G5	Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids
ACUTE PHASE REACTANTS	**C-reactive protein:** SD1 M ↑ 9.47 G2	Fibrinogen (also under coagulation),

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
	SD1 M ↑ 9.64 G3 SD1 M ↑ 9.32 G4 SD1 M ↑ 11.7 G5 SD43 M ↑ 3.34 G2 SD43 M ↑ 4.86 G3 SD43 M ↑ 5.22 G4 SD43 M ↑ 11.3 G5 SD1 F ↑ 5.22 G2 SD1 F ↑ 4.61 G3 SD1 F ↑ 4.00 G4 SD1 F ↑ 6.18 G5 SD38 F ↓ 0.37 G4 SD38 F ↓ 0.43 G5 SD43 F ↑ 1.91 G2 SD43 F ↑ 3.04 G3 SD43 F ↑ 2.38 G4 SD43 F ↑ 5.43 G5	
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	**Creatine kinase:** SD49 M ↑ 1.94 G4 SD70 M ↓ 0.45 G2 SD1 F ↑ 1.70 G4 SD7 F ↑ 1.73 G4	Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Fasting triglycerides

Table 34: *Clinical chemistry results (study #* (b) (4) *V 20094)*

Clinical chemistry results showed decrease in AST levels in groups 2 and 3 males on study day 7. Bilirubin levels were increased in groups 2, 3, 4, and 5 males on study day 1. Bilirubin levels were increased in groups 3 and 5 males on study day 43. Bilirubin level was increased in group 5 males on study day 49. Bilirubin levels were increased in groups 2 and 5 females on study day 7. C-reactive protein levels were increased in groups 2, 3, 4, and 5 males on study day 1. C-reactive protein levels were increased in groups 2, 3, 4, and 5 males on study day 43. C-reactive protein levels were increased in groups 2, 3, 4, and 5 females on study day 1. C-reactive protein levels were increased in groups 2, 3, 4, and 5 females on study day 43. C-reactive protein levels were decreased in groups 4 and 5 females on study day 38. Creatine kinase levels were increased in group 4 males on study day 49. Creatine kinase levels were decreased in group 2 males on study day 70. Creatine kinase levels were increased in group 4 females on study days 1 and 7.

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	**Neutrophil count:** SD43 M ↑ 1.93 G5 SD43 F ↑ 1.97 G5 **Monocyte count:** SD1 M ↑ 1.83 G5 SD70 M ↑ 1.70 G3 SD70 M ↑ 1.70 G4 SD70 M ↑ 1.60 G5 SD38 F ↓ 0.52 G4 **Basophils:** SD71 F ↓ 0.51 G4 **Eosinophils count:** SD7 F ↑ 1.82 G3 SD43 F ↓ 0.64 G2	Lymphocyte count Total Leukocytes (WBC) Large Unstained Cells (LUC)
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

Table 35: *Hematology results (study # (b) (4) V 20094)*

Hematology results showed increase in neutrophil levels in group 5 males and females on study day 43. Monocyte levels were increased in group 5 males on study day 1. Monocyte levels were increased in groups 3, 4, and 5 males on study day 70. Monocyte levels were decreased in group 4 females on study day 38. Basophils levels were decreased in group 4 females on study day 71. Eosinophils levels were increased in group 3 females on study day 7. Eosinophils levels were decreased in group 2 females on study day 43.

Systemic toxicity:
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, body temperature, or organ weight were reported.

Due to a punctured subcutaneous blood vessel upon injection, a hematoma at the site of injection was observed in 6 males and 5 females of the saline control group, in 1 male and 4 females of the gE/AS01B (SC) group, in 3 males and 3 females of the AS01B (SC) group, in 1 male of the gE/AS01B (IM) group, and in 3 females of the AS01B (IM) group. The hematoma generally cleared within few days.

Organ Weight (SD45/SD70):

SEX	MALES				
GROUPS	1 (SD45/SD70) (CONTROL)	2 (SD45/SD70)	3 (SD45/SD70)	4 (SD45/SD70)	5 (SD45/SD70)
NUMBER OF ANIMALS	5/5	5/5	5/5	5/5	5/5
BODY WEIGHT (terminal)	3603/4125	3885/4090	3719/3970	3697/4003	4040/4160
BRAIN	8.69/9.90	9.81/9.54	9.01/9.52	9.30/9.78	9.29/10.0
ADRENALS	0.17/0.22	0.20/0.21	0.20/0.20	0.18/0.25	0.18/0.24
EPIDIDYMIDES	1.82/2.77	2.22/2.59	2.21/2.39	1.91/2.80	2.32/2.40
HEART	8.17/9.29	9.80/9.04	8.78/8.72	9.21/9.01	9.26/8.64
KIDNEYS	17.7/19.9	19.3/18.5	20.0/17.6	18.4/19.0	19.3/18.9
LIVER	134.6/118.2	127.3/124.1	129.9/114.8	116.0/117.2	139.1/121.9
Lungs	12.8/14.1	15.0/14.8	14.1/13.8	13.7/13.8	15.0/14.3
PITUITARY	0.021/0.020	0.021/0.021	0.016/0.021	0.022/0.022	0.022/0.023
POP-LN-L*	0.272/0.221	0.306/0.331	0.288/0.207	0.220/0.320	0.233/0.282
POP-LN-R**	0.204/0.237	0.267/0.273	0.293/0.220	0.252/0.210	0.250/0.286
PROSTATE	2.92/4.80	3.84/4.00	2.52/3.80	3.15/4.85	3.49/5.30
SPLEEN	1.22/1.32	1.32/1.41	1.06/1.28	1.43/1.25	1.43/1.38
TESTES	3.70/5.74	4.56/4.78	4.21/4.49	3.85/5.45	5.16/5.55
THYROID and PARATHYROID	0.20/0.21	0.20/0.22	0.24/0.20	0.19/0.21	0.22/0.22
THYMUS	4.06/4.80	5.02/5.16	4.80/4.10	3.87/4.08	4.48/4.51
OVARIES					
UTERUS					

Absolute weights are expressed as mean (grams). Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase (SD 45) and recovery phase (SD 70) of the study (main phase organ weight/recovery phase organ weight). * Left popliteal lymph node. ** Right popliteal lymph node.

Table 36: *Organ weight results in males (study #* (b) (4) *V 20094)*

Body weights were increased 12% in group 5 males at study day 45. Brain weight was increased 13% in group 2 males at study day 45. Adrenal weights were increased 18% in groups 2 and 3 males at study day 45. Adrenal weights were increased 14% in group 4 males at study day 70. At study day 45, epididymides weights were increased 22%, 21%, and 27% in groups 2, 3, and 5, respectively. At study day 70, epididymides weights were decreased 14% and 13% in groups 3 and 5, respectively. At study day 45, heart weights were increased 20%, 13%, and 13% in groups 2, 4, and 5, respectively. Kidneys weights were increased 13% in group 3 males at study day 45. Kidneys weights were decreased 12% in group 3 males at study day 70. Liver weights were

decreased 14% in group 4 males at study day 45. At study day 45, lung weights were increased 17%, 10%, and 17% in groups 2, 3, and 5, respectively. Pituitary weights were decreased 24% in group 3 males at study day 45.

At study day 45, left popliteal lymph node weights were increased 13% in group 2 males. At study day 45, left popliteal lymph node weights were decreased 19% and 14% in groups 4 and 5 males, respectively. At study day 70, left popliteal lymph node weights were increased 50%, 45%, and 28% in groups2, 4, and 5 males, respectively. At study day 45, right popliteal lymph node weights were increased 31%, 44%, 24%, and 23% in groups 2, 3, 4, and 5 males, respectively. At study day 70, right popliteal lymph node weights were increased 15% and 21% in groups 2 and 5 males, respectively. At study day 45, prostate weights were increased 32% and 20% in groups 2 and 5, respectively. At study day 45, prostate weights were decreased 14% in group 3. At study day 70, prostate weights were decreased 17% and 21% in groups 2 and 3, respectively. At study day 70, prostate weights were increased 10% in group 5. At study day 45, spleen weights were increased 17% in groups 4 and 5. At study day 45, spleen weights were decreased 13% in group 3.

At study day 45, testes weights were increased 23%, 14%, and 39% in groups 2, 3, and 5, respectively. At study day 70, testes weights were decreased 17% and 22% in groups 2 and 3, respectively. Thyroid weights were increased 20% in group 3 males at study day 45. At study day 45, thymus weights were increased 24% and 18% in groups 2 and 3, respectively. At study day 70, thymus weights were decreased 15% in groups 3 and 4.

SEX	FEMALES				
GROUPS	1 (SD45/SD71) (CONTROL)	2 (SD45/SD71)	3 (SD45/SD71)	4 (SD45/SD71)	5 (SD45/SD71)
NUMBER OF ANIMALS	5/5	5/5	5/5	5/5	5/5
BODY WEIGHT (terminal)	3732/4053	3935/4097	4013/4130	3976/4394	4029/4233
BRAIN	9.00/9.61	9.40/9.21	9.52/9.26	8.94/10.4	9.31/9.86
ADRENALS	0.19/0.21	0.20/0.22	0.19/0.24	0.21/0.21	0.20/0.18
EPIDIDYMIDES					
HEART	8.90/8.65	8.69/8.43	9.39/8.03	8.64/8.69	9.23/8.58
KIDNEYS	16.9/17.7	18.4/16.8	18.1/17.0	19.2/17.5	19.1/16.7
LIVER	116.9/121.2	141.8/111.2	138.3/119.6	130.7/111.0	153.1/109.1
LUNGS	12.7/14.0	13.4/13.4	13.7/13.6	14.1/14.4	13.9/14.1
PITUITARY	0.020/0.030	0.024/0.022	0.030/0.026	0.028/0.029	0.025/0.024
POP-LN-L*	0.353/0.365	0.413/0.316	0.333/0.334	0.333/0.332	0.253/0.390
POP-LN-R**	0.274/0.313	0.415/0.271	0.350/0.335	0.336/0.355	0.368/0.366
PROSTATE					
SPLEEN	1.42/1.46	1.85/1.39	1.50/1.43	1.70/1.84	1.87/1.56
TESTES					
THYROID and PARATHYROID	0.22/0.27	0.22/0.23	0.24/0.29	0.20/0.26	0.23/0.19
THYMUS	4.05/3.78	4.18/4.50	5.05/4.77	4.67/4.69	4.97/4.33
OVARIES	0.24/0.35	0.40/0.25	0.30/0.33	0.29/0.30	0.26/0.32
UTERUS	5.15/7.86	7.19/6.72	6.26/5.69	6.10/7.18	6.64/6.75

Absolute weights are expressed as mean (grams). Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase (SD 45) and recovery phase (SD 71) of the study (main phase organ weight/recovery phase organ weight). * Left popliteal lymph node. ** Right popliteal lymph node.

Table 37: *Organ weight results in females (study #* (b) (4) *V 20094)*

Adrenal weights were increased 14% in group 3 females at study day 71. Adrenal weights were decreased 14% in group 5 females at study day 71. At study day 45, kidneys weights were increased 14% and 13% in groups 4 and 5 females, respectively. At study day 45, livers weights were increased 21%, 18%, 12%, and 31% in groups 2, 3, 4, and 5 females, respectively. At study day 45, pituitary weights were increased 20%, 50%, 40%, and 20% in groups 2, 3, 4, and 5 females, respectively. At study day 71, pituitary weights were decreased 27%, 13%, and 20% in groups 2, 3, and 5 females, respectively. At study day 45, left popliteal lymph node weights were increased 17% in group 2 females. At study day 45, left popliteal lymph node weights were decreased 28% in group 5 females. At study day 71, left popliteal lymph node weights were decreased 13% in group 2 females. At study day 45, right popliteal lymph node weights were increased 51%, 28%, 23%, and 34% in groups 2, 3, 4, and 5, respectively. At study day 71, right popliteal lymph node weights were decreased 13% in group 2 females. At study day 71, right popliteal lymph node weights were increased 13% and 17% in groups 4 and 5, respectively.

At study day 45, spleen weights were increased 30%, 20%, and 32% in groups 2, 4, and 5, respectively. At study day 71, spleen weight were increased 26% in group 4 females. At study day 45, thyroid weights were decreased 15% and 30% in groups 2 and 5, respectively. At study

day 45, thymus weights were increased 25%, 15%, and 23% in groups 3, 4, and 5 females, respectively. At study day 71, thymus weights were increased 19%, 26%, 24%, and 15% in groups 2, 3, 4, and 5 females, respectively.

At study day 45, ovaries weights were increased 67%, 25%, and 21% in groups 2, 3, and 4, respectively. At study day 71, ovaries weights were decreased 29% and 14% in groups 2 and 4, respectively. At study day 45, uterus weights were increased 40%, 22%, 18%, and 29% in groups 2, 3, 4, and 5, respectively. At study day 71, uterus weights were decreased 15%, 28%, and 14% in groups 2, 3, and 5, respectively.

Gross Pathology:

No test article-related effects on the macroscopic findings were reported.

Group	Findings (Day 45)
1M	Uni-lateral small epididymides (1/5); uni- or bi-lateral discharge in eyes (1/5); uni-lateral small testes (1/5)
2M	Uni- or bi-lateral discharge in eyes (1/5); small lobe most probably caused by torsion in liver (1/5)
3M	NF
4M	Uni-lateral small epididymides (1/5); uni-lateral cyst in mandibular lymph node (1/5); uni-lateral red appearance in mandibular lymph node (1/5); uni-lateral small testes (1/5)
5M	Red area in right anterior thigh muscle (1/5); uni- or bi-lateral discharge in eyes (1/5); uni-lateral red spot in mandibular lymph node (1/5)
1F	Superficial red area in right anterior thigh muscle (1/5)
2F	Uni-lateral black area and enlarged ovary (1/5); encrustation in skin/subcutis (1/5)
3F	Superficial hemorrhage in injection site at left anterior thigh (1/5); uni-lateral cyst in mandibular lymph node (1/5)
4F	Red discolored mesenteric lymph node (1/5); encrustation in skin/subcutis (1/5)
5F	Superficial petechial in right anterior thigh muscle (1/5); red area in right anterior thigh muscle (1/5); uni-lateral small adrenals (1/5); jejunum partly trapped in umbilical hernia, dark red, filled with hemorrhagic fluid (1/5); uni-lateral cyst in oviduct (1/5); hemorrhagic fluid in vagina (1/5)

(NF = no findings)
Table 38: *Gross pathology results (study #* (b) (4) *V 20094)*

Microscopic finding are listed below:

Groups	Findings (Day 45)
1M	Focal encrustations at left injection site (1/5); uni-lateral devoid of spermatozoa epididymides (1/5); uni-lateral conjunctivitis in eyes (1/5); focal atrial mononuclear cell infiltrate in heart (1/5); focal aortic medial mineralization in heart (1/5); multi-focal mononuclear cell infiltrate in kidneys (1/5); mineralization in kidneys (3/5); basophilic tubules in kidneys (2/5); multi-focal mononuclear cell infiltrate in larynx (3/5); focal mononuclear cell infiltrate in liver (1/5); focal alveolitis in lungs (1/5); cystically dilated sinus in mandibular LN (1/5); remnant rathkes pouch in pituitary (1/5); focal epithelial hyperplasia in prostate (1/5); multi-focal mononuclear cell infiltrate in stomach (1/5); focal mononuclear cell infiltrate in testes (1/5); uni-lateral seminiferous tubular atrophy in testes (1/5); microhemorrhage in thymus (1/5); focal mononuclear cell infiltrate in thyroid (1/5); focal mononuclear cell infiltrate in trachea/bronchi (1/5)
2M	Slight diffuse mixed inflammatory cell infiltration at left injection site (1/5); very slight focal mixed inflammatory cell infiltration at left injection site (2/5); focal ulceration at left injection site (1/5); focal encrustations at left injection site (2/5); focal acanthosis at left injection site (1/5); increased polymorphonuclear leukocytic infiltration in left inguinal LN (2/5); uni-lateral conjunctivitis in eyes (1/5); focal atrial mononuclear cell infiltrate in heart (1/5); focal aortic medial mineralization in heart (2/5); sinusoidal blood in iliac LN (1/5); multi-focal mononuclear cell infiltrate in kidneys (3/5); mineralization in kidneys (1/5); basophilic tubules in kidneys (4/5); multi-focal mononuclear cell infiltrate in larynx (4/5); focal laryngitis in larynx (1/5); focal mixed inflammatory-cell infiltration in liver (1/5); lobar necrosis in liver (1/5); focal alveolitis in lungs (1/5); focal bronchitis in lungs (1/5); cystically dilated sinus in mandibular LN (1/5); focal mononuclear cell infiltrate in pancreas (1/5); focal polymorphonuclear leukocytic infiltration in stomach (2/5); multi-focal mononuclear cell infiltrate in stomach (1/5); microhemorrhage in thymus (1/5); focal mononuclear cell infiltrate in trachea/bronchi (2/5); urolithiasis in urinary bladder (1/5)
3M	Very slight focal mixed inflammatory cell infiltration at left injection site (1/5); very slight localized mononuclear cell infiltrate at triceps (1/5); sinusoidal blood in right inguinal LN (1/5); increased polymorphonuclear leukocytic infiltration in left inguinal LN (1/5); multi-focal mononuclear cell infiltrate in kidneys (3/5); mineralization in kidneys (3/5); basophilic tubules in kidneys (3/5); multi-focal mononuclear cell infiltrate in larynx (4/5); focal alveolitis in lungs (2/5); cystically dilated sinus in mandibular LN (2/5); remnant rathkes pouch in pituitary (2/5); focal epithelial hyperplasia in prostate (1/5); focal polymorphonuclear leukocytic infiltration in stomach (3/5); multi-focal mononuclear cell infiltrate in stomach (2/5); microhemorrhage in thymus (1/5); increased number of activated macrophages in thymus (1/5); focal mononuclear cell infiltrate in thyroid (1/5); focal mononuclear cell infiltrate in trachea/bronchi (1/5); urolithiasis in urinary bladder (1/5)

Groups	Findings (Day 45)
4M	Slight inflammatory cell infiltration wide spread in right anterior thigh muscle (5/5); very slight inflammatory muscle fiber degeneration associated with the inflammation in right anterior thigh muscle (1/5); hemorrhage in right anterior thigh muscle (1/5); very slight localized mononuclear cell infiltrate at triceps (1/5); sinusoidal blood and erythrophagocytosis in left popliteal lymph nodes (1/5); uni-lateral devoid of spermatozoa epididymides (1/5); focal myocardial mononuclear cell infiltrate in heart (2/5); increased polymorphonuclear leukocytic infiltration in iliac LN (2/5); multi-focal mononuclear cell infiltrate in kidneys (1/5); mineralization in kidneys (2/5); basophilic tubules in kidneys (2/5); multi-focal mononuclear cell infiltrate in larynx (1/5); focal laryngitis in larynx (1/5); focal mixed inflammatory-cell infiltration in liver (1/5); focal alveolitis in lungs (1/5); focal accumulation of alveolar macrophages in lungs (1/5); cystically dilated sinus in mandibular LN (3/5); focal sinusoidal blood in mandibular LN (1/5); remnant rathkes pouch in pituitary (1/5); focal polymorphonuclear leukocytic infiltration in stomach (1/5); multi-focal mononuclear cell infiltrate in stomach (2/5); focal lymphangiectasis in stomach (1/5); uni-lateral seminiferous tubular atrophy in testes (1/5); microhemorrhage in thymus (1/5); focal mononuclear cell infiltrate in thyroid (1/5); urolithiasis in urinary bladder (3/5)
5M	Slight inflammatory cell infiltration wide spread in right anterior thigh muscle (1/5); slight inflammatory cell infiltration multifocal mixed in right anterior thigh muscle (5/5); slight inflammatory cell infiltration localized mixed in right anterior thigh muscle (3/5); hemorrhage in right anterior thigh muscle (1/5); increased polymorphonuclear leukocytic infiltration in right popliteal lymph nodes (1/5); focal histiocytosis in right popliteal lymph nodes (1/5); increased polymorphonuclear leukocytic infiltration in left popliteal lymph nodes (1/5); increased polymorphonuclear leukocytic infiltration in left inguinal LN (1/5); focal myocardial mononuclear cell infiltrate in heart (1/5); mineralization in kidneys (3/5); basophilic tubules in kidneys (2/5); multi-focal mononuclear cell infiltrate in larynx (3/5); focal mixed inflammatory-cell infiltration in liver (1/5); focal alveolitis in lungs (1/5); cystically dilated sinus in mandibular LN (3/5); focal sinusoidal blood in mandibular LN (1/5); remnant rathkes pouch in pituitary (2/5); focal epithelial hyperplasia in prostate (1/5); focal squamous metaplasia and adenitis in seminal vesicles (1/5); focal polymorphonuclear leukocytic infiltration in stomach (1/5); multi-focal mononuclear cell infiltrate in stomach (2/5); microhemorrhage in thymus (2/5); focal mononuclear cell infiltrate in trachea/bronchi (1/5)
1F	Slight localized mononuclear cell infiltrate in right anterior thigh muscle (2/5); very slight inflammatory muscle fiber degeneration associated with the inflammation in right anterior thigh muscle (1/5); hemorrhage in right anterior thigh muscle (1/5); slight focal mixed inflammatory cell infiltration at left injection site (4/5); focal ulceration at left injection site (2/5); focal encrustations at left injection site (5/5); focal acanthosis at left injection site (1/5); focal cortical mononuclear cell infiltrate in adrenals (2/5); focal

Groups	Findings (Day 45)
	myocardial mononuclear cell infiltrate in heart (2/5); sinusoidal blood in iliac LN (1/5); multi-focal mononuclear cell infiltrate in kidneys (1/5); mineralization in kidneys (4/5); tubular dilatation in kidneys (3/5); basophilic tubules in kidneys (3/5); multi-focal mononuclear cell infiltrate in larynx (2/5); focal bile duct hyperplasia in liver (1/5); cystically dilated sinus in mandibular LN (1/5); cystically dilated sinus in mesenteric LN (1/5); increased luteinized appearance in ovaries (2/5); remnant rathkes pouch in pituitary (2/5); multi-focal mononuclear cell infiltrate in stomach (1/5); mucosal cyst in stomach (1/5); focal lymphangitis in thymus (1/5); focal c-cell hyperplasia in thyroid (1/5); focal follicular dilatation in thyroid (1/5); focal mononuclear cell infiltrate in trachea/bronchi (1/5)
2F	Slight diffuse mixed inflammatory cell infiltration at left injection site (1/5); slight diffuse mononuclear cell infiltrate at left injection site (1/5); very slight focal mixed inflammatory cell infiltration at left injection site (2/5); focal encrustations at left injection site (4/5); very slight localized mononuclear cell infiltrate at triceps (1/5); increased polymorphonuclear leukocytic infiltration in right inguinal LN (1/5); increased polymorphonuclear leukocytic infiltration in left inguinal LN (1/5); medullary brown pigment accumulation in adrenals (1/5); focal myocardial mononuclear cell infiltrate in heart (1/5); increased polymorphonuclear leukocytic infiltration in iliac LN (1/5); mineralization in kidneys (2/5); tubular dilatation in kidneys (1/5); basophilic tubules in kidneys (3/5); multi-focal mononuclear cell infiltrate in larynx (3/5); increased BALT in lungs (1/5); focal bronchitis in lungs (1/5); cystically dilated sinus in mandibular LN (5/5); corpora lutea in ovaries (1/5); increased luteinized appearance in ovaries (2/5); focal mononuclear cell infiltrate in pancreas (1/5); remnant rathkes pouch in pituitary (3/5); focal hyperkeratosis and focal dermatitis in skin/subcutis (1/5); focal polymorphonuclear leukocytic infiltration in stomach (2/5); ulceration in stomach (1/5); mucosal cyst in stomach (1/5); focal mononuclear cell infiltrate in thyroid (1/5); urolithiasis in urinary bladder (1/5); intraluminal polymorphonuclear leukocytic infiltration in uterus (1/5); endometrial hyperplasia in uterus (1/5)
3F	Very slight focal mixed inflammatory cell infiltration at left injection site (2/5); focal encrustations at left injection site (2/5); very slight localized mixed inflammatory cell infiltration in triceps (1/5); very slight localized mononuclear cell infiltrate at triceps (1/5); increased polymorphonuclear leukocytic infiltration in left inguinal LN (1/5); focal corneal mononuclear cell infiltrate in eyes (1/5); multi-focal mononuclear cell infiltrate in kidneys (2/5); mineralization in kidneys (2/5); tubular dilatation in kidneys (1/5); basophilic tubules in kidneys (2/5); multi-focal mononuclear cell infiltrate in larynx (3/5); focal alveolitis in lungs (3/5); cystically dilated sinus in mandibular LN (1/5); focal sinusoidal blood in mandibular LN (1/5); focal mononuclear cell infiltrate in pancreas (1/5); remnant rathkes pouch in pituitary (4/5); focal polymorphonuclear leukocytic infiltration in stomach (2/5); multi-focal mononuclear cell infiltrate in stomach (1/5); focal

Groups	Findings (Day 45)
	mononuclear cell infiltrate in thyroid (1/5); focal mononuclear cell infiltrate in trachea/bronchi (2/5); urolithiasis in urinary bladder (1/5)
4F	Slight inflammatory cell infiltration wide spread mix in right anterior thigh muscle (5/5); hemorrhage in right anterior thigh muscle (1/5); very slight localized mononuclear cell infiltrate at triceps (2/5); increased polymorphonuclear leukocytic infiltration in left popliteal lymph nodes (2/5); focal corneal mononuclear cell infiltrate in eyes (1/5); focal myocardial mononuclear cell infiltrate in heart (1/5); focal atrial mononuclear cell infiltrate in heart (1/5); increased polymorphonuclear leukocytic infiltration in iliac LN (1/5); focal synovial mononuclear cell infiltrate in joint [knees] (1/5); multi-focal mononuclear cell infiltrate in kidneys (1/5); mineralization in kidneys (3/5); tubular dilatation in kidneys (2/5); basophilic tubules in kidneys (3/5); multi-focal mononuclear cell infiltrate in larynx (3/5); focal alveolitis in lungs (2/5); focal alveolar blood in lungs (1/5); cystically dilated sinus in mandibular LN (2/5); accumulation of brown macrophages in mandibular LN (1/5); sinusoidal blood in mesenteric LN (1/5); increased luteinized appearance in ovaries (1/5); remnant rathkes pouch in pituitary (4/5); focal escharosis and focal dermatitis in skin/subcutis (1/5); focal polymorphonuclear leukocytic infiltration in stomach (2/5); multi-focal mononuclear cell infiltrate in stomach (2/5); mucosal hemorrhage and ulceration in stomach (1/5); mucosal cyst in stomach (2/5); microhemorrhage in thymus (2/5); urolithiasis in urinary bladder (2/5)
5F	Slight (1/5) and moderate (1/5) inflammatory cell infiltration wide spread in right anterior thigh muscle; very slight (1/5) and slight (1/5) inflammatory cell infiltration localized mixed in right anterior thigh muscle; diffuse atrophy in right anterior thigh muscle (1/5); hemorrhage in right anterior thigh muscle (1/5); very slight localized mononuclear cell infiltrate at triceps (1/5); diffuse atrophy at triceps (1/5); increased polymorphonuclear leukocytic infiltration in right popliteal lymph nodes (1/5); increased polymorphonuclear leukocytic infiltration in left popliteal lymph nodes (1/5); congestion in right inguinal LN (1/5); focal polymorphonuclear leukocytic infiltration in heart (1/5); necrosis in jejunum (1/5); focal perivascular mononuclear cell infiltrate in joint [knees] (1/5); multi-focal mononuclear cell infiltrate in kidneys (3/5); mineralization in kidneys (4/5); tubular dilatation in kidneys (3/5); basophilic tubules in kidneys (2/5); multi-focal mononuclear cell infiltrate in larynx (3/5); focal accumulation of alveolar macrophages in lungs (1/5); focal alveolar blood in lungs (1/5); cystically dilated sinus in mandibular LN (3/5); focal sinusoidal blood in mandibular LN (1/5); increased luteinized appearance in ovaries (1/5); remnant rathkes pouch in pituitary (2/5); multi-focal mononuclear cell infiltrate in stomach (4/5); ulceration in stomach (1/5); mucosal cyst in stomach (1/5); decreased cortex/medulla ratio in thymus (1/5); focal mononuclear cell infiltrate in trachea/bronchi (1/5); urolithiasis in urinary bladder (1/5); intraluminal blood in vagina (1/5); mixed inflammatory-cell infiltration in vagina (1/5)

LN = Lymph node.

Table 39: *Microscopic examination results (study # (b) (4) V 20094)*

An extensive number of tissues were examined for histology. No increased incidences of histological findings indicative of potential adverse events were observed in the treated groups relative to the controls.

Due to injection procedure, microscopic findings were reported at the injection site.

Due to the anticipated immune responses, popliteal and inguinal lymph nodes of the gE/AS01B (IM and SC) males showed an enhanced activated appearance. This was clear in the gE/AS01B animals (SC and IM) as it has been shown in the statistically significant increase of the weight of the left popliteal and the left inguinal lymph nodes. The incidence of increased polymorphonuclear leukocytic infiltration in the left popliteal lymph node of gE/AS01B (IM) females was statistically significantly higher than in the controls.

Body temperature:

No body temperature above 40º was reported in males or females. No statistically significant changes were reported in post-dose body temperatures of the gE/AS01B (SC), AS01B (SC), gE/AS01B (IM) and AS01B (IM) males and females.

Local toxicity:

Hematoma at the site of injection (due to a punctured subcutaneous blood vessel upon injection) was reported in 6 males and 5 females of the saline control group, in 1 male and 4 females of the gE/AS01B (SC) group, in 3 males and 3 females of the AS01B (SC) group, in 1 male of the gE/AS01B (IM) group, and in 3 females of the AS01B (IM) group. The hematoma generally cleared within a few days.

On day 37, one group 5 female was found dead. From day 16 onwards, this animal showed an umbilical hernia which had no adverse effect on its general health condition at that time. The cause of death was due to the partial entrapment of the jejunum in this umbilical hernia, that led to the necrosis of the jejunum and consecutive fatal toxaemia, which is an acute process. The skeletal muscles showed general diffuse atrophy due to wasting syndrome associated with the poor health condition of the animal.

Serology:

To measure gE-specific antibodies total Ig antibodies in serum, ELISA was developed using gE protein as the coating antigen. No gE-specific antibody response was reported in the saline control animals and in animals injected with AS01B (SC and IM) at the different time points, and in the gE/AS01B (SC and IM) animals pre-dose.

Animals treated with gE/AS01B (SC and IM) showed 100% seroconversion. Geometric means of the titer (anti-gE) are reported in the following table:

Groups	Formulation	Timing	Anti-gE	
			GMT (EU/ml)	Seroconversion
1	Saline (NaCl 0.9%)	D0 (n = 20)	<cutoff	0%
		D45 (n=10)	<cutoff	0%
		D70/71(n = 10)	<cutoff	0%
2	gE/AS01B (SC)	D0 (n = 20)	<cutoff	0%
		D45 (n = 10)	815926	100%
		D70/71 (n = 10)	410501	100%
3	AS01B (SC)	D0 (n = 20)	<cutoff	0%
		D45 (n = 10)	<cutoff	0%
		D70/71 (n = 10)	<cutoff	0%
4	gE/AS01B (IM)	D0 (n = 20)	<cutoff	0%
		D45 (n = 10)	1754873	100%
		D70/71 (n = 10)	851127	100%
5	AS01B (IM	D0 (n = 20)	<cutoff	0%
		D45 (n = 10)	<cutoff	0%
		D70/71 (n = 10)	<cutoff	0%

n: number of animals tested
GMTs calculated on responders
D45: 3 days post IV
D70/71: 28/29 days post IV

Table 40: *Serology results (study # (b) (4) V 20094)*

Test article related effects and assessment:

Test article related effects
↑ Bilirubin
↑ CRP
↑ Monocytes
↑ Popliteal LN weight
↑ Spleen weight
↑ Pituitary LN weight
↑ Thymus weight
↑ Immune responses
Injection site findings

Table 41: *Test article related effects (study # (b) (4) V 20094)*

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, or body temperature were reported.

Bilirubin is the yellow breakdown product of normal heme catabolism, caused by the body's clearance of aged red blood cells which contain hemoglobin.[5] Bilirubin is excreted in bile and urine, and elevated levels may indicate certain diseases. It is responsible for the yellow color of bruises and the yellow discoloration in jaundice. It is also responsible for the brown color of feces, via its conversion to stercobilin, and the background straw-yellow color of urine via its breakdown product, urobilin. Studies have revealed that levels of serum bilirubin are inversely related to risk of certain heart diseases.[6, 7] Bilirubin testing is used to check liver function and watch for signs of liver disease, such as hepatitis or cirrhosis, effect of medicines that can damage the liver, bile ducts blockage, or increased destruction of red blood cells.

CRP is protein synthesized by the liver, found in the blood, and is a member of the class of acute-phase reactants as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes. CRP binds to phosphocholine on microbes. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages, which express a receptor for CRP. It is also believed to play another important role in innate immunity, as an early defense system against infections. Also, a positive CRP test that indicates an inflammation in the body may be used to detect a variety of different conditions, including: cancer, connective tissue disease, heart attack, infection, inflammatory bowel disease (IBD), lupus, pneumococcal pneumonia, rheumatoid arthritis, rheumatic fever, or tuberculosis.

Monocytosis could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair. The increases in the monocyte count reported in this study might be related to test article-treatment.

Adverse gross findings that could be indicative of systemic or local toxicity were not observed.

The increase in the popliteal lymph nodes weight might be related to the immune responses due to test article-treatment.

Spleen weight increases might be related to the intended immune response. The spleen plays important roles in regard to red blood cells and the immune system[9]. It removes old red blood cells and holds a reserve of blood in case of hemorrhagic shock while also recycling iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes. The globin portion of hemoglobin is degraded to its constitutive amino acids, and the heme portion is metabolized to bilirubin, which is subsequently shuttled to the liver for removal[10]. It synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation.

[9] Spleen, Internet Encyclopedia of Science.
[10] Mebius RE, Kraal G. (2005). Structure and function of the spleen. Nat Rev Immunol. 5(8):606-16.

Pituitary gland is a protrusion off the bottom of the hypothalamus at the base of the brain. The hypophysis rests upon the hypophysial fossa of the sphenoid bone in the center of the middle cranial fossa and is surrounded by a small bony cavity (sella turcica) covered by a dural fold (diaphragma sellae).[11] The anterior pituitary (or adenohypophysis) is a lobe of the gland that regulates several physiological processes (including stress, growth, reproduction, and lactation). The intermediate lobe synthesizes and secretes melanocyte-stimulating hormone. The posterior pituitary (or neurohypophysis) is a lobe of the gland that is functionally connected to the hypothalamus by the median eminence via a small tube called the pituitary stalk. Hormones secreted from the pituitary gland help control: growth, blood pressure, certain functions of the sex organs, thyroid glands and metabolism as well as some aspects of pregnancy, childbirth, nursing, water/salt concentration at the kidneys, temperature regulation and pain relief.[12]

The thymus is a specialized primary lymphoid organ of the immune system. Within the thymus, T cells or T lymphocytes mature. T cells are critical to the adaptive immune system, where the body adapts specifically to foreign invaders. The thymus is composed of two identical lobes and is located anatomically in the anterior superior mediastinum, in front of the heart and behind the sternum.[13] One of the major characteristics of vertebrate immunology is thymic involution, the shrinking of the thymus with age, resulting in changes in the architecture of the thymus and a decrease in tissue mass.[14] T-cells are named for the thymus where T-lymphocytes migrate from the bone marrow to mature. Its regression has been linked to the reduction in immunosurveillance in the elderly.[15]

Immunology performed in this study verified that an active dose was administered.

Due to a punctured subcutaneous blood vessel upon injection, a hematoma at the site of injection and microscopic findings were reported.

Based on the overall findings in this study, it can be concluded that in (b) (4) rabbits repeated intramuscular or subcutaneous administration of gE/AS01B vaccine had no adverse effects in terms of systemic toxicity.

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

[11] Mancall, Elliott L.; Brock, David G., eds. (2011). "Cranial Fossae". Gray's Clinical Anatomy. Elsevier Health Sciences. p. 154. ISBN 9781437735802.
[12] https://en.wikipedia.org/wiki/Pituitary_gland
[13] https://en.wikipedia.org/wiki/Thymus.
[14] Shanley D.P.; Danielle A.W.; Manley N.R.; Palmer D.B.; et al. (2009). "An evolutionary perspective on the mechanisms of immunosenescence". Trends Immunol. **30** (7): 374–381. doi:10.1016/j.it.2009.05.001. PMID 19541538
[15] Linton P.J.; Dorshkind K. (2004). "Age-related changes in lymphocyte development and function". Nat. Immunol. **5** (2): 133–139. doi:10.1038/ni1033. PMID 14749784

Study # 5: The Acute Intraperitoneal Toxicity of Monophosphoryl Lipid A (MPL) in Rats (Study No. DT127 –Reported in BLA 125259)

Performing Laboratory: (b) (4) .
Study Initiation Date: December 4, 1987
Final Report Date: May 17, 1988
Animal species and Strain: (b) (4) rats
Breeder supplier: (b) (4)
Animals per sex and group: 6 males & 6 females per group
Age: approximately 8-11 weeks of age at the initiation of dosing
Body weight range: 212-286 g
Route: Intraperitoneal
Site of administration: Intraperitoneum
Volume of injection: 4 ml test article/kg followed by 36 ml (b) (4) /kg = 40 ml/kg animal
Method of administration: Single IP dose of test article or control article followed by (b) (4) with 1.5% (b) (4) (was used as a delayed, intraperitoneal (IP) diluent of the reconstituted test/control article and was administered at an IP dosage of 36 ml/kg approximately 10-15 minutes after the test article was administered.
Dose: Single dosages of approximately 0, 10, 40, 400, and 4000 mcg per kg body weight
Test Article: 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL) manufactured by (b) (4) The test article was identified with Lot No. (b) (4) .
Vehicle/Formulation: The control article (MPLA diluent) for this study was (b) (4)
Duration of Postdose: 14-day observation period
GLP Compliance: Yes
Report status: Final

Methods:
Laboratory Methods
This study was conducted using five treatment groups, each consisting of six male and six female rats. Animals in the four test article groups received a single 4 ml/kg intraperitoneal dosage of MPLA at 10 (manufacturers recommended safe human dose), 40 (active human dose/clinical dose = IX), 400 (l0X), or 4000 (l00X) mcg/kg of body weight, respectively, followed 10-15 minutes later by a 36 ml/kg intraperitoneal dosage of (b) (4) (combined volume dosage = 40 ml/kg). Rats in the control group received a single 4 ml/kg intraperitoneal dosage of the control article (MPLA diluent) followed 10-15 minutes later by a 6 ml/kg dosage of (b) (4) Clinical observations were recorded for fourteen days post-treatment.

In order to accommodate the necropsy procedure and the hematology laboratory schedule, each group of animals was randomly divided into two equally numbered replicates. Treatment of the two replicates was initiated over successive days. Ophthalmic examinations were performed on all animals pretreatment and prior to sacrifice. Each rat was weighed immediately prior to injection, and the calculated dosage was administered intraperitoneally. All rats were observed for signs of toxicity within approximately 60 minutes after treatment and again one to three hours after treatment. Unfasted body weights were recorded on study day 0 (for calculation of dosage volume), and day 13. A fasted body weight was recorded at necropsy (day 14). Clinical

observations were conducted daily for a 14 day period. On day 13, subsequent to recording body weight, rats were fasted and placed into metabolism cages for overnight urine collection.

Prior to necropsy, animals were anesthetized with ether and a terminal blood sample was drawn (from the abdominal aorta) for hematologic and clinical chemistry analyses. The animals were then killed by exsanguination. Urine samples were scheduled to be aspirated from the bladder at the time of necropsy if no previous sample was collected. A complete necropsy was performed and major organs and tissues fixed for histopathologic evaluation.

Measurements and Records

The following measurements were made:

General Health Status

Clinical Observations

Overt signs of toxicity were recorded within approximately 60 minutes after treatment and again 1-3 hours after treatment. In addition, clinical signs of toxicity were recorded once daily throughout the 14 day observation period.

Ophthalmic examinations were performed on each rat prior to the first treatment and again prior to completion of the 14 day observation period. Ophthalmic examinations were performed using a slit-lamp biomicroscope and an indirect ophthalmoscope to assess all ocular structures.

Body Weights

Unfasted body weights were measured on days 0 and 13. Fasted body weights were measured at necropsy (day 14). Because of scheduling problems, body weights could not be recorded on day 6 as proposed.

Urinalysis

An overnight urine sample was collected from each rat just prior to necropsy. (b) (4) was placed in each collection vessel as a preservative. Urinalyses were performed. (b) (4) (b) (4) were used to semiquantitatively determine pH, protein, glucose, ketones, bilirubin, occult blood, and urobilinogen. The urine sediment was examined microscopically, and the specific gravity of the urine was measured using a (b) (4)

Blood Sample Collection

A fasted blood sample from the abdominal aorta was collected from each rat at necropsy.

Hematology Assays

Blood was collected in tubes containing either (b) (4) . Blood collected in tubes containing (b) (4) was used for the determination of prothrombin time (PT), activated partial thromboplastin time (APTI), and fibrinogen level. Blood collected in tubes containing (b) (4) was used for determination of all other hematologic parameters. Samples were collected on the day of necropsy.

Clinica1 Chemistry Assays

Blood was collected in tubes containing no anticoagulant. Following centrifugation, the serum was used to perform clinical chemistry assays. Samples were collected on the day of necropsy.

Necropsy Procedures

At the time of necropsy, each rat was weighed, anesthetized, and subsequently killed by exsanguination. A necropsy was performed immediately thereafter.

Organ Weights

The brain, heart, lungs, spleen, liver, kidneys (right and left), and adrenals (right and left) were weighed at the time of necropsy.

Gross Pathology

Macroscopic lesions detected at necropsy were recorded on necropsy worksheets. Tissues and organs taken at necropsy were fixed in (b) (4) The following tissues were examined microscopically: brain, heart, spleen, liver (two portions from opposing lobes), lungs, kidneys (right cross section and left longitudinal section), eyes (right and left), adrenals (right and left), diaphragm, abdominal aorta, thymus, urinary bladder, gonads (right and left), stomach, duodenum. jejunum, ileum, cecum, omentum, mesentery, ventral abdominal wall, lymph nodes (mesenteric and bronchial) and gross pathological lesions.

All histopathologic specimens were submitted, received, and archived according to standard procedures.

Results:

Survival and Clinical Signs

None of the test article animals died during the course of the study and there were no adverse clinical signs associated with the intraperitoneal administration of MPLA.

Ophthalmic Examinations

No lesions were detected at the pre- or post-treatment examination periods.

Urinalysis

Objective analysis of post-treatment urinalysis profiles indicated that no toxicologically significant changes resulted from administration of the test or control articles.

Body Weight

Absolute body weight and cumulative body weight change were not affected by the acute intraperitoneal administration of MPLA

Hematology

No toxicologically significant differences were apparent for any of the hematology parameters. Random group differences were exhibited with respect to fibrinogen, Burr cells and mean corpuscular hemoglobin (MCH), but no dosage-response effects were apparent. An increasing dosage-response effect and higher group means were exhibited for hemoglobin and hematocrit (both sexes) of the test article groups relative to controls. However, since the magnitude of change was small and not correlated with other biochemical or histopathologic effects, no meaningful toxicological significance was attached to this observation.

Assessment:

Test-article related observations were small changes in terms of the animal's hemoglobin and hematocrit. All animals gained weight over the test period and there were no deaths.

Study # 6: 8-Day Intravenous Toxicity Study of MPL in Rats (Study No. (b) (4) 3262.2 –Reported in BLA 125259)

Test Article: 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL)
Species/Strain: CD Rat. **Duration of Dosing:** 8 days
Initial Age: 7 weeks. **Duration of Postdose:** N/A

Date of First Dose: 16 October 1991. **Method of Administration:** Intravenous
Vehicle/Formulation: MPL in (b) (4) in water for injection
GLP Compliance: Yes
Performing Laboratory: (b) (4)
In-life Portion of Study Initiation Date: October 16, 1991
Final Report Date: July 23, 1992.
Report status: Final

Methods:
This 8-day intravenous toxicity study consisted of four groups of CD rats with 10 rats/sex/group. Initially, MPL was administered by daily intravenous injection of 0, 100, 1000, 5000 μg/kg/day. On study day 2, the high dose level was decreased from 5000 to 2500 μg/kg/day due to excessive treatment-related toxicity (mortality). The rats were observed daily and weighed on days 1, 2, and 8. Individual food consumption was measured daily. Clinical pathology determinations were performed on all study animals on the day of scheduled sacrifice. All study animals were subjected to a per protocol gross necropsy at the time of death or sacrifice. A complete set of tissues and organs was preserved from each rat and selected tissues were processed for microscopic examination.

Results:

Survival:
Two high-dose rats (#83/F and #91/F) died after receiving a single 5.0 mg/kg dose of MPL. A third high-dose rat (#61 /M) was sacrificed moribund on day 4 due to severe debility. These deaths were considered treatment related. One control rat (#91/F) died shortly after dosing on day 8. The specific cause of this death was not determined; however, it is suspected to be unrelated to vehicle treatment since similar mortality was not observed in other control rats.
Clinical observations:
Clinical signs of toxicity were observed in the MPL treated rats at each level. The most severe changes were recognized at the 2.5 mg/kg/day level and included decreased activity, prostration, soft stools, few feces, mucoid stools, rough coat, unkempt appearance, piloerection, fecal and urine staining, dehydration, hypothermia, reddened pinna(e), partially closed eye lids, corneal opacity, and dark material around the eyes, nose and/or mouth. As compared to the 2.5 mg/kg/day level, the overall incidence of clinical signs was lower in the 1.0 and 0.1 mg/kg/day rats. At the 1.0 mg/kg/day level, MPL-related clinical signs included rough coat, urine staining, decreased activity, tail discoloration, and dark material around the eyes. MPL-related clinical signs at the 0.1 mg/kg/day level consisted of fecal staining, corneal opacity, decreased activity and lacrimation. Both control and MPL-treated rats exhibited a high incidence of wobbly gait following dosing. This change was thought to be associated with intravenous administration of the vehicle, (b) (4) in water for injection, USP.
Body weights and weight gain:
Statistically significant, dose-dependent reductions in mean body weight gain were observed in the 0.1, 1.0 .and 2.5 mg/kg/day males and females on study day 2. All groups exhibited a net loss in body weight between days 1 and 2, with the exception of the 0.1 mg/kg/day males that showed a slight but statistically lower net weight gain. The reductions in body weight gain for days 1-2 led to statistically decreased mean body weights in the 2.5 mg/kg/ day males and females, and

1.0 mg/kg/day males on day 2. Mean body weight of the 2.5 mg/kg/day males remained statistically lower than controls on day 8. For all groups, mean weight gain returned to normal levels or exceeded control values during days 2-8 indicating that a recovery had occurred.

Food consumption:

Statistically significant decreases in food consumption (g/animal/day and g/kg/day) were observed in the 0.1, 1.0 and 2.5 mg/kg/day males and females. These reductions were first observed in all groups for days 1-2 and followed a dose-related pattern in both magnitude and duration. In males, the persistence of statistically reduced food consumption (g/animal/day) ranged from 3 days (0.1 mg/kg/day group) to 5 days (2.5 mg/kg/day group). In females, the persistence of statistically reduced food consumption (g/animal/day) ranged from 1 day (0.1 mg/kg/day group) to 3 days (2.5 mg/kg/day group). After these periods, daily food consumption returned to normal levels or exceeded control values indicating that a recovery had occurred.

Clinical Pathology:

1. Hematology

RBC Parameters: RBC count, hemoglobin and hematocrit were statistically decreased in the 0.1, 1.0 and 2.5 mg/kg/day males and females. These reductions followed a dose-related pattern and were most severe in the 2.5 mg/kg/day males and females. Other statistically significant RBC parameters consisted of increased reticulocytes in 2.5 mg/kg/day males and 1.0 and 2.5 mg/kg/day females, decreased MCV in the 1.0 mg/kg/day females, and increased mean corpuscular hemoglobin concentration (MCHC) in the 2.5 mg/kg/day females.

Platelets: Statistically significant decreases in platelets were observed in the 1.0 and 2.5 mg/kg/day males. Although these decreases were relatively minor, they did occur in a dose-related pattern. The mean platelet level of the 2.5 mg/kg/day females also appeared to be decreased slightly, but was not statistically different from the controls.

Total and Differential Leukocytes: Total leukocytes, segmented neutrophils, lymphocytes and monocytes were statistically increased in the 2.5 mg/kg/day males and segmented neutrophils were statistically increased in the 1.0 mg/kg/day males. Total leukocytes, lymphocytes and monocytes also appeared to be increased in the 2.5 mg/kg/day females, but were not statistically different from the controls. Segmented neutrophils were statistically increased in the 1.0 and 2.5 mg/kg/day females. With regard to red cell morphology, apparent increases in slight to moderate polychromasia and anisocytosis were observed in the 2.5 mg/kg/day males and females. Similar changes in red cell morphology were also noted in the 1.0 mg/kg/day females.

2. Coagulation

PT and APTT: No statistically significant or biologically meaningful differences in PT or APTT were observed among the groups.

Fibrinogen: Fibrinogen levels were slightly but statistically increased in the 0.1, 1.0 and 2.5 mg/kg/day males and females. The biological significance of these increases was not clear. The increased fibrinogen values were generally similar for MPL-treated males and females and followed no apparent dose response pattern.

3. Clinical Chemistry

BUN and Creatinine: BUN was slightly but significantly increased in the 2.5 mg/kg/day males and females. In the 2.5 mg/kg/day males, serum creatinine was slightly but statistically decreased as compared to controls. This decrease was minor and appeared to be incidental.

Alkaline Phosphatase: Alkaline phosphatase was statistically decreased in the 0.1, 1.0 and 2.5 mg/kg/day males. The significance of this difference was not determined. Similar decreases in alkaline phosphatase were not observed in the female treatment groups.

Total Protein, Albumin and Globulin: There were no statistical differences in total protein among the groups, however, in both males and females, an apparent dose-related trend toward decreased total protein was observed. This trend correlated with statistically significant, dose-dependent decreases in serum albumin in the 0.1, 1.0 and 2.5 mg/kg/day females. A similar pattern of reduced albumin was observed in MPL-treated males, however, only the albumin level of the 2.5 mg/kg/day males was statistically different from the controls. This latter decrease led to a slight but statistically significant decrease in A/G ratio of the 2.5 mg/kg/day males. A/G ratios were not statistically different in the other study groups and no statistical differences in globulin levels were observed.
AST and ALT: Serum ALT levels were slightly but statistically decreased in the 1.0 and 2.5 mg/kg/day females. These decreases were very minor and appeared to be unrelated to MPL treatment. There were no apparent differences in serum AST levels among the groups.
Glucose: Glucose levels were statistically increased in the 0.1, 1.0 and 2.5 mg/kg/day females as compared to controls. The biological significance of this change was not clear since high fasting glucose levels were also observed in the control, 0.1, 1.0 and 2.5 mg/kg/day males.
Amylase: Amylase was statistically decreased in the 2.5 mg/kg/day males. This difference was not considered to be biologically significant since the reduced amylase level (1493.6 IU/L) remained similar to the pretest amylase level for males (1557.8 IU/L).

Gross Necropsy
At necropsy, enlarged spleens were observed in 2 rats of the 0.1 mg/kg/day group (2 males), 10 rats of the 1.0 mg/kg/day group (6 males and 4 females), and 15 rats of the 2.5 mg/kg/day group (9 males and 6 females). Necropsy findings in the remaining animals were generally unremarkable.
Organ Weights
Statistically significant and apparent changes in absolute and relative organ weight data are described below.
Spleen: Dose-dependent increases in absolute and relative spleen weights were observed in the 0.1, 1.0 and 2.5 mg/kg/day males and females. As compared to controls, the magnitude of the spleen weight increases ranged from approximately two-fold (0.1 mg/kg/day level) to approximately three-fold (2.5 mg/ kg/day). All spleen weights were statistically increased as compared to controls, with the exception of the absolute spleen weights of the 0.1 mg/kg/day females.
Liver: Absolute and relative liver weights were increased in a dose-related fashion in the 0.1, 1.0 and 2.5 mg/kg/ day females. In males, absolute and relative liver weights also appeared to be increased at all three MPL treatment levels; however, a clear dose response relationship was not observed and only the liver weight (absolute and relative) of the 2.5 mg/kg/day males was statistically increased.
Adrenal Glands: Absolute and relative adrenal gland weights were statistically increased in the 2.5 mg/kg/day males. In 2.5 mg/kg/day females, absolute and relative adrenal weights also appeared to be increased slightly; however, they were not statistically different from the control group.
Thymus Gland: Absolute and relative thymus gland weights of the 2.5 mg/kg/day females were slightly, but statistically decreased as compared to controls. The biological significance of this change was not determined.

Kidney: Relative kidney weights of the 0.1, 1.0 and 2,5 mg/kg/day males and females were slightly but statistically increased. These differences followed no apparent dose-response pattern.
Heart: Relative heart weight was statistically increased in the 1.0 and 2.5 mg/kg/day males, and 0.1, 1.0 and 2.5 mg/kg/day females. Relative heart weight of the 0.1 mg/kg/day males also appeared to be increased, although it was not statistically different from the controls.
Histopathology
Test article-related microscopic changes were observed in the eyes, heart, kidneys, liver, lung and spleen of rats from each of the MPL treated groups which were sacrificed at study termination. The changes were generally characterized by minimal to mild infiltrations of mononuclear inflammatory cells and are probably related to the pharmacologic action of the test article. Similar changes were observed in rats which died or were sacrificed moribund during the study. However, the extents of the changes were less in these rats because of their brief treatment. In addition, either edema or hemorrhage was observed in the brain and spinal cord in the three 2.5 mg/kg/day rats which died or was sacrificed moribund. This change was also considered to be test article related. No cause of death could be established for the control group female which was found dead on day 8.

Assessment:
A no-observed-effect level for MPL was not established in this study because treatment-related effects were reported at all MPL treatment levels tested.

Study # 7: 7-Day Intravenous Dose Range-Finding Toxicity Study in (b) (4) Rats with MPL (Study No. (b) (4) 3262.4 - Reported in BLA 125259)

Test Article: 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL)
Species/Strain: (b) (4) Rat. **Duration of Dosing:** 7 days.
Initial Age: 9 weeks. **Duration of Postdose:** N/A.
Date of First Dose: 23 September 1992. **Method of Administration:** Intravenous.
Vehicle/Formulation: MPL in (b) (4) in water for injection, (b) (4)
Number of animals: 24 (3 male and 3 female per group)
GLP Compliance: Yes
Performing Laboratory: (b) (4)
In-life Portion of Study Initiation Date: September 23, 1992
Final Report Date: February 24, 1993
Report status: Final

Methods:
This 7-day intravenous toxicity study consisted of four groups of (b) (4) rats with 3 rats/sex/group receiving 0, 40, 200, and 1000 mcg/kg/day in a dosage volume of 4.0 ml/kg. Animals were administered the test article or control material by intravenous injection, via the lateral tail vein, once daily for seven consecutive days. Doses were administered at a slow, constant rate, approximately 1 ml/60 seconds. Individual doses were calculated based on the most recent body weight data.
Clinical Observations

During the treatment period, all animals were observed a minimum of once daily for clinical signs of toxicity, including physical or behavioral abnormalities. Mortality and moribundity checks were performed twice daily, in the morning and afternoon. In addition, during the treatment period, the rats were observed within one hour following dosing for overt signs of toxicity.

Body Weights

Individual body weights were measured prior to dose administration on days 1, 2, 4 and 7. Terminal body weights were measured prior to scheduled euthanasia on day 8.

Food Consumption

Individual food consumption was measured on study days 1, 2, 4 and 7. Food consumption was calculated and reported as grams/animal/day.

Clinical pathology

Blood samples were collected from all animals on the day of scheduled euthanasia (day 8) for evaluation of selected hematology and clinical chemistry parameters. The animals were fasted overnight prior to blood sample collection. Blood samples were obtained via the orbital plexus while the rats were under light isoflurane anesthesia. The following parameters were evaluated:

Hematology

- Erythrocyte count (RBC)
- Hematocrit (Hct)
- Hemoglobin concentration (Hgb)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Mean corpuscular volume (MCV)
- Platelet count
- Reticulocyte count
- Total and differential leukocyte counts

Clinical Chemistry

- A/G ratio (calculated)
- Alanine aminotransferase (ALT)
- Albumin
- Alkaline phosphatase
- Aspartate aminotransferase (AST)
- Calcium
- Creatinine
- Electrolyte balance (sodium, potassium, chloride)
- Globulin (calculated)
- Glucose (fasting)
- Phosphorus
- Total bilirubin
- Total protein
- Urea nitrogen (BUN)

Gross Necropsy

All animals were euthanized on study day 8 by CO2 inhalation followed by exsanguination. The rats were subjected to a complete gross necropsy examination at the time of death or euthanasia.

The gross necropsy included examination of the external surfaces of the body and all internal viscera.

Organ weights
Fresh organ weights were obtained from all animals at scheduled euthanasia for the spleen, heart, kidneys, liver and brain. Paired organs were weighed together. Relative organ weights were subsequently calculated.

Results:

Survival
All animals survived to scheduled euthanasia on day 8.
Clinical Observations
There were no remarkable clinical signs of toxicity in the MPL treated rats. Tail discoloration was noted occasionally at the 40 and 1000 mcg/kg/day levels.
Body Weights and Weight Gain
Dose-dependent decreases in mean body weight gain were noted in both males and females of the 40, 200 and 1000 mcg/kg/day groups during days 1-2. Subsequent weight gain in these groups (days 2-4 and 4-7) was comparable to or exceeded control values.
Food Consumption
Dose-dependent reductions in mean food consumption (grams/animal/day) were observed in both males and females of the 40, 200 and 1000 mcg/kg/day groups during days 1-2. Additional reductions in food consumption were observed in the 200 mcg/kg/day males and 1000 mcg/kg/day females during days 2-4 and in the 1000 mcg/kg/day males during days 2-4 and 4-7.
Clinical pathology
Hematology
In MPL treated males, a possible, slight decrease in platelets was observed at the 1000 mcg/kg/day level. In addition, in males of the 40 mg/kg/day group, slightly higher total leukocytes, segmented neutrophils and lymphocytes were observed; however, similar changes were not observed in the 200 and 1000 mcg/kg/day males.
In MPL treated females, erythrocytes, hemoglobin and hematocrit appeared to be decreased slightly, but in a dose-dependent manner, at the 200 and 1000 mcg/kg/day levels. Similar trends toward decreased erythrocytes, hemoglobin and hematocrit were noted in the 40 mcg/kg/day females; however, these differences were only marginal and may have been due to biological variation. A slight but dose-related increase in segmented neutrophils was noted in the 40, 200, and 1000 mcg/kg/day females. In the 1000 mcg/kg/day females, possible slight increases in nucleated RBCs and reticulocytes were noted. In addition, in the 200 and 1000 mcg/kg/day females, apparent slight increases in the occurrence of polychromasia (slight to moderate) were observed.
Clinical Chemistry
There were no apparent differences in clinical chemistry data among the groups for either sex.
Gross Necropsy Observations
Enlarged spleens were observed at necropsy in 2/6 rats at the 40 mcg/kg/day level (2 males); 3/6 rats at the 200 mcg/kg/day level (2 males and 1 female); and 6/6 rats at the 1000 mcg/kg/day level (3 males and 3 females). Other necropsy findings were generally unremarkable.

Organ Weights
Dose-dependent increases in absolute and relative spleen weights (relative to final body weights and relative to brain weights) were observed in the MPL treated males and females at the 40, 200, and 1000 mcg/kg/day levels. Mean liver weights of the MPL treated males and females also appeared to be increased as compared to controls; however, these increases did not follow any consistent dose-related pattern.

Assessment
No test article-related effects on clinical signs and clinical pathology were reported. Dose-dependent decreases in food consumption and mean body weight gain were reported. Increases in spleen weight might be related to the immune responses.

Study # 8: 14-Day Intravenous Toxicity Study of MPL in Dogs (Study No. (b) (4) 3262.1 - Reported in BLA 125259)

Test Article: 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL)
Species/Strain: Dog/(b) (4). **Duration of Dosing:** 14 days.
Initial Age: **A**pproximately 8 months. **Duration of Postdose:** N/A.
Date of First Dose: 4 November 1991. **Method of Administration:** Intravenous.
Vehicle/Formulation: (b) (4) in water for injection
GLP Compliance: Yes
Report status: Final

Methods:
Study Design and Treatment
Three animals per sex/group received 0, 6, 120, and 1200 mcg/kg/day in a dosage volume of 1.2 ml/kg. Animals were administered the test or control materials by slow intravenous injection via the cephalic veins once a day for at least 14 days. The first day of administration was designated as study day 1. Intravenous injection of the test article was selected since it was a potential route of administration in humans.
Clinical Observations
Detailed clinical observations were performed and recorded at least once daily starting on day -7. In addition, the animals were observed twice daily for mortality and moribundity. During the dosing period, animals were observed at least once during the 2 hour post-dose period for overt signs of toxicity.
Body Weights
Individual body weights were measured on days -7, -1, 1, 2, 8 and 14. A terminal body weight was determined on the day of scheduled sacrifice.
Food Consumption
Individual food consumption was measured daily beginning on day -7 and continuing to day 14. Each dog was offered 400 g of food for a period of approximately 4 hours per day beginning approximately 2 hours after dosing. Food consumption was calculated in daily intervals as grams/animal/day and grams/kg/day.
Clinical Pathology
Hematology and biochemistry parameters were evaluated for all animals once prior to study initiation (on day -7), and on days 2, 8 and 15/16. The dogs were fasted overnight prior to each

sampling day. An additional blood sample was collected from each dog on day 3 (nonfasted) for evaluation of prothrombin time. All blood samples were taken via the jugular vein. On each day of blood collection for clinical pathology (days 2, 8 and 15/16), an extra serum sample was obtained and sent to the sponsor.
An 18-hour urine sample was collected from each dog once prior to study initiation (day -6) and on days 2 and 14. During the collection, food was withheld, but water was available. The following parameters were evaluated:

Hematology
- Erythrocyte Count (RBC)
- Hematocrit (Hct)
- Hemoglobin (Hgb)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Mean corpuscular volume (MCV)
- Platelet count
- Reticulocyte count
- Total and differential leukocyte counts

Coagulation
- Activated partial thromboplastin time
- Fibrinogen
- Prothrombin time

Biochemistry
- Albumin/globulin (A/G) ratio
- Alanine aminotransferase (ALT)
- Albumin
- Alkaline phosphatase
- Aspartate aminotransferase (AST)
- Calcium
- Creatinine
- Electrolyte balance (sodium, potassium, chloride)
- Globulin (calculated)
- Glucose (fasting)
- pH
- Phosphorus
- Serum amylase
- Total bilirubin
- Total protein
- Urea nitrogen (BUN)

Urinalysis
- 18-hour volume
- Bilirubin (qualitative)
- Blood (qualitative)
- Glucose (qualitative)
- Gross appearance
- Ketone (qualitative)
- Microscopic examination of sediment

pH
Protein (qualitative)
Specific gravity

Cardiology
Electrocardiogram (ECG) recordings of lead 2, heart rate, and blood pressure were recorded for each dog once during the pretest period (day -5 or -4) and at approximately 1 hour following dosing on days 1 and 14.

Gross Necropsy
All animals were subjected to a complete gross necropsy examination which included examination of the external surfaces of the body and all viscera. Fasted dogs were exsanguinated following an intravenous overdose of sodium pentobarbital. The following organs and tissues were collected from all animals and preserved in (b) (4) :

- Accessory genital organs (epididymides, prostate or uterus and vagina)
- Adrenals
- All gross lesions
- Aorta
- Bone marrow (smear from femur)
- Brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex)
- Cecum
- Colon
- Duodenum
- Ear (for identification only)
- Esophagus
- Eyes (including optic nerve)
- Femur (including articular surface)
- Gall bladder
- Heart
- Ileum
- Jejunum
- Kidneys
- Liver
- Lungs (infused with fixative)
- Mammary gland
- Mesenteric lymph node
- Pancreas
- Peripheral nerve (sciatic)
- Pituitary
- Rectum
- Skeletal muscle (thigh)
- Skin: Site 1 (nonfrictional surface-dorsal thorax)

Site 2 (frictional surface - elbow)
Site 3 (injection site - including vein)

- Spinal cord (cervical, midthoracic, lumbar)
- Spleen
- Sternum with bone marrow

- Stomach
- Submaxillary salivary gland
- Testes/Ovaries (including oviducts)
- Thymus
- Thyroid/parathyroid
- Trachea
- Urinary bladder

Organ Weights

The adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thymus and thyroid and parathyroids from all surviving animals were weighed wet; paired organs were weighed together. Organ weights relative to final body weights were subsequently calculated.

Histopathology

All tissues collected from each animal on study were processed for histopathological examination. Tissue sections were cut from paraffin blocks, mounted on microscope slides and (b) (4) . Histology was performed by (b) (4) The tissues were subsequently examined by a board certified veterinary pathologist.

Results:

Survival

All animals survived to scheduled sacrifice at study termination.

Clinical Observations

No overt clinical signs of toxicity were observed during the study. Signs of gastrointestinal disturbance such as vomitus, mucoid stools and soft stools were observed among the groups. Possible slight increases in the occurrence of these signs were observed at the 120 and 1200 mcg/kg/day levels.

Body Weights and Weight Gain

No statistically significant differences in mean body weights were noted for males or females during the study. Mean weight gain was statistically reduced in the 1200 mcg/kg/day females during study days 1-2, however, for both males and females there were no consistent patterns of reduced weight gain that would indicate an effect of MPL treatment.

Food Consumption

No statistically significant differences were observed in male food consumption. However, food consumption of the 1200 mcg/kg/day males calculated as grams/animals/day and grams/kg/day was moderately decreased during the first few days of treatment when compared to the control group. In females, occasional statistical reductions in food consumption (grams/animal/day and grams/kg/day) were noted at the 120 and 1200 mcg/kg/day levels. Although statistical significance was not demonstrated at other times, daily food consumption of the 120 and 1200 mcg/kg/day females was slightly, but consistently lower than controls throughout the study. No treatment-related differences in food consumption were observed for the 6 mcg/kg/day females.

Clinical Pathology Examinations

Hematology

Very slight to moderate reductions in platelets were observed in the 120 and 1200 mcg/kg/day males and females on days 2, 8 and 15/16, however, only the day 6 platelet level of the 1200 mcg/kg/day females was statistically different from the control group. The lowest platelet levels were observed in the 1200 mcg/kg/day males on day 8 (116.7 x 103/cmm) and day 15 (133.3 x

103/cmm). All other measured platelet levels were > 170 x 103/cmm. Marked increases in serum fibrinogen levels were noted in the 1200 mcg/kg/day males (day 2) and females (days 2 and 8). These increases were statistically significant in the female, but not the male dogs. A possible trend toward increased fibrinogen levels was also observed in the 120 mcg/kg/day males and females on day 2. The biological significance of these changes was not determined.
Marked increases in leukocytes were noted in the 1200 mcg/kg/day males and females on day 2. For each sex, the leukocytosis was attributed to an increase in segmented neutrophils. According to the sponsor, these changes are expected pharmacological effects of MPL. On days 8 and 15/16, leukocyte and segmented neutrophil counts remained slightly higher than controls, but were not statistically different.
Other statistical differences in hematology data included decreased prothrombin time in the 1200 mcg/kg/day males on day 3 and decreased segmented neutrophils in the 6 mcg/kg/day females on day 2. These differences appeared to be incidental and unrelated to treatment with MPL.
Clinical Chemistry
Statistical differences in clinical chemistry data included decreased phosphorus in the 1200 mcg/kg/day males on day 2, decreased AST in the 1200 mcg/kg/day females on day 8, decreased total bilirubin in the 1200 mcg/kg/day females on day 16, decreased pH in the 120 mcg/kg/day females on day 2, increased calcium in the 1200 mcg/kg/day females on day 2, increased sodium in the 1200 mcg/kg/day females on day 16, increased pH level in the 1200 mcg/kg/day females on day 8, increased albumin in the 1200 mcg/kg/day females on day -7, and increased calcium in the 6 mcg/kg/day females on day 2. These differences were not considered to be biologically meaningful since they were relatively minor, did not occur in any dose-related pattern, and they did not correlate with abnormal histopathology.
Urinalysis
Urine specific gravity was statistically decreased in the 120 and 1200 mcg/kg/day females on day 2. This change, coupled with increased urine volume in the 120 and 1200 mcg/kg/day females, was indicative of slight treatment-related hypoosmolar diuresis. No other apparent differences in urinalysis data were noted among the groups.
Cardiology
There were no indications of test article-related cardiovascular changes in the MPL-treated dogs.
Gross Necropsy Observations
Gross necropsy examinations did not reveal any specific changes attributable to MPL treatment.
Organ Weights
No statistically significant differences were noted in absolute or relative organ weight data. However, both absolute and relative spleen weight of the 1200 mcg/kg/day males and females were increased when compared to the control group.
Histopathology Observations
No test article-related lesions were observed microscopically in the MPL-treated dogs.
Assessment:
No observed adverse effect level (NOAEL) of 6 mcg/kg/day was determined in this study in dogs.

Overall conclusions from toxicology studies:
The results from the toxicology studies conducted with MPL alone confirmed that MPL is a detoxified form of bacterial LPS. Significant difference in the results from the toxicology studies conducted with MPL alone (included in the BLA [#125259]) as compared to the results reported

for LPS by Ribi et al, 1986 were noted. Repeat-dose toxicity studies were performed in which MPL was given once daily for up to 8 days in rats and 14 days in (b) (4) dogs, by the intravenous route to maximize the exposure. These studies showed a no-observed effect level at 6 mcg/kg/day in (b) (4) dogs and that the 40 mcg/kg/day dose was well-tolerated in rats. Effects reported were generally dose-related. The very few effects seen at the lowest dose of 40 mcg/kg/day in rat were minor and consistent with an immunostimulatory action of MPL, such as increased WBC count and spleen weight. These results may be contrasted with those reported for LPS by Ribi et al, 1986 where doses as low as 1 mcg/kg body weight of LPS induce death in rabbits.
MPL caused death when administered intravenously to rats at very high doses (5 mg/kg/day), which was attributed to endotoxic shock because of the findings of edema and hemorrhage in the brains of the dead animals. In this case, the MPL was administered intravenously and it was thought to be in a soluble (monomeric) form as it was dissolved in (b) (4) This is in contrast to the MPL in Cervarix, which rather than being administered intravenously and in a soluble form, is being administered intramusucularly and in a non-soluble, particulate form (i.e., (b) (4)). In addition, in Cervarix, MPL is to be administered at a much lower dose (i.e., 50 mcg per dose, which equates to 1.7 to 0.7 mcg/kg dose for 30-70 kg-weighing individuals). In addition, the peak systemic exposure to MPL after a human intramuscular vaccination with an AS04-containing vaccine, administered according to a 0, 1, 6 month immunization schedule, for example, is expected to be significantly reduced compared to daily intravenous MPL injections used in the repeated-dose toxicity studies.

In conclusion, the data reported here showed expected findings associated with a strong immunostimulant (increased spleen weight and white blood cell value), and for these changes, there is a high safety margin for the proposed use of MPL. These data therefore support the use of MPL as a vaccine adjuvant in human papillomavirus vaccine, AS04 adjuvant-adsorbed (Cervarix) for human vaccination against HPV infections and related clinical outcomes.

Study # 9: Repeated Dose Toxicity Study with DQ Administered Intramuscularly to Male and Female Rabbits (Study No. V 20155)

Performing laboratory: (b) (4)

Study initiation date: 13 and 14 August, 2012 for males and females respectively.
Final Report date: 03 April, 2013
Test article batch/lot:

Test article	Batch number	Expiration date
DQ (QS-21 = 10 µg/dose)	(b) (4)	
DQ (QS-21 = 50 µg/dose)	(b) (4)	
DQ (QS-21 = 100 µg/dose)	(b) (4)	
Saline	(b) (4)	

Animal species and strain: (b) (4) rabbits
Breeder/supplier: (b) (4)
Number of animal per group and sex: 33 males and 33 females

Age: Approximately 12 week at start
Body weight range: 2000-2300 g
Route and site of administration: Intramuscular
Volume of injection: 1 mL per animal (two injections of 0.5 mL per occasion)
Frequency of administration and study duration:
Day 0, posterior thigh muscle;
Day 4, calf muscle;
Day 7, posterior thigh muscle;
Day 11, calf muscle;
Day 14, posterior thigh muscle;
Day 18, anterior thigh muscle.
Dose: See study design
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study.

Statistical analysis: Yes
GLP: Yes
Means of administration: Intramuscular
Report status: Final

Methods:

Experimental design:

Groups	Number of animals		Animal nos.		Treatment (intramuscular; 0.5 mL per site)		Dose[1] of QS21 [µg/Kg bw]
	Subgroup 1	Subgroup 2	Males Even nos.	Females Odd nos.	Left leg	Right leg	
1	5 males + 5 females	5 males + 5 females	2-10 (S1) 12-20 (S2)	1-9 (S1) 11-19 (S2)	Saline	Saline	-
2	5 males + 5 females		22-30 (S1)	21-29 (S1)	DQ; 20 µg/mL	DQ; 20 µg/mL	7
3	5 males + 5 females		32-40 (S1)	31-39 (S1)	DQ; 100 µg/mL	DQ; 100 µg/mL	33
4	5 males + 5 females	5 males + 5 females	42-50 (S1) 52-60 (S2)	41-49 (S1) 51-59 (S2)	DQ; 200 µg/mL	DQ; 200 µg/mL	67

[1] Dose level of QS-21 per occasion (two injections)

Table 42: *Experimental design (study # V 20155)*

The following parameters were evaluated: clinical signs (twice daily), skin reactions at the intramuscular site of injection (approximately at 3, 24 and 48 hours after each injection), body weights (in subgroup 1; days -7, -4, 0, 3, 7, 14 and 21; in subgroup 2; days -7, -4, 0, 3, 7, 14, 21, 28, 35, 42 and 45), food consumption (in subgroup 1; days -4-0, 0-3, 3-7, 7-14, 14-21; in subgroup 2; days -4-0, 0-3, 3-7, 7-14, 14-21, 21-28, 28-35, 35-42, 42-45), ophthalmoscopy (predose and on days 17 and 43 of the study), body temperature (prior to and circa 4 and 24 hours after the first and sixth injection), hematology and clinical chemistry (predose and on days 1, 3, 17, 19 and 21, and in S2 animals on day 45), pathology (subgroup 1 on day 21; subgroup 2 on day 45).

Tissues collected:

Tissue/organ sampled	Weighed	Examined
Adrenals	X	X
Aorta (thoracic)		X
Bone and bone marrow (sternum, femur and joints)		X
Brain (3 levels, including hypothalamus) and meninges	X	X
Caecum		
Colon		X
Inguinal lymph nodes		X
Iliac lymph nodes		X
Popliteal lymph nodes	X	X
Duodenum		
Epididymides (males)	X	X
Eyes and optic nerve		X
Heart	X	X
Ileum		
Jejunum		
Kidneys	X	X
Knee joint		X
Lacrimal glands		X
Liver and gall-bladder	X	X
Lungs including larynx, trachea and bronchi	X	X
Lymph nodes (mandibular and mesenteric)		X
Mammary glands (females only)		
Muscle at injection sites[1]		X
Muscle (skeletal) = triceps		X
Mesenteric artery		X
Oesophagus		
Ovaries (females)	X	X
Pancreas		X
Pituitary	X	X
Prostate (males)	X	X
Rectum		
Salivary glands[2]		X
Sciatic nerve		X
Seminal vesicles (males)		
Skin/subcutis (hind limb)		
Spinal cord (cervical, thoracic and lumbar)		X
Spleen	X	X
Stomach		X
Testes (males)	X	X
Thymus	X	X
Thyroid/parathyroids	X	X
Tongue		
Urinary bladder		X
Uterus (females)	X	X
Vagina (females)		X
Gross lesions		X
Tissue masses or tumors (if found)		X

[1] Histopathological examination was performed on the anterior thigh muscles (left and right) injected on day 18 only. [2] Submaxillary, sublingual and parotid

Table 43: *Tissues collected (study # V 20155)*

With the preserved anterior thigh muscles three areas were processed, i.e. central and adjacent left and right areas. These areas were also examined macroscopically for gross findings. The three areas of the injection site were (b) (4) sectioned at 5 µm and (b) (4) All other tissue/organs submitted to histopathological examination were also (b) (4), sectioned at 5 µm and (b) (4). Tissue/organs not submitted to histopathological examination were kept in (b) (4).

Results:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, body temperature, or organ weight were reported.

Body Weights and Weight Gain

On day 0 (prior to injection), a higher mean body weight was observed in the DQ 200 µg/mL males. Post-dose, a higher mean body weight was observed in the DQ 200 µg/mL males on days 7 and 45 of the study, and in DQ 200 µg/mL females on days 3 and 7 of the study. Compared to the respective pre-dose (day 0) body weights, the day 7 mean body weight of the DQ 200 µg/mL males had slightly less increased compared to the saline control. No toxicological significance was attached to these minor changes in body weight.

Clinical Pathology Examinations

Hematology

Fibrinogen: A higher fibrinogen concentration was reported in group 3 males on day 1 after the first injection and in group 4 males on days 1 and 3 after the first injection and on day 1 after the sixth injection. The fibrinogen concentration tended to be higher in group 4 males on day 3 after the sixth injection and in group 2 males on day 1 after the first injection and in group 3 males on day 3 after the first injection and on day 1 after the sixth injection.

A higher fibrinogen concentration was reported in group 4 females on days 1 and 3 after the first and the sixth injection and on day 1 prior to the sixth injection.

The increases in fibrinogen were considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

Increases in white blood cells, neutrophils, and lymphocytes were reported in group 4 males on day 1 after the first injection. The absolute neutrophil count tended to be higher in group 3 males on day 1 after the first injection. On day 1 after the sixth injection, a dose-dependent increase in neutrophils was reported in groups 2, 3, and 4 males. A higher absolute monocyte count was reported in group 4 males on day 3 after the sixth injection. A higher absolute basophil count was reported in group 4 males on day 3 after the first injection. A lower absolute basophil count was reported in group 4 males on day 27 after the sixth injection.

Increases in white blood cells and neutrophils were reported in group 4 females on day 1 after the first injection. Increases in esoinophils and monocytes were reported in group 4 females on day 27 after the sixth injection

Clinical Chemistry

A higher TP and albumin concentrations was reported in group 4 females on day 1 after the first injection. A higher TP concentration was reported in group 4 females on day 3 after the first injection. In general, a dose dependent decrease of the A/G ratio was reported in groups 2, 3, and 4 females after the first and the sixth injection. A dose-dependent increase in CRP concentration was reported in groups 2, 3, and 4 females on days 1 and 3 after the first injection and on day 1 after the sixth injection in groups 3 and 4 females. This increase was not statistically significant in the group 2 females and not statistically significant in group 3 females on day 3 after the first injection. On day 1, prior to the sixth injection, the CRP concentration was still increased in group 4 females. On day 3, after the sixth injection, the CRP levels in groups 2, 3, and 4 females had returned to the saline control level. The transient increases in CRP were considered to be part of the inflammatory process upon injection.

A higher bilirubin concentration in groups 2 and 3 females on day 3 after the sixth injection, and in group 4 females on day 27 after the sixth injection was reported.

Organ weights
No test article-related effect on organ weights were reported in subgroup 1 (sacrificed 3 days after the sixth injection).

Subgroup 2 (sacrificed 27 days after the sixth injection)
When compared to control group, higher absolute and relative mean prostate weight was reported in group 4 males. When compared to control group, lower relative mean brain weight was reported in group 4 males. Histopathology of the prostate and the brain did not reveal any treatment-related abnormalities.

Macroscopic examination
Hemorrhage at the injection site at the right anterior thigh muscle at necropsy (3 days after the sixth injection) was reported in 3/10, 2/10, and 4/10 in groups 1, 3, and 4, respectively. At the injection site of the left anterior thigh muscle a hemorrhage was reported in 3/10, 1/10, 1/10, and 2/10 in groups 1, 2, 3, and 4, respectively. In group 1, hemorrhage was reported at the injection site in 2/10 and 1/10 at the left posterior thigh muscle and the left calf muscle, respectively. This hemorrhage was considered a reaction to insertion of the injection needle.

No hemorrhage at the injection site was reported in all subgroups 2.

Microscopy
Subgroup 1, sacrificed day 21, 3 days post sixth injection

Right treated anterior thigh muscle (3 days post sixth injection);

At the injection site, minimal to mild localized mononuclear inflammatory responses were reported in 1/5, 3/5, and 4/5 of groups 1, 2, and 3 males, respectively. At the injection site, minimal to mild localized mononuclear inflammatory responses were reported in 4/5, 4/5, and 1/5 in groups 1, 2, and 3 females, respectively.

The mononuclear inflammatory reaction was widespread (i.e. extended along the epimysium and diffusely between the muscle fibers) in 1/5 of group 3 males, 5/5 of group 4 males, 2/5 of group 3 females and 3/5 of group 4 females. The mononuclear inflammatory reaction consisted of lymphocytes and small macrophages.

Widespread mixed inflammatory response was reported (besides lymphocytes and macrophages, polymorphonuclear inflammatory cells were also present) in 2/5 of group 3 females and in 2/5 of group 4 females. Minimal to mild hemorrhage(s) were reported at the injection site of 1/5 of group 3 males, 1/5 group 4 males, 2/5 group 1 females, and 2/5 group 4 females. Minimal mineralization was reported, as part of the local response, in 2/10 group 4 animals but also in 1/10 group 1 animals.

Left treated anterior thigh muscle (3 days post sixth injection);

Minimal to mild localized mononuclear inflammatory responses were reported at the injection site in 2/5, 2/5, and 1/5 in groups 1, 2, and 3 males, respectively. Minimal to mild localized mononuclear inflammatory responses were reported at the injection site in 3/5, 4/5, and 1/5 in groups 1, 2, and 4 females, respectively. The mononuclear inflammatory reaction was widespread (i.e. extended along the epimysium and diffusely between the muscle fibers) in 4/5 of group 3 males, 5/5 of group 4 males, and 2/5 of group 4 females. Mononuclear inflammatory reaction consisted of lymphocytes and small macrophages.

Widespread mixed inflammatory response was reported (besides lymphocytes and macrophages, polymorphonuclear inflammatory cells were also present) in 5/5 and 2/5 in groups 3 and 4 females, respectively. Minimal to mild hemorrhage(s) were reported at the injection site of 1/5 and 2/5 of groups 3 and 4 males, respectively. Minimal to mild hemorrhage(s) were reported at the injection site of 1/5 of groups 1, 2, and 4 females. Minimal mineralization was reported as part of the local response in 1/5 of group 3 males and group 2 females.

The minimal to mild localized response in the right and left anterior thigh muscle is considered as a reaction to insertion of an injection needle. This response was considered test article-related in the animals showing a widespread inflammatory response, either mononuclear or mixed.

Subgroup 2, sacrificed day 45, 27 days post sixth injection
Minimal localized mononuclear inflammatory response was reported in 1/5 group 1 males (left) and 1/5 group 4 females (right). This finding was not considered test article-related.

Assessment
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, body temperature, or organ weight were reported. However, test article-related effects at the injection site (widespread inflammatory response, either mononuclear or mixed) were reported.

Study # 10: Repeated-dose Toxicity Study with AS01B Administered Intramuscularly (Seven Times) to Male and Female Rats Followed by a 4-Week Treatment Free Period (Study No. V 20165)

Performing laboratory: (b) (4)

Study initiation date: 24 April, 2012.
Final Report date: 20 March, 2013
Test article batch/lot:

Test article	Batch number	Expiration date
AS01B	(b) (4)	
Saline	(b) (4)	

Animal species and strain: (b) (4) rat; (b) (4):CD(b) (4)
Breeder/supplier: (b) (4)
Number of animal per group and sex: 33 males and 33 females
Age: Approximately 12 week at start
Body weight range: 370-400 g males, 225-250 g females
Route and site of administration: Intramuscular
Volume of injection: 0.2 mL per animal (two injections of 0.1 mL per occasion)
Frequency of administration and study duration:

Day 0, left and right posterior thigh muscles;
Day 14, left and right calf muscles;
Day 28, left and right posterior thigh muscles;
Day 42, left and right calf muscles;
Day 29, left and right posterior thigh muscles;
Day 70, left and right calf muscles;
Day 84, left and right anterior thigh muscles.

Dose: 700 µl monodose vial containing 50 µg QS-21 and 50 µg MPL in a liposome-based formulation per 500 µl.

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study.
Statistical analysis: Yes
GLP: Yes
Means of administration: Intramuscular
Report status: Final

Methods:
Experimental design:
Males:

Group	Color code	Treatment	Volume	Animal numbers of males (even nos.)	
				S1 (n=10)	S2 (n=5)
20165/1	White	Saline	0.2 mL	2-20	22-30

Group	Color code	Treatment	Volume	Animal numbers of males (even nos.)	
				S1 (n=10)	S2 (n=5)
20165/2	Blue	AS01B	0.2 mL	32-50	52-60

Females:

Group	Color code	Treatment	Volume	Animal numbers of females (odd nos.)	
				S1 (n=10)	S2 (n=5)
20165/1	White	Saline	0.2 mL	1-19	21-29
20165/2	Blue	AS01B	0.2 mL	31-49[1]	51-59

[1] animal no. 49 did not survive blood collection on day 85 of the study
S1 = subgroup 1 sacrificed on day 87 (i.e. 3 days post 7th injection)
S2 = subgroup 2 sacrificed on day 113 (i.e. 29 days post 7th injection)
Table 44: *Experimental design (study # V 20165)*

The following parameters were evaluated: clinical signs (twice daily), skin reactions at the intramuscular site of injection (approximately at 3, 24 and 48 hours after each injection), body weights (in subgroup 1; days -7, -4, 0, 3, 7, weekly until 84, and day 87; in subgroup 2; days -7, -4, 0, 3, 7, weekly until day 113), food consumption (in subgroup 1; days -4, 0, 3, 7, and weekly until 84; in subgroup 2; days -4, 0, 3, 7, and weekly until day 112), ophthalmoscopy (pre-dose and on days 84 and 112 in subgroup 2 animals only), body temperature (prior to and circa 4, 24, and 48 hours after the first and seventh injection [subgroup 1 only]), hematology and clinical chemistry (by orbital puncture: on days 1, 3 and 85 [subgroup 1 only], and by abdominal aorta on day 87 [subgroup 1] and on day 113 [subgroup 2]), pathology (subgroup 1 on day 87; subgroup 2 on day 113).

Tissues collected:

Tissue/organ sampled	Weighed	Examined
Adrenals	X	X
Aorta (thoracic)		X
Bone and bone marrow (sternum, femur and joints)		X
Brain (3 levels, including hypothalamus) and meninges	X	X
Caecum		
Colon		X
Inguinal lymph nodes		X
Iliac lymph nodes		X
Popliteal lymph nodes	X	X
Duodenum		
Epididymides (males)	X	X
Eyes and optic nerve		X
Heart	X	X
Ileum		
Jejunum		
Kidneys	X	X
Knee joint		X

Lacrimal glands		X
Liver	X	X
Lungs including larynx, trachea and bronchi	X	X
Lymph nodes (mandibular and mesenteric)		X
Mammary glands (females only)		
Muscle at injection sites1		X
Muscle (skeletal) = triceps		X
Mesenteric artery		X
Oesophagus		
Ovaries (females)	X	X
Pancreas		X
Pituitary	X	X
Prostate (males)	X	X
Rectum		
Salivary glands2		X
Sciatic nerve		X
Seminal vesicles (males)		
Skin/subcutis (hind limb)		
Spinal cord (cervical, thoracic and lumbar)		X
Spleen	X	X
Stomach		X
Testes (males)	X	X
Thymus	X	X
Thyroid/parathyroids	X	X
Tongue		
Urinary bladder		X
Uterus (females)	X	X
Vagina (females)		
Gross lesions		X
Tissue masses or tumours (if found)		X

[1] Histopathological examination was performed on the anterior thigh muscles (left and right) injected on day 84 only.
[2] Submaxillary, sublingual, and parotid

Table 45: *Tissues collected (study # V 20165)*

With the preserved anterior thigh muscles (left and right) three areas were processed, i.e. central and adjacent left and right areas. These areas were also examined macroscopically for gross findings. The three areas of the injection sites were (b) (4) , sectioned at 5 µm and (b) (4) . All other tissue/organs not submitted to histopathological examination were kept in (b) (4) .

Results:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, body temperature, or organ weight were reported.

Clinical signs

A swollen calf muscle was reported in all group 2 males after its first and second injection and in 5/15 males after its third injection. In all group 2 females, a swollen calf muscle was reported after its first injection only. Hematoma at the site of injection was reported in 4 males and 4 females of group 1, and in 1 male and 1 female of group 2.

Body temperature
Four hours after the first and the seventh injection, higher mean body temperature was reported in group 2 males. Twenty four hours after the seventh injection, lower mean body temperature was reported in group 2 males. Four hours after the first injection, higher mean body temperature was reported in group 2 females. Four and 24 hours after the seventh injection, higher mean body temperature was also reported in group 2 females.

Clinical Pathology Examinations
Hematology
Males:
Prothrombin time (PT): On day 1 after the seventh injection, shorter PT in group 2 was reported. Longer PT in group 2 was reported on days 3 and 29 after the seventh injection. The individual values were within or very close to the control range.

Activated partial thromboplastin time (APTT): A shorter APTT on day 3 after the seventh injection was reported in group 2.

Fibrinogen: In group 2, higher fibrinogen concentration on days 1 and 3 after the first and the seventh injection was reported. The increases in fibrinogen were considered to be part of the inflammatory process upon injection.

Total white blood cell count (WBC): In group 2, higher WBC on day 1 after the seventh injection was reported. The increases in WBC were considered to be part of the inflammatory process upon injection.

Absolute neutrophil count: In group 2, higher absolute neutrophil count on day 1 after the first and the seventh injection was reported. The increases in neutrophils were considered to be part of the inflammatory process upon injection.

Females:
Prothrombin time (PT): On day 3 after the first and the seventh injection, longer PT in group 2 was reported. The toxicological significance of these transient findings was considered negligible.

Fibrinogen: On days 1 and 3 after the first and the seventh injection, higher fibrinogen concentration in group 2 was reported. The increases in fibrinogen were considered to be part of the inflammatory process upon injection.

Total white blood cell count (WBC): In group 2, higher WBC on day 3 after the first and on day 1 after the seventh injection was reported. The WBC tended to be higher on day 1 after the first injection. The increases in WBC were considered to be part of the inflammatory process upon injection.

Absolute eosinophil count: On day 1 after the first and the seventh injection, higher absolute eosinophil count in group 2 was reported. The eosinophil count tended to be higher on day 3

after the first injection. The increases in eosinophils were considered to be part of the inflammatory process upon injection.

Absolute neutrophil count: On days 1 and 3 after the first and on day 1 after the seventh injection, higher absolute neutrophil count in group 2 was reported. The increases in neutrophils were considered to be part of the inflammatory process upon injection.

Clinical Chemistry
Males:
Albumin: On days 1 and 3 after the first and on day 3 after the seventh injection, lower albumin concentration in group 2 was reported. The individual values were within or very close to the control range. Therefore, the toxicological significance of these transient findings was considered negligible.

Albumin/Globulin ratio (A/G ratio): On days 1 and 3 after the first and the seventh injection, lower A/G ratio in group 2 was reported. The lower A/G ratios were related to the increase in globulin levels since the albumin concentrations had decreased and TP concentrations were, in general, not affected. The lower A/G ratios were considered to be part of the inflammatory process upon injection of AS01B.

Females:
Albumin/Globulin ratio (A/G ratio): On days 1 and 3 after the first and the seventh injection, lower A/G ratio in group 2 was reported. The lower A/G ratios were related to increasing globulin levels since the albumin concentrations had decreased and TP concentrations were, in general, not affected. The lower A/G ratios were considered to be part of the inflammatory process upon injection of AS01B.

Bilirubin: On day 1 after the first and on days 1 and 3 after the seventh injection, higher bilirubin concentration in group 2 was reported.

<u>Organ weights</u>
Subgroup 1 (sacrificed day 87, 3 days after the seventh injection)
When compared to control groups, high absolute and relative mean spleen weights and high absolute and relative mean pituitary weights in group 2 females were reported.

Subgroup 2 (sacrificed 27 days after the sixth injection)
No test article-related effect on organ weights was reported in subgroup 2.

<u>Macroscopic examination:</u>
Subgroup 1, sacrificed day 87, 3 days post seventh injection
Discoloration (white stripe) at the left posterior thigh muscle of 1/10 of group 2 females and a hemorrhage at the left anterior thigh muscle in 1/10 of group 1 females was reported.

Subgroup 2, sacrificed day 113, 29 days post seventh injection

Discoloration (stripe/spot) at the right anterior thigh muscle of 1/5 of group 2 males, and at the left anterior thigh muscle of 1/5 of group 2 males and females, and 1/5 group 1 males and females was reported.

Microscopy:
Right treated anterior thigh muscle (3 days post 7th injection)
Minimal to mild localized mononuclear cell inflammatory response was reported in 7/10 group 1 males, 5/10 group 1 females, 1/10 group 2 males and females. In 8/10 group 2 males and 9/10 group 2 females, the mononuclear cell inflammatory reaction was widespread (i.e. extending along the epimysium and diffusely between the muscle fibers) and graded minimal to marked. Minimal to mild multifocal mononuclear cell inflammatory response was reported in 1/10 group 2 males and 2/10 group 1 females. Mononuclear cell inflammation reaction consisted of lymphocytes and small macrophages. Minimal focal muscle fiber degeneration was reported in 2/10 group 1 males and 1/10 group 2 males. Mild edema was reported in 1/10 group 2 females. Moderate accumulation of extramuscular fibrin was reported in 1/10 group 2 males.

Left treated anterior thigh muscle (3 days post 7th injection)
Minimal to mild localized mononuclear cell inflammatory response was reported in 7/10 group 1 males, 6/10 group 1 females, 1/10 group 2 males and 1/10 group 2 females. The mononuclear cell inflammatory reaction was widespread (i.e. extending along the epimysium and diffusely between the muscle fibers) and graded mild to moderate in 8/10 group 2 males and 9/10 group 2 females. Mild multifocal mononuclear cell inflammatory response was reported in 1/10 group 1 males. Mononuclear cell inflammation consisted of lymphocytes and small macrophages. Minimal focal muscle fiber degeneration was reported in 3/10 group 1 males and 1/10 group 2 males. Moderate accumulation of extramuscular fibrin was reported in 1/10 group 2 males.

An increase in basophilic tubules in group 2 males was reported. An increase in extramedullary hematopoiesis and brown pigment accumulation in group 2 males' spleen was reported.

Subgroup 2, sacrificed day 113, 29 days post seventh injection:
Right treated anterior thigh muscle (29 days post 7th injection)
Minimal localized mononuclear cell inflammatory response was reported at the injection site in 3/5 group 1 males, 2/5 group 1 females, 3/5 group 2 males, and 2/5 group 2 females. Minimal multifocal mononuclear cell inflammatory response was reported in 1/5 group 2 males. Mononuclear cell inflammation consisted of lymphocytes and small macrophages. Minimal focal muscle fiber degeneration was reported in 1/5 group 1 males, 1/5 group 1 females, 2/5 group 2 males, and 1/5 group 2 females.

Left treated anterior thigh muscle (29 days post 7th injection)
Minimal localized mononuclear cell inflammatory response was reported at the injection site in 2/5 group 1 males, 1/5 group 1 females, 4/5 group 2 males and 3/5 group 2 females. Minimal multifocal mononuclear inflammatory response was reported in 1/5 group 2 males. Mononuclear inflammation consisted of lymphocytes and small macrophages. Minimal focal muscle fiber degeneration was reported in 1/5 group 2 males.

Assessment:
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, body temperature, or organ weight were reported. However, test article-related effects at the injection sites were reported.

Study # 11: Repeated Dose Toxicity Study with DQ Administered Intramuscularly to Male and Female Rats (Study No. V 20154)

Performing laboratory: (b) (4)

Study initiation date: 23 August, 2012.
Final Report date: 03 April, 2013
Test article batch/lot:

Test article	Batch number	Expiration date
DQ (QS-21 = 10 µg/dose)	(b) (4)	
DQ (QS-21 = 50 µg/dose)	(b) (4)	
DQ (QS-21 = 100 µg/dose)	(b) (4)	
Saline	(b) (4)	

Animal species and strain: (b) (4) rat; (b) (4):CD
Breeder/supplier: (b) (4)
Number of animal per group and sex: 53 males and 53 females
Age: Approximately 12/13 (males/females) week at start
Body weight range: 375-400 g males, 225-250 g females
Route and site of administration: Intramuscular
Volume of injection: 0.2 mL per animal (two injections of 0.1 mL per site)
Frequency of administration and study duration:

- Day 0, posterior thigh muscle;
- Day 4, calf muscle;
- Day 7, posterior thigh muscle;
- Day 11, calf muscle;
- Day 14, posterior thigh muscle;
- Day 18, anterior thigh muscle.

Study duration is 46 days.
Dose: See study design

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study.

Statistical analysis: Yes
GLP: Yes
Means of administration: Intramuscular
Report status: Final

Methods:

Experimental design:

Groups	Number of animals		Animal nos.		Treatment (intramuscular; 0.1 mL per site)		Dose[1] of QS21 [µg/Kg/bw]	
	Subgroup 1	Subgroup 2	Males Even nos.	Females Odd nos.	Left leg	Right leg	Males	Females
1	10 males + 10 females	5 males + 5 females	2-20 (S1) 22-30 (S2)	1-19 (S1) 21-29 (S2)	Saline	Saline	-	-
2	10 males + 10 females		32-50 (S1)	31-49 (S1)	DQ; 20 µg/mL	DQ; 20 µg/mL	10	16
3	10 males + 10 females		52-70 (S1)	51-69 (S1)	DQ; 100 µg/mL	DQ; 100 µg/mL	50	80
4	10 males + 10 females	5 males + 5 females	72-90 (S1) 92-100 (S2)	71-89 (S1) 91-99 (S2)	DQ; 200 µg/mL	DQ; 200 µg/mL	100	160

[1] Dose level of QS-21 per occasion (two injections)

Table 46: *Experimental design (study # V 20154)*

The following parameters were evaluated: clinical signs (twice daily), skin reactions at the intramuscular site of injection (approximately at 3, 24 and 48 hours after each injection), body weights (subgroup 1; days -3, 0, 4, 7, 14 and 21; subgroup 2; days -3, 0, 4, 7, 14, 21, 28, 35, 42 and 46), food consumption (subgroup 1; days -3-0, 0-4, 4-7, 7-14, 14-21; subgroup 2; days -3-0, 0-4, 4-7, 7-14, 14-21, 21-28, 28-35, 35-42, 42-46), ophthalmoscopy (pre-dose and on days 19 and 42), body temperature (prior to and circa 4, 24, and 48 hours after the first and sixth injection [subgroup 1 only]), hematology and clinical chemistry (by orbital puncture on day 19 and by abdominal aorta on days 21 and 46), pathology (subgroup 1 on day 21; subgroup 2 on day 46).

Tissues collected:

Tissue/organ sampled	Weighed	Examined
Adrenals	X	X
Aorta (thoracic)		X
Bone and bone marrow (sternum, femur and joints)		X
Brain (3 levels, including hypothalamus) and meninges	X	X
Caecum		
Colon		X
Inguinal lymph nodes		X
Iliac lymph nodes		X
Popliteal lymph nodes	X	X
Duodenum		
Epididymides (males)	X	X
Eyes and optic nerve		X
Heart	X	X
Ileum		
Jejunum		
Kidneys	X	X
Knee joint		X
Lacrimal glands		X
Liver	X	X

Tissue/organ sampled	Weighed	Examined
Lungs including larynx, trachea and bronchi	X	X
Lymph nodes (mandibular and mesenteric)		X
Mammary glands (females only)		
Muscle at injection sites[1]		X
Muscle (skeletal) = triceps		X
Mesenteric artery		X
Oesophagus		
Ovaries (females)	X	X
Pancreas		X
Pituitary	X	X
Prostate (males)	X	X
Rectum		
Salivary glands[2]		X
Sciatic nerve		X
Seminal vesicles (males)		
Skin/subcutis (hind limb)		
Spinal cord (cervical, thoracic and lumbar)		X
Spleen	X	X
Stomach		X
Testes (males)	X	X
Thymus	X	X
Thyroid/parathyroids	X	X
Tongue		
Urinary bladder		X
Uterus (females)	X	X
Vagina (females)		X
Gross lesions		X
Tissue masses or tumors (if found)		X

[1] Histopathological examination performed on the anterior thigh muscles (left and right) injected on day 18 only.
[2] Submaxillary, sublingual and parotid

Table 47: *Tissues collected (study # V 20154)*

With the preserved anterior thigh muscles three areas were processed, i.e. central and adjacent left and right areas. These areas were also examined macroscopically for gross findings. The three areas of the injection site were (b) (4), sectioned at 5 µm and (b) (4). All other tissue/organs not submitted to histopathological examination were kept in (b) (4).

Results:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, body temperature, or organ weight were reported.

Clinical signs

Swollen muscle was reported in 3/15 group 4 males and 1/10 group 2 females after the first injection of the calf muscle on day 4. Hematoma at the site of injection was reported in 1/15 group 1 males, in 1/10 group 2 males and females and in 1/15 group 4 males.

Body temperature
Higher mean body temperature was reported in the three male DQ dose groups. Four hours post-dose the sixth injection; a slightly higher mean body temperature was reported in the group 4 males.

Clinical Pathology Examinations
Hematology
Males:

Fibrinogen: On day 1 after the sixth injection, higher fibrinogen levels were reported in groups 3 and 4. On day 3 after the sixth injection, higher fibrinogen levels were reported in group 4. Fibrinogen levels tended to be higher in group 2 on day 1 after the sixth injection and in groups 2 and 3 on day 3 after the sixth injection. The increases in fibrinogen levels were not considered frank toxicity but rather an anticipated effect associated with an immunological response.

Females:
Thrombocytes: On day 3 after the sixth injection, higher number of thrombocytes was reported in groups 3 and 4. Thrombocytes levels tended to be higher in group 2 on day 3 after the sixth injection.

Prothrombin time (PT): On day 1 after the sixth injection, shorter PT was reported in group 4. Decreases in PT were related to the increases in fibrinogen levels.

Fibrinogen: On day 1 after the sixth injection, higher fibrinogen concentrations were reported in groups 2, 3, and 4. On day 3 after the sixth injection, fibrinogen levels were increased in group 4. Fibrinogen concentration tended to be higher in groups 2 and 3 on day 3 after the sixth injection. The increases in fibrinogen levels were not considered frank toxicity but rather an anticipated effect associated with an immunological response.

Male:
Total white blood cell count (WBC): On day 1 after the sixth injection, higher WBC was reported in group 4. On day 1 after the sixth injection, WBC tended to be higher in groups 2 and 3.

Absolute eosinophil count: On day 1 after the sixth injection, higher absolute eosinophil count was reported in groups 3 and 4.

Absolute neutrophil count: On day 1 after the sixth injection, higher absolute neutrophil count was reported in groups 2 and 4. On day 1 after the sixth injection, absolute neutrophil count tended to be higher in group 2. On day 28 after the sixth injection, lower absolute neutrophil count was reported in group 4.

Absolute monocyte count: On day 1 after the sixth injection, higher absolute monocyte count was reported in group 4. The monocyte count tended to be higher in groups 2 and 3 on day 1 after the sixth injection.

The increases in WBC, eosinophils, neutrophils, and monocytes levels were considered to be part of the inflammatory process.

Females:
Absolute neutrophil count: On day 1 after the sixth injection, higher absolute neutrophil count was reported in groups 3 and 4. The absolute neutrophil count tended to be higher in group 2 on day 1 after the sixth injection.

Clinical Chemistry
Males:
Bilirubin: On day 1 after the sixth injection, higher bilirubin concentration was reported in groups 3 and 4.

Creatinine: On day 3 after the sixth injection, higher creatinine concentration was reported in groups 2, 3, and 4.

Females:
Gamma-glutamyl transferase (GGT): On day 1 after the sixth injection, higher GGT activity was reported in groups 3 and 4.

Bilirubin: On day 1 after the sixth injection, significant increases (3.6X, 3.4X, and 4.7X) in bilirubin levels were reported in groups 2, 3, and 4, respectively.

Total protein (TP): On day 1 after the sixth injection, lower TP concentration was reported in groups 3 and 4. On day 3 after the sixth injection, lower TP concentration was reported in group 4.

Albumin: On days 1 and 3 after the sixth injection, lower albumin concentration was reported in groups 3 and 4.

Albumin/Globulin ratio (A/G ratio): On days 1 and 3 after the sixth injection, lower A/G ratio was reported in groups 3 and 4.

Phosphate (PO4): On day 1 after the sixth injection, higher PO4 concentration was reported in group 4.

Organ weights
Subgroup 1 (sacrificed 3 days after the sixth injection)
Lower relative mean kidneys weight was reported in group 3 males. Higher relative mean left popliteal lymph node weight was reported in group 3 females. Without a dose response effect relationship and because histopathology of the kidneys and the popliteal lymph nodes did not reveal any treatment-related abnormalities, these changes were not considered toxicologically significant.

Subgroup 2 (sacrificed 28 days after the sixth injection)
No test article-related effect was reported in groups 2, 3, and 4.

Macroscopic examination:
Subgroup 1, sacrificed day 21, 3 days post sixth injection
No test article-related effect was reported in groups 2, 3, and 4.

Subgroup 2, sacrificed day 45, 28 days post sixth injection
No test article-related effect was reported in groups 2, 3, and 4.

Microscopy:
Subgroup 1, sacrificed day 21, 3 days post sixth injection
Right treated anterior thigh muscle: Minimal to mild localized mononuclear cell inflammatory response was reported in 7/10 group 1 males, 8/10 group 2 males, 1/10 group 3 males, 8/10 groups 1 and 2 females. A mild to moderate widespread (i.e. extended along the epimysium and diffusely between the muscle fibers) mononuclear cell inflammatory reaction was reported in 9/10 group 3 males, 10/10 group 4 males, 10/10 group 3 females and group 4 females. A minimal to mild multifocal mononuclear cell inflammatory response was reported in 3/10 group 1 males and in 1/10 group 1 females. Mononuclear cell inflammatory reaction consisted of lymphocytes and small macrophages. Minimal hemorrhage or focal muscle fiber degeneration were reported as part of the local response in 1/20 group 1 animals, 3/20 group 2 animals, and 3/20 group 3 animals. Because these reactions also occurred in saline control animals, they were considered not related to the DQ treatment.

Left treated anterior thigh muscle: Minimal to mild localized mononuclear cell inflammatory response was reported in 7/10 group 1 males, 5/10 group 2 males, 7/10 group 1 females, and 10/10 group 2 females. A mild to marked widespread (i.e. extended along the epimysium and diffusely between the muscle fibers) mononuclear cell inflammatory reaction was reported in 10/10 group 3 males, 10/10 DQ group 4 males, 10/10 DQ group 3 females, and 10/10 group 4 females. A minimal multifocal mononuclear cell inflammatory response was reported in 2/10 group 2 males and in 1/10 group 1 females. Mononuclear cell inflammatory reaction consisted of lymphocytes and small macrophages. Minimal focal muscle fiber degeneration was reported as part of the local response in 2/20 group 1 animals, 1/20 group 2 animals, and 4/20 group 3 animals. Because these reactions also occurred in saline control animals, they were considered not to be related to the DQ treatment.

The minimal to mild localized response in the right and left anterior thigh muscle of the DQ animals did in fact not exceed the minimal to mild localized response in the saline. The response was considered to be associated with DQ treatment in the animals showing a wider distribution of the mononuclear cell inflammation (reported in all but one DQ 100 µg/mL and DQ 200 µg/mL animals). The severity of the local response in these animals was slightly higher than that in the group 1 animals.

Sciatic nerve: A minimal to moderate inflammatory reaction in the interstitial tissue surrounding the sciatic nerve was reported in 2/20 group 1 animals, 5/20 group 2 animals, 16/20 group 3 animals, and 15/20 group 4 animals. The inflammatory reactions in the interstitial tissue surrounding the sciatic nerve in several animals were associated with the local reaction in the nearby muscle tissue.

Mesenteric artery: A minimal to mild inflammatory reaction in the interstitial tissue surrounding the mesenteric artery (periarteritis) was reported in 2/20 group 1 animals, 9/20 group 2 animals, 5/20 group 3 animals, and 7/20 group 4 animals. The mesenteric artery was not affected. The periarteritis surrounding the mesenteric artery in several animals could not be explained. Since there was no apparent dose effect relationship and the lesion also occurred in 2/20 saline control animals, the periarteritis was not ascribed to the DQ treatment.

Hemorrhages, degeneration and/or inflammation in the optic nerve were reported in a few animals of all groups. These findings were ascribed to the orbital puncture 3 days prior to necropsy.

Subgroup 2, sacrificed day 45, 28 days post sixth injection

Right treated anterior thigh muscle: Minimal localized or multifocal mononuclear cell inflammatory response was reported in 5/5 group 1 males, 3/5 group 4 males, 5/5 group 1 females and 3/5 group 4 females. Minimal focal muscle fiber degeneration was reported as part of the local response in 4/10 group 1 animals.

Left treated anterior thigh muscle: Minimal localized mononuclear cell inflammatory response was reported in 5/5 group 1 males, 5/5 group 4 males, 4/5 group 1 females and 3/5 group 4 females. Minimal focal muscle fiber degeneration was reported as part of the local response in 4/10 group 1 animals and in 3/10 group 4 animals.

Assessment
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, body temperature, or organ weight were reported. However, test article-related effects at the injection site were reported.

Study # 12: Repeated Dose Toxicity and Local Tolerance Study with QS-21 and with DQ Administered Intramuscularly to Male and Female Rats (Study No. (b) (4) V20041)

Performing laboratory: (b) (4)

Study initiation date: 7 June, 2011.
Final Report date: 18 October, 2011
Test article batch/lot:

Test article	Batch number	Expiration date
QS-21	(b) (4)	
DQ	(b) (4)	
Saline	(b) (4)	

Animal species and strain: (b) (4) rat; (b) (4):CD
Breeder/supplier: (b) (4)
Number of animal per group and sex: 6 males and 6 females
Age: Approximately 12 weeks upon arrival
Body weight range: 275-300 g males, 225-240 g females

Route and site of administration: Intramuscular
Volume of injection: 0.2 mL per animal (two injections of 0.1 mL per site)
Frequency of administration and study duration:

Injection sites subgroup 1: day 0, anterior thigh muscle (R and L)
Injection sites subgroup 2: day 0, posterior thigh muscle(R and L); day 4, calf muscle (R and L); day 8, posterior thigh muscle (R and L); day 12, anterior thigh muscle (R and L).

Study duration was 15 days.
Dose: See study design

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study.

Statistical analysis: Yes
GLP: Yes
Means of administration: Intramuscular
Report status: Final

Methods:

Experimental design:
The experimental design was as follows:

Groups	Number of animals[1]	Animal nos.		Treatment (intramuscular; 0.1 mL per site)	
		Males Even nos.	Females Odd nos.	Left leg	Right leg
1	6 males + 6 females	2-12	1-11	Saline	Saline
2	6 males + 6 females	14-24	13-23	QS21; 20 µg/mL	QS21; 20 µg/mL
3	6 males + 6 females	26-36	25-35	QS21; 100 µg/mL	QS21; 100 µg/mL
4	6 males + 6 females	38-48	37-47	QS21; 200 µg/mL	QS21; 200 µg/mL
5	6 males + 6 females	50-60	49-59	DQ; 20 µg/mL	DQ; 20 µg/mL
6	6 males + 6 females	62-72	61-71	DQ; 100 µg/mL	DQ; 100 µg/mL
7	6 males + 6 females	74-84	73-83	DQ; 200 µg/mL	DQ; 200 µg/mL

[1] Each group consisted of 2 subgroups of 3 males and 3 females each

The animals were dosed intramuscularly in duplicate (right and left hind leg muscle) on a single occasion (subgroup 1) or on four occasions (subgroup 2). On each occasion, two 0.1 mL injections were given with the three test concentrations (20, 100 and 200 µg/mL) of QS-21 and with the three test concentrations (20, 100 and 200 µg/mL) of DQ. The actual dose given to the animals of the three dose groups was 4, 20 and 40 µg of QS-21 or 4, 20 and 40 µg of DQ per animal on each occasion.

The following parameters were evaluated: clinical signs (twice daily), skin reactions at the intramuscular site of injection (approximately at 3, 24 and 48 hours after each injection), body weights (subgroup 1; days -5, 1 and 3; subgroup 2; days -5, 1, 5, 9, 13, and 15), food consumption

(subgroup 1; days -5 - 0, 0 - 3; subgroup 2; days -5 - 0, 0 - 7, 7 - 15), ophthalmoscopy (Not performed), body temperature (Not measured), hematology and clinical chemistry (by orbital puncture on days 1 and 13 [subgroups 1 and 2, respectively] and by abdominal aorta on days 3 and 15 [subgroups 1 and 2, respectively]), pathology (subgroup 1 on day 3; subgroup 2 on day 15). Tissues collected:

Tissue/organ sampled	Weighed	Examined
Adrenals	X	X
Bone and bone marrow (sternum, femur and joints)		X
Brain (3 levels, including hypothalamus) and meninges	X	X
Inguinal lymph nodes		X
Popliteal lymph nodes	X	X
Heart	X	X
Iliac lymph nodes		X
Kidneys	X	X
Liver	X	X
Lungs including larynx, trachea and bronchi	X	X
Muscle at injection sites		X
Muscle (skeletal) = triceps		X
Spleen	X	X
Thymus	X	X
Gross lesions		X
Tissue masses or tumours (if found)		X

With the preserved anterior thigh muscles three areas were processed, i.e. central and adjacent left and right areas. These areas were also examined macroscopically for gross findings. The three areas of the injection site were (b) (4), sectioned at 5 µm and (b) (4) All other tissue/organs not submitted to histopathological examination were kept in (b) (4).

Results:
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, or organ weight were reported.

Clinical signs
Erythema reported at the injection site of one 20 µg/mL QS-21 female. Due to a punctured subcutaneous blood vessel upon injection a hematoma at the site of injection was reported in one 200 µg/mL QS-21 male and one 100 µg/mL DQ male.

Clinical Pathology Examinations

Hematology
Red blood cells-Males:

Significantly lower red blood cells were reported in group 3 subgroup (S) 1 on day 1. Hemoglobin levels were significantly lower in groups 2 and 3 of subgroup 1 on day 1. Hemoglobin levels were significantly lower in groups 3 and 4 of subgroup 1 on day 3. Mean corpuscular hemoglobin (MCH) levels were significantly lower in groups 3 and 4 of subgroup 2

on day 3. Thrombocyte levels were significantly higher in group 3 of subgroup 1 on day 1. Significantly lower red blood cells were reported in group 7 subgroup 1 on day 1. Hemoglobin levels were significantly lower in groups 6 and 7 of subgroup 1 on day 1. Packed cell volume (PCV) levels were significantly lower in group 7 of subgroup 1 on day 1. A longer APTT was reported in groups 6 and 7 of subgroup 2 on day 3 after the fourth injection.

Without a clear dose-response relationship and because the individual values were within or very close to the saline control range, the toxicological significance of these findings was considered negligible.

Fibrinogen: Significant, dose-dependent, increase in fibrinogen levels were reported in all QS-21 dose groups (except 20 μg/mL S2; day 3 after the fourth injection) on all sampling dates. A higher, dose-dependent, fibrinogen levels were reported in all DQ groups, but only statistically significant in group 7 of subgroup 1 on day 3 and in groups 6 and 7 of subgroup 2 on day 1 after the fourth injection.

White blood cells-Male:
Absolute neutrophil count: Dose dependent higher neutrophil levels were reported in groups 2, 3, and 4 of subgroup 1 on day 1. This increase was not significant in group 2. Dose dependent higher neutrophil levels were reported in group 4 of subgroup 2 on day 1 after the fourth injection. The neutrophil count tended to be higher in group 3 of S2 on day 1 and in groups 3 and 4 of S2 on day 3 after the fourth injection. Increases (not to significance) in neutrophil levels were reported in groups 5, 6, and 7 of S1 on days 1 and 3.

Absolute monocyte count: Significantly higher monocyte levels were reported in group 3 of S2 on day 1. A higher monocyte count was reported in group 7 of S1 on day 1 after injection.

The increases in neutrophils and monocytes levels were considered to be part of the inflammatory process.

White blood cells-Females:
Absolute white blood cells (WBC) count: Dose dependent higher WBC levels were reported in groups 3 and 4 of S1 on day 1 and in group 4 of S2 on day 3 after the fourth injection. Dose dependent higher WBC levels were reported in group 7 of S1 on day 1.

Absolute eosinophil count: A higher absolute eosinophil count was reported in group 4 of S2 on days 1 and 3 after the fourth injection. Dose dependent higher eosinophil levels were reported in groups 6 and 7 of S2 on day 1 after the fourth injection.

Absolute neutrophil count: Dose dependent higher neutrophil levels were reported in groups 3 and 4 of S1 and S2 on day 1 and in group 4 of S2 on day 3 after the fourth injection. Dose dependent higher neutrophil levels were reported in groups 6 and 7 of S1 on day 1. Dose dependent higher neutrophil levels were reported in groups 5 and 7 of S2 on day 1 after the fourth injection.

Absolute monocyte count: A higher absolute eosinophil count was reported in group 4 of S2 on day 1 after the fourth injection.

The relative counts (expressed as a percentage of the total WBC count) also showed several statistically significant differences. Both absolute and relative counts indicate a dose-dependent WBC response in QS-21 males and females. The response consisted predominantly of an increase in neutrophils mainly on day 1 after the single injection (S1) and the fourth injection (S2), and in the S2 high-dose QS-21 females was accompanied by an increase in lymphocytes, eosinophils and monocytes.

In the DQ males and females the WBC response was distinctly less pronounced and mainly consisted of an increase in neutrophil and eosinophil counts in the S1 high-dose DQ females. In the DQ males only a tendency towards an increase of neutrophils was observed.

Clinical Chemistry
Males:
Aspartate aminotransferase (ASAT): An increase in ASAT levels was reported in group 2 of S1on day 1. An increase in ASAT levels was reported in groups 2 and 4 of S2 on day 1 after the fourth injection.

Gamma-glutamyl transferase (GGT): An increase in GGT levels was reported in group 3 of S1on day 3. An increase in ASAT levels was reported in groups 2 and 4 of S2 on day 1 after the fourth injection.

Total protein (TP): Dose dependent increase in TP levels was reported in groups 3 and 4 of S1on day 1. Dose dependent increase in TP levels was reported in groups 2, 3, and 4 of S2 on days 1 and 3 after the fourth injection. This increase was significant only in group 4 on day 3 after the fourth injection.

Albumin/Globulin ratio (A/G ratio). A lower dose-dependent A/G ratio was reported in groups 3 and 4 of S1 on day 1 after injection, and in groups 2, 3, and 4 of S2 dose groups on days 1 and 3 after the fourth injection. A lower dose-dependent A/G ratio was reported in groups 6 and 7 of S1 on day 1 after the fourth injection. A decrease in A/G ratio was reported in group 7 of S1 dose groups and in groups 5, 6, and 7 of S2.

Bilirubin: On day 1 after the injection, higher bilirubin concentration was reported in group 4 of S1. On day 1 after the injection, higher bilirubin concentration was reported in groups 5, 6, and 7 of S1.

Urea: On day 1 after the injection, lower urea concentration was reported in group 4 of S1.

Inorganic phosphate (PO4). A higher PO4 concentration was reported in groups 5, 6, and 7 of S1 dose groups on day 1 after injection.

Females:
Aspartate aminotransferase (ALAT): An increase in ALAT levels was reported in groups 2 and 3 of S1on day 1.

Aspartate aminotransferase (ASAT): An increase in ASAT levels was reported in groups 2, 3, and 4 of S1 and S2 on day 1.

Lactate dehydrogenase (LDH): A decrease in LDH levels was reported in group 7 of S1on day 1.

Albumin: A decrease in albumin levels was reported in groups 2 and 4 of S1on day 1. A decrease in albumin levels was reported in groups 2, 3, and 4 of S1 on day 3. A decrease in albumin levels was reported in groups 2, 3, and 4 of S2 on days 1 and 3 after the fourth injection. Albumin concentration tended to be lower in groups 6 and 7 of S1 and S2 on all sampling dates.

Albumin/Globulin ratio (A/G ratio). A lower dose-dependent A/G ratio was reported in groups 2, 3, and 4 of S1 on day 1 after injection and in groups 3 and 4 of S2 dose groups on day 1 after the fourth injection. The A/G ratio tended to be lower in groups 2, 3, and 4 of S1 dose groups on day 3 after injection, and in groups 2, 3, and 4 of S2 dose groups on day 3 after the fourth injection. The A/G ratio tended to be dose-dependently lower in groups 5, 6, and 7 of S1 and S2 dose groups on all sampling dates. This decrease was statistically significant in group 7 of S1 and S2 dose groups, respectively on days 1 and 3 after injection and on day 3 after the fourth injection.

Bilirubin: On day 1 after the injection, higher bilirubin concentration was reported in group 3 of S1. On day 1 after the fourth injection, higher bilirubin concentration was reported in group 2 of S2.

Urea: A higher urea concentration was reported in group 6 of S2 dose groups on day 3 after the fourth injection.

Calcium (Ca) and Sodium (Na): A lower Ca and Na concentrations were reported in group 6 of S1 dose groups on day 1 after injection.

Chloride (Cl): A higher Cl concentration was reported in group 6 of S1 on day 1 after injection. A higher Cl concentration was reported in groups 6 and 7 of S2 on day 1 after the fourth injection.

Organ weights
Subgroup 1 (sacrificed 3 days after single injection)
Males (QS-21 and DQ groups):
Absolute mean heart weight was increased in group 3 males. This increase was not toxicologically significant because the histopathology of the heart did not reveal any treatment-related abnormalities and no dose response relationship was present. No statistically significant changes were observed in groups 5, 6, and 7 males

QS-21 females (groups 2, 3, and 4):
Absolute and relative mean spleen weights were increased in groups 2 and 4 females. This increase was not toxicologically significant because the histopathology of the spleen did not reveal any treatment-related abnormalities.

DQ females (groups 5, 6, and 7):

Absolute mean spleen weight was increased in group 6 females. This increase was not toxicologically significant because the histopathology of the spleen did not reveal any treatment-related abnormalities.

Subgroup 2 (sacrificed 3 days after the fourth injection)
QS-21 males:
Relative mean liver weight was increased in group 2 males. This increase was not toxicologically significant because the histopathology of the liver did not reveal any treatment-related abnormalities and no dose response relationship was present.

Compared to the saline control group, no statistically significant changes were observed in the QS-21 (groups 2, 3, and 4) females, the DQ (groups 5, 6, and 7) males and the DQ (groups 5, 6, and 7) females.

Macroscopic examination
Subgroup 1, sacrificed on day 3, 3 days post single injection:
Brown/discolored area at the injection site was reported in most QS-21 males and females. This gross finding correlated with the microscopical findings. This finding was also reported in a single group 7 male, few DQ (groups 5, 6, and 7) females, and in most saline control females. No microscopical finding was correlated to this finding.

The gross changes observed in the other organs were unremarkable.

Subgroup 2, sacrificed on day 15, 3 days post fourth injection:
Brown/discolored area at the injection site was reported in most QS-21 males and females. This gross finding correlated with the microscopical findings. This finding was also reported in few DQ females and in a single saline control male. No microscopical finding was correlated to this finding. Discoloration of the popliteal space was reported in one group 7 male and in one group 5 female. This finding was correlated with microscopical findings. The gross changes observed in the other organs were unremarkable.

Microscopy
Subgroup 1, sacrificed on day 3, 3 days post single injection:
Left and right treated anterior thigh muscle (3 days post injection).

QS-21 (groups 2, 3, and 4):
Segmental muscular necrosis at the injection sites was reported in all QS-21 animals. The majority of cases were graded moderate; in a few animals the process was graded as slight or severe. The necrotic area in all animals was surrounded by a mononuclear cell infiltrate consisting of lymphocytes and small macrophages. The inflammatory process was called localized when it was confined to the necrotic area. The process will be called widespread if there was also inflammatory cell infiltration in other parts of the muscle. In most cases, the necrotic area of the muscle contained minimal hemorrhage(s) but virtually no inflammatory cells. Small group of polymorphonuclear cells in the center of the necrotic area (in addition to the mononuclear infiltrate surrounding the necrotic area) was reported in few animals. These cases were called mixed inflammatory cell infiltrates. The inflammatory reaction was relatively

mild and it was graded slight in most cases. The inflammatory reaction was present at the endomysium, perimysium, epimysium as well as extramuscular in most animals. A dose-effect relationship was not established.

DQ:
Muscular necrosis and hemorrhage(s) at the injection sites of the DQ (groups 5, 6, and 7) animals was not reported. The histopathological changes were characterized by mononuclear cell infiltrates extending in between and along the muscle fibers and groups of muscle fibers. In most cases the infiltrates were graded slight. Dose-effect relationship was reported and shown by a shift in severity from very slight to slight as well as by a shift in distribution from localized to widespread.

The other histopathological changes reported were unremarkable.

Subgroup 2, sacrificed on day 15, 3 days post fourth injection:
Left and right treated anterior thigh muscle (3 days post fourth injection).

QS-21 (groups 2, 3, and 4):
The histopathological changes reported at the injection sites of the S2 QS-21 animals were comparable to those reported in the S1 QS-21 animals.

DQ (groups 5, 6, and 7):
Few necrotic muscle fibers were reported in one group 6 male. No muscular necrosis and hemorrhage(s) was reported in any of the other DQ animals. The histopathological changes reported at the injection sites of the S2 DQ animals were comparable to those reported in the S1 DQ animals. Treatment related discoloration of the popliteal space, caused by a focal inflammatory infiltrate, was reported in two DQ animals.

The other histopathological changes reported were unremarkable.

Assessment
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, or organ weight were reported.

Groups 2, 3, and 4 (S1 and S2):
Dose-dependent increase in fibrinogen on days 1 and 3 after the injection, and a dose-dependent WBC response, (mainly consisting of an increase in neutrophils predominantly on day 1 after injection) was reported. The increase in fibrinogen levels and the WBC response was considered to be part of the inflammatory process following injection. Dose-dependent increase in TP concentration on day 1 after injection and a decreased A/G ratio predominantly on day 1 after injection was reported in groups 2, 3, and 4 males. Increase in ASAT activity on day 1 after injection, a lower albumin concentration and a dose-dependent decreased A/G ratio predominantly on day 1 after injection were reported in groups 2, 3, and 4 females. The increased ASAT was consecutive to the muscular necrosis, since it is present in high concentrations in skeletal muscle and its level in blood can be elevated after muscle injury.

Microscopic examination of the injection sites revealed histopathological changes characterized by a distinct area of segmental muscular necrosis with minimal hemorrhages, surrounded by a relatively mild mononuclear inflammatory cell infiltrate. There was no clear dose-effect relationship.

Groups 5, 6, and 7 (S1 and S2):
Dose-dependent increase in fibrinogen and WBC response, predominantly on day 1 after injection, was reported in groups 5, 6, and 7 males and females. The WBC response was considered to be part of the inflammatory process following injection. The increased fibrinogen was also considered related to the inflammatory process, since it is a marker for the acute phase of tissue damage and inflammation linked to the local reaction.
Dose-dependent decrease in A/G ratio (on days 1 and 3 after injection) and decreased albumin concentration was reported in groups 5, 6, and 7 females.

Microscopic examination of the injection sites revealed histopathological changes characterized by a mild, mononuclear inflammatory cell infiltrate extending in between the muscle tissue. There was a clear dose-effect relationship shown by a shift in severity from very slight to slight as well as by a shift in distribution from localized to widespread.

In conclusion, QS-21 was distinctly more reactive than DQ when considering the response on fibrinogen, neutrophils, A/G ratio and especially ASAT. The same degree of local reactogenicity was shown after single or multiple injections of test materials.

Repeat-Dose Studies
Repeated-Dose Toxicity Study with DQ administered Intramuscularly to Male and Female Rats
In this study, 15 animals/sex/group will receive either saline, 20 µg/mL DQ, 100 µg/mL DQ or 200 µg/mL DQ intramuscularly in the left and right legs at a volume 0.1 mL. Each group will be divided into a subgroup of 10 males and 10 females. The saline control group and the high-dose DQ group will have a second subgroup of 5 males and 5 females. The animals of S1 will be sacrificed on study day 21 (3 days post-6th injection) and those of S2 on study day 46 (28 days post-6th injection). Animals will be injected on day 0, posterior thigh muscle; day 4, calf muscle; day 7, posterior thigh muscle; day 11, calf muscle; day 14, posterior thigh muscle; and day 18, anterior thigh muscle. Cage-side observations will be performed twice daily and a full physical examination will be carried out in case of abnormal clinical signs. Injection sites will be observed approximately 3, 24 and 48 hours after injection and skin effects, if occurring, will be graded according to the method of Draize (erythema, eschar formation and edema formation). Ophthalmoscopy observations will be conducted on 10 animals/sex/group pre-dose and on days 19 and 42 of the study. All visible structures of the eyes (cornea, eye chambers, iris, lens, vitreous body and the fundus) will be examined. Body temperature will be recorded prior to and circa 4 and 24 hours after the first and the sixth injection (subgroup 1 only) via subcutaneous temperature

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY STUDIES:

Study #1: Zoster Candidate Vaccine (gE/AS01B): Study of Effects on Embryo-Fetal, Pre- and Post-natal Development in CD Rats by Intramuscular Administration (Including Pre-Mating Immunization Phase)

Reviewer: Dr. Claudia Wrzesinski

Summary:
Female CD rats with were treated with either saline or the candidate vaccine gE/AS01B or adjuvant AS01B at 40% of the full human dose per occasion, on 28 and 14 days before pairing and then on Days 3, 8, 11 and 15 of gestation and on Day 7 of lactation. The vaccine was well tolerated by the F0 females with effects restricted to slight, transient swelling at the injection site and did not adversely affect embryo-fetal or pre- and post-natal survival, growth or development of the offspring up to Day 25 of age.

Study no.: (b) (4) 0005
Conducting laboratory and location: GlaxoSmithKline Biologicals S A
Date of study initiation: 18 May 2010
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
gE: batch number: (b) (4) (Monodose vial containing 62.5 µg of freeze-dried gE antigen per cake)
AS01B: batch number: (b) (4) (700 µl monodose vial containing 50 µg QS-21 and 50 µg MPL in a liposome-based formulation per 500 µl)

Doses:
AS01B: 200µl (2 x 100 µl) was injected per rat, equivalent to two fifths of a human dose.
gE/AS01B: 200 µl (2 x 100 µl) was injected per rat, equivalent to two fifths of a human dose.

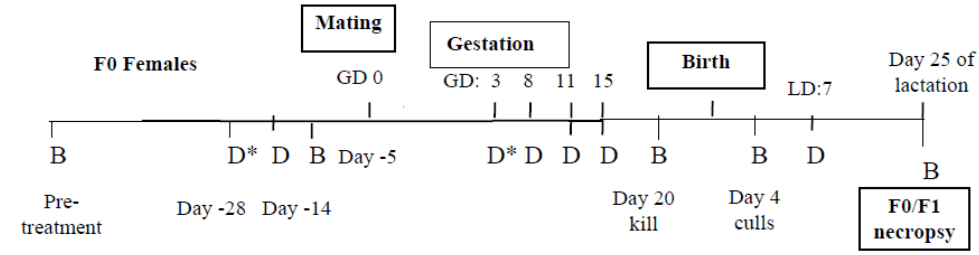
Group	Treatment	Dose (volume/occasion)	Treatment day	Number of females ‡	Animal numbers
1	Saline	200 µl	Day -28 and -14 before pairing, then Days 3, 8, 11, 15 after mating and Day 7 of lactation	44	1-44
2	AS01B	200 µl	Day -28 and -14 before pairing, then Days 3, 8, 11, 15 after mating and Day 7 of lactation	44	45-88
3	gE/AS01B	200 µl	Day -28 and -14 before pairing, then Days 3, 8, 11, 15 after mating and Day 7 of lactation	44	89-132

‡ 44 animals per group were allocated and treated, in order to obtain 40 females with a positive indication of mating. 40 females per group were treated during gestation and 20 females per group were treated during lactation.

***Table 48**: Dosing in study (b) (4) 0005, table provided by the sponsor.*

Species/strain: (b) (4) :CD (b) (4) rats
Number/sex/group: 40 females per dosage group
Route, formulation, volume, and infusion rate: intramuscular

Study design: Three groups of 44 female rats were allocated initially to study and treated on Days -28 and -14 (before pairing). The females were paired with stock males of the same strain. Forty females in each group with a positive indication of mating were treated on Days 3, 8, 11 and 15 after mating and the excess animals were killed on Day 6 or 7 after mating. For each group, 20 animals were killed on Day 20 after mating (embryo-fetal phase) and the remaining 20 animals were allowed to litter and rear their young to Day 25 of age (postnatal phase). Females in the postnatal phase were then treated on Day 7 of lactation. The F1 offspring received no direct administration of the test substances: any exposure was in utero or via the milk.



GD Gestation day
LD Lactation day
D Day of dosing
B Blood sampling
* Although the majority of females mated within 4 days of initial pairing, some matings fell outside this timeframe. There was therefore a natural inter-animal variation between the first injection and third injection.

Figure 2: *Study design study* (b) (4) *0005, figure provided by the sponsor.*

Parameters and endpoints evaluated:

Clinical observations: Animals were inspected visually at least twice daily for evidence of ill-health or reaction to treatment.
At each treatment, detailed observations were recorded at the following times in relation to dose administration:

Immediately before dosing including injection sites
Immediately after dosing on return of the animal to its cage
On completion of dosing of each group
Between one and two hours after completion of dosing of all groups
As late as possible in the working day

Injection sites were examined on each day of dosing, until 4 days after the injection and weekly thereafter and at termination.
In addition, a more detailed physical examination was performed on each F0 animal weekly before pairing, on Days 0, 6, 13 and 20 after mating and Days 1, 7, 14, 21 and 25 of lactation to monitor general health.

Body weight: The weight of each F0 female was recorded on the day of treatment and weekly until mating was detected, on Days 0, 3, 6, 8, 11, 15, 17 and 20 after mating, daily until parturition, and on Days 1, 4, 7, 11, 14, 18, 21 and 25 of lactation.

Food consumption: For each F0 female was recorded weekly before pairing, for the periods Days 0-2, 3-5, 6-7, 8-10, 11-14, 15-16 and 17-19 after mating and on Days 1-3, 4-6, 7-10, 11-13,

14-17, 18-20 and 21-24 of lactation. From these records the mean weekly consumption (g/animal/week) before pairing and daily consumption (g/animal/day) after mating and during lactation was calculated for each animal.

Parturition observations and gestation length: From Day 20 after mating, females were inspected three times daily for evidence of parturition. The progress and completion of parturition was monitored, numbers of live and dead offspring were recorded and any difficulties observed were noted. The duration of gestation was calculated as the time elapsing between the detection of mating and commencement of parturition.

Records made during littering phase: All litters were examined at approximately 24 hours after birth (Day 1 of age) and then daily thereafter. The records maintained were as follows:

- **Clinical signs:** Daily records were maintained for evidence of ill health or reaction to treatment; these were on an individual offspring basis or for the litter as a whole, as appropriate.
- **Litter size:** Daily records were maintained of mortality and consequent changes in litter size from Days 1-25 of age. On Day 4 of age, litters containing more than ten offspring were reduced to ten by random culling, leaving, whenever possible, five male and five female offspring in each litter.
- **Sex ratio:** The sex ratio of each litter was recorded on Days 1, 4 (before and after culling) and on Day 25 of age.
- **Bodyweight:** Individual offspring bodyweights were recorded on Days 1, 4 (before culling), 7, 11, 14, 18, 21 and 25 of age.

Pre-weaning examination:
The following pre-weaning reflex developmental tests were performed on each offspring:

- **Surface righting:** assessed daily from Day 2 of age until achieved.
- **Air righting:** assessed daily from Day 16 of age until achieved but not beyond Day 21 of age.
- **Auditory function**: the startle response to a sudden sharp sound was assessed on Day 20 of age.
- **Visual function**: the pupil closure response of dark adapted eyes to a bright point source of light was assessed on Day 20 of age.

Biosampling (antibody assay):

- Blood samples were obtained from the F0 females pretreatment, on Day -5 before pairing, Day 20 after mating (embryo-fetal phase) and Day 25 of lactation (postnatal phase).
- Umbilical cord blood samples were obtained from the fetuses in 10 litters per group on Day 20 of gestation.
- Blood samples were obtained from all offspring killed on Day 4 of age and up to 3 male and 3 female offspring per litter on Day 25 of age.

Necropsy and fetal processing:

- F0 animals allocated to the embryo-fetal and postnatal phases of the study were subject to a detailed necropsy.

- **Animals allocated to the embryo fetal phase**: 20 per group were killed on Day 20 after mating.
  For each animal, the number of corpora lutea in each ovary and the number of implantation sites, the number and distribution of resorption sites (classified as early or late) and live and dead fetuses were recorded for each uterine horn.
  For apparently non-pregnant animals, and for apparently empty uterine horns, the number of uterine implantation sites was checked after (b) (4)
  Fetal examination and processing: All fetuses and placentae were dissected from the uterus and weighed individually. Fetuses were individually identified within the litter, using a coding system based on their position in the uterus. Each fetus and placenta was externally examined and any abnormalities were recorded.
  Free-hand serial sections were prepared from the Bouin's fixed fetuses and were examined under the microscope for visceral abnormalities.
- **Animals allocated to the postnatal phase:** 20 were allowed to litter and rear their young to Day 25 of age. The F0 females and remaining F1 offspring were killed on Day 25 of lactation.
- **F0 females:** For F0 females, the numbers of implantation sites in each uterine horn was counted. For females failing to produce a viable litter, the number of uterine implantation sites was checked after (b) (4) .
- **F1 offspring:** Offspring culled on Day 4 of age which were externally abnormal were examined at necropsy. The offspring considered to be externally normal were discarded without examination

Reproductive assessment:
Individual values are presented for the numbers of corpora lutea, implantations, resorptions (early, late and total), live fetuses (male, female, total) and sex ratio (percentage male) for litters at Day 20 of gestation.
Pre-natal losses are separated into pre- and post-implantation phases. Pre-implantation loss includes losses due to non-fertilisation of ova, in addition to post-fertilisation losses before implantation. It was calculated from the formula:

Pre-implantation loss (%) = (Number of corpora lutea – Number of implantations)/Number of corpora lutea x 100

Where the number of implantations exceeded the number of corpora lutea observed, pre-implantation loss was assumed to be zero (i.e. no pre-implantation loss was considered to have occurred).

Post-implantation loss was considered to exclude the first two to three days post-implantation as deaths occurring at this stage are considered to leave no remains visible at Day 20 of gestation. It was calculated from the formula:

Post-implantation loss (%) = (Number of implantations – Number of live fetuses)/ Number of implantations x 100

All group values and SD (as appropriate) were calculated from the individual litter values

Fetal, litter and placental weights: Mean fetal weights were calculated for each litter. Values were presented for male, female and overall fetal weight. Litter weight was calculated as the sum of all fetal weights. Mean placental weight was also calculated for each litter.

Detailed fetal examination:
Findings from external, visceral and skeletal examination of fetuses are presented on an individual basis for affected litters and fetuses, linking the results of initial external examinations with subsequent visceral and/or skeletal examinations and fetal weight. Group incidences of observations on fetuses and litters are summarized in terms of major or minor abnormalities or as skeletal variants. The incidence of structural changes is presented as numeric fetal and litter incidences.

Findings observed were classified, according to severity and incidence, as:

Major abnormalities: normally rare, definitely detrimental to normal subsequent development, possibly lethal, e.g. ventricular septal defect

Minor abnormalities: minor differences from normal that are detected relatively frequently considered to have little detrimental effect and may be a transient stage in development e.g. bipartite centrum, dilated ureter.

Variants: alternative structures or stages of development occurring regularly in the control population, e.g. number of ribs, incomplete ossification of 5th and 6th sternebrae.

Pre-coital interval: Individual intervals were tabulated for females only, for the time elapsing between initial pairing and mating. Percentage of females with pre-coital intervals were calculated for durations of 1-4, 5-8, 9-12 or 13-14 days of pairing.

Gestation length:
Gestation length was calculated as the number of gestation days up to and including the day on which offspring were first observed, with Day 1 = day of mating for calculation purposes.

Gestation index:
Gestation index (%) = Number of live litters born/Number pregnant x 100

Mating performance and fertility:

Individual data was tabulated. Group values were calculated for males and females separately for the following:

Percentage mating : Number animals mating/ Animals paired x100
Conception rate (%) : Number animals achieving pregnancy/Animals mated x100
Fertility index (%) : Number animals achieving pregnancy /Animals paired x100

Litter size: Individual litter values were tabulated for the number of implantation sites, total at Day 1 after littering (live and dead) and live at Days 1, 4 (before and after culling), 7, 11, 14, 18, 21 and 25 of age. Group mean litter size and SD were calculated from the individual litter values.

Survival indices:

The following were calculated for each litter:

Post-implantation survival index (%) = Total number of offspring born/Total number of uterine implantation sites x 100

Post-implantation survival index was expressed as 100% where the number of offspring exceeded the number of implantation sites recorded.

Live birth index (%) = Number of live offspring on Day 1 after littering/ Total number of offspring born x 100
Viability index (%) = Number of live offspring on Day 4 before culling/Number live offspring on Day 1 after littering x 100
Lactation index (%) = Number of live offspring on day of examination/Number live offspring on Day 4 (after culling) x 100

Sex ratio:
The percentage of male offspring in each litter was calculated at Day 1 after littering (live and dead), and for live offspring on Days 1, 4 (before and after culling) and 25 of age.

Percentage males = Number of males in litter/Total number of offspring in litter x 100

Pre-weaning examinations: Surface and righting reflexes were presented as the age that the reflexes were observed. Auditory and visual functions were presented as percentage passing the test. Group mean values were calculated from the individual values presented.

Macroscopic findings:
The individual findings for F0 animals have been presented in an Appendix (only females/litters with findings are presented).
Findings from examination of offspring are presented in an Appendix on an individual basis for affected litters and offspring before and at scheduled termination.

Statistical methods
Statistical analyses were performed on the majority of data presented and results of these tests, whether significant or non-significant, are presented in the relevant tables. For some parameters, including mating performance, the similarity of the data was such that analyses were not considered to be necessary.

All statistical analyses were carried out separately for males and females. Data relating to food consumption was analysed on a cage basis. For all other adult parameters, the analyses were carried out using the individual animal as the basic experimental unit. For litter findings the litter was taken as the treated unit and the basis for statistical analysis.

The following data types were analyzed at each time point separately:
Bodyweight, using absolute weights and gains over appropriate study periods
Food consumption, using means over appropriate study periods
Litter size and survival indices
Fetal, placental and litter weight
Pre-weaning examinations
Mating performance and fertility

The following sequence of statistical tests was used for bodyweight, food consumption, litter size and survival indices, fetal, placental and litter weight and pre-weaning examinations data:
A parametric analysis was performed. If Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level. Groups were compared using t-tests. A non-parametric analysis was performed . If Bartlett's test was still significant at the 1% level following both logarithmic and square-root transformations. Groups were compared using Wilcoxon rank sum test. For survival indices and live fetuses, if 75% of the data (across all groups) were the same value, Fisher's Exact tests were performed. Treatment groups were compared using pairwise comparisons of each dose group against the control both for i) values <c versus values ≥c, and for ii) values ≤c versus values >c, as applicable.
Pre- and post-implantation losses were analyzed by generalized mixed linear model with binomial errors, a logit link function and litter as a random effect. Each treated group was compared to control using a Wald chi-square test. For resorptions, each treated group was compared to control by exact Wilcoxon rank sum test.
Sex ratio were analyzed by generalized mixed linear model with binomial errors, a logit link function and litter as a random effect. Each treated group was compared to control using a Wald chi-square test. The numerator was Number of males, the denominator was Number of live fetuses. Significant differences between Control and treated groups were expressed at the 5% (p<0.05) or 1% (p<0.01) level.

Results:

F0 Generation

Mortality/Clinical signs:

Parameter		**F0 generation**		
		Control	**AS01B**	**gE/AS01B**
Injection site observation (summary of all events)	Swelling-both	0	13	34
	Swelling-left	0	16	25
	Swelling right	0	11	28
	Right hind limb elevated	0	3	0
Appearance		unaffected	unaffected	unaffected
Abnormal Stool		ND	ND	ND
Mortality (fetuses dying before scheduled termination		4	0	4
Neurotoxicity		ND	ND	ND

Table 49: *Mortality and clinical signs (study (b) (4) 00005): ND: not determined*

During the pre-pairing phase, after administration on Day -28 and Day -14, there were no signs that could be related to dosing. During gestation animals were dosed on 4 occasions (Days 3, 8, 11 and 15 after mating), signs observed in association with dosing were limited to transient swelling at the injection sites for animals receiving the adjuvant and the whole vaccine and the incidence of signs was slightly higher for animals receiving gE/AS01B. Two animals receiving AS01B had limited use of the right hindlimb on Days 5 and/or 6 of gestation after dose administration on Day 3 but this sign did not persist. Following the final dose on Day 7 of lactation there was a very low incidence of swelling at the injection site on Day 8 of lactation for animals that received AS01B or gE/AS01B.

Body weight:

Observations during pre-pairing period (female): Overall there was no effect on bodyweight gain during the 4 week pre-pairing period.

Observations during gestation (female): Bodyweight change during gestation showed no clear effect of treatment with either AS01B or gE/AS01B. Overall (Days 0-20 of gestation) the mean bodyweight gain for females receiving AS01B was slightly but significantly lower when compared with the controls and bodyweight gain for females receiving gE/AS01B was slightly but significantly higher when compared with females receiving AS01B.

Observations during lactation (female): Bodyweight gain during lactation was unaffected by treatment with either AS01B or gE/AS01B.

Food consumption: Food consumption measurement during the four week pre-pairing phase, during gestation and during lactation showed no adverse effect of treatment with either AS01B or gE/AS01B.

There were incidences of statistical differences relative to the control group and the adjuvant group, however the differences were slight. During the pre-pairing phase and the gestation phase animals receiving AS01B tended to show slightly lower food consumption compared to the controls; however the group receiving gE/AS01B had mean food consumption that was similar to the controls and slightly higher when compared with females receiving AS01B. These differences were not considered to be of toxicological significance

During lactation food consumption was essentially similar amongst the groups until late lactation (Days 21-25) when both treated groups had food consumption that was significantly higher when compared with the controls. At this stage of lactation the majority of the consumption can be attributed to the offspring and as such this is unlikely to relate to administration of either the adjuvant or vaccine.

Necropsy:

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Reproductive parameters examined (in F_0 animals), natural birth group:

Mating performance and fertility: Mating performance and fertility was unaffected by treatment with either AS01B or gE/AS01B.

Parameter		F0 generation		
		Control	AS01B	gE/AS01B
Female Fertility Index (both groups)		98%	100%	98%
Gestation Index (natural birth group)		100%	100%	100%
Gestation Length	22 days	55%	53%	55%
(natural birth group)	22.5 days	25%	20%	26%
	23 days	20%	25%	21%
Post Implantation Survival Index		93.7%	89.0%	95.3%
Live Birth Index (natural birth group)		99.0%	98.8%	98.7%
Viability Index		100%	100%	99.5%
Lactation Index	day 7	100%	100%	99.5%
	day 14	99.5%	100%	99.5%
	day 21	99.5%	100%	99.5%
Number (Total and Per Liter) of Stillbirths at Day 0		ND	ND	ND
Natural birth: Number (Total and Per Liter) of Live Births at Day 0		(294/14.7)	(253/12.7**)	(275/14.5)
Implantation loss	pre	4.7%	5.8%	3.4%
	post	4.6%	5.0%	3.1%
Sex ration (5M)		44.0%	50.8%	53.9%*
Number of Corpora Lutea		15.8%	16.1%	15.7%
Number of Implantation Sites		15.7	14.2**	15.2
Number of Resorptions	Early	0.6	0.8	0.4
	Late	0.1	0	0.1
	Total	0.7	0.8	0.5

Table 50: *Reproductive parameters (study (b) (4) 0005), natural birth group, ND: Not Determined;*p<0.05; **p<0.01*

The gestation length was within the normal range of 22 to 23½ days and there was no evidence for a treatment-related shift in the distribution of gestation lengths. The gestation index was unaffected by treatment, with all pregnant females producing live litters.

Results F1 generation (natural birth group)

Reproductive parameters:

GENERATION		F_1 LITTER		
		Control	AS01B	gE/AS01B
LITTER SIZE				
Number Born Day 1– Total[1] Per Litter	N MEAN (S.D.)	14.7 (1.5)	12.7** (1.9)	14.5 (1.5)
Day 1 – Total Per Litter	N MEAN (S.D.)	14.6 (1.6)	12.5** (1.9)	14.3 (1.7)
Day 4 (before culling) – Total Per Litter	N MEAN (S.D.)	14.6 (1.5)	12.5** (1.9)	14.3 (1.7)
Day 7 – Total[2] Per Litter	N MEAN	10.0 (0.0)	9.9 (0.4)	9.9 (10.0)

GENERATION		F_1 LITTER		
		Control	AS01B	gE/AS01B
	(S.D.)			
Day 14 – Total[2] Per Litter	N MEAN (S.D.)	10.0 (0.4)	9.9 (0.2)	9.9 (0.2)
Day 21 – Total[2] Per Litter	N MEAN (S.D.)	10.0 (0.2)	9.9 (0.4)	9.90 (0.2
LITTER WEIGHT IN G (♂/♀)				
Day 1	N MEAN [S.D.]	(6.7/6.4) [0.5/0.6]	(7.1/6.7) [0.7/0.7]	(6.9/6.6) [0.5/0.5]
Day 4 (before cull)	N MEAN [S.D.]	(9.6/9.0) [0.8/0.8]	(10.3*/9.9) [1.1/1.1]	(9.8/9.4) [1.0/0.9]
Day 7	N MEAN [S.D.]	(15.9/15.0) [1.2/1.2]	(16.5/15.8) [1.4/1.5]	(16.1/15.3) [1.2/1.8]
Day 14	N MEAN [S.D.]	(32.1/30.7) [2.0/2.0]	(32.5/31.4) [2.0/2.2]	(32.1/30.7) [1.5/2.0]
Day 21	N MEAN [S.D.]	(50.6/48.3) [3.4/3.5]	(52.7/50.3) [4.5/4.4]	(50.9/48.6) [3.8/4.1]
VIABILITY INDICES				
Day 1-4		99.7%	100%	100%
Day 4-25		99.5%	100%	99.5%
WEANING INDEX	N MEAN S.D.	ND	ND	ND
SEX RATIO (M%)	Day 1	44.0%	50.8%	53.9%*

Table 51: *Fertility parameter (study (b) (4) 0005): 1) Includes offspring that died prior to the designated Day 1 of age, 2) Culling: On Day 4 of age, litters containing more than ten offspring were reduced to ten by random culling, leaving, whenever possible, five male and five female offspring in each litter; ND: Not Determined, *p<0.05; **p<0.01*

The number of implantations, and litter size was slightly but significantly lower for the group receiving adjuvant AS01B alone compared to the control group. However, animals that received the complete vaccine gE/AS01B were similar to the control group. Further, the study report stated that this difference in implantation counts in the adjuvant alone group was not observed in females allocated to the embryo-fetal phase. When the number of implantations for both phases are combined there is no significant difference of implantation sites between the treatment groups (mean for control group: 15.4, for AS01B group: 14.6; for gE/AS01B group: 15.1).

Number of implantations	Number of animals			Percentage of animals		
	Group 1 Control	Group 2 AS01B	Group 3 gE/AS01B	Group 1 Control	Group 2 AS01B	Group 3 gE/AS01B
12	0	4	1	0	10	3
13	2	8	3	5	20	8
14	7	7	10	18	18	26
15	12	9	11	31	23	28
16	11	5	7	28	13	18
17	5	5	4	13	13	10
18	1	2	2	3	5	5
19	1	0	1	3	0	3

Table 52: *Overall number of implantation sites for both combined embryo-fetal and post-natal phase, table provided by sponsor*

The sex ratio (% male pups) in the gE/AS01B group was significantly higher than the saline control group, but neither sex ratio was grossly different from the expected 50% male and the apparent effect was partially due to a low control value and was considered to be of no toxicological importance.

Offspring bodyweight on Day 1 of age and subsequent bodyweight gain up to Day 25 of age showed no adverse effects of maternal treatment with either AS01B or gE/AS01B.

Fetal alterations: F_1 generation

			Control	**AS01B**	**gE/AS01B**
Litters evaluated		N	20	20	20
Fetuses evaluated		N	139	144	146
Minor skeletal abnormalities					
Cranial: structural bone/additional suture	Litter incidence	N	1	1	1
	Fetal incidence	N	1	1	1
Cranial: interparietal fissure	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Cranial: unossified areas	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Vertebral abnormality (thoracic)	Litter incidence	N	0	0	1
	Fetal incidence	N	0	0	1
Sternebrae misaligned ossification site	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Cervical rib short supernumerary	Litter incidence	N	1	2	**5**
	Fetal incidence	N	1	2	2
13^{th} rib short	Litter incidence	N	1	2	2
	Fetal incidence	N	1	2	2
Number of ribs 13/14 or 14/14	Litter incidence	N	12	6	**20**
	Fetal incidence	N	7	3	9
14^{th} rib full supernumerary	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Thoracolumbar vertebrae 18	Litter incidence	N	0	0	1
	Fetal incidence	N	0	0	1
Thoracolumbar vertebrae 20	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0

Incomplete ossification/unossified					
Head/neck: cranial centers	Litter incidence	N	8	9	17
	Fetal incidence	N	5	6	8
Head/neck: hyoid	Litter incidence	N	11	12	15
	Fetal incidence	N	5	8	7
Head/neck: presphenoid	Litter incidence	N	0	0	2
	Fetal incidence	N	0	0	1
Vertebrae: cervical	Litter incidence	N	0	2	4
	Fetal incidence	N	0	1	2
Vertebrae: thoracic	Litter incidence	N	8	7	6
	Fetal incidence	N	4	6	4
Vertebrae: lumbar	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Vertebrae: sacrocaudal	Litter incidence	N	8	8	15
	Fetal incidence	N	5	3	5
Sternebrae: 5^{th} and/or 6^{th}	Litter incidence	N	74	87	86
	Fetal incidence	N	18	18	19
Sternebrae total	Litter incidence	N	74	90	87
	Fetal incidence	N	18	19	19
Sternebrae other	Litter incidence	N	4	15	9
	Fetal incidence	N	4	8	5
Gridles pelvic bones	Litter incidence	N	6	5	12
	Fetal incidence	N	5	3	5
Limbs metacarpal	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Limbs metatarsal	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Limbs long bones	Litter incidence	N	0	1	3
	Fetal incidence	N	0	1	2
Precocious ossification cervical vertebral centra more than 4 ossified	Litter incidence	N	1	2	0
	Fetal incidence	N	1	2	0
Minor visceral abnormality					
Lens variation in shape	Litter incidence	N	2	1	0
	Fetal incidence	N	1	1	0
Thyroid absent lobe	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Thymus partially undescended lobe	Litter incidence	N	1	2	2
	Fetal incidence	N	1	2	2
Thinning of diaphragm with liver protrusion	Litter incidence	N	0	0	2
	Fetal incidence	N	0	0	2
Liver bilobed/fissured posterior caudate lobe folded posterior caudate lobe,	Litter incidence	N	0	0	4
	Fetal incidence	N	0	0	3
Kidney small renal papilla	Litter incidence	N	0	2	1
	Fetal incidence	N	0	1	1
Ureter dilated	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Testis displaced	Litter incidence	N	2	5	3
	Fetal incidence	N	2	3	3
Umbilical artery left	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Hemorrhages head brain	Litter incidence	N	4	7	7
	Fetal incidence	N	4	6	5
Hemorrhages head	Litter incidence	N	4	7	7

aqueous/vitreous humor eye	Fetal incidence	N	4	6	5
Hemorrhages neck/thorax intra-thoracic	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Hemorrhage abdominal cavity	Litter incidence	N	11	13	7
	Fetal incidence	N	7	8	7
Hemorrhage liver lobes	Litter incidence	N	4	3	2
	Fetal incidence	N	3	3	2
Hemorrhage general subcutaneous	Litter incidence	N	0	4	0
	Fetal incidence	N	0	2	0
Major abnormality findings					
Heart and Major vessels Membranous ventricular septal defect	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Limbs and Girdles: Short scapula(e)	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Limbs and Girdles: Short pelvic bones	Litter incidence	N	0	2	0
	Fetal incidence	N	0	1	0
Limbs and Girdles: Short/thickened long bones	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Limbs and Girdles: Short long bones	Litter incidence	N	0	2	0
	Fetal incidence	N	0	1	0

Table 53: *Fetal alterations (study (b) (4) 0005): Caesarian delivered live fetuses; F1 generation*; [a] *excludes values for fetus #10398-7 and #10398-12 only the heads had been examined at the tissue examination (appeared normal)*

The incidence of major and minor abnormalities and skeletal variants did not show any relationship to treatment. In the group that received AS01B there were three fetuses in two litters with shortened/thickened/bent scapula(e), long bones and associated short pelvic bones but this was within historical control data range. In the group that received gE/ AS01B there appeared to be a slightly higher incidence of fetuses with 14 ribs, compared with concurrent Control, this was within historical control data range, although on the high end of it. The group receiving gE/ AS01B also showed a slightly higher incidence of unossified gridles pelvic bones, head/neck cranial centers and sacrocaudal vertebrae, but these changes were not considered to be of toxicological relevance.

Pre-weaning examination:

The following pre-weaning reflex developmental tests were performed on each offspring:

Surface righting: assessed daily from Day 2 of age until achieved.
Air righting: assessed daily from Day 16 of age until achieved but not beyond Day 21 of age.
Auditory function: the startle response to a sudden sharp sound was assessed on Day 20 of age.
Visual function: the pupil closure response of dark adapted eyes to a bright point source of light was assessed on Day 20 of age.

Pre-weaning examinations F1				
		Control	**AS01B**	**gE/AS01B**
Surface righting	Days of age	3.5	3.5	3.8
Air righting	Days of age	17.0	16.9	17.2
Pupil reflex	% passed	100%	100%	100%
Startle response	% passed	100%	100%	100%

Table 54: *Pre-weaning examinations -group values for offspring (F1)(study (b) (4) 0005)*

Age of attainment for air and surface righting, the pupil reflex and startle response were unaffected by maternal treatment.

Macropathology of offspring killed/dying before scheduled termination		
group	Days of age	Macroscopic observations
Control	9	No abnormalities
	<1	No abnormalities
	<1	No milk in stomach
	<1	No milk in stomach
3	6	No abnormalities
	<1	No milk in stomach
	<1	No milk in stomach
	<1	No milk in stomach

Table 55: *Macropathology -individual findings for offspring killed or dying before scheduled termination (F1)(study* (b) (4) *0005)*

Macroscopic examination of offspring that died before scheduled termination and examination of offspring at scheduled termination on Day 25 of age did not reveal any findings that could be related to maternal treatment.

Serology:

The study report mentioned that serology was evaluated by the sponsor for this study but the serology report was not included in the BLA submission. An information request was submitted to the sponsor and the sponsor submitted the serology report under amendment 23 (6/16/2017) to the original BLA submission. The anti-gE antibody responses measured using ELISA.

The serological analysis showed that anti-gE antibody response was observed in 100% of dams after 7 intra-muscular administrations of the gE/$AS01_B$ VZV candidate vaccine. Anti-gE antibody response was observed in 100% of fetuses, as well as in 100% of the pups from dams receiving 7 administrations of VZV vaccine candidate. No anti gE antibodies were detected in the sera collected from dams or fetuses in $AS01_B$ adjuvant-treated control group animals. Weak anti-gE antibody response was observed in 10% of dams receiving 7 injections of saline. These weak responses were at least 2000 fold lower than those observed for dams injected with the VZV candidate vaccine. As consequence, some seroconversion was also observed in the related fetuses and pups. Investigations are ongoing at (b) (4) and GSK to find a plausible explanation to that observation.

Overall, the serological data confirm the exposure to and vaccine take of dams prior and/or during pregnancy. The results also demonstrate the transfer of antibodies from vaccinated dams to fetuses and offspring during in utero development and lactation.

Conclusions:
Treatment of female CD rats with the candidate vaccine gE/AS01B or adjuvant AS01B at 40% of the full human dose per occasion, on 28 and 14 days before pairing and then on Days 3, 8, 11 and 15 of gestation and on Day 7 of lactation was well tolerated by the F0 females and did not

adversely affect embryo-fetal or pre- and post-natal survival, growth or development of the offspring up to Day 25 of age.

Study # 2: Zoster Candidate Vaccine: Study of Effects on the Fertility of Male CD Rats by Intramuscular Administration

Reviewer: Claudia Wrzesinski

Summary:
Male rats received either gE/AS01B (100 µl/occasion, equals 1/5th of a human dose), AS01B (100 µl/occasion, equals 1/5th of a human dose) or saline by intramuscular injection on Days -42, -28 and -14 prior to pairing. Forty two days after the first dose administration, treated males were paired with untreated females for assessment of potential effects on fertility and early embryonic development. Treatment with either gE/AS01B or AS01B was well tolerated. Local swelling/edema was observed at the injection site in groups treated with vaccine and adjuvant, the day following each injection. Mating performance and fertility, were unaffected by treatment with gE/AS01B or AS01B. No adverse effects on sperm motility, or morphology were apparent after treatment with the adjuvant AS01B or the Zoster candidate vaccine gE/AS01B. However, values for the epididymal sperm concentration, testicular spermatid concentration and total testicular spermatid were statistically significantly lower in the vaccine group compared to saline control, while no significant differences were observed for the adjuvant group. Historical control data (HCD) ranges indicated that the concurrent control values for total testiticular spermatid, testicular spermatid count and epididymal sperm count were near the maximum range or slightly higher than expected and that all the statistically low values obtained for the animals treated with the gE/AS04B vaccine were within the HCD range. Microscopic examination did not highlight any treatment related changes affecting the right testis, right epididymis, prostate and seminal vesicles. Mating performance and fertility, as assessed by percentage mating, conception rate and fertility index, were unaffected by treatment with gE/AS01B or AS01; 100% was reached for each group in each assessed variable. There was no evidence of an effect of treatment of the males with gE/AS01B or AS01B on early embryonic development.

In conclusion, the treatment of male CD rats with the Zoster candidate vaccine gE/AS01B or adjuvant AS01B alone on three occasions prior to pairing (on Day -42, Day -28 and Day -14) did not affect male mating performance, fertility or early embryonic development.

Study no.: (b) (4) **0004**

Conducting laboratory and location: (b) (4)

Date of study initiation: 1 Feb 2010

GLP compliance: yes

QA reports: yes

Drug, lot #: gE: batch number: (b) (4)

AS01B: batch number: (b) (4)

Methods:

Doses: Group1: 0.9% saline, Group 2 - AS01B adjuvant formulation: 100 µl (10 µg QS-21 and 10 µg MPL, equals 1/5th of a human dose) was injected per rat Group 3 - gE/AS01B candidate vaccine: 100 µl (10 µg gE, 10 µg QS-21 and 10 µg MPL, equals 1/5th of a human dose) was injected per rat

Frequency of dosing: Days -42, -28 and -14 prior to pairing

Dose volume: 100 µl

Route of administration: Intramuscular injection

Species/Strain: (b) (4):CD® (b) (4) rats

Number/Sex/Group: 22

Study design:

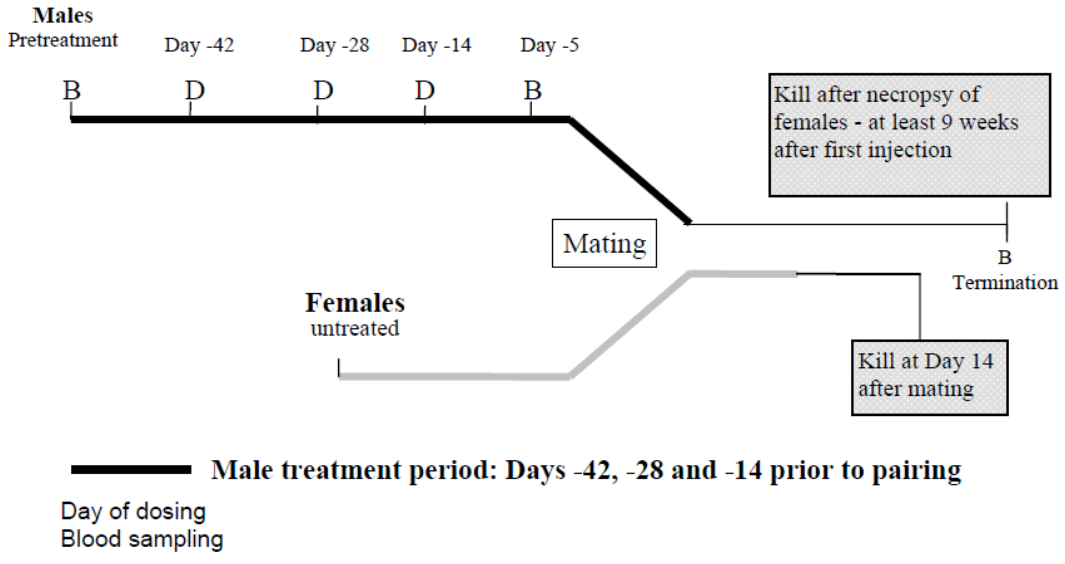


Figure 3: *Study design (study (b) (4) 0004), figure provided by the sponsor.*

Group	Treatment	Dose	Volume	Number of males
1	Saline	0.9% saline	100 µl	22
2	AS01B	10 µg QS-21, 10 µg MPL	100 µl	22
3	gE/AS01B	10 µg gE, 10 µg QS-21, 10 µg MPL	100 µl	22

Table 56: *Study design and dosing (study* (b) (4) *0004)*

Observations and Results:

Mortality: There were no deaths.

Clinical Signs:

Animals were inspected visually at least twice daily for evidence of ill-health or reaction to treatment. Cages and cage-trays were inspected daily for evidence of ill-health amongst the occupant(s). A detailed physical examination was performed weekly on each male and for females on Days 0, 7 and 14 after mating, to monitor general health. Injection sites were examined daily on each day of dosing, until two days subsequent and then approximately weekly thereafter and at termination, for signs of reaction (e.g. local swelling and reddening). If signs were still apparent two days after injection, daily monitoring continued until the signs had resolved.

Injection site observation of local swelling/edema was observed the day following the first, second and final injections and this sign generally resolved in 1-2 days. The sign was observed in both animals treated with vaccine gE/AS01B and adjuvant AS01B, and it was observed at the highest incidence following the second injection. This sign was no longer apparent after Day 33 of study (Day -10 prior to pairing). No swelling or edema was observed at the injection site of the control animals.

Body Weight

Males were weighed on the day that treatment commenced (Day -42), at twice-weekly intervals to termination. Females were weighed on Days 0, 4, 7, 11 and 14 after mating.

There were no adverse effects on bodyweight. Bodyweight gain for the vaccine (gE/AS01B) and adjuvant (AS01B) treatment groups was slightly lower during the first three days following the

first dose administration, the difference attained statistical significance for the adjuvant treatment group. Bodyweight gain was comparable with the control group for the remainder of the study.

Feed Consumption

The weight of food supplied to each cage of males, that remaining and an estimate of any spilled was recorded on a twice-weekly basis from the start of treatment until the animals were paired for mating. From these records the mean daily consumption per animal (g/rat/day) was calculated for each phase, for each cage.

There were no adverse effects on food consumption prior to pairing from either vaccine gE/AS01B or adjuvant AS01B administration. Mean food consumption was marginally but statistically significantly lower than in controls during the first three days after the first and second doses of the adjuvant (Days 1-3 and 15-17 of study). Mean food consumption was also marginally low during the first three days after the first dose of the vaccine (Days 1-3 of study).

Mating procedure:

Following the scheduled period of treatment (42 days after first pre-pairing injection), males and females were paired on a one-to-one basis for a period of up to 2 weeks. If there was no positive indication of mating after seven days, the female partner was replaced by a spare female.

Once mating occurred, the males and females were separated and smearing was discontinued. The pre-coital interval was calculated for each male as the time elapsing between initial pairing and detection of mating.

Biosampling (antibody assay):

Blood samples were obtained from the males pre-treatment and on Day -5 (prior to pairing) and at termination. Each 0.8 mL sample collected during the in-life phase was taken from the sublingual vein and collected into plain tubes. Samples collected at termination were taken from the retro-orbital sinus without recovery into plain tubes. Each animal was held under (b) (4) anesthesia during the sampling procedure.

Necropsy

Females were killed on Day 14 after mating. Males were killed after at least nine weeks after the first injection on Day -42 prior to pairing, following completion of the necropsy of the females and assessment of the Day 14 litter parameters. All adult animals were subject to a detailed necropsy, which involved the following: After a review of the history of each animal, a full macroscopic examination of the tissues was performed including injection sites. All external features and orifices were examined visually. For males, samples for sperm analysis (as detailed below) were taken as soon as possible after death. After ventral mid-line incision, the neck and

associated tissues and the thoracic, abdominal and pelvic cavities and their viscera were exposed and examined in situ. Any abnormal position, morphology or interaction was recorded.

The requisite organs were weighed and external and cut surfaces of the organs and tissues were examined as appropriate. Any abnormality in the appearance or size of any organ and tissue was recorded and the required tissue samples preserved in appropriate fixative.

The following reproductive assessment was made for all females: The number of corpora lutea in each ovary and the number of implantation sites, the number and distribution of resorption sites (classified as early or late) and live embryos.

The following organs were taken and weight from each male: epididymitis, prostate, seminal vesicles, testes.

Groups	Epididymis (g)	Prostate (g)	Seminal Vesicles (g)	Testes (g)
Saline	1.269	1.289	2.233	3.60
AS01B	1.332	1.278	2.247	3.73
gE/AS01B	1.336	1.194	2.222	3.69

***Table 57**: Organ weight - mean group values (study* (b) (4) *0004)*

The weights of the testes, prostate and seminal vesicles were not affected by treatment with either the vaccine gE/AS01B or adjuvant AS01B.

Histopathology:

	Saline	**AS01B**	**gE/AS01B**
Prostate (22 animals examined)			
Lymphoid Aggregates	1	2	3
Oedema	0	1	0
Reduced Colloid/ Colloid Alteration	0	1	1
Rt. Epididymis (22 animals examined)			
Degenerate Spermatogenic Cells in Duct(s)	0	0	1

Lymphoid Aggregates	0	1	1
Rt. Testis (22 animals examined)			
Eosinophilic Globules	0	0	1
Multinucleate Giant Cells	0	2	1
Seminiferous Tubular Vacuolation	0	1	0
Seminal Vesicles (22 animals examined)			

Table 58: *Histopathologic evaluation of prostate and testis (study* (b) (4) *0004)*

In the testis multinucleate giant cells involving single tubules within the parenchyma were observed in 2 males from the adjuvant AS01B treatment group and one male from the vaccine gE/AS01B treatment group

Sperm analysis:

Immediately after scheduled sacrifice of each male, the left vas deferens, epididymis and testis was removed and the epididymis and testis were weighed. The following tests were performed: sperm motility, sperm morphology, sperm count, homogenization-resistant spermatids count.

Groups	**Motility sperm (%)**	**Progressively motility sperm**	**Cauda epididymis**			**Testis**		
			Weight	Sperm count (millions/g)	Total (millions)	Weight	Sperm count (millions/g)	Total (millions)
Saline	92	54	0.239	1076	257	1.80	199	357
AS01B	92	50	0.251	1028	258	1.86	183	343
gE/AS01B	90	51	0.254	970**	246	1.84	167**	306**

Table 59: *Sperm analysis - group mean values (study* (b) (4) *0004)*

Groups	Normal sperm morphology (%)	Abnormal sperm morphology (%)

Groups	Normal sperm morphology (%)	Abnormal sperm morphology (%)
Saline	97.4	2.6
AS01B	97.5	2.5
gE/AS01B	97.9	2.1

Table 60*: Sperm morphology analysis - group mean value (study* (b) (4) *0004)*

No adverse effects on sperm motility, concentration or morphology were apparent after treatment with the adjuvant AS01B. No adverse effects on sperm motility, or morphology were apparent after treatment with the Zoster candidate vaccine gE/AS01B. However, values for the epididymal sperm concentration, testicular spermatid concentration and total testicular spermatid were statistically significantly lower in the vaccine group compared to saline control, while no significant differences were observed for the adjuvant group. Historical control data (HCD) ranges indicated that the concurrent control values for total testicular spermatid, testicular spermatid count and epididymal sperm count were near the maximum range or slightly higher than expected and that all the statistically low values obtained for the animals treated with the gE/AS04B vaccine were within the HCD range. Therefore, it was concluded that these differences were not related to treatment with the vaccine.

Mating performance and fertility:

Reproductive assessment:

Pre-implantation loss (%): (number of corpora lutea – number of implantation)/number of corpora lutea x 100

Post-implantation loss (%): (number of implantation – number of live embryos)/number of implantations x 100

Groups	Corpora lutea	Implantations	Live embryos	Resorption (mean)		Implantation loss (%)	
	mean	mean	mean	Early	Late	Pre-	Post-
Saline	17.2	16.6	15.5	1.1	0	4.1	6.5
AS01B	16.5	16.4	14.7	1.7	0	1.8	10.6

Groups	Corpora lutea	Implantations	Live embryos	Resorption (mean)		Implantation loss (%)	
gE/AS01B	16.6	16.2	15.1	1.0	0	3.6	6.5

Table 61: *Reproductive assessment - group mean values (study* (b) (4) *0004)*

Vaccine treatment of the males showed no effect on litter data, as assessed by the mean number of corpora lutea, implantations, resorptions, live embryos and pre- and post-implantation losses. All females were pregnant with live embryos on Day 14 of gestation.

A slightly higher post-implantation loss was seen in the adjuvant only group. This was mainly due to one litter with an atypically high number of resorptions. The vaccine group did not show higher post-implantation loss compared to the saline control group.

Statistical analyses:

All statistical analyses were carried out separately for males and females. Data relating to food consumption was analysed on a cage basis. For all other adult parameters, the analyses were carried out using the individual animal as the basic experimental unit. For litter findings the litter was taken as the treated unit and the basis for statistical analysis.

The following data types were analyzed at each timepoint separately: Bodyweight, using absolute weights and gains over appropriate study periods; food consumption, using means over appropriate study periods; litter size and survival indices; organ weights, absolute and adjusted for terminal bodyweight; sperm analysis, motility and count.

The following sequence of statistical tests was used for bodyweight, food consumption, litter size and survival indices, organ weights and sperm analysis, motility and count: A parametric analysis was performed if Bartlett's test for variance homogeneity was not significant at the 1% level. Groups were compared using *t*-tests. A non-parametric analysis was performed if Bartlett's test was still significant at the 1% level following both logarithmic and square-root transformations. Groups were compared using Wilcoxon rank sum tests.

For corpora lutea, implantations, live embryos and sperm analysis, motility and count data, if 75% of the data (across all groups) were the same value, Fisher’s Exact tests were performed. Treatment groups were compared using pairwise comparisons of each dose group against the control both for i) values $<c$ versus values $\geq c$, and for ii) values $\leq c$ versus values $>c$, as applicable.

Pre- and post-implantation losses were analyzed by generalized mixed linear model with binomial errors, a logit link function and litter as a random effect. Each treated group was compared to control using a Wald chi-square test. For resorptions, each treated group was compared to control by exact Wilcoxon rank sum test. For organ weight data, analysis of

covariance was performed using terminal bodyweight as covariate. The treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights.

Antibody assay report:

Blood samples were taken from male rats at day and antibodies against gE were measured by ELISA. Anti-gE antibody response was observed in 100% of male rats after three IM administrations of gE/AS01B. No anti-gE antibodies were detected in sera collected from rats receiving either saline or AS01B. The serological data confirm the exposure to the vaccine prior to pairing.

Conclusions:

Treatment of male CD rats with the Zoster candidate vaccine gE/AS01B or adjuvant AS01B alone at 20% of the full human dose on three occasions prior to pairing (on Day -42, Day -28 and Day -14) did not affect male mating performance, fertility or early embryonic development. At termination, no treatment-related differences were detected in males in respect to reproductive organ weights, seminology parameters or macroscopic and microscopic appearance of the reproductive tissues.

Study 3 ((b) (4) 1729/7) and 4 (b) (4) : 1729/8) evaluated MPL in reproductive toxicology studies. These studies have been reviewed by Dr. Marion Gruber under the BLA 123259, the full review can be found there. Here only a summary of these studies is included.

Study 3: (b) (4) 1729/7: MPL: Subcutaneous Study of Embryo-Fetal Development in the Rat

Reviewer: Dr. Marion Gruber
This study has been originally submitted to BLA 125259 and has been reviewed by Dr. Marion Gruber, see her review below.

Summary:

In this study the effects on MPL on the embryonic and fetal development of the rat was evaluated. Three groups of 24 mated female rats were given MPL at dose levels of 1, 10 and 100 µg/kg/day daily, from Days 6 to 17 of gestation, inclusive. A further group of 24 mated females, dosed with the vehicle (phosphate buffered saline) by the same route and over the same period, served as controls. All animals were maintained to Day 20 of gestation, when they were killed and their uterine contents examined.

No mortalities were observed during the study. Clinical signs, body weights and food intakes were unaffected by treatment and there were no treatment-related effects noted at necropsy.

There were no adverse effects of treatment on the pregnancy rate or on the uterine/implantation or fetal data. 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 conclusion, administration of MPL to pregnant rats at dose levels of up to 100 µg/kg/day from day 6 to 17 of gestation showed no indication of maternal or embryo toxicity or of teratogenicity.

Study 4: (b) (4) 1729/8: MPL: Subcutaneous Study of Embryo-Fetal Development in the Rabbit

Reviewer: Dr. Marion Gruber
This study has been originally submitted to BLA 125259 and has been reviewed by Dr. Marion Gruber, here only a short summary of her review is included, her full review can be found under BLA 125259.

Summary:

In this study the effect of MPL on the embryonic and fetal development of the rabbit when administered subcutaneously was evaluated. Three groups of 24 mated female rabbits were given MPL at dose levels of 1, 10 and 100 µg/kg/day, from Days 7 to 19 of gestation, inclusive. A further group of 24 mated females, dosed with the vehicle (phosphate buffered saline) by the same route and over the same period, served as controls. All animals were maintained to Day 29 of gestation, when they were killed and their uterine contents examined.

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. 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 death and 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 (see tables 6 and Appendix 8 reproduced below). 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. Mean sex ratio was unaffected by treatment.

The observed malformations 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. Overall there were no adverse effects of treatment on the pregnancy rate or on the uterine/implantation, fetal or fetal defect data.

In conclusion, administration of MPL to pregnant rabbits at dose levels of up to 100 µg/kg/day from Day 7 to Day 19 of gestation, showed no indication of maternal or embryo toxicity or of teratogenicity.

Comment from the reviewer of BLA 125258 (for more details see Dr. Gruber's review of BLA 125258):
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 [This is a reproductive toxicology study evaluating CERVARIX submitted to BLA 125258. In this study two observations of small membranous intraventricular septal defect (IVSD) were described under minor visceral fetal abnormalities in rats, one pub from the group receiving the full vaccine and one pub from the group receiving the adjuvant alone showed this finding.) 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 testes 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. The reviewer concluded that GSK has satisfactorily refuted a potential association of IVSD with HPV/AS04 vaccine and noted that GSK is planning a pregnancy registry following licensure of Cervarix in the US which will capture outcomes of registered pregnancies. For more details see the review of the reproductive toxicology studies by Dr. Gruber under BLA 125258.

Study 5: (b) (4) 1729/17: MPL: Subcutaneous Study of Pre- and Postnatal Development in the Rat-In (b) (4)

Reviewer: Dr. Marion Gruber
This study has been originally submitted to BLA 125259 and has been reviewed by Dr. Marion Gruber, see her review below.

Summary:

This study assessed the effects of MPL on the pre- and postnatal development, including maternal function, m the rat when administered subcutaneously. Twenty-four (b) (4):CD(b) (4) rats/group and were dosed with MPL by subcutaneous injection at 0, 1, 10 and 100 µg/kg/day. The parental females were dosed daily from Day 6 of gestation to Day 21 *postpartum*, inclusive. The females were allowed to litter and rear their offspring to weaning. Twenty animals of each sex were randomly selected from each group to form the F_1 generation. These
animals were maintained untreated for 12 weeks post-weaning (maturation phase) before being paired for up to 15 days. Mated F_1 females were killed on Day 13 of gestation and their uterine contents examine. The F_1 males were killed in Week 17.

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 effect 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; 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.

In conclusion, administration of 1, 10 and 100 µg/kg/day MPL from Day 6 of gestation to Day 21 of lactation elicited no maternal toxicity and there were no adverse effects of maternal treatment on the F1 generation.

Study 6: DQ* Immunostimulant: Study for Effects on Female Fertility, Embryo-Fetal and Pre- and Postnatal Development in the CD Rat by Intramuscular Administration (Including Pre-Mating Immunization Phase) (b) (4) 0020)

Summary:
Female (b) (4) :CD (b) (4) rats received DQ at doses of 4, 20 and 40 µg of QS-21/occasion by intramuscular administration or received saline control. Animals were treated on days -28 and -14 before pairing as well as on Days 3, 8, 11 and 15 after mating and on day 7 of lactation.

There was no adverse effect on bodyweight or bodyweight gain during the pre-pairing, gestation and lactation period. A slight, but statistically significant reduction in food consumption and body weight gain was observed in animals receiving DQ at 40 µg of QS-21/occasion between Days 3 and 6 of gestation (after the 3rd administration). Pre-coital interval, mating performance and fertility were unaffected by treatment with DQ. No effects on litter data and placental, fetal and litter weights were observed. Detailed fetal examination did not reveal any major or minor abnormalities or skeletal variants considered to be related to treatment. No treatment related effects on gestation and parturition were observed; litter size, offspring survival and sex ratio were unaffected by DQ up to 40 µg of QS-21/occasion. Offspring bodyweight on Day 1 of age and bodyweight gain up to Day 25 of age showed no adverse effects of maternal treatment with DQ, and there were no effects on the development of normal reflexes in the offspring

In conclusion, DQ, at doses of 4, 20 and 40 µg of QS-21/occasion did not adversely affect female fertility, embryo-fetal or pre- and post-natal survival, growth or development of the offspring up to Day 25 of age.

Study 7: (b) (4) AB14898: DQ –Developmental Toxicity Study (Including Teratogenicity and Postnatal Investigations) by the Intramuscular Route in the Rabbit

Summary:
Three groups of female (b) (4) rabbits were administered intramuscular injections of DQ adjuvant containing 20, 100 or 200 µg/mL of QS2, 28 and 14 days before the start of mating and on days 3, 8, 11, 15 and 24 of gestation, then on day 7 of lactation (littering sub-groups only). A control group of female (b) (4) rabbits was administered sterile physiological (b) (4) Animals were either assigned to a caesarean sub-group (all females were necropsied on day 29 *post-coitum)* or littering sub-group.

There were no treatment-related clinical signs before mating or during the gestation and lactation periods. At 200 µg/mL of QS-21, a slight reduction in mean body weight gain (not statistically significant) between days 0 and 14 of the pre-mating period (after the first dosing) was observed. A statistically significant mean body weight loss occurred between days 24 and 29 of gestation, leading to a statistically significant reduction in mean body weight gain between days 0 and 29 of gestation. Conversely, a statistically significant increase in mean body weight

gain was observed between days 4 and 35 of lactation, mainly due to a higher mean body weight gain between days 7 and 14 of lactation. There were no treatment-related effects on body weight or body weight gain in the 20 or 100 μg/mL QS-21 dose groups during the pre-mating, gestation or lactation periods. At 200 μg/mL of QS-21, a slight but statistically significant decrease in mean food consumption was observed between days 0 and 7 of pre-mating period. A slight decrease in mean food consumption was also noted from day 11 of gestation onwards (caesarean sub-group), that was statistically significant between days 24 and 29 of gestation (littering sub-group). No effects on mean food consumption were observed in the 20 or 100 μg/mL QS-21 dose groups during the pre-mating or gestation periods. No treatment-related effects on food consumption were seen during the lactation period.
The adjuvant produced no effects on mating performance or fertility of the females in either the caesarean or littering sub-groups. No treatment-related effects on gravid uterine weight were observed in the caesarean sub-group, nor were treatment-related effects on ovary weight seen in the caesarean or littering sub-groups. A dose-related lower net (minus uterine weight) mean body weight change was observed in the treated groups, compared with control, attaining the statistical significance at the 200 μg/mL QS-21 dose level. Necropsy examination of the females did not reveal any treatment-related lesions.

In the caesarean sub-group, 23, 22, 23 and 19 females were pregnant in the control, 20, 100 and 200 μg/mL QS-21 groups, respectively, and all pregnant females had viable fetuses, with the exception of two control females. No effects of the DQ adjuvant on the pre- and post-implantation parameters were observed. Slightly statistically significant lower mean fetal weight was observed in the 200 μg/mL QS-21 group, however, no effects of treatment on mean fetal weight were seen in the 20 or 100 μg/mL QS-21 dose groups. No effects of treatment on the fetal sex ratio were observed in any DQ dose group.

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

In conclusion, under the defined experimental conditions of the study, the dose level of DQ adjuvant containing 100 μg/mL of QS-21 was selected as the for maternal, pre- and post-natal toxicity.

Study no.: (b) (4) 4898
Conducting laboratory and location: (b) (4)
Date of study initiation: 20 December 2012
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:

DQ (QS-21-10 µg/500 µL) at 10 µg: (b) (4)
DQ (QS-21-50 µg/500 µL) at 50 µg: (b) (4)
DQ (QS-21-100 µg/500 µL) at 100 µg: (b) (4)

Doses: DQ adjuvant containing QS-21, DQ is the detoxified formulation of QS-21, animals received 20, 100 or 200 µg given in 2 injections of 500µL (concentration: 20, 100 or 200 µg/mL, respectively), ratio between QS-21: cholesterol : DOPC (b) (4)
DQ at 10 µg: 20 µg/mL QS-21, 400 µg/mL DOPC and 100 µg/mL cholesterol.
DQ at 50 µg: 100 µg/mL QS-21, 2000 µg/mL DOPC and 500 µg/mL cholesterol.
DQ at 100 µg: 200 µg/mL QS-21, 4000 µg/mL DOPC and 1000 µg/mL cholesterol
Human DQ: 50 µg/mL QS-21, 1000 µg/mL DOPC and 250 µg/mL cholesterol

Frequency: Once, 28 days (i.e. day 0 of study) and 14 days (i.e. day 14 of study) before mating, on days 3, 8, 11, 15 and 24 of gestation (i.e. G 3, G 8, G 11, G 15 and G 4) then on day 7 of lactation (i.e. L 7; for littering sub-groups only).

Species/strain: (b) (4)
Number/sex/group: 55 females per dosage group (25 caesarean and 30 littering)
Route, and volume: intramuscular, 500 µL; Bolus injection in the dorsal lumbar muscles (right and left sites for each occasion) using sterile syringe and needle after disinfection with an antiseptic solution.

Study design:

Group number/ Treatment	Dose level per injection (µg of QS21/0.5mL)	Dose level per occasion (µg of QS21/mL)	Dose volume per injection (mL/site)	Dose volume per occasion (mL/occasion)
1. Control	0	0	0.5	1
2. Low dose	10	20	0.5	1
3. Intermediate dose	50	100	0.5	1
4. High dose	100	200	0.5	1

Females received 2 injections of 0.5 mL for a total of 1 mL injected/occasion.

Table 62: *Study design (study AB14898): test article was given 28 days and 14 days before mating and on days 3, 8, 11, 15 and 24 of gestation, den on day 7 of lactation (for littering sub-groups), table submitted by the sponsor.*

Parameters and endpoints evaluated:

Morbidity/mortality: All adults were observed twice daily at the beginning and at the end of each working day. Offspring were examined daily from postnatal day 1 (PND 1) with minimal interference to nursing between postnatal days 1 and 7.

Clinical observations: All females were observed daily for clinical signs. At the end of gestation, the females in the littering phase were inspected at least twice daily for signs of

parturition. A physical examination was performed weekly (including pretest). Offspring were observed daily from postnatal day 1 (PND 1). The clinical observation was performed from outside the nesting box during the early phase of lactation (between PND 1 and 7)

Injection site observations: Any local reactions at the injection sites were assessed daily until disappearance. However, since reduced food consumption was noted in some females from all groups at the end of the gestation period, it was decided to not perform the injection site observations from day 27 of gestation to day 6 of lactation in the littering sub-groups, to avoid any interference with mating, gestation, littering and other reproductive parameters. During the lactation phase, the local reactions were observed on day 7 of lactation to minimize any interference with the nursing behavior, then they were observed daily until disappearance of the signs.

Body weight:
Individual body weights for females were recorded:
- once pretest on day -8 or -7 (at randomization)
- on day 0 (28 days before mating) and day 14 (14 days before mating) of the study
- on days 0 (day of mating), 6, 9, 11, 16, 20, 24 and 29 of gestation
- on days 4, 7, 11, 14, 17, 21, 28 and 35 of lactation (littering sub-group only).

Food consumption:
Food consumption of each female was recorded daily from the day of arrival to day 29 of gestation for the caesarean sub-groups and to day 35 of lactation for the littering sub-groups and reported as follows:
- daily pretest (from arrival)
- the mean (g/animal/day) was calculated for the periods (days)
- 0 to 7, 7 to 14, 14 to 21 and 21 to 28 before mating
- 0 to 6, 6 to 9, 9 to 11, 11 to 16, 16 to 20, 20 to 24 and 24 to 29 of gestation
- 0 to 4, 4 to 7, 7 to 11, 11 to 14, 14 to 17, 17 to 21, 21 to 28 and 28 to 35 of lactation (littering sub-group only).

Mating:
Cohabitation started 28 days after the first administration. Each day, a number of females was paired with males of the same strain for up to 10 minutes or until copulation occurred. Following observed copulation, the males and females were left together for at least 1 hour. Unmated females were paired with a different male on the same day or on subsequent days until copulation occurred (up to 10 days).

Parturition observations and gestation length:

Pregnancy and parturition – Littering subgroup only:
From day 30 of gestation, each female in the littering sub-groups was observed at least 4 times a day for the onset and duration of parturition.
The following data were recorded:
- date of mating
- date of parturition (day 0 of lactation or L 0)

- duration of gestation
- abnormalities of delivery, nesting or nursing behavior
- number of implantation sites (at necropsy).

Litter data – Littering subgroup only
For each litter, the following data were recorded:
- number of pups born (live and dead)
- external abnormalities of the pups
- number and weight of live pups on PND 4, 7, 11, 14, 21, 28 and 35
- physical development of the offspring, as assessed by the intra-litter onset and duration of incisor eruption, fur growth and eye opening on PND 4 then from PND 7 (any pup failing the eye opening test was examined the following days until attainment)
- behavioural and functional tests in all pups as follows:
- pupillary reflex and auditory reflex on PND 35
- external and necropsy findings of dead pups.

Necropsy schedule:

Surviving females were necropsied according to the following schedule:
Caesarean sub-groups:
- on day 29 of gestation
- two females (group 1 female no. 7484 and group 4 female no. 7550) that failed to mate (mating not detected) were killed on study day 39 (end of the mating period).

Littering sub-groups:
- on L 35 (with litter)
- three females (group 1 female no. 7379, group 2 female no. 7385 and group 3 female no. 7435) that failed to mate (mating not detected) were killed on study day 39 (end of the mating period)
- mated females that failed to produce a viable litter were necropsied on day 35 or 37 *post-coitum*
- one female with undetected mating (group 4 female no. 7458): after completion of the mating period on study day 39
- females with total litter death were necropsied on L 0 (group 2 female no. 7411), PND 2 (group 2 female no. 7386 and group 4 female no. 7451), or PND 7 (group 4 female no. 7452)
- one female with total litter resorption (group 4 female no. 7454) was necropsied on day 35 of gestation.

Necropsy of gestating and lactating females:
All females, at the scheduled sacrifice (G 29 for the caesarean sub-groups and L 35 for the littering sub-groups) were weighed and submitted to a macroscopic examination, including the thoracic, abdominal and pelvic viscera, and injection sites. Abnormal organs or tissues were sampled and preserved in 10 % neutral formalin but were not examined further.
The number and distribution of uterine implantation scars were recorded for all littering sub-group females that gave birth.

Organ weights
The ovaries from all surviving females from both sub-groups were weighed paired.

Caesarean examinations - Caesarean sub-groups
The ovaries and uterus of each female were removed and examined. The placentae were also examined. The following data were recorded:
- pregnancy status
- number of corpora lutea
- number of implantations
- number and distribution of live fetuses
- number and distribution of embryonic/fetal deaths, classified as follows:
- early: only placenta visible at termination
- late: both placenta and embryonic tissue visible at termination
- dead fetus
- gravid uterus weight
- individual fetal weights
- fetal sex.

Fetal examinations - Caesarean sub-groups:

All live fetuses were examined for external defects and killed by oral intubation of (b) (4) Dead fetuses were examined externally, preserved in (b) (4) but not examined further. All live fetuses were examined viscerally and sexed at the time of caesarean section. Following this, the heads of approximately half of the fetuses in each litter were removed and fixed for subsequent examination by serial sectioning. The eviscerated fetal carcasses were fixed and processed for skeletal examination. The ossified skeleton was (b) (4) .

Necropsy of pups - Littering sub-groups

All pups were given a macroscopic examination of the thoracic, abdominal and pelvic viscera for structural or pathological changes following an intracardiac injection of (b) (4) Abnormal organs or tissues were sampled and preserved in (b) (4) but were not examined further. The pups (including decedents) were sexed, where possible, by internal inspection.

Reproductive assessment:

Fetal abnormalities are categorized as follows:
- Malformations - structural defects which are rare in the control population and are thought to be life threatening or of major physiological consequence.
- Anomalies - minor abnormalities or defects which are relatively rare in the control population and/or are considered not to be of major physiological consequence.
- Variations - minor abnormalities, defects or alternative forms which are either common

in the control population or are of no known physiological consequence.

For the caesarean sub-groups, the following parameters were calculated:

$$\text{Pre-implantation loss (in \%):} \quad \frac{\text{Number of corpora lutea - Number of implantations}}{\text{Number of corpora lutea}} \times 100$$

$$\text{Post-implantation loss (in \%):} \quad \frac{\text{Number of implantations - Number of viable fetuses}}{\text{Number of implantations}} \times 100$$

The following reproductive indices were calculated for the littering sub-groups:

$$\text{Gestation index (in \%):} \quad \frac{\text{number of females with live pups}}{\text{number of pregnant females}} \times 100$$

$$\text{Lactation index (in \%):} \quad \frac{\text{number of pups alive on PND 35}}{\text{number of pups alive on PND 4}} \times 100$$

$$\text{Live birth index (in \%):} \quad \frac{\text{number of pups born alive}}{\text{number of pups born}} \times 100$$

The following indices were calculated for both caesarean and littering sub-groups:

$$\text{Pre-coital interval (in days):} \quad \frac{\text{sum of days until successful insemination}}{\text{number of inseminated females}}$$

$$\text{Copulation (mating) index (in \%):} \quad \frac{\text{number of inseminated females}}{\text{number of paired females}} \times 100$$

$$\text{Fertility index (in \%):} \quad \frac{\text{number of pregnant females}}{\text{number of inseminated females}} \times 100$$

$$\text{Sex ratio (proportion of male) (in \%):} \quad \frac{\text{number of males}}{\text{number of pups}} \times 100$$

Statistical methods

Gestation, lactation and fetus/pup-related data analysis:

The data were checked for homogeneity of variance across groups using Bartlett's test.
- Homogeneous data were then analyzed by parametric methods, i.e. one-way analysis of variance (ANOVA) followed by Dunnett's test if the ANOVA was significant.
- Non-homogeneous data were analyzed by non-parametric methods, i.e. Kruskal-Wallis test followed by Dunn's test if the Kruska-Wallis was significant.

The numbers of resorptions and all litter-based percentages were analyzed using the above non-parametric methods. Selected incidence data were analyzed using a chi^2 test for all groups followed by two-tailed Fisher's exact test with Bonferroni correction for each treated group versus the control if the chi^2 was significant.

Pretest body weight, pretest food consumption, terminal body weight and organ weight data analysis

For pretest body weights, pretest food consumption, terminal body weights, absolute and relative organ weights, statistical analyses were performed by the Provantis data acquisition system as follows:
The best transformation for the data (none, log or rank) was determined depending upon the kurtosis of the data, the probability of the Bartlett's test for homogeneity of the variances and the similarity of the group sizes.
- Non- or log-transformed data were analyzed by parametric methods. Rank transformed data were analyzed using non-parametric methods.
- The homogeneity of means was assessed by one-way analysis of variance (ANOVA).

Data were then analyzed to test for a dose-related trend to detect the lowest dose at which there was a significant effect, based on the Williams test for parametric data or Shirley's test for non-parametric data.
If no trend was found and the means were not homogeneous, the data were analyzed by a stepwise parametric or non-parametric Dunnett's test to look for significant differences from the control group.

Pre-coital interval data analysis

The pre-coital interval were analysed using the following methods:
- Levene's test was used to test the equality of variance across groups and Shapiro-Wilk's test was used to assess the normality of the data distribution in each group.
- Data with homogeneous variances and a normal distribution in all groups were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's test if the ANOVA was significant.
- Data showing non-homogeneous variances or a non-normal distribution in at least one group were analysed using Kruskal-Wallis test followed by the Wilcoxon's rank sum test if the Kruskal-Wallis test was significant.

Results:

F0 Generation
Mortality/Clinical signs:

One female given 100 µg/mL of QS-21 was prematurely sacrificed on day 18 of gestation following severe clinical signs, mainly characterized by diarrhea and subdued behavior, marked body weight loss (-451 g between days 9 and 16 of gestation) associated with no food intake between days 11 and 18 of gestation. At necropsy, no treatment-related macroscopic findings were observed. Since this premature death was not observed in the high dose group, this isolated case was considered to be not treatment-related.

One female given 200 µg/mL of QS-21 (caesarean sub-group no. 7570) was sacrificed after aborting on day 28 of gestation. No specific clinical signs were observed during the gestation period. A body weight loss of -103 g was noted between days 11 and 20 associated with reduced food consumption between days 11 and 28 of gestation. No macroscopic findings were noted at necropsy. This female had 13 implantation sites but no live fetus. The sponsor considered this case to be isolated and not treatment-related, since abortion can be noted in the historical control data (2/25 pregnant females aborted and 1/21 pregnant females aborted in two different studies in 2010). However, since this animal also showed significant weight loss it could also be a consequence of the observed maternal toxicity in the high dose group.

Red fluid in the cage, red vaginal discharge, few, soft or no feces, hair loss and/or scratches on the back (outside the treatment area) were observed on some occasions during the study and were considered to be incidental since they were also observed in controls or during the pretest period and can occur spontaneously in the rabbit.

Some local reactions, including hematoma (grade 1 to 4), very slight to slight edema, small induration and/or very slight to well defined erythema were noted after each injection in all groups, including the control group. The incidence and the duration of these reactions were similar between the control and treated groups and were considered to be related to the administration procedure.
However, in three female animals severe edema was observed:

- 2 days after the fourth injection (day 8 of gestation) in one female treated at 100 µg/mL of QS-21
- 1 day after the fifth injection (day 11 of gestation) in one female treated at 100 µg/mL of QS-21
- 2 days after the third injection (day 3 of gestation) in one female treated at 20 µg/mL of QS-21

This local reaction disappeared three to five days after the injection for the first two females and 19 days after the injection for the third.

Observation at the injection site (summary of all	Injection	**F0 generation**			
		Control	**10 µg/admin***	**50 µg/admin***	**100 µg/admin***

events)					
Bleeding		56	59	50	61
Induration (grade:1/2/3/4)	1st	(4/0/0/0)	(0/0/0/0)	(1/0/0/0)	(2/0/0/0)
	2nd	(0/0/0/0)	(4/0/0/0)	(0/0/0/0)	(1/0/0/0)
	3rd	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)
	4th	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)
	5th	(0/0/0/0)	(0/0/0/0)	(2/0/0/0)	(0/0/0/0)
	6th	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)
	7th	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)
	8th	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)
Edema/Erythema (grade:1/2/3/4)	1st	(9/2/0/0)	(6/1/0/0)	(2/0/0/0)	(2/1/0/0)
	2nd	(/0/0/0)	(0/0/0/0)	(5/0/0/0)	(3/0/0/0)
	3rd	(0/0/0/0)	(1/7/6/4)**	(1/0/0/0)	(4/0/0/0)
	4th	(0/0/0/0)	(13/2/0/0)	(3/1/0/2)	(7/6/0/0)
	5th	(0/0/0/0)	(4/2/1/0)	(2/0/0/2)	(4/0/0/0)
	6th	(3/0/0/0)	(1/3/0/0)	(2/0/0/0)	(0/0/0/0)
	7th	(2/0/0/0)	(2/0/0/0)	(5/0/0/0)	(12/0/0/0)
	8th	(0/0/0/0)	(1/0/0/0)	(1/0/0/0)	(5/0/0/0)
Hematoma (grade:1/2/3/4)	1st	(4/8/1/1)	(16/0/1/0)	(15/1/0/0)	(6/1/2/0)
	2nd	(6/0/0/0)	(11/2/0/0)	(6/4/0/0)	(12/3/2/0)
	3rd	(5/0/0/0)	(7/0/0/0)	(4/0/0/0)	(13/0/0/0)
	4th	(11/0/0/0)	(1/0/0/0)	(3/0/0/0)	(8/0/0/0)
	5th	(5/0/0/0)	(19/7/0/0)	(17/2/0/0)	(13/5/0/0)
	6th	(16/6/0/0)	(10/3/0/0)	(15/3/0/0)	(10/8/0/0)
	7th	(12/3/0/0)	(26/0/0/0)	(13/0/0/0)	(20/0/0/0)
	8th	(3/4/0/0)	(10/3/0/0)	(7/0/0/0)	(2/0/0/0)

Table 63: *Injection site observations (study AB14898) in the littering and caesarian sub-group combined; * each animal received 2 administration on each dosing day** data for grade 2, 3 and 4 edema are seen in one animal over several days*

Erythema and eschar formation

No erythema	0
Very slight erythema (barely visible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beetroot red) or eschar formation (deep lesion) making observation of intensity of erythema impossible	4

Oedema formation

No oedema 0	
Very slight oedema (barely visible)	1
Slight oedema (edges of area well-defined)	2
Moderate oedema (edges raised approximately 1 mm)	3
Severe oedema (edges raised more than 1 mm and extended)	4

Induration

No induration	0
Induration of an area < 1 cm^2	1
Induration of an area > 1 cm^2 and < 2 cm^2	2
Induration of an area > 2 cm^2 and < 3 cm^2	3

Induration of an area > 3 cm^2	4

Hematoma

No hematoma	0
Hematoma of an area < 1 cm^2	1
Hematoma of an area > 1 cm^2 and < 2 cm^2	2
Hematoma of an area > 2 cm^2 and < 3 cm^2	3
Hematoma of an area > 3 cm^2	4

Body weight:

There was a slight and not statistically significant reduction in mean body weight gain between days 0 and 14 of the pre-mating period in the 200 µg/mL QS-21 group from both sub-groups (mean body weight gain of 225 g and 257 g in females from caesarean and littering sub-groups, respectively, compared to a gain of 253 or 322 g in the controls). There was a statistically significant mean body weight loss between days 24 and 29 of gestation (after the last dosing on day 24 of gestation) in the 200 µg/mL QS-21 group sub-group; a mean loss in body weight of 88g (-88g) was observed between day 24 and 29 in animals receiving 200 µg/mL QS-21 while a mean weight gain of 72 g (+72 g) was seen in the control group. This effect resulted in a statistically significantly lower mean body weight gain between days 0 and 29 of gestation (mean overall body weight gain of 296 g and 299 g in females from the caesarean and littering sub-groups, respectively, compared to a gain of 515 or 449 g in the controls). There was a statistically significantly higher mean body weight gain between days 4 and 35 of lactation in the 200 µg/mL QS-21 group, mainly due to a higher mean body weight gain between days 7 and 11 of lactation and between days 11 and 14 of lactation.

There were no relevant effects of treatment on body weight or body weight gain in the 20 or 100 µg/mL QS-21 dose groups during the pre-mating, gestation or lactation periods.

Food consumption:

There was a slight but statistically significant decrease in mean food consumption in the 200 µg/mL QS-21 group between days 0 and 7 of pre-mating period (-9 % and -15 % in the caesarean and littering sub-group, respectively, compared with controls). This reduced mean food consumption was consistent with the body weight effect.
In the caesarean sub-group, there was a slight decrease in mean food consumption from day 11 of gestation onwards in the 200 µg/mL QS-21 group, compared with the control group and/or the historical control data, leading to a statistically significantly lower mean food consumption between days 24 and 29 of gestation (-37 %, when compared with controls). Consequently, average mean food consumption was slightly reduced during the gestation period (days 0 and 29) in this dose group, compared with controls (-12 %).
The same tendency was noted in the littering high dose group but between days 24 and 29 of gestation only (-20 %, compared with controls). However, the mean food consumption was comparable with the control group during the gestation period (days 0 and 29 of gestation). This reduced mean food consumption was consistent with the body weight effect.

There was no effect on mean food consumption in the lower dose groups during the pre-mating and gestation periods. There were no effects of treatment on food consumption during the lactation period.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Reproductive parameters examined (in F_0 animals), natural birth group:

GROUP TREATMENT	1 0 µg/adm	2 10 µg/adm	3 50 µg/adm	4 100 µg/adm
LITTERING AND CAESAREAN SUB-GROUPS:				
NUMBER OF FEMALES:				
Paired	55	55	55	55
Failed to mate	2	1	1	1
Inseminated	53	54	54	54
Pregnant	51	51	50	48
Not pregnant	2	3	4	6
Aborted	0	0	0	1
With viable fetuses at caesarean section	21	22	23	19
No viable fetuses at caesarean section	2	0	0	0
Pregnant females allowed to litter	28	29	27	28
Total litter death *post-partum*	0	2	0	2
Total litter resorption	0	0	0	1
Elective sacrifice	0	0	1	0
Mistimed pregnancy	0	0	0	1
Reared pups to weaning	28	27	26	24
PRE - COITAL INTERVAL - DAYS				
MEAN	1.62	1.52	1.46	1.74
S.D.	1.30	1.48	1.46	1.37
N	53	54	54	53
COPULATION INDEX (%)	96	98	98	98
FERTILITY INDEX (%)	96	94	93	89
LACTATION INDEX (%)	100	93	96	86

Table 64: *Summary of cohabitation data and maternal performance (study AB14898) in the littering and caesarean sub-group*

All paired females mated with the exception of 2, 1, 1 and 1 in the control, 20, 100 and 200 µg/mL QS-21 groups, respectively. The majority of animals mated on the first day of pairing and the mean pre-coital interval was consequently comparable in all groups.
Most mated females became pregnant with the exception of 2, 3, 4 and 6 in the control, 20, 100 and 200 µg/mL QS-21 groups, respectively. The slightly higher incidence of non-pregnant females in the group given 200 µg/mL QS-21 was due to 2 of the mating males (nos. 28 and 30) that between them failed to induce pregnancy in 5 females. The fertility index was consequently

incidentally slightly lower (89 %) in the high dose group compared with the control (96 %), without statistical significance. .

One female given 100 µg/mL QS-21 was prematurely sacrificed for ethical reasons on day 18 of gestation but was pregnant. One female given 200 µg/mL QS-21 for which mating was not detected (mistimed date of mating) was prematurely sacrificed, as soon as possible after the end of the mating period, while pregnant. For both females, fetuses were not submitted to examination due to their small size. One female given 200 µg/mL QS-21 was prematurely sacrificed following abortion.

Maternal organ weights and terminal body weights:

There were no treatment-related effects on ovary weights from females in the caesarean or littering sub-groups. The ovary weights in the adjuvant groups were comparable with the concurrent control.

There were no treatment-related effects on gravid uterine weight in the caesarean sub-groups. One female given 200 µg/mL QS-21 had a lower gravid uterus weight (173.1 g, compared with a mean control value of 489.2 g), however, this female only had two fetuses. A dose-related lower net (minus uterine weight) mean body weight change was observed in the treated groups, compared with control, attaining statistical significance at the 200 µg/mL QS-21 dose level. This is a reflection of the reduced maternal body weight gain and observed maternal toxicity in the high dose group.

	Control	**10 µg/admin***	**50 µg/admin***	**100 µg/admin***
Net mean body weight change (g)	515	511	458	296**
Mean gravid uterine weight (g)	489	526	491	440
Net weight change minus uterine weight (g)	26	-15	-33	-144**

Table 65: *Summary of gravid uterus weight and body weight change (g) (study AB14898): * each animal received 2 administration on each dosing day; **p<0.01; Net body weight change + terminal body weight minus day 0 body weight; net weight change = net body weight change minus uterine weight change*

Parameter	**F0 generation**			
	Control	**10 µg/admin***	**50 µg/admin***	**100 µg/admin***
Mating index (natural birth group)	96.7%	96.7%	96.7%	100.0%
Female Fertility Index (natural birth group)	96.6%	100.0%	93.1%	93.3%
Gestation Index (natural birth group)	100.0%	100.0%	96.3%	92.9%
Gestation Length (natural birth group)	31.5 days	31.4 days	31.6 days	31.3 days
Females completing delivery % (N)	93.3% (28)	96.7% (29)	86.7% (26)	86.7% (26)

Table 66: *Reproductive parameters (study AB14898), natural birth group, ND: Not Determined; * each animal received 2 administration on each dosing day*

Results F1: Caesarean data:

Parameter	F0 generation			
	Control	**10 µg/admin***	**50 µg/admin***	**100 µg/admin***
Pregnant dams with no viable fetuses	2	0	0	0
Pregnant dams with viable fetuses	21	22	23	19
Corpora lutea per animal	9.9	10.3	10.2	9.8
Implantation sites per animal	8.4	9.0	8.6	8.9
Preimplantation loss per animal (% per animal)	1.5 (17.2%)	1.4 (12.5%)	1.7 (14.4%)	0.9 (9.0%)
Live fetuses per animal (% males/% female)	7.7 (47.0/53.0)	8.5 (43.4/56.7)	8.4 (48.2/51.8)	8.1 (51.5/48.5)
Postimplantation loss	15	11	3	16
Dead fetuses	0	0	0	0
Resorption: early	10	2	1	11
Resorption: late	5	9	2	5
Fetal body weight	39.8	41.8	40.1	36.2**

Table 67: *Summary of caesarean section data (study AB14898): * each animal received 2 administration on each dosing day;*
***p<0.05;*

There were 23, 22, 23 and 19 pregnant females in the control, 20, 100 and 200 µg/mL QS-21 groups, respectively, at the terminal caesarean examinations. All pregnant females had viable fetuses, with the exception of two control females.
One other pregnant female in the 200 µg/mL QS-21 group aborted earlier in gestation. This female had 13 implantation sites but no live fetus. This finding could be a background finding since abortion can be noted in the historical control data (2/25 pregnant females aborted and 1/21 pregnant females aborted in two different studies in 2010) or an consequence of maternal toxicity.

The pre-implantation data (mean numbers of corpora lutea, implantation sites and the percentage of pre-implantation loss) were comparable with the concurrent control and/or historical control range in all treated groups. The percentage pre-implantation loss was incidentally slightly higher in the control group (17.2 %) but remained within the historical control range (7.6 to 18.2 %).

The mean number of early resorptions was slightly higher in the 200 µg/mL QS-21 group (0.6 per female) in comparison with the mean historical control value (0.2) and the concurrent control value (0.4). However, the difference was essentially due to one atypical female within the group (no. 7565) with 8 early resorptions. It should be noted that 7 early resorptions were observed in one control female in one study performed in 2011 in the same laboratory. Consequently, this isolated finding was considered to be incidental. Mean live litter size was marginally higher in all treated groups compared with the control due to the incidentally high pre-implantation loss in the control group.

There was a slightly statistically significant lower mean fetal weight (36.2 g) in the 200 µg/mL QS-21 group (-9 %, compared with control 39.8 g; P<0.05) and historical control range (38.6 to 44.0 g).

Results F1 generation (natural birth group)

Reproductive parameters:

GENERATION		F_1 LITTER			
		Control	**10 µg/admin***	**50 µg/admin***	**100 µg/admin***
FEMALES ON STUDY	N	30	30	30	30
MATING INDEX	(%)	96.7	96.7	96.7	100.0
GESTATION INDEX	N	100.0	100.0	96.3	92.9
FEMALES COMPLETING DELIVERY	N (%)	28 (100)	29 (96.7)	26 (86.7)	26 (86.7)
FEMALES WITH STILLBORN PUPS	N (%)	8 (28.6)	7 (24.1)	5 (19.2)	4 (15.4)
LITTERS WITH LIVEBORN BUT NO PUPS ALIVE DAY4		0	2	0	1
DAY 35		0	2	0	2
MEAN DURATION OF GESTATION	days	31.5	31.4	31.6	31.3
LITTER SIZE					
Litters with liveborn pups	N MEAN	28	29	26	26
Liveborn Live Birth Index	N %	226 93.8	216 91.9	212 97.7	218 97.8
Stillborn	N %	15 (6.2)	19 (8.1)	5 (2.3)	5 (2.2)
Pups dying. Missing an/or cannibalized Day 0	N %	0 0.0	3 1.4	1 0.5	0 0.0
Day 1-7	N %	19 8.4	21 9.7	13 6.1	38* 17.4
Day 8-35	N %	13 5.8	14 6.5	16 7.5	10 4.6
Day 0-4	N %	5 2.2	17* 7.9	10 4.7	25** 11.5
Day 0-35	N %	32 14.2	38 17.6	30 14.2	48 22.0
Pups surviving 4 days **Viability Index**	N %	221 97.8	199** 92.1	202 95.3	193*** 88.5
Pups surviving 35 days **Lactation Index**	N %	194 87.8	178 89.4	182 90.1	170 88.1
LITTER WEIGHT IN G (♂/♀)					
Day 4	GRAM	83.0	83.3	83.5	77.1
Day 7	GRAM	111.7	111.7	113.4	108.8
Day 11	GRAM	115.4	155.4	155.7	152.1
Day 14	GRAM	185.2	183.8	189.8	182.9
Day 21	GRAM	256.2	267.4	276.4	266.9
Day 28	GRAM	454.1	178.0	479.6	472.1
Day 35	GRAM	752.9	744.3	771.1	753.9
SEX RATIO (M%)	Day 1	53.5	50%	49.1%	53.1%

Table 68: *reproductive parameter (study AB14898):* each animal received 2 administration on each dosing day; * each animal received 2 administration on each dosing day;, ** p<0.05; *** p<0.01*

There was no treatment-related effect on parturition and gestation length in any group. There were 28, 29, 26 and 26 females that completed delivery in the control, 20, 100 or 200 µg/mL QS-21 groups, respectively. The mean duration of gestation was comparable

(approximately 31 days) in the treated and control groups. The mean numbers of implantation sites and delivered pups were comparable in all groups. As a consequence, there was no influence of treatment in any group on pre-birth loss.

The total number of live pups (226, 216, 212 and 218 in the control, 20, 100 or 200 µg/mL QS-21 groups, respectively) was comparable in all groups and the corresponding live birth index (93.8, 91.9, 97.7 and 97.8 %, respectively) were slightly superior in the 100 and 200 µg/mL QS-21 groups due to fewer stillborn pups when compared with the control group (15, 19, 5 and 5 stillborn pups in the control, 20, 100 or 200 µg/mL QS-21 groups, respectively).

During the first week post-partum, there was a statistically significantly greater number of pups dying or missing in the 200 µg/mL QS-21 group, compared with the concurrent control (38 pups compared with 19 in the control group). This finding was due to two females (nos. 7451 and 7452) with total litter loss accounting for 16 of the affected pups. In the absence of an increase in pup death amongst the other females in the high dose group and since the pup viability index on PND 4 and the lactation index on PND 35 remained within the historical control range (84.7 % to 99.3 % for the viability index and 83.5 % to 93.7 % for the lactation index), no clear association with treatment was considered to have occurred. However, a treatment association can also not be completely excluded, especially since maternal toxicity was observed at this dose.
There was also a statistically significantly higher incidence of pups dying or missing in the 20 µg/mL QS-21 groups over the first four days post-partum, which was due to two females with total litter loss (i.e. female nos. 7386 and 7411).
The number of live pups per litter after PND 7 and through to PND 35 remained comparable between the treated groups and controls. Litter sizes in the treated groups were comparable with controls throughout lactation.

There was a slightly statistically significant lower mean fetal weight (36.2 g) in the 200 µg/mL QS-21 group (-9 %, compared with control 39.8 g; $P<0.05$) and historical control range (38.6 to 44.0 g). There was no effect of treatment on mean fetal weight in the lower dose groups.
There was no effect of treatment on fetal sex ratio in any dose group.

Fetal alterations: F_1 generation: caesaran data

			Control	**10 µg/admin ***	**50 µg/admin***	**100 µg/admin***
Litters evaluated		N	21	22	23	19
Fetuses evaluated		N	178	186	194	153
Fetal external observations						
Anasacra (M)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Cranial: acephalia (M)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Limbs: malrotated (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Fetal visceral observations						
Thymus large (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1

Ventricular Septum defect (M)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Carotid: Narrowed (M)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Great vessels: malformed (M)	Fetal incidence	N	0	1	0	**3**
	Litter incidence	N	0	1	0	3
Common Carotid trunk: absent (V)	Fetal incidence	N	96	106	117	85
	Litter incidence	N	20	20	23	19
Innominate: absent	Fetal incidence	N	0	0	0	**2**
	Litter incidence	N	0	0	0	2
Lung: azygos love absent (V)	Fetal incidence	N	3	1	7	3
	Litter incidence	N	3	1	4	3
Lung: small	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Diaphragmatic hernia (M)	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Liver: dark raised area (A)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Liver: discolored (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Liver: additional fissure (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Liver: supernumerary lobe (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Liver: pale area (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Kidney: malposition	Fetal incidence	N	0	1	0	1
	Litter incidence	N	0	1	0	1
Kidney: malformed	Fetal incidence	N	0	0	0	2
	Litter incidence	N	0	0	0	0
Kidney: dilated renal pelvis, slight (A)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Kidney: pale (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Ovary: cyst (A)	Fetal incidence	N	4	0	1	1
	Litter incidence	N	3	0	1	1
Eyes: discolored (A)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Fetal skeletal observations						
Phalanx: unossified 1st or 5th digit, forepaw (V)	Fetal incidence	N	9	19	16	24
	Litter incidence	N	8	6	8	11
Phalanx: incomplete ossification, forepaw (V)	Fetal incidence	N	0	0	0	3
	Litter incidence	N	0	0	0	3
Metacarpal: unossified 1st digit, (A)	Fetal incidence	N	8	4	6	9
	Litter incidence	N	5	2	4	6
Phalanx: incomplete ossification, hindpaw (V)	Fetal incidence	N	1	1	1	3
	Litter incidence	N	1	1	1	2
Phalanx: unossified hindpaw (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Tarsal bone: incomplete ossification (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Sternebra: incomplete ossification (V)	Fetal incidence	N	19	25	12	14
	Litter incidence	N	9	12	9	9

Sternebra: 5th unossified (V)	Fetal incidence	N	18	34	33	15
	Litter incidence	N	9	15	16	9
Sternebra: bibartite ossification (V)	Fetal incidence	N	3	2	1	3
	Litter incidence	N	3	2	1	2
Sternebra: incomplete ossification of 2nd/4th (V)	Fetal incidence	N	16	3	1	11
	Litter incidence	N	4	3	1	6
Sternebra: minor fusion (V)	Fetal incidence	N	2	0	0	0
	Litter incidence	N	2	0	0	0
Sternebra: asymmetric (V)	Fetal incidence	N	3	1	1	0
	Litter incidence	N	1	1	1	0
Sternebra: 6th unossified (V)	Fetal incidence	N	5	2	2	3
	Litter incidence	N	3	2	2	2
Sternebra: 2nd/4th unossified (V)	Fetal incidence	N	1	0	0	1
	Litter incidence	N	1	0	0	1
Sternebra: extra ossification site (V)	Fetal incidence	N	1	0	1	0
	Litter incidence	N	1	0	1	0
Sternebra: incomplete ossification of 1st and 3rd (V)	Fetal incidence	N	4	0	0	0
	Litter incidence	N	2	0	0	0
Number of full ribs 12/13 (V)	Fetal incidence	N	37	25	40	11
	Litter incidence	N	17	15	18	7
Rib: short (A)	Fetal incidence	N	48	55	59	33
	Litter incidence	N	18	19	20	14
Rib: detached (A)	Fetal incidence	N	6	8	5	6
	Litter incidence	N	5	5	2	6
Number of full ribs 12/12 (V)	Fetal incidence	N	62	77	49	45
	Litter incidence	N	16	22	18	11
Rib: incomplete ossification (A)	Fetal incidence	N	8	6	5	6
	Litter incidence	N	5	6	5	5
Rib: thickened (A)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Rib: absent (A)	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Rib: unossified area (A)	Fetal incidence	N	0	1	1	1
	Litter incidence	N	0	1	1	1
Rib: cervical (A)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Cervical vertebra supernumerary (A)	Fetal incidence	N	3	0	1	0
	Litter incidence	N	3	0	1	0
Cervical vertebra: incomplete ossification (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Thoracic vertebra: incomplete ossification (A)	Fetal incidence	N	0	1	0	1
	Litter incidence	N	0	1	0	1
Thoracic vertebra: malformed (M)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Lumbar vertebra: misshaped arch (A)	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Lumbar vertebra: number 7 (A)	Fetal incidence	N	2	0	1	4
	Litter incidence	N	2	0	1	3
Lumbar vertebra: number 5 (A)	Fetal incidence	N	1	2	1	0
	Litter incidence	N	1	1	1	0
Lumbar vertebra: bipartite ossification (A)	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Caudal vertebra fused (A)	Fetal incidence	N	0	0	1	0

	Litter incidence	N	0	0	1	0
Caudal vertebra misshapes (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Pelvis: incomplete ossification of pubis (A)	Fetal incidence	N	2	0	2	10
	Litter incidence	N	2	0	2	5
Pelvis: unossified pubis (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Arcania (M)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Cranium: parietal: uunossified area (A)	Fetal incidence	N	2	1	0	0
	Litter incidence	N	2	1	0	0
Cranium: frontal: fused (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Cranium: frontal: structural bone (A)	Fetal incidence	N	0	1	1	0
	Litter incidence	N	0	1	1	0
Nasal: structural bone (A)	Fetal incidence	N	0	1	0	2
	Litter incidence	N	0	1	0	2
Mandibular: hyoid: incomplete ossification (V)	Fetal incidence	N	5	6	0	5
	Litter incidence	N	4	6	0	4
Maxilla: incomplete ossification (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1

Table 69: *Fetal alterations (study AB14898): M-Malformation, V-Variation, A-Anomaly,*

Dose level of QS21 (µg/mL)	Female number	Male number	Fetus number	Malformation(s)#
0	7477	5	6	Diaphragmatic hernia
20	7501	13	1	Malformed thoracic vertebrae
	7504	15	5	Ventricular septum defect, malformed great vessels
	7515	10	7	Malpositioned kidney (left)
100	/		/	/
200	7551	25	6	Acephaly/acrania, narrowed carotid arteries
	7553	27	10	Malformed great vessels
	7557	31	2	Malpositioned kidneys (both), malformed kidney (right)
			8	Malpositioned kidney (right)
	7558	29	8	Malformed great vessels
	7566	25	2	Anasarca
	7574	31	1	Malformed great vessels Fused, malformed and malpositioned kidneys (both)

Figure 4: *Summary of malformations (study AB14898) - Individual descriptions; # including external, visceral and skeletal examinations, /: no malformation observed*

External observations:

There were two malformed fetuses from separate litters in the 200 µg/mL QS-21 group. One fetus had acephaly and the other had anasarca. There were no external malformations in the lower dose groups. These malformations were considered to be not treatment-related in view of the isolated nature of these findings (0.7 %, 1/153 fetuses) and since they are part of the

background of changes noted in the strain of rabbit used (1 fetus had acrania and 1 fetus had local edema between 2007 and 2012).

Further, one fetus from the 200 μg/mL QS-21 group had malrotated hindlimbs. This anomaly was considered to be not treatment-related in view of the isolated nature of this finding restricted to a single litter and since it is part of the background of changes noted in the strain of rabbit used (5 fetuses between 2007 and 2012).

Visceral observations:

There were 1 (1), 2 (2), 0 (0) and 6 (5) fetuses (litters) with visceral malformations in the control, 20, 100 and 200 μg/mL QS-21 groups, respectively.

Common findings included three fetuses from separate litters with malformed great blood vessels with defects of the aortic arch (retro or high arched) in the 200 μg/mL QS-21 group (female nos. 7553, 7558 and 7574) in comparison with none in the control and intermediate dose groups. One fetus from female no. 7504 in the 20 μg/mL QS-21 group also had malformed great blood vessels associated with persistent truncus arteriosus with pulmonary arteries arising from the descending aorta, and a ventricular septal defect. Two fetuses in the 200 μg/mL QS-21 group had malformed kidneys (one fetus from female no. 7557 had small right kidney and one fetus from female no. 7574 had fused kidneys on midline).
Other findings amongst the treated groups included malpositioned kidneys for three fetuses in the 200 μg/mL QS-21 group (two fetuses from female no. 7557 and one fetus from female no. 7574) and one fetus (female no. 7515) in the 20 μg/mL QS-21 group and narrowed carotid arteries in another fetus in the 200 μg/mL QS-21 group (female no. 7551). One control fetus had a diaphragmatic hernia (female no.7477). These findings are isolated, part of the background of changes noted in the strain of rabbit used and were considered to be spontaneous in origin (historical control data: mal positioned kidneys and diaphragmatic hernia were observed in 2/1699 fetuses (0.12 %) between 2010 and 2012, 1/3915 fetuses (0.03 %) between 2007 and 2009, respectively).

There were no fetuses with visceral malformations in the 100 μg/mL QS-21 group.

The incidences of other less severe soft tissue anomalies/variations, including absent common carotid trunk, absent innominate artery, absent azygos lobe of the lungs, small lungs, dark or pale areas/additional fissure/supernumerary lobe in the liver, dilated renal pelvis, pale kidneys, ovarian cystic areas and/or discoloured eyes did not suggest any association with treatment.

Skeletal observations:

There was no treatment-related effect on fetal skeletal development.

Skeletal malformations were observed in one fetus in each of the 20 and 200 μg/mL QS-21 groups. The fetus in the 20 μg/mL QS-21 group (female no. 7501) had malformed thoracic vertebrae with associated scoliosis and the fetus in the 200 μg/mL QS-21 (female no. 7551) had acrania. These findings are part of the background of changes noted in the strain of rabbit used (6 fetuses between 2010 and 2012 with a malformed thoracic vertebrae, 2 fetuses between

2010 and 2012 with scoliosis and 1 fetus between 2007 and 2012 with acrania) and were considered to be incidental.
The incidence of fetuses with incomplete ossification of the pubis was slightly higher in the 200 µg/mL QS-21 group (6.5 %) than in the control group (1.1 %) and the historical control data (2.4 %). Since this anomaly is part of the background of changes noted in the strain of rabbit used and was considered to be spontaneous in origin, this delay in ossification was considered to be of no toxicological significance.

The incidence of other skeletal anomalies and variations in the treated groups were comparable with the concurrent control and/or historical control data and therefore did not suggest any association with treatment.

Pre-weaning examinations F1					
		Control	**10 µg/admin***	**50 µg/admin***	**100 µg/admin***
Incisor eruption	Day 4 (% positive of pups)	100%	100%	100%	100%
Fur growth	Day 4 (% positive of pups)	100%	100%	100%	100%
Pupil reflex	Day 35 (% positive of pups)	100%	100%	100%	100%
Auditory reflex	Day 35 (% positive of pups)	100%	100%	100%	100%
Eye opening	Day 8 (% positive of pups)	0%	0%	0%	0%
	Day 9 (% positive of pups)	0%	7%	5%	3%
	Day 10 (% positive of pups)	14%	33%	34%	27%
	Day 11 (% positive of pups)	50%	73%	76%	51%
	Day 12 (% positive of pups)	77%	92%	95%	82%
	Day 13 (% positive of pups)	96%	96%	99%	92%
	Day 14 (% positive of pups)	100%	100%	100%	95%
	Day 15 (% positive of pups)	100%	100%	100%	97%*

Table 70: *Summary of reflex and physical development (study AB14898), *less than 100% due to death of pups.*

Pup physical development, as assessed by the day of occurrence of incisor eruption, fur growth and eye opening, was similar in the control and treated groups. The evaluation of pup reflexes (surface righting, pupil and auditory responses) did not reveal any evidence of functional defects in the adjuvant groups.

Pub necropsy observations:

			Control	**10 µg/admin***	**50 µg/admin***	**100 µg/admin***
Litters evaluated		N	28	27	26	24
Fetuses Live evaluated		N	226	207	211	200
Fetuses Stillborn evaluated		N	15	10	5	5
Gross Exam: Omphaclocele	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Lung: dark area	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Kidney: malpositioned	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Kidney: small	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Kidney: abnormal shape	Fetal incidence	N	0	0	0	1

	Litter incidence	N	0	0	0	1
Dilated renal pelvis	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Abnormal cavity: autolysis	Fetal incidence	N	5	2	6	6
	Litter incidence	N	3	2	4	4
Abnormal cavity: clear fluid	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Urinary bladder dilatation	Fetal incidence	N	1	1	0	0
	Litter incidence	N	1	1	0	0
Abnormal cavity: cannibalized	Fetal incidence	N	0	2	0	4
	Litter incidence	N	0	2	0	3
Limb/paw: cannibalized	Fetal incidence	N	0	3	1	0
	Litter incidence	N	0	3	1	0
Limb/paw: hyperextension	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Uterus: enlarged	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Thoracic cavity: autolysis	Fetal incidence	N	4	2	5	7
	Litter incidence	N	3	2	3	4
Thoracic cavity: cannibalized	Fetal incidence	N	0	0	0	2
	Litter incidence	N	0	0	0	1
Tale: cannibalized	Fetal incidence	N	1	0	0	1
	Litter incidence	N	1	0	0	1

Neither the incidence nor type of pup observations noted suggested any association with treatment in any group. One pup from female no. 7409 given 20 µg/mL QS-21 was prematurely sacrificed on PND 0 due to an omphalocele with protusion of the intestines. Since this malformation was observed in only one pup at the low dose level and was not seen for any fetus in the caesarean sub-groups, this finding was considered to be incidental.
One pup from female 7448 given 200 µg/mL QS-21 was found dead on PND 4. At necropsy, a malpositioned, small and abnormally shaped kidney was observed. One pup from female 7457 given 200 µg/mL QS-21 was prematurely sacrificed on PND 6 and dilated renal pelvis with reduced papillae was observed at necropsy. These isolated findings were considered to be incidental.

Conclusions

Intramuscular administrations of DQ adjuvant containing 200 µg/mL of QS-21 to (b) (4) rabbits starting 28 and 14 days before the start of mating and on gestation days 3, 8, 11, 15 and 24 and on day 7 of lactation induced a significant maternal mean body weight loss associated with reduced mean food consumption at the end of the gestation period. In addition lower mean fetal weight was noted at this dose level. Defects of the aortic arch (retro or high arched) were observed in three fetuses from separate litters suggestive of a possible association with treatment at this dose. Doses of DQ adjuvant containing 100 or 20 µg/mL of QS-21 did not induce any adverse effects on maternal condition or embryo-fetal and post-natal development. Under the defined experimental conditions of the study, the dose level of DQ adjuvant containing 100 µg/mL of QS-21 (corresponding to 30 µg of QS-21/kg body weight considering a mean body weight of 3.33 kg for female rabbits) was selected as the NOAEL (No Observed Adverse Effect Level) for maternal, pre- and post-natal toxicity.

GENOTOXICOLOGY STUDIES:

Genotoxicology studies: in vivo

Reviewer: Nabil Al-Humadi

Study # 1: Comparison of Different Test Formulations in the Rat Micronucleus Test. Genetic Toxicology (Micronucleus Test) (Reviewed by Nabil in IND 13857)

Study title: Comparison of different test formulations in the rat micronucleus test.
Study no.: (b) (4) 317/032657
Conducting laboratory and location: (b) (4)
Date of study initiation: March 04, 2003.
Date of study completion: June 05, 2003.
GLP compliance: Yes
Drug, lot #, and % purity:

IV1, (b) (4)). Stable for 15 days.
IM1, (b) (4) end of filling (pool).
IM2, (b) (4) Stable for 15 days.
IM3, (b) (4) end of filling (pool).

Strains/species/cell line: (b) (4) (CD) Rat.
Breeder/supplier: (b) (4) .
Number of animal per group and sex: 5/sex/group
Age: Not provided.
Body weight range: 120-150 g males, 120-140 g females
Route, site, and frequency of administration:

Animals in the vehicle and IV1 groups were dosed by intravenous injection at a volume dosage of 0.5 mL.
Animals in IM1, IM2, and IM3 groups were dosed by intramuscular injection at a volume dosage of 0.1 mL in each hind limb. Dosing was administered on two occasions, the second dose administered approximately 24 hours after the first dose.
No positive control was used.

Volume of injection: For vehicle and IV1 groups 0.5 mL/day, and for IM1, IM2, and IM3 groups 0.2 mL/day.
Methods
Study design:

Toxicity Test 1: used to determine the suitable dose level for use in the micronucleus test.

Group Number	Description	Dose Route	Dosage Volume* (mL/day)	Dose Concentration (µg/animal)	Animal Numbers	
					Male	Female
1	Vehicle (Saline)	Intravenous	0.5	0	5	5
2	IV1	Intravenous	0.5	1.05	5	5
3	IM1	Intramuscular	0.2[a]	0.66	5	5

Group Number	Description	Dose Route	Dosage Volume* (mL/day)	Dose Concentration (µg/animal)	Animal Numbers	
					Male	Female
4	IM2	Intramuscular	0.2 [a]	8.92	5	5
5	IM3	Intramuscular	0.2 [a]	0.50	5	5

[*] Constant volume irrespective of individual bodyweight.
[a] 0.1 mL in each hind limb
Table 71: *Experimental design* (b) (4) *study #* (b) (4) *317/032657)*

Doses used: (b) (4) CD rats in the vehicle control group (sterile 0.9% w/v physiological saline) and IV1 treatment group were dosed by intravenous injection at a volume dosage of 0.5 ml. The aim of the study was to evaluate the possible risk of a contamination with (b) (4) Concentration levels of this compound were measured in the batches used in the micronucleus test and the compound was found to be stable for 15 days in the concentration range between 1.5 and 42.4 µg/ml. Animals in the IM1, IM2 and IM3 groups were dosed by intramuscular injection at a volume dosage of 0.1 ml in each hind limb. Dosing was administered on two occasions, the second dose administered approximately 24 hours after the first dose. All animals were sacrificed approximately 24 hours after the second treatment.

Positive controls: No positive control was used.

Sample preparations: The femurs were cleared of tissue and the proximal epiphysis removed from each bone. The bone marrow of both femurs from each animal was flushed out. Six bone marrow smears from each animal were prepared and examined microscopically. The (b) (4) smears were examined by (b) (4) to determine the incidence of micronucleated cells per 2000 polychromatic erythrocytes per animal. One smear per animal was examined and the remaining smears were held temporarily in reserve in case of technical problems with the first smear.

Results

Study validity: The study met all criteria for validity. Five animals/sex/group was used for bone marrow analyses. Up to 2000 immature erythrocytes were scored for the presence of micronuclei for each animal. The proportion of immature erythrocytes was assessed by examination of a total of at least 1000 erythrocytes per animal and the number of micronucleated mature erythrocytes was recorded.

A positive response is normally indicated by a statistically significant increase in the incidence of micronucleated immature erythrocytes for the treatment group compared with the vehicle control group ($P<0.01$); individual and/or group mean values should exceed the laboratory historical control range.

A negative result is indicated where individual and group mean incidences of micronucleated immature erythrocytes for the group treated with the test substance are not significantly greater than incidences for the vehicle control group ($P>0.01$) and where these values fall within the historical control range.

An equivocal response is obtained when the results do not meet the criteria specified for a positive or negative response. Bone marrow cell toxicity (or depression) is normally indicated by a substantial and statistically significant decrease in the proportion of immature erythrocytes ($P<0.01$).

Study outcome: Following administration of the second treatment, animals treated with IM3 showed clinical signs including underactivity, raised gait and reluctant, slow use of hind limbs. No other adverse clinical signs were reported in the treated groups over the duration of the test. All animals had recovered by the time of scheduled termination.

During the slide preparation process, cell pellets from animals treated with IM3 formed gelatinous clumps after the addition of (b) (4) and did not form a homogenous cell suspension for smearing on to the microscope slides. Various methods were tried to break up the clumps (e.g. agitation, stirring) but without success. The clumping effect observed in the IM3 treated group was not observed in cells from any animal in the vehicle control or IV1, IM1 and IM2 treated groups, indicating the possibility of a treatment related effect. Slides were prepared from all animals and on examination, prior to coding, the smears prepared from animals treated with IM3 did not appear to show any morphological differences compared to the slides for the vehicle control group.

IV1, IM1 and IM2 did not show any evidence of causing chromosome damage or bone marrow cell toxicity in this in vivo test procedure. IM3 showed no evidence of causing micronuclei but did show evidence of causing bone marrow cell toxicity in this in vivo test procedure.

Sampling time after 2nd dose	Treatment	Dose volume (ml/day)	% ie/(ie+me) †	ncidence mie (mean)	ncidence mme (group mean)[b]
24 Hours	Vehicle	0.5	40	1.2	0.3
	V	0.5	37	1.7	0.3
	M	0.2a	38	0.4	0.3
	M2	0.2a	41	1 .0	0.3
	M3	0.2a	31***	0.3	0.3

Vehicle (b) (4)
a 0. ml in each hind limb
% ie/(ie+me) Proportion of immature erythrocytes
ie Immature erythrocytes
mie Number of micronucleated cells observed per 2000 immature erythrocytes examined
me Mature erythrocytes
mme Number of micronucleated cells calculated per 2000 mature erythrocytes

Results of statistical analysis using the appropriate nonparametric method of analysis based on permutation (one-sided probabilities):

*** $P < 0.001$ (highly significant)
Otherwise $P > 0.01$ (not significant)

† Occasional apparent errors of ± % may occur due to rounding of values for presentation in the table

[b] Formula for calculation of incidence mme (group mean):

Sum of group incidence mme scored x 2000/Sum of group me scored

Test article related effects and assessments:

IV1, IM1 and IM2 did not show any evidence of causing chromosome damage or bone marrow cell toxicity in this in vivo test procedure. IM3 showed no evidence of causing micronuclei but did show evidence of causing bone marrow cell toxicity in this in vivo test procedure.

Study # 2: Assessment of Effect on Blood Cells and Bone Marrow Following Intramuscular Administration to CD Rats. Genetic Toxicology (Bone Marrow and Blood Cells Assessment) (Reviewed by Nabil in IND 13857)

Study title: Assessment of effect on blood cells and bone marrow following intramuscular administration to CD rats.

Purpose of the study: To investigate the effect of adjuvant AS01B on erythroid cell production and maturation in the bone marrow seen in a previous study.

Key findings: The ability of the bone marrow to produce red blood cells in male rats was not affected by the treatment, for 1 or 2 days, with the adjuvant AS01B. A decrease in the proportion of immature erythrocytes in the bone marrow and some cell clumping during preparation of the smear was identified in the previous study. No decrease in the proportion of immature erythrocytes was apparent in this study; however, cell clumping was seen on day 3 of study for the majority of animals treated with AS01B.

Study no.: (b) (4) 681/043748
Conducting laboratory and location: (b) (4)
Date of study initiation: October 27, 2004
Date of study completion: March 23, 2005.
GLP compliance: Yes
QA reports: Yes (x) No ( )
Drug, lot #, and % purity: AS01B, Batch No. (b) (4), No purity reported.
Strains/species: (b) (4):CD® (b) (4) (b) (4) rats
Breeder/supplier: (b) (4).
Number of animal per group and sex: 10/group
Age: Not provided.
Body weight range: 132-146 g males
Route, site, and frequency of administration:

Each animal received 0.2 mL/rat/day, this was administered as one injection of 0.1 mL into each anterior thigh muscle.
No positive control was used.

Volume of injection: For vehicle and AS01B adjuvant 0.2 mL/rat/day.

Methods

Study design

Group Number	Description	Days of Dosing	Animal Numbers Male	Dosage Concentration (µg/rat/day)	Dosage Volume (mL/rat/day)	Days of kill
1	Vehicle (Saline)	1, 2	10	0	0.2 [a]	3
2	Vehicle (Saline)	1	10	0	0.2 [a]	13
3	AS01B adjuvant	1, 2	10	20	0.2 [a]	3
4	AS01B adjuvant	1, 2	10	20	0.2 [a]	13
5	AS01B adjuvant	1	10	20	0.2 [a]	3
6	AS01B adjuvant	1	10	20	0.2 [a]	13

[a] 0.1 mL in each anterior thigh muscle

Table 72: *Experimental design* (b) (4) *study #* (b) (4) *681/043748)*

Doses used in definitive study: 0.2 mL/rat/day.
Compostition per vial (0.5 mL):
KH2PO4: 41 mM
Na2HPO4: 9mM
NaCl: 100mM

Dose used and sample collections: Treatment groups, of ten male rats, were treated with 0.2 mL/rat/day on either day 1 or on days 1 and 2. The rats were observed for three or thirteen days for treatment-related deaths, clinical signs (twice daily), and body weights (week -1 and days 1, 3, 6, 9, 12 and before necropsy). Blood samples were collected from the sublingual vein on days 3, 6, 9, and 12 of study for hematology assessment. The following were measured: hematocrit (Hct), hemoglobin conc. (Hb). mean corp. Hb (MCH), mean corp. Hb conc. (MCHC), mean corp. volume (MCV), total erythrocyte count (RBC), total white cell count (WBC), differential WBC count (neutrophils, lymphocytes, eosinophils, basophils, monocytes and large unstained cells), and platelet count (Plt). Bone marrow samples were obtained, on either day 3 or day 13, from the tibia of all animals and smears were prepared from these samples. All animals were subject to a detailed necropsy.

Negative controls: No negative control was used
Positive controls: No positive control was used

Results

There were no treatment-related clinical signs recorded. There were significant decrease in body weight of animals treated with AS01B on day 1 only or on days 1 and 2 (56 and 68% of group 2 for groups 5 and 6, respectively, and 19 and 31% of group 1 for groups 3 and 4). Thus, effect was substantially greater in animals dosed twice in comparison with those dosed once.
Low hematocrit, hemoglobin concentration, mean cell volume and mean cell hemoglobin and abnormal red cells forms apparent but no effect on red cell numbers were reported in the treated animals (day 3/6). Differences were minimal but there was evidence that the degree of these differences were dose related (2 doses > 1 dose). As the post-dose period progressed, these effects lessened and some evidence of a response to low oxygen carrying capacity was apparent as marginally higher reticulocyte counts and macrocytosis. This may reflect early release of immature red cells into the circulation. Pro-erythrocyte counts were slightly high and no treatment-related differences in the normocyte counts were reported in the bone marrow. This suggests the early stages of up-regulation of red cell production and indicates that AS01B does not impair the ability of the body to produce red cells. A direct effect on AS01B on circulating red cells could be occurring and interaction of AS01B with red cell membranes may be the cause of the cell clumping observed during preparation of blood smears on day 3. The recovery of hemoglobin concentration by day 12 and the absence of cell clumping in the smears produced on day 13 indicate this is a short-term effect. Total white cell counts were increased (day 6 onwards) due to increases in neutrophil and lymphocyte counts.

Test article related effects and assessments:
AS01B does not affect the ability of the bone marrow to produce red blood cells in male rats after 1 or 2 days treatment.

Study # 3: MPL (b) (4)) Rat Micronucleus Test (Reviewed by Steve Kunder in 2009)
Test article: 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL)
Test for induction of chromosome damage, aneuploidy
Treatment schedule: 2 doses/24 hr
Study No. (b) (4) 730/052198
Species/Strains: CD Rats. **Sampling time:** 24h post dose 2. **Location:** (b) (4)
Animal weight: 162-180 g. **Method of administration:** intramuscular.
Cells evaluated: Bone marrow immature erythrocytes. **Vehicle formulation:** Saline solution **GLP Compliance:** Yes.
Results

No of cells analyzed/animal: 2000 **Date of dosing:** Dec 2004 **Test article**	**Dose**	**Proportion immature erythrocytes (%)**	**Incidence of No of micronucleated cells / 2000 immature erythrocytes (mean)**
Saline	0.2 ml	44 %	2.8
MPL (1.05 mg/ml)	0.2 ml (approx 0.4mg/kg)	41%	2.8
Cyclophosphamide (2 mg/ml)	20 mg/kg	37%	35.1***

*** P < 0.001. Note: the MPL dose used in this study compares to the dose used in toxicology studies of about 0.1 mg/kg in rats
Table 73: *Erythrocytes results* (b) (4) *study #* (b) (4) *730/052198)*

MPL (b) (4)) did not cause any statistically significant increases in the number of micronucleated immature erythrocytes when compared to vehicle control values. MPL (b) (4) did not cause any substantial increases in the incidence of micronucleated mature erythrocytes. MPL was not positive in the rat micronucleus assay.

Study # 4: An Assessment of the Effects of AS01B on Red Blood Cells in Peripheral Blood and Bone Marrow (study number GVB0026/070209). Genetic Toxicology (Reviewed by Nabil).

Purpose of the study: To investigate the effect of adjuvant AS01B on red blood cells in peripheral blood and bone marrow.

Key findings: In rats, AS01B did not cause any, reproducible, effect on the proportion immature erythrocytes in bone marrow and no effect on erythrocyte count in peripheral blood was reported. Rabbits in six repeated dose studies produced marginal reductions in hemoglobin, hematocrit, and red cell count which were inconsistent with respect to time after dose administration, but did occur with some reproducibility between the studies. No evidence of AS01B effect on cell counts in the bone marrow in rabbits was reported.

Study no.: GVB0026/070209
Conducting laboratory and location: (b) (4)
Date of first study: June 06, 2003
Date of study completion: January 25, 2008
GLP compliance: Yes
QA reports: Yes (x) No ( )
Drug, lot #, and % purity: AS01B (contains 100 µg/mL monophosphoryl lipid A (MPL), a non-toxic derivative of lipopolysaccharide (LPS), 100µg/mL QS- 21, a purified form of saponin and 500 µg/mL cholesterol in a liposomal formulation)

Introduction: A rat micronucleus study (study 1 below) on AS01B resulted in a statistically significant reduction in the percentage of immature erythrocytes in the bone marrow. To further investigate this, a more extensive rat study (study 2 below) was performed which assessed red cells in bone marrow and peripheral blood. Six repeated dose rabbit studies have been performed using AS01B and various novel vaccine antigens, during which red cell indices were assessed. The objective of this report is to summarize the effects of AS01B on red blood cells in peripheral blood and bone marrow in eight studies, reported between 2003 and 2007, and to assess the toxicological significance of those effects.

Study summaries:
1- Comparison of Different Test Formulations in the Rat Micronucleus Test. (b) (4) Report Number (b) (4) 317/032657, 6 June 2003.

A group of 5 male and 5 female (b) (4) rats was treated with AS01B by intramuscular injection into both hind limbs on two occasions, one day apart. They were given a dose volume of 200 µL (100µL/limb) of the formulation and thus received 20 µg MPL, 20 µg QS-21 and 100 µg cholesterol. Control group was treated with 0.9% saline intravenously at 500 µL/rat (as a control specifically for one of the other adjuvants administered).

Bone marrow smears were prepared and examined for the presence of micronuclei.

During the slide preparation process, cell pellets from animals treated with AS01B formed gelatinous clumps after the addition of fetal calf serum and did not form a homogenous cell suspension for preparation of smears for microscopic evaluation. Various methods were tried to break up the clumps (e.g. agitation, stirring) but without success. This clumping effect was not reported in cells from any animal in either the vehicle control or other adjuvants assessed.

No evidence of micronuclei formation was reported. However, reduction in the proportion of immature erythrocytes in rats treated with AS01B was reported. The mean percentage of immature erythrocytes decreased from 40% in the controls to 31% in the AS01B treated group ($p<0.001$). This shows that the values for AS01B treated rats are the lowest in comparison with the controls and the other three adjuvant groups assessed (IV1, IM1 and IM2). This is an indication of bone marrow cell toxicity.

2- AS01B Assessment of Effect on Blood Cells and Bone Marrow Following Intramuscular Administration to CD Rats. (b) (4) Report Number (b) (4) 681/043748. 23 March 2005.

Groups of 10 male (b) (4) rats were treated with AS01B by intramuscular injection into both hind limbs on one or two occasions and euthanized 1 or 12 days after the last dose. They were treated with a dose volume of 200 µL of the formulation and thus received 20 µg MPL, 20 µg QS-21 and 100 µg cholesterol per dose.

Hematological investigations were performed on days 3, 6, 9 and 12, that is 2, 5, 8 and 11 days after one dose or 1, 4, 7 and 10 days after two daily doses.

Hematocrit and hemoglobin concentration were reduced from day 3 to between 0.94 and 0.97X control with recovery on days 9 or 12. Red blood cell count was not affected by treatment. Consequently, mean cell hemoglobin and mean cell volume were low. Abnormalities (hypochromasia from day 3 and macrocytosis and anisocytosis from day 6) were reported. These abnormalities were not recovering by day 12. Minimal evidence of increase in reticulocytes was reported on days 9 and 12.

Bone myelograms were assessed on days 3 or 13 (two or twelve days after one dose or one or eleven days after the second dose) for proportion (percentage) of each cell type.

No treatment-related effects were reported on erythroid series cell lines in rats euthanized on day 3. Statistically significantly higher percentage pro-erythrocytes were reported in rats euthanized on day 13 (dosed once or twice). Lower percentage late normoblasts (dosed twice [$P<0.05$ or $P<0.01$]) was also reported.

In marrow smears, no decreases in proportion of immature erythrocytes were reported. However, cell clumping during the preparation of the slides from rats euthanized on day 3 was reported for all rats that had received one or two doses of AS01B. This effect was not reported in slides prepared from rats euthanized on day 13.

3- RTS,S/AS01B Versus RTS,S/AS02V Malaria Candidate V[redacted]y Repeated (4 times) Intramuscular Administration to Rabbits. [redacted] Report Number [redacted]033/022086, 15 April 2005.

Two groups of 10 male and 10 female [redacted] rabbits were treated with the malaria vaccine RTS.S/AS01B by intramuscular injection into the gastrocnemius muscles of each hind limb in turn on four occasions 14 days apart. The formulation administered contained 100 µg/ml RTS.S antigen, in AS01B. The low dose group was given a dose volume of 125 µL and the high dose group 500 µL of the formulation. The low dose rabbits thus received 12.5 µg MPL, 12.5 µg QS-21 and 62.5 µg cholesterol per dose. The high dose rabbits received 50 µg MPL, 50 µg QS-21 and 250 µg cholesterol per dose. Five rabbits per sex per group were euthanized 3 or 28 days after the last dose.

Only data for rabbits given 500 µL has been assessed for comparability with other studies below. No test article-related effect on red blood cell count, hemoglobin, hematocrit or reticulocytes, assessed by routine hematology, was reported. No test article-related effect on bone marrow, assessed histopathologically, was reported. There were no statistically significant differences from control in these parameters at any time point. Mean values were very similar to control (generally no lower than 0.96X control). However, there were some occasions when the degree of difference from control (0.94-0.95X) was similar to that described in rabbit studies below as being test article related when statistically significant. These were; hematocrit for males 1 day after the first dose; hemoglobin for females 1 day after the first dose and 28 days after the fourth dose; and red cell count for females 1 day after the first dose.

4- AS01B Versus AS02V Toxicity Study by Repeated (5 times) Intramuscular Administration to Rabbits. [redacted] Report Number [redacted]045/022412, 15 December 2006

Group of 10 male and 10 female [redacted] rabbits was treated with AS01B by intramuscular injection into the gastrocnemius muscles of each hind limb over five occasions 14 days apart. They were given a dose volume of 500 µL of the formulation and thus received 50 µg MPL, 50 µg QS-21 and 250 µg cholesterol. Five rabbits per sex were euthanized 3 or 28 days after the last dose. Control group treated with 0.9% saline was also included and were euthanized at the same times.

No test article-related effect on red blood cell count, haemoglobin, haematocrit, or reticulocytes was reported. No test article-related effect on bone marrow, assessed histopathologically, was reported. There were no statistically significant differences from control in these parameters at any time point. Mean values were very similar to control (generally no lower than 0.96X control). However, there were some occasions when the degree of difference from control (0.91-0.95X) was similar to that described in rabbit studies below as being test article related when statistically significant.

5- [redacted] /AS01B Versus [redacted] /AS02V Toxicity Study by Repeated (5 times) Intramuscular Administration to Rabbits. [redacted] Report Number [redacted]049/022268, 4 January 2007.

Group of 10 male and 10 female (b) (4) rabbits were treated with HIV vaccine (b) (4) in combination with AS01B by intramuscular injection into the gastrocnemius muscles of each hind limb on five occasions 14 days apart. The formulation administered contained 200 µg/ml (b) (4) antigen and 40 µg/ml (b) (4) antigen, in AS01B. The rabbits were given a dose volume of 500 µL of formulation and thus received 50 µg MPL, 50 µg QS- 21 and 250 µg cholesterol per dose. Five rabbits per sex per group were euthanized 3 or 28 days after the last dose. A similarly sized control group was given 0.9% saline at the same dose volume and euthanized at the same times.

No test article-related effect on red blood cell count, hemoglobin, hematocrit, or reticulocytes was reported. No test article-related effect on bone marrow, assessed histopathologically, was reported. The only statistically significant differences from control in these parameters were $P<0.05$ for hemoglobin in males 1 and 3 days after the first dose and 3 and 28 days after the last dose.

6- Repeated-dose Toxicity Study with (b) (4) ASCI Candidates ((b) (4)/AS01B and (b) (4)/AS15) Administered Intramuscularly (Seven times) to Male and Female Rabbits. (b) (4) Report Number V7464, 7 November 2007

Group of 10 male and 10 female (b) (4) rabbits were treated with AS01B by intramuscular injection into the muscles of each hind limb on seven occasions 14 days apart. They were given a dose volume of 500 µL of the formulation and thus received 50 µg MPL and 50 µg QS-21 in a liposomal formulation per dose. Five rabbits per sex per group (including control group) were euthanized 3 or 28 days after the last dose.

Red cell count, hemoglobin, and hematocrit levels were reduced in male rabbits only. This reduction was one and three days after administration of the first dose (typically 0.95X control) with statistical significance attained ($p<0.05$ or $p<0.01$). There were no effects of similar magnitude at other time points in males or at any time point in females. There were no effects on reticulocyte proportions. There were no effects on bone marrow assessed histopathologically.

7- Repeat Dose Toxicity Study with HIV Candidate Vaccines (HIV(b) (4) versus HIV-(b) (4)/ASo1B) Administered Intramuscularly (Four times) to Male and Female Rabbits. (b) (4) report number V6794, 3 April 2007.

Group of 10 male and 10 female (b) (4) rabbits were treated with AS01B by intramuscular injection into the m d limb on four occasions 14 days apart. This group was acting as a control in the assessment of a cancer vaccine containing AS01B. They were given a dose volume of 500 µL of the formulation and thus received 50 µg MPL and 50 µg QS-21 in a liposomal formulation per dose. Five rabbits per sex per group (including control group) were euthanized 3 or 28 days after the last dose.

Red cell count, hemoglobin, and hematocrit levels were reduced in female rabbits only. Red cell count was reduced 28 days after the last dose only. Hemoglobin and hematocrit were reduced 1 day after the first dose and 1 and 28 days after the last dose (but not 3 days after the last dose). These differences were considered as test article related because statistical significance was attained ($p<0.05$ or $p<0.01$). No similar differences were reported in males. There were no effects on reticulocyte proportions.

8- Repeat Dose Toxicity Study with a VZV Candidate Vaccine (gE 100 µg 01B) Administered Intramuscularly (Three times) to Male and Female Rabbits. (b) (4) report number V6721, 3 April 2007.

Group of 10 male and 10 female (b) (4) rabbits were treated with AS01B by intramuscular injection into the muscles of each hind limb on three occasions 14 days apart. They were given a dose volume of 500 µL of the formulation and thus received 50 µg MPL and 50 µg QS-21 in a liposomal formulation per dose. Five rabbits per sex per group (including control group) were euthanized 3 or 28 days after the last dose.

Red cell count, hemoglobin, and hematocrit levels were reduced intermittently in both sexes, typically 0.95X control. In males these parameters were reduced 1 and 3 days after the first dose. In females they were reduced 1 day after the first dose and 3 days after the last dose. Reticulocyte proportion was increased in males only three days after the first dose only. These differences were considered as test article related because statistical significance was attained ($p<0.05$ or $p<0.01$). The data for other time points shows no effect.

Assessment of results

Rats

AS01B produced a reduction in the proportion of immature erythrocytes in bone marrow smears generated during a micronucleus study (study 1). As a result, another study was carried out to assess peripheral blood hematology parameters and bone marrow in rats (study 2). This study failed to repeat the effect on proportion of immature erythrocytes in blood smears reported in study 1. In study 1, gelatinous clumping was reported when preparing blood smears from rats killed one day after the last dose. This effect was also reported in study 2 for rats killed one or two days after the last dose but not for rats killed 13 days after the last dose. It is possible that this clumping was caused by the presence of AS01B in the blood. It affected the quality of the smears and consequently the assessment of red cell proportion in study 1. In Study 2, hematocrit and hemoglobin levels were reduced to between 0.94X and 0.97X control between 1 and 7 days after one or two doses, with recovery from 10 days after dosing. Red cell count was not affected by treatment (no statistically significant differences, means never less than 0.97X control).

Rabbits

In the six rabbit studies reviewed, hematocrit, hemoglobin concentration and red blood cell count were assessed 1 and 3 days after the first dose, 10 days after an intermediate dose and 1, 3 and 28 days after the last dose when the number of doses varied from 3 to 7 between studies, all at 14 day intervals. The effects on hematological parameters other than red cells, that is white cells and fibrinogen, were similar in the studies in which AS01B was given intramuscularly to rabbits alone (studies 4, 6, 7, 8) or in combination with (b) (4) (study 3) or (b) (4) (study 5). This indicates that the findings were principally attributable to AS01B, that the presence of the vaccine antigen did not generate any findings that might have masked an effect of AS01B on red cells and consequently, studies 3 and 5 are also useful in the assessment of the effect of AS01B on red cells.

Bone marrow was examined during routine histopathology in four of the rabbit studies and no effects of treatment were reported.

Hematocrit, hemoglobin, and red cell count showed marginally lower values (typically 0.91X-0.95X control) intermittently and inconsistently in five of the six studies. Many of the differences were statistically significant.

It was concluded that the incidence of marginally lower mean hematocrit, hemoglobin, and red cell count levels were affected by treatment with AS01B in rabbit.

Conclusion:
Treatment with AS01B did not reproducibly effect the proportion immature erythrocytes in the bone marrow of the rats. In addition, AS01B did not affect the erythrocyte count in peripheral blood. Rabbits in six repeated dose studies produced marginal reductions in hemoglobin, hematocrit, and red cell count which were inconsistent with respect to time after dose administration, but did occur with some reproducibility between the studies. No evidence of AS01B effect on cell counts in the bone marrow in rabbits was reported.

Study # 5: Bone Marrow Micronucleus Test with DQ in Rats (Study no.: V20204/04). Genetic toxicology (Reviewed by Nabil in IND 13857)

Purpose of the study: To investigate the potential of DQ to cause damage to the chromosomes and/or the mitotic apparatus of erythroblasts by analysis of erythrocytes as sampled in bone marrow of rats.

Key findings: In male rats treated by IV administration of the maximum (tolerable) dose of DQ containing 160 µg QS-21/kg-bw, no test article-related chromosomal damage and/or damage to the mitotic spindle apparatus of the bone marrow target cells was reported.

Study no.: V20204/04
Conducting laboratory and location: (b) (4)
Date of study initiation: November 13, 2012
Date of study completion: March 03, 2013.
GLP compliance: Yes
QA reports: Yes (x), No ( )
Drug, lot #, and % purity:
DQ (QS-21 = 100 µg/dose), Batch No.; (b) (4), expiration date; (b) (4), No purity reported.
Water for injection, Batch No.; (b) (4), expiration date; (b) (4).
Negative control: physiological saline, expiration date; (b) (4).
Positive control: (b) (4) Batch No.; (b) (4), expiration date; (b) (4)
Strains/species: (b) (4) outbred) male rats
Breeder/supplier: (b) (4).
Number of animal per group and sex: 43 rats
Age: 6 weeks

Body weight range: Not provided
Route, site, and frequency of administration: Intravenous.
Volume of injection: See study design below.
Methods
Study design

The experimental design of the bone marrow micronucleus test was as follows:

[1]

Group	Treatment	Color	Dosing volume[1]	Dose level	n
1	Negative control[2]	White	10	-	5
2	DQ[3]	Blue	10	40 µg QS-21/kg-bw	5
3	DQ[3]	Green	10	80 µg QS-21/kg-bw	5
4	DQ[3]	Red	10	160 µg QS-21/kg-bw	7
5	Positive control[4]	Yellow	10	1.5 mg/kg-bw	5

Dosing volume in ml/kg-bw/day
[2] Rats of this group were dosed twice intravenously with physiological saline on two consecutive days with an interval of approximately 24 h
[3] Rats of these groups were dosed twice intravenously with DQ on two consecutive days with an interval of approximately 24 h
[4] Rats of this group were dosed once intraperitoneally with 1.5 ml/kg-bw (b) (4) freshly formulated in physiological saline

Table 74: *Study design (*(b) (4) *study # V20204/04)*

The following criteria were used for the scoring of cells:

1- A polychromatic erythrocyte (PE) is an immature erythrocyte that still contains ribosomes and can be distinguished from mature, normochromatic erythrocytes by a (b) (4) (b) (4) .
2- A normochromatic erythrocyte (NE) is a mature erythrocyte that lacks ribosomes and can be distinguished from immature, polychromatic erythrocytes by a (b) (4) .
3- A micronucleus is a small, normally round, nucleus with a diameter of circa 1/20 to 1/5 of an erythrocyte, distinguished from the cytoplasm by a (b) (4) .

The numbers of PE and NE were recorded in a total of at least 200 erythrocytes (E) per animal. If micronuclei were observed, these were recorded as micronucleated polychromatic erythrocytes (MPE) or micronucleated normochromatic erythrocytes (MNE). Once a total of 200 E (PE + NE) were scored, an additional number of PE was scored for the presence of micronuclei until a total of 2000 PE was scored.

Results

Preliminary dose range finding study

First, animals were treated with a single dose of DQ containing 500 µg QS-21/kg-bw (diluted in water for injection, dosing volume 10 ml/kg-bw) by intravenous administration. Approximately

4 h after dosing, lethargy, hunched posture, blepharospasm, and piloerection were reported. Approximately 7 h after dosing, dyspnoea and nasal swelling were also reported. Based on these clinical signs the animal was killed for ethical reasons.
Second, another rat received a dose of DQ containing 125 µg QS-21/kg-bw (diluted in water for injection, dosing volume 10 ml/kg-bw) by intravenous administration twice on two consecutive days with an interval of approximately 24 h between doses. No abnormalities were observed up to approximately 72 h after administration of the second dose.

Third, one rat received a single dose of DQ containing 250 µg QS-21/kg-bw (diluted in water for injection, dosing volume 10 ml/kg-bw) by intravenous administration. Approximately 7 h after dosing, piloerection was reported and approximately 24 h after dosing hunched posture and blepharospasm were also reported. In addition, a >11% body weight reduction compared to the body weight prior to dosing was reported.

Fourth, another rat received a dose of DQ containing 160 µg QS-21/kg-bw (diluted in water for injection, dosing volume 10 ml/kg-bw) by intravenous administration twice on two consecutive days with an interval of approximately 24h between doses. Approximately 24h after the first dose and 1h after the second dose piloerection was reported. Approximately 4h after the second dose, nasal encrustations were also reported. No clinical signs were reported approximately 24h after the second dose. Prior to the second dose, a body weight reduction of approximately 7% compared to the body weight prior to the first dose was reported. No further weight loss was observed after the second dose.

Based on these observations, the maximum tolerable intravenous dose for the bone marrow micronucleus test was established as a DQ dose containing 160 µg QS-21/kg-bw per day for two consecutive days with an interval of approximately 24h between doses.

<u>Bone marrow micronucleus test</u>

Clinical signs
Approximately 7 h after the final treatment, one animal treated with DQ containing 160 µg QS-21/kg-bw showed nasal and eye encrustations. This finding disappeared approximately 24 h after the final treatment.

Body weights
One day after the first treatment, body weight reduction (mean body weight reduction of 7.2%) was reported in all animals treated with DQ containing 160 µg QS-21/kg-bw. No other changes were reported.

Bone marrow micronucleus test
The groups mean numbers of MPE/2000E and PE/200E are presented in Table 53.

The number of micronucleated polychromatic erythrocytes (MPE) per 2000 polychromatic erythrocytes (PE) and number of PE per 200 erythrocytes (E) reported in the micronucleus test (group mean ± SD) are listed in the following table:

Group	Treatment	Dose level	MPE/2000PE	PE/200E
1	Negative control[1]	-	2.0 ± 1.6	114 ± 18
2	DQ[2]	40 µg QS-21/kg-bw	3.0 ± 0.7	112 ± 17
3	DQ[2]	80 µg QS-21/kg-bw	1.6 ± 0.5	101 ± 8
4	DQ[2]	160 µg QS-21/kg-bw	1.8 ± 1.3	95 ± 9
5	Positive control[3]	1.5 mg/kg-bw	41.4 ± 13.3*	86 ± 12*

[1] Rats of this group were dosed twice intravenously with physiological saline on two consecutive days with an interval of approximately 24 h
[2] Rats of these groups were dosed twice intravenously with DQ on two consecutive days with an interval of approximately 24 h
[3] Rats of this group were dosed once intraperitoneally with 1.5 ml/kg-bw (b) (4) freshly formulated in physiological saline
* Statistically significant difference from negative control group
Table 75: *MPE and PE results (*(b) (4) *study # V20204/04) Table provided by the sponsor.*

Validity of the study
Statistically significant increase (p=0.0060) in the mean number of MPE/2000PE were reported in the positive control animals (group 5) compared to the negative control animals (group 1). The mean number of MPE/2000E reported in the positive control mitomycin C was within the range of means of the historical data.

Statistically significant decrease (p=0.0208) in the mean number of PE/200E was reported in the positive control animals (group 5) compared to the negative control animals (group 1). This confirms that the positive control substance (b) (4) reached the bone marrow.

The positive control (b) (4) demonstrated the expected response and the negative control was within the range of historical data. Therefore the study was considered valid.

Treatment groups DQ (40, 80 and 160 µg QS-21/kg-bw)
No statistically significant increase in the mean number of MPE/2000PE was reported in groups 2, 3, or 4 when compared to group 1.

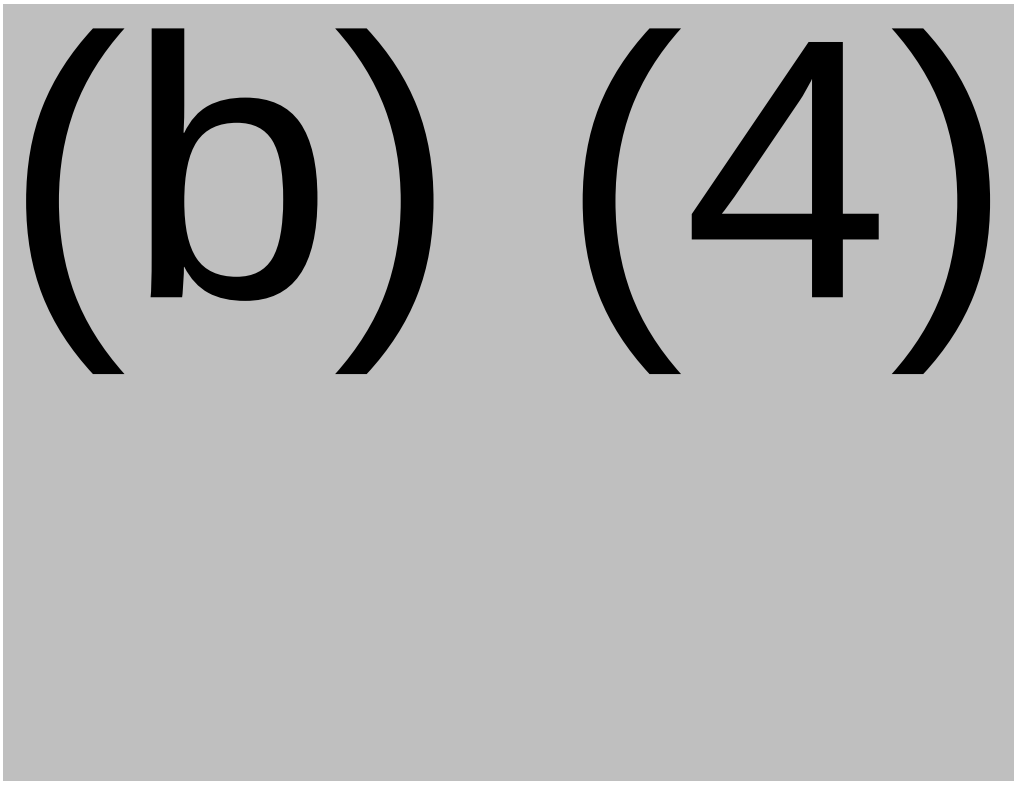
No statistically significant decrease in the mean number of PE/200E was reported in groups 2, 3, or 4 when compared to group 1.

Conclusion
No test article-related chromosomal damage and/or damage to the mitotic spindle apparatus of the bone marrow target cells was reported in male rats treated by IV administration of the maximum (tolerable) dose of DQ containing 160 µg QS-21/kg-bw.

Genotoxicology studies: in vitro

Reviewer: Claudia Wrzesinski

(b) (4)



4 pages have been determined to be not releasable: (b)(4)

Local tolerance studies:

Study 1: Single Dose Toxicity and Local Tolerance Study with Zoster Candidate Vaccine (gE/AS01B) Administered Subcutaneously to Male and Female Rabbits (V 9912/05)

The Zoster candidate vaccine (gE/AS01B) was evaluated for acute toxicity and local reactogenicity after a single subcutaneous injection in a group of three male and three female (b) (4) rabbits, sacrificed 3 days after the injection. The animals received 0.5 ml per injection, equivalent to the intended full human dose. The reactions of the Zoster candidate vaccine was compared to an adjuvant alone group (AS01B) and to a saline control group. Local reactions at the injection site were recorded approximately 3, 24, 48 and 72 hours after injection. General and local clinical signs, body weights, macroscopic changes at necropsy and histopathology of the injection site collected 3 days after injection (necropsy) were evaluated in this study.

No treatment-related changes were observed in general and local clinical signs and body weights of the animals treated with the Zoster candidate vaccine. Macroscopically, a hemorrhage at the injection site in treated animals as well as control animals. This was most likely the result of a punctured blood vessel during injection. Microscopically, a slight to severe diffuse mixed inflammatory cell infiltration (small and medium sized macrophages, plasma cells and granulocytes) was seen at the injection site of all animals receiving gE/AS01B as well as a slight to moderate diffuse mixed inflammatory cell infiltration (small and medium sized macrophages, plasma cells and granulocytes) in 2/3 male and female animals receiving the adjuvant alone. A slight acanthosis was observed in 1/3 male animal receiving gE/AS01B or the adjuvant alone. The severity of the histopathological changes was slightly lower in females than in males and slightly lower in animals treated with the adjuvant alone (AS01B) than in those treated with gE/AS01B.

Study 2: Single Dose Toxicity and Local Tolerance Study with a VZV Candidate Vaccine (gE 100 gE/AS01B) Administered Intramuscularly to Male and Female Rabbits (v 6812/02)

The VZV candidate vaccine (gE 100 *µg*/ASO1B) was examined for its local reactogenicity after a single subcutaneous injection in a group of three male and three female (b) (4) rabbits, sacrificed 3 days after the injection. The animals received 0.5 ml per injection (right calf muscle), equivalent to the intended full human dose. The reactions of the Zoster candidate vaccine was compared to an adjuvant alone group (AS01B) and to a saline control group. Local reactions at the injection site were recorded approximately 3, 24, 48 and 72 hours after injection. General and local clinical signs, body weights, macroscopic changes at necropsy and histopathology of the injection site collected 3 days after injection (necropsy) were evaluated in this study.

No treatment-related changes were observed in general and local clinical signs and body weights of the animals treated with the Zoster candidate vaccine. Microscopically, a treatment related

very slight to slight widespread, predominantly extramuscular, mononuclear cell infiltrate was observed in all adjuvant and vaccine formulation treated animals; while mononuclear cell infiltrates were observed in the control animals.

Study 3: Single Dose Toxicity and Local Tolerance Study with DQ Administered Intramuscularly to Male and Female Rabbits (V 20212/02)

Three male and 3 female (b) (4) rabbits received a single intramuscular dose of 20, 100 and 200 µg/mL DQ (0.5 mL at the right and left tight) and were compared to a saline control group. General and local clinical signs, body weights, as well histopathology of the injection sites collected 3 days after injection were evaluated in this study. No treatment related changes were observed regarding clinical signs or body weights. During the necropsy, three days after the administration discoloration (white area) at the injection site was observed in one male and one female animal receiving 100 µg/mL and a hemorrhage at the injection site in one male animal receiving 200 µg/mL of DQ.

Microscopically, animals in the treatment and control group showed minimal to mild localized mononuclear (lymphocytes and small macrophages) inflammatory response. In 1/3 saline control females, 2/3 DQ 100 µg/mL females and 1/3 DQ 200 µg/mL females, a minimal multifocal mononuclear inflammatory cell infiltrate was observed, characterized by scattered small foci of inflammatory cells. A mild widespread (extended along the epimysium and diffusely between the muscle fibers) mononuclear cell infiltration was seen in animal receiving 100 or 200 µg/mL DQ (1/3 DQ 100 µg/mL males, 2/3 DQ 200 µg/mL males and 1/3 DQ 200 µg/mL females at the right anterior thigh muscle, and 1/3 DQ 200 µg/mL males and 2/3 DQ 200 µg/mL females at the left anterior thigh muscle) which also involved a mild mixed cell infiltration (besides lymphocytes and macrophages, polymorphonuclear inflammatory cells were present) in animals receiving 200 µg/mL DQ (1/3 DQ 200 µg/mL males and 2/3 DQ 200 µg/mL females at the right anterior thigh muscle). Minimal to mild hemorrhage were observed at the injection site of male animals receiving 200 µg/mL DQ. Minimal mineralization and muscle fiber degeneration were observed as part of the local response in few treated animals but also in a few saline control animals.

Study 4: Single Dose Toxicity and Local Tolerance Study with DQ Administered Intramuscularly to Male and Female Rats (V 20212/01)

DQ was evaluated for acute toxicity and local l tolerance in 5 male and 5 female (b) (4) rats after a single intramuscular dose 4, 20 or 40 µg/rat DQ compared to a sham-treated saline control group. Animals received 0.2 mL of the various formulations, given as two injections of 0.1 mL in the right and left anterior thigh muscles and were sacrificed 3 days after the injection. Clinical signs, body weight, hematology, clinical chemistry, gross changes at necropsy and histopathological examination of the injection sites collected 3 days after injection were evaluated.

No treatment related changes were observed in general and local clinical signs, body weight and clinical chemistry. Hematological, a dose-dependent increase in fibrinogen was observed in animals receiving DQ on day 1 and on day 3 after injection. In the DQ 100 µg/mL (20 µg/rat) and the DQ 200 µg/mL (40 µg/rat) males a dose-dependent WBC response, consisting of an

increase in neutrophils and a decrease in lymphocytes, were observed on day 1 and on day 3 after injection. In the DQ 100 µg/mL and the DQ 200 µg/mL females, an increase in neutrophils and a decrease in lymphocytes were observed on day 1 after. The changes were considered to be part of the inflammatory process following injection of an immunostimulant.

Three days post injection, the main treatment related microscopic findings at the injection sites were either a localized or a widespread mononuclear inflammatory response. Generally, the response increased in severity and extension with increasing DQ dose, i.e. minimal to mild localized in DQ 20 µg/mL animals, mild to moderate localized in DQ 100 µg/mL animals, and mild to moderate widespread in DQ 200 µg/mL animals. In the saline control animals a minimal localized response was observed.

The sponsor considered the NOEL to be below 4 µg DQ/rat since a minimal to mild inflammatory reaction was observed at the 20 µg/mL injection site (4 µg DQ/rat), and systemic increases in fibrinogen, neutrophils and lymphocytes. However, the observed local effects and the systemic effects were all considered non-adverse and, therefore, the NOAEL of DQ was considered to be above 40 µg DQ/rat when administered as a single injection.

OVERALL SUMMARY:

Safety Pharmacology:

The sponsor submitted 4 safety pharmacology studies. Safety pharmacology studies were performed with the gE (100 μg)/AS01B vaccine formulation, AS01B and MPL. The study with the gE (100 μg)/AS01B vaccine formulation was performed in the anesthetized rat, studies with AS01B were performed in the anesthetized rat and in the conscious dog, and the safety pharmacology study with MPL was performed in the anesthetized dog. No concerning findings were reported in these studies.

In the anaesthetized dog IV administration of MPL induced a small and gradual increase in mean heart rate and a small increase in mean respiratory rate was seen after administration of the top dose of MPL. These changes were not considered to be physiologically relevant. The studies with the either gE (100 μg)/AS01B vaccine formulation or AS01B performed in the anesthetized rat did not affect the cardiovascular and respiratory parameters, all parameters within the normal range expected for this type of study. A full human dose of AS01B adjuvant administered intramuscularly to a conscious dog, did not affect the cardiovascular function and the respiratory function but induced a slight increase in body temperature 6 hours after treatment, compared with control (saline).

General toxicology:

Twelve studies were submitted to support this BLA. Rats, rabbits, and dogs, as animal models, were used in 5, 6, and 1 studies, respectively.

Overall findings of the candidate vaccine showed no treatment-related effects on mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, or body temperature.

Overall, the vaccine was well tolerated, but induced systemic as well as local reactogenicity. A transient but statistically significant increase in CRP levels was observed in rabbits receiving the Shingrix vaccine with levels up to 9 times (male animals) and 5 times (female animals) higher compared to control animals. These changes in CRP levels reflect an activation of the acute-phase response and indicate increasing levels of systemic inflammation, which potentially may be correlated with clinical adverse events like malaise and fatigue. Further, increases in bilirubin (up to 2x), popliteal lymph node weight (up to 50%), spleen weight (up to 17%), and thymus weight (up to 24%) were reported. Locally, mixed inflammatory cell infiltrate in the muscle and an enhanced activated appearance in the draining popliteal lymph nodes were observed.

CRP is protein synthesized by the liver, found in the blood, and is a member of the class of acute-phase reactants as its levels rise dramatically during inflammatory processes occurring in the body. Monocytosis could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair. The increase in the popliteal lymph nodes weight, spleen weight, and thymus weight might be related to the immune responses due to test article-treatment. The increase in fibrinogen levels

and the WBC response was considered to be part of the inflammatory process following injection.

Pituitary gland is a protrusion off the bottom of the hypothalamus at the base of the brain. Hormones secreted from the pituitary gland help control: growth, blood pressure, certain functions of the sex organs, thyroid glands and metabolism as well as some aspects of pregnancy, childbirth, nursing, water/salt concentration at the kidneys, temperature regulation and pain relief.[16]

Adequate nonclinical toxicology data were included in the 12 studies. Based on nonclinical toxicity assessments of the above mentioned studies there were no significant safety issues to preclude the BLA from approval. The delivery of an active dose of the product was verified.

Reproductive toxicology:

The Shingrix vaccine was evaluated in a male fertility study in rats as well as in a reproductive developmental toxicity study in female rats. The influence of intramuscular administration of the Shingrix on embryo-fetal, pre- and postnatal development and female fertility was evaluated in (b) (4) rats. Treatment of female CD rats with the candidate vaccine gE/AS01B or adjuvant AS01B at 40% of the full human dose per occasion, on 28 and 14 days before pairing and then on Days 3, 8, 11 and 15 of gestation and on Day 7 of lactation was well tolerated by the F0 females with effects restricted to slight, transient swelling at the injection site and did not adversely affect embryo-fetal or pre- and post-natal survival, growth or development of the offspring up to Day 25 of age.

The influence of Shingrix on fertility and early embryonic development in CD rats was assessed in male rats exposed by intramuscular administration before mating. Treatment of male CD rats with the Zoster candidate vaccine gE/AS01B or adjuvant AS01B alone at 20% of the full human dose on three occasions prior to pairing (on Day -42, Day -28 and Day -14) did not affect male mating performance, fertility or early embryonic development. No adverse effects on sperm motility, or morphology were apparent after treatment with the adjuvant AS01B or the Zoster candidate vaccine gE/AS01B. However, values for the epididymal sperm concentration, testicular spermatid concentration and total testicular spermatid were statistically significantly lower in the vaccine group compared to saline control, while no significant differences were observed for the adjuvant group. Historical control data (HCD) ranges indicated that all the statistically low values obtained for the animals treated with the gE/AS04B vaccine were within the HCD range. Microscopic examination did not highlight any treatment related changes affecting the right testis, right epididymis, prostate and seminal vesicles. Mating performance and fertility, as assessed by percentage mating, conception rate and fertility index, were unaffected by treatment with gE/AS01B or AS01. There was no evidence of an effect of treatment of the males with gE/AS01B or AS01B on early embryonic development.

Further, the sponsor submitted studies evaluating the effect of MPL on embryofetal development in rats and rabbits. No maternal toxicity or female fertility, embryo-fetal or pre- and post-natal survival, growth or development were observed at doses up to 2 times (100 µg/kg/day) the

[16] https://en.wikipedia.org/wiki/Pituitary_gland

human MPL dose contained in the Shingrix vaccine. These studies had been previously reviewed under the BLA 125259.

Additionally, the sponsor included studies evaluating the effects of DQ (QS-21) on the reproductive function as well as embroyfetal development in the rat and rabbit. In the rat, DQ, at doses up to 40 μg of QS-21/occasion (80% of the QS-21 dose contained in Shingrix) did not adversely affect female fertility, embryo-fetal or pre- and post-natal survival, growth or development of the offspring up to Day 25 of age. However, intramuscular administrations of DQ adjuvant containing 200 μg/mL of QS-21 to (b) (4) rabbits starting 28 and 14 days before the start of mating and on gestation days 3, 8, 11, 15 and 24 and on day 7 of lactation induced a significant maternal mean body weight loss associated with reduced mean food consumption at the end of the gestation period. In addition lower mean fetal weight was noted at this dose level. Defects of the aortic arch (retro or high arched) were observed in three fetuses from separate litters suggestive of a possible association with treatment at this dose. Doses of DQ adjuvant containing 100 or 20 μg/mL of QS-21 did not induce any adverse effects on maternal condition or embryo-fetal and post-natal development. Since Shingrix has been shown to induce significant systemic reactogenicity not only in rabbits (CRP increase, maternal toxicity) but also in humans (grade 3 fatigue, headache, myalgia and shivering) which could adversely affect pregnancy outcome it might be warranted to evaluate a full human dose of the vaccine in a reproductive-developmental toxicity study in rabbits if this vaccine should be recommended to females of child bearing age.

Genotoxic toxicology:

In vivo studies

Five *in vivo* genotoxicology studies were submitted to support this BLA. These studies included three micronucleus tests and two studies assessing the test article or the adjuvant effect on red blood cells and bone marrow.

IV1, IM1 and IM2 did not show any evidence of causing chromosome damage or bone marrow cell toxicity. IM3 showed no evidence of causing micronuclei but did show evidence of causing bone marrow cell toxicity.

MPL (b) (4)) did not cause any statistically significant increases in the number of micronucleated immature erythrocytes when compared to vehicle control values. MPL (b) (4)) did not cause any substantial increases in the incidence of micronucleated mature erythrocytes. MPL was not positive in the rat micronucleus assay.

AS01B did not affect the ability of the bone marrow to produce red blood cells in male rats after 1 or 2 days treatment.

Treatment with AS01B did not reproducibly affect the proportion immature erythrocytes in the bone marrow of the rats. In addition, AS01B did not affect the erythrocyte count in peripheral blood. Rabbits in six repeated dose studies produced marginal reductions in hemoglobin,

hematocrit, and red cell count which were inconsistent with respect to time after dose administration, but did occur with some reproducibility between the studies. No evidence of AS01B effect on cell counts in the bone marrow in rabbits was reported.

No test article-related chromosomal damage and/or damage to the mitotic spindle apparatus of the bone marrow target cells was reported in male rats treated by IV administration of the maximum (tolerable) dose of DQ containing 160 µg QS-21/kg-bw.

In vitro studies:

The sponsor submitted 5 in vitro gentoxicology studies. No genotoxicity was observed for MPL, DQ and QS in an *in vitro* (b) (4) test; MPL in a (b) (4) test and DQ in a (b) (4) cells.

Package Insert Part Review

In compliance with PLLR, section 8.1 is recommended to revise as marked below:

USE IN SPECIFIC POPULATIONS

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to Shingrix during pregnancy. Healthcare providers are encouraged to register women by calling XXXXXXXX.

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. There are no available human data to establish whether there is vaccine-associated risk with SHINGRIX in pregnant women.

A reproductive and developmental toxicity study was performed in female rats administered SHINGRIX or the $AS01_B$ Adjuvant System alone prior to mating, during gestation and lactation periods. The total dose was 0.2 mL at each occasion (a single human dose is 0.5 mL). This study revealed no adverse effects on fetal or pre-weaning development due to SHINGRIX *[see Data]*.

Data

Animal Data: In a reproductive and developmental toxicity study, female rats were administered SHINGRIX or the $AS01_B$ Adjuvant System alone by intramuscular injection 4 and 2 weeks prior to mating, on gestation Days 3, 8, 11, and 15, and on lactation Day 7. The total dose was 0.2 mL at each occasion (a single human dose is 0.5 mL). No adverse effects on pre-weaning

development up to post-natal Day 25 were observed. There were no vaccine-related fetal malformations.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

SHINGRIX has not been evaluated for its carcinogenic or mutagenic potential. In a male fertility study, rats were vaccinated with 0.1 mL SHINGRIX (a single human dose is 0.5 mL) on 42, 28 and 14 days prior to mating. There were no effects on male fertility.

Overall conclusion:

Based on the nonclinical toxicity assessments of the Zoster (non-live) vaccine submitted in this BLA, there are no significant safety issues to preclude the BLA from being approved.

Concurrence: Martin D. Green