

BLA 125428

Toxicology Review of BLA/STN 125428 Hepatitis B Vaccine (Recombinant) HEPLISAV

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File: BLA 125428/0

Product: HEPLISAV vaccine consisting of recombinant yeast cell-derived (Hansenula polymorpha) hepatitis B virus surface antigen (HBsAg) adjuvanted with 1018 ISS immunomodulatory sequences

Subject: Review of toxicology studies (combined HBsAg + 1018 ISS adjuvant)

Reviewer: Steven Kunder, Ph.D., DABT

Sponsor: Dynavax Technology Corporation

Cross references: IND 13332, IND 12692, MF (b) (4)

Reference: BLA sections reviewed (toxicology studies with the combined HBsAg + 1018 ISS adjuvant vaccine):

Repeat-dose Toxicity

Reproductive and Developmental Toxicity

Proposed use: Prevention of Hepatitis B Virus Infection

Executive Summary

A GLP toxicity study with 1018 ISS Adjuvant + HBsAg was conducted in mice given 3 IM injections (on Days 0, 14, and 28) with dose levels of 1 to 50 mcg/mouse 1018 ISS Adjuvant (approximately 0.04 to 2 mg/kg 1018 ISS Adjuvant on a body weight basis) in combination with a fixed dose of 0.5 mcg/dose/mouse HBsAg (approximately 0.02 mg/kg). The highest dose level of 1018 ISS Adjuvant was equivalent to a 43-fold clinical multiple, and the lowest dose approximates the human dose on a body weight basis. There were mild decreases in the group mean serum albumin, total protein, and triglyceride values for the 50 mcg 1018 ISS Adjuvant (2 mg/kg) + HBsAg dose compared to HBsAg alone. In addition, for the 50 mcg 1018 ISS Adjuvant + HBsAg group the mean red blood cell (RBC) parameters were slightly decreased relative to those for HBsAg alone. The reductions in circulating RBC mass and albumin are class effects of oligonucleotides in rodents, and appear to be treatment-related. Differences in albumin, protein, triglycerides, and red blood cell parameters were not observed at recovery. Terminal necropsy findings included increases in spleen weight, and histopathologic changes in the spleen, liver, and injection-sites. Enlarged spleens were observed at necropsy in all animals treated with the high-dose (2.0 mg/kg/dose) of 1018 ISS Adjuvant and in 2 mid-dose animals (0.2 mg/kg/dose 1018 ISS Adjuvant). Extramedullary hematopoiesis (EMH) was observed microscopically in the spleen of high-dose animals and largely accounted for the splenic enlargement. This effect was reversible, as no gross enlargement or EMH and only minimal organ weight increases were observed in the spleens of high-dose mice at the end of the recovery period (following 3 weeks from terminal necropsy). EMH was also observed microscopically in the liver, with the greatest incidence in high-dose mice. EMH in the liver largely resolved over the recovery period, persisting with decreased severity in high-dose females. Inflammation and mineralization were observed microscopically at the injection-sites, and the incidence and severity were 1018 ISS Adjuvant dose-related, with partial resolution in high-dose animals at the end of the recovery period. Immunostimulatory responses included cellular inflammation at injection-sites the lowest dose levels of 1018 ISS Adjuvant (0.04 mg/kg). After a 3-week recovery (following terminal necropsy) findings in this study in mice had fully resolved or partially resolved (in the 50 mcg 1018 ISS Adjuvant animals there were increased spleen weights (1.3-fold compared to antigen alone in males), hepatic EMH (minimal in females), and injection-site chronic inflammation and mineralization [generally minimal severity in males and females]). A No-Observable-Adverse-Effect-Level (NOAEL) was not defined in the study report, but no severe toxicity was observed, and all effects reflected the expected immunostimulatory properties of the vaccine.

1018 ISS Adjuvant + HBsAg was also tested in rats in a reproductive and developmental toxicity study which assessed the potential effects on mating behavior, fertility, gestation, embryo-fetal development, parturition, lactation and maternal behavior (from implantation through lactation and weaning) and on the development of the offspring (F1 generation) of the treated female rats, including postnatal behavioral/functional and immunological evaluation. The combination of 1018 ISS Adjuvant + HBsAg was administered as 4 IM injections at appropriate intervals before and during gestation. The highest dose level of 1018 ISS Adjuvant was the human dose of 3000 mcg, which is approximately 200-fold

greater than clinical dosage on a body weight basis (10 mg/kg/dose). The HBsAg dose was at 2.5 mcg (approximately 25-fold greater than clinical dosage on a body weight basis). There were no adverse effects on maternal reproductive performance, fetal development, the growth and development of the offspring, or any of the other parameters evaluated, even though the highest dose level displayed maternal toxicity. A NOAEL for reproductive and developmental toxicity and the growth and development of the F₁ generation was observed at the highest dose levels tested, 3000 mcg 1018 ISS Adjuvant + HBsAg.

Introduction

The 1018 ISS Adjuvant component of HEPLISAV is a 22-mer phosphorothioate oligodeoxynucleotide (PS ODN) prepared by (b) (4) techniques. The molecular mass of 1018 ISS is (b) (4). The sequence of 1018 ISS Adjuvant is 5' TGA CTG TGA ACG TTC GAG ATG A 3'. The molecular formula of 1018 ISS Adjuvant free acid is (b) (4). 1018 ISS Adjuvant was selected from a large panel of oligodeoxynucleotides for immunostimulatory activity in vitro and in vivo and for activity in both humans and important animal species. The 1018 ISS Adjuvant has been demonstrated to be immunostimulatory in vivo in mice, rabbits, dogs, baboons, and cynomolgus monkeys and in vitro in human peripheral blood cells. Please refer to the review by Claudia Wrzesinski, Ph.D., DVM, for toxicology studies using the 1018 ISS Adjuvant alone.

The Heplisav vaccine by Dynavax is intended for immunization against infection caused by all known subtypes of hepatitis B virus in adults 18 through 70 years of age. Intended dosage is one dose administered at an elected date; a second dose administered one month later (2 doses total; 1 dose each given at 0, 1 month).

Clinical studies (completed):

The clinical studies submitted for this BLA for safety and efficacy were previously conducted and submitted together with the nonclinical studies.

Dynavax enrolled 5870 subjects in 9 completed clinical trials of HEPLISAV, including 4438 subjects randomized to receive any of the 3 formulations of HEPLISAV and 1432 subjects randomized to receive Engerix-B. The 3 formulations of HEPLISAV are:

- HEPLISAV (F1), which comprises 20 mcg HBsAg subtype *adw* and variable concentrations of 1018 ISS Adjuvant in a 2-vial presentation;
- HEPLISAV (F2), which comprises 20 mcg HBsAg subtype *adr* and 3000 mcg 1018 ISS Adjuvant in a single-vial or 2-vial presentation; and,
- HEPLISAV, also known as HEPLISAV (F3), which comprises 20 mcg HBsAg subtype *adw* and 3000 mcg 1018 ISS Adjuvant in a single-vial presentation and is the proposed commercial formulation. Two doses were administered by intramuscular injection 4 weeks apart to adults 18 to 55 years of age.

Toxicology Study Review
Repeat dose toxicology

Title and study number: A 5-week toxicity study of a recombinant hepatitis B vaccine combined with an immunostimulatory phosphorothioate oligonucleotide, with a 3-week recovery, in mice given intramuscular injections every other week. (b) (4)

Study Number: Q-838; Sponsor Study Number: 00-95

Performing laboratory: (b) (4)

Study initiation date: January 31, 2000

Final Report date: July 27, 2000

Test article batch/lot: recombinant hepatitis B vaccine (HBV), obtained from (b) (4)

(b) (4) (Lot # NJ28), and an immunostimulatory phosphorothioate oligonucleotide (ISS; Lot # (b) (4), Batch # (b) (4)); control HBV vaccine, Engerix-B (SmithKline Beecham, Lot # ENG2803A4).

Animal species and strain: Mice, (b) (4)

Breeder/supplier: (b) (4)

Number of animal per group and sex: 10

Age: 5-7 weeks at dosing initiation

Body weight range: 16.1-24.7 g

Route and site of administration: intramuscular

Volume of injection: 0.05 mL

Frequency of administration and study duration: days 0, 14, 28, 4 week dosing period followed by 3 week recovery period

Dose: see table below

Study design

group	# main study mice	# recovery mice	HBV dose (µg/mouse)	ISS dose (µg/mouse)	Engerix-B dose (µg/mouse)	Dose volume (mL/mouse)
1	10m/10f	0	0	0	0.5	0.05
2	10m/10f	5m/5f	0.5	0	0	0.05
3	10m/10f	5m/5f	0.5	1	0	0.05
4	10m/10f	5m/5f	0.5	5	0	0.05
5	10m/10f	5m/5f	0.5	50	0	0.05

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 on the same batches of vaccine and adjuvant control as used in this study. Both showed stability for at least the length of the study.

Means of administration: intramuscular injection via syringe, right thigh muscle

Report status: final

Methods:

Endpoint	Methodology
Hematology	Blood specimens for serum chemistry and hematology analysis were collected from animals at termination of both the main study and the recovery groups. Due to the small amount of blood collected from (b) (4) mice of this size, the mice were subdivided into 2 subgroups, with one subgroup being designated for clinical chemistry assessment and the other subgroup for hematology evaluation. For the Day 34 and 35 necropsies, mice were divided into 2 subgroups of 5/sex/group. At the Day 56 necropsy of recovery animals, mice were divided into 2 subgroups with 2/sex/group designated for clinical chemistry and 3/sex/group for hematology. Blood specimens were collected under carbon dioxide-induced anesthesia by cardiac puncture and exsanguination.
Clinical chemistry	See hematology above
Coagulation	Not performed

Randomization procedure: Animals were assigned to groups prior to initiation of dosing by a stratified randomization scheme designed to achieve similar group mean body weights, and the groups were randomly assigned to treatment, which provided for control of bias.

Statistical analysis plan: All statistical comparisons were performed at the 0.05 level of significance using SAS version (b) (4) (SAS Institute2). Continuous normal data, such as body weight, organ weight, organ weight ratios, clinical pathology, were analyzed by two-way analysis of variance (ANOVA) with Dose and Sex as factors. If a significant term was identified, unadjusted contrasts were performed to locate those dose groups that differed from the control group, within a gender for significant interaction terms. For any parameter, if Groups 3, 4, or 5 were significantly different from Group 2, a comparison of these groups to Group 1 was performed to determine the potential role of adjuvant (alum in the Engerix-B to ISS in Heplisav) in any effects.

Parameters	Frequency of Testing
Cageside observation ¹	Daily
Clinical observations ²	daily
Body weight	Prior to first dose, then weekly
Food consumption	Prior to first dose, then weekly
Body temperature	NC
Ophthalmologic exam	NC
Clinical chemistry*	At necropsy (days 34-35, 58), collected by cardiac puncture and exsanguination

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.

Hematology**	At necropsy (days 34-35, 58), collected by cardiac puncture and exsanguination
Coagulation	NC
Immunological response	NC
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Not scored
Necropsy	Days 34-35, 58
Tissues for histopathology	At necropsy (days 34-35, 58)

*(collected by cardiac puncture) **(collected by cardiac puncture) (NC = not collected)

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)	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)		
Gall bladder	X!	
Gross lesions (if any)	X!	
Harderian gland (if rat, mouse or hamster)		X
Heart	X*!	
Ileum	X!	
Injection site(s)	X!	
Jejunum	X!	
Kidneys	X*!	
Lacrimal glands		X
Larynx		X
Liver	X!	
Lung (main-stem; bronchi)	X!	

Lymph nodes (cervical)		X
Lymph nodes (mandibular)		X
Lymph nodes (mesenteric)	X!	
Mammary glands	X!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		
Ovaries	X*!	
Pancreas	X!	
Peyer's patch (if applicable)		
Pituitary gland	X!	
Prostate	X!	
Rectum	X!	
Salivary glands (mandibular)	X!	
Sciatic nerve	X!	
Skeletal muscle	X!	
Skin	X!	
Spinal cord (cervical, lumbar, thoracic)	X!	
Spleen	X*!	
Stomach (squamous and glandular)	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!	

Table of Histology – Tissues examined: All group 2 dose group and Heplisav high dose and control. Additionally group upon identification of lesions attributable to the test article

Results:

Morbidity and mortality: A group 2 male mouse was found dead on day 27. All other mice **survived** to their scheduled termination. No abnormal clinical findings preceded death. The cause of death was not determined from histopathological examination of the tissues. This male received HBV and no ISS.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase ND Sorbitol dehydrogenase ND Total bile acids ND
B) HEPATOBILIARY	Alkaline phosphatase (ALP): ↓, 4m, 5m Total bilirubin: ↓, 3f, 3m, 4f, 4m, 5f, 5m	Gamma-glutamyl transferase (GGT) Total bile acids ND
ACUTE PHASE REACTANTS		C-reactive protein, ND Fibrinogen, ND
KIDNEY FUNCTION	Creatinine: ↓, 3f, 0.5x	Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Albumin ↓, 4m, 4f, 5m, 5f Triglycerides ↓, 4m, 5m, 5f Globulin: ↓, 2f rec Total protein: ↓, 5m	or A/G Ratio ND Total cholesterol Cholinesterase ND
MUSCLE INJURY		Creatine phosphokinase (CPK) ³ ND

Table of Clinical Chemistry Results

↓ or ↑, decreased or increased <1.5x: if >1.5x, actual value provided

Rec= recovery

³ Serum CPK activities in the range of 2000 to 3000 IU/liter following intramuscular dosing should be considered to have significant potential for human toxicity (Gray, Fundamental and Applied Tox 1:290, 1981). Minor increases in CPK serum levels (2 to 3 fold elevations) may be indicative of a febrile response (Mukhutdinova, Bulletin of Experimental Bio Med: 128: 674, 1999)

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
RED BLOOD CELLS	Hemoglobin Conc.: 5m↓, 5f↓ Hematocrit (Hct): 5m↓, 5f↓ Mean Corp. Volume: 4m↓, 4f↓, 5m↓, 5f↓ Mean Corp. Hb.: 5m↓, 5f↓ Mean Corp. Hb. Conc.: ↓, 5f↓	Total Erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	Eosinophils: 3f↓3x, 4f↓3x, 5m↓10x, 5f↓1.8x; 5m rec↑1.8x, 5f rec↑5x Macrophage/monocyte count: 4m↑3x, 4f↑, 5m↑2.5x, 5f↑2x Monocyte%: 5m↑3x, 5f↑1.9x Lymphocyte %: 4m↓, 4f↑; 4m rec↑	Basophils, count lymphocyte count Neutrophil count Total leukocytes (WBC) Large unstained cells (LUC)
CLOTTING POTENTIAL	Platelet count: ↓5m, 5f↓	Activated partial-thromboplastin time clotting time ND Prothrombin time ND Mean platelet volume ND Fibrinogen ND
OTHERS		Bone marrow cytology ND

Table of Hematology Results. ND = not determined

↓or ↑, decreased or increased <1.5x: if change >1.5x, actual value provided

Rec= recovery

Systemic toxicity:

Organ Weights: (table, following page)

SEX	MALES					FEMALES				
	1	2	3	4	5	1	2	3	4	5
GROUPS										
NUMBER per GROUP	10	9	10	10	10	10	10	10	10	10
STUDY/RECOVERY	---	5	5	5	5	---	5	5	5	5
BODY WEIGHT (terminal)	25.44	25.96 28.64	25.51 27.70	25.88 27.16	25.77 27.60	21.43	22.02 23.16	21.57 22.76	21.36 22.26	22.75 22.20
BRAIN	0.43606	0.43216 0.44506	0.45085 0.45262	0.43608 0.42164	0.43284 0.43584	0.44396	0.44158 0.45464	0.44689 0.44818	0.43981 0.46488	0.44212 0.44840
ADRENALS	0.00525	0.00509 0.01132	0.00543 0.00736	0.00770 0.01128	0.00581 0.01194	0.00927	0.01092 0.01230	0.00938 0.01226	0.00919 0.01274	0.01100 0.01274
EPIDIDYMIDES	ND	ND	ND	ND	ND	-----	-----	-----	-----	-----
HEART	0.14977	0.13880 0.15130	0.14264 0.15654	0.14477 0.14468	0.14093 0.15740	0.12409	0.12516 0.11754	0.11929 0.12178	0.11909 0.12478	0.12922 0.11860
KIDNEYS	0.48718	0.45704 0.54096	0.46254 0.51328	0.46283 0.50846	0.49164 0.52492	0.29642	0.30832 0.33346	0.30999 0.33612	0.30393 0.32812	0.35317 0.33508
LIVER	1.49718	1.55472 1.55854	1.51056 1.58878	1.33783 1.50736	1.66248 1.57536	1.19942	1.26644 1.28240	1.20910 1.30712	1.19932 1.28118	1.63783 1.30326
SPLEEN	0.09813	0.09419 0.09346	0.10270 0.10628	0.11419 0.10006	0.26387 0.13546	0.09915	0.09675 0.11312	0.09784 0.10628	0.10205 0.10006	0.33706 0.12524
TESTES	0.19352	0.19464 0.20798	0.19746 0.20282	0.20414 0.21082	0.19409 0.19884	-----	-----	-----	-----	-----
THYROID and PARATHYROID	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
THYMUS	0.05179	0.05148 0.04126	0.05161 0.04414	0.05390 0.04806	0.05339 0.04464	0.05537	0.05594 0.05134	0.05105 0.04562	0.05906 0.04374	0.05455 0.05432
OVARIES	-----	-----	-----	-----	-----	0.01653	0.01942 0.01924	0.01887 0.02128	0.01878 0.02332	0.01964 0.02554
UTERUS	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table of organ weight. Day 35/day 56

Absolute weights are expressed as mean (grams). *different from controls at $P \leq 0.05$; **different from controls at $P \leq 0.01$.

Gross Pathology:

Group	Animal #	Study Day	Organ	Findings
1M	83069	34	Liver	White masses 1x1mm
	83070	34	liver	Diffuse white masses 1x1mm
2M	83066	34	Liver	White masses
	83089	56	liver	Multiple white masses
3M	81218	34	Liver	White masses
	83055	34	Liver	White masses
	83063	34	liver	White masses
4M	83068	34	Liver	White masses
	83081	34	Spleen	Enlarged, minimal
	83083	34	Liver	White masses
	83107	34	Liver	White masses
	83087	56	Thymus	White masses
5M	83060	34	Heart	Diffuse white patches
			Skin	Red area near injection site
	83064	34	Spleen	Enlarged, mild
			Heart	Diffuse white patches
	83075	34	Spleen	Enlarged, mild
			Heart	Diffuse white patches
	83082	34	Spleen	Enlarged, mild
	83090	34	Spleen	Enlarged, mild
	83094	34	Spleen	Enlarged, mild
	83100	34	Lymph node	Enlarged, axillary
			Spleen	Enlarged, mild
	83105	34	Liver	White mass 1x1mm
			Spleen	Enlarged, mild
83106	34	Spleen	Enlarged, mild	
83121	34	Spleen	Enlarged, moderate	
83077	56	Heart	Multifocal white patches	
83124	56	Heart	Multifocal white patches	
1F	83152	35	Heart	White patches
	83172	35	Injection site	Red areas
2F	83175	35	Liver	White mass 3x3 mm
	83176	56	Heart	White patches
	83202	56	liver	White masses 1x2 mm
3F	83171	35	Heart	White patches
			Injection site	Red areas
4F				NF
5F	83136	35	Spleen	Enlarged, moderate
	83139	35	Spleen	Enlarged, mild
	83147	35	Spleen	Enlarged, moderate
	83159	35	Spleen	Enlarged, mild
	83180	35	Spleen	Enlarged, moderate
	83182	35	Spleen	Enlarged, mild

	83189	35	Spleen	Enlarged, mild
	83194	35	Liver	White mass 1x1 mm
	83204	35	Spleen	Enlarged, moderate
			Injection site	Yellow brown area 5x5mm
	83206	35	Spleen	Enlarged, moderate
			spleen	Enlarged, moderate

NF=no findings

Microscopic findings are listed below:

Finding	Group 1	Group 2	Group 3	Group 4	Group 5
LIVER					
Necrosis	2/10m 2/10f	1/10f 1/5m rec 2/5f rec	1/10m 1/10f	2/10m	1/10f 3/5m rec
Inflammation, subacute	2/10m 4/10f	1/10f 1/5m rec	1/10f	2/10m 1/10f	
Infiltrate, monocyte	4/10m 2/10f	1/10m 2/10f 1/5m rec 1/5f rec	1/10m 1/10f 1/5f rec	1/10m 8/10f	
Inflammation, chronic		1/10m			1/5 m rec
Extramedullary hematopoiesis (EMH)					10/10m 10/10f 4/5f rec
Necrosis, single cell	1/10m			1/5f rec	
Infiltrate ,mixed cell			1/10m		
SPLEEN					
Extramedullary hematopoiesis	2/10m	1/10m 1/5m rec 2/5f rec	1/10m 1/10f 1/5m rec	1/10m	10/10m 10/10f 2/5f rec
KIDNEY					
Basophilia,tubular epithelium		2/10f 1/5m rec			2/10f 1/5m rec
HEART					
Mineralization,epicardium		2/10m 1/10f 1/5m rec 1/5f rec			6/10m 4/10f 1/5m rec 2/5f rec
Inflammation, chronic					1/10f

ADRENAL					
Vacuolation, cytoplasm cortex		3/10f 2/5f rec			1/10f
PARATHYROID					
Infiltrate, monocyte					1/3f
MANDIBULAR SALIVARY GLAND					
Infiltrate, monocyte	1/5f rec	1/10f			
Inflammation, chronic	1/5m rec				
UTERUS					
dilation		2/10f 3/5f rec			1/10f 1/5f rec
STOMACH					
Inflammation, subacute		1/7m			
Infiltrate, monocyte					1/5m rec
SKIN					
Hemorrhage, subcutis					1/10m
PROSTATE					
Infiltrate, monocyte		3/9m			2/10m
INJECTION SITE					
Inflammation, subacute		2/9m 2/10f			
Inflammation, chronic	10/10m 9/10f	4/9m 4/9f	5/10m 8/10f 1/5f rec	10/10m 9/10f	10/10m 9/10f 4/5m rec 4/5f rec
Mineralization	1/10m	1/10f 2/5m rec	1/10m 2/10f	6/10m 4/10f	7/10m 7/10f 5/5m rec 4/5f rec
Infiltrate, monocyte			3/10m 1/10f 2/5m rec	1/10f	
Myofiber degeneration		1/5m rec	1/10f 1/5f rec	1/5f rec	
Hemorrhage	1/10f				

All findings from main study day 34/35 necropsy unless indicated by rec (recovery)

A group 2 male was found dead on Day 27. Microscopically, this animal had epicardial mineralization and cytoplasmic vacuolation in the adrenal cortex, which may be spontaneous lesions. The cause of death for this animal was not determined.

In the main study mice sacrificed on days 34/35, treatment-related histopathologic findings were observed in the spleen, liver and injection sites. The incidences and severities of the treatment-related findings are summarized in the above table.

Treatment-related increased EMH, involving spleen and liver occurred in Group 5 mice (50 mcg ISS/0.5 mcg HBV). In the spleen, EMH was characterized by increased hematopoietic tissue in sinusoids. Although present in the spleen in Groups 1, 2, 3 and 4, the incidence and severity (mild to moderate) of EMH were increased only in Group 5. In the liver, EMH consisted of focal to multifocal aggregates of hematopoietic tissue randomly scattered throughout the parenchyma. Minimal to mild EMH occurred in all Group 5 animals, but did not occur in any animals in Groups 1, 2, 3 or 4. Based on these incidences, EMH was considered to be treatment-related in Group 5 animals. Since Group 5 animals received the combination vaccine (recombinant HBV and ISS), while Group 1 received commercial vaccine (HBV and alum) and Group 2 (recombinant HBV without ISS) animals received only recombinant HBV, EMH was considered related to ISS administration. This finding is consistent with immunostimulatory properties of phosphorothioate oligonucleotides previously reported in rodents .

Injection sites in all treatment groups contained inflammation within the skeletal muscle and the surrounding connective tissue. The inflammation consisted of fibroplasia, fibrosis, cellular infiltrates and scattered degenerate or necrotic myofibers. In animals receiving the test vaccine (HBV and ISS), this change was consistent with chronic inflammation as the cellular infiltrates were predominately macrophages, lymphocytes and plasma cells with fewer neutrophils or eosinophils. The incidence and severity of chronic inflammation in Groups 3 (females), 4 and 5 were related to the dose of ISS. Both the incidence and severity of chronic inflammation were less in Group 2 (HBV without ISS) than in Groups 3, 4 and 5. Chronic inflammation in injection sites in Groups 3 (females), 4 and 5 appears to be partially due to the 1018 ISS adjuvant.

In Group 1 animals receiving the commercial hepatitis B vaccine (HBV and alum), the cellular infiltrates in chronic inflammation were primarily neutrophils and eosinophils with few macrophages, lymphocytes or plasma cells. This cellular infiltrate was consistent with chronic-active inflammation, which was minimal to moderate in all animals in this group. The incidence and severity of inflammation were similar in Group 1 and Group 5, indicating that the local response to the adjuvant (ISS) component of the combination vaccine (test article) was comparable to the adjuvant (alum) component of the control vaccine (Energix).

Minimal focal to multifocal mineralization occurred within areas of inflammation at injection sites in all treatment groups. The mineralization appeared to be associated with necrotic myofibers in these sites. The incidence of injection site mineralization was low in Group 1 and Group 2, but increased with the dose of ISS in Groups 3, 4 and 5 (3,10 and 14 of 20, in Groups 3, 4 and 5, respectively). This is consistent with the 1018 ISS relative to the injection site mineralization.

Other histopathologic findings observed in terminal sacrifice animals included liver necrosis and epicardial mineralization in the heart. In this study, liver necrosis had a low and similar incidence and severity in affected groups, was not dose-related, and was associated with inflammation. The incidence of epicardial mineralization was similar in both sexes in Groups 2 and 5, and predominately affected the right ventricular epicardium. Liver necrosis and epicardial mineralization are common spontaneous lesions in mice and appear not to be treatment related.

At the recovery on day 56, the incidence and severity of splenic EMH in Groups 2, 3, 4 and 5 were similar and were consistent with those in Groups 1, 2, 3 and 4 on Days 34/35, indicating complete resolution of this change. In the liver, EMH had also resolved in males on Day 56, but minimal hepatic EMH persisted in females in Group 5, consistent with incomplete resolution of hepatic EMH in these females. Incidences and severities of injection site inflammation and mineralization were slightly decreased in Group 5 animals on Day 56 as compared to days 34/35, appearing to partially resolve.

Body temperature. Not determined

Local toxicity: Injection sites were examined microscopically and were not scaled according to Draize method.

Serology: Not conducted

Test article related effects
-Extramedullary hematopoiesis in the liver and spleen -Liver necrosis -Mild splenomegaly Reduction in RBC Inflammation, myofiber degeneration, mineralization and mononuclear cell infiltrate at the injection site

Assessment

There was no mortality in this study attributable to 1018 ISS Adjuvant + HBsAg vaccine. A Group 2 male was found dead on Day 27. This male received only HBsAg and no 1018 ISS Adjuvant; hence, death was in no way related to the combination vaccine, and the absence of any other deaths makes the relationship to HBsAg administration also unlikely. Administration of 1018 ISS Adjuvant + HBsAg was not associated with any clinical signs and had no effect on body weight or food consumption.

There were mild decreases in the group mean serum albumin, total protein, and triglyceride values for the 50 mcg 1018 ISS Adjuvant + HBsAg (Group 5) mice, relative to Engerix or HBsAg alone (Groups 1 and 2, respectively); These changes were small, and the biological significance and relationship to treatment was uncertain. For the high-dose 1018 ISS Adjuvant animals (Group 5), mean red blood cell parameters were slightly decreased relative to those for Engerix or HBsAg alone (Groups 1 and 2, respectively). Reductions in circulating red blood cell mass and albumin are class effects of phosphorothioate oligonucleotides in rodents these minor changes appear to be treatment-related. None of these differences in Group 5 mean values for albumin, protein, triglycerides, and red blood cell parameters were observed at the recovery necropsy.

Absolute and relative spleen weights at the terminal necropsy of Group 5 mice (50 µg ISS/0.5 µg HBV) were significantly increased relative to either of the Group 1 or Group 2 controls. The spleen weight in Group 5 mice was approximately 3-fold greater than the means of the control Groups 1 and 2. This increase in spleen weight correlated with the observation of grossly enlarged spleens at necropsy and with the observation microscopically of EMH in these mice. This effect was largely reversible upon cessation of treatment. Group 5 males at recovery had increased spleen weights approximately 1.3-fold greater than the control Group 2 males, while no difference was observed in Group 5 females from the control Group 2.

A statistically significant increase in spleen weight was also observed in Group 4 male mice (5 mcg ISS/0.5 mcg HBV). This increase in group mean, and its statistical significance, was attributable to the two Group 4 males with grossly enlarged spleens at necropsy (#s 83081 and 83083). Again this effect was correlated with the microscopic observation of EMH and was reversible within the 28 days of recovery.

Absolute and relative liver weights of Main Study Group 5 mice were significantly increased relative to both of the control Groups 1 and 2. The increase in liver weight was mild, with absolute liver weights roughly 20% greater in Group 5 mice relative to Group 2 mice. This increase in liver weight was attributed to administration of ISS and correlated microscopically with the liver alterations observed in Group 5 mice. Liver weights were similar among treatment groups at end of the recovery period.

An inflammatory response to treatment with 1018 ISS Adjuvant + HBsAg at the injection-site was also observed microscopically in mice at the terminal necropsy. This inflammatory response was considered to be treatment-related in 1 mcg 1018 ISS Adjuvant (Groups 3 females only), 5 mcg 1018 ISS Adjuvant (Group 4), and 50 mcg 1018 ISS Adjuvant (Group 5), as there was a dose-related increase in the incidence and severity relative to HBsAg alone (Group 2). In animals receiving the 1018 ISS Adjuvant + HBsAg, this change was consistent with chronic inflammation, as the cellular infiltrates were predominately macrophages, lymphocytes and plasma cells with fewer neutrophils or eosinophils. Inflammation is an expected finding in vaccine injection-sites and was of similar incidence and severity in the Engerix (Group 1) and 50 mcg 1018 ISS Adjuvant + HBsAg vaccine (Group 5) animals in this study, indicating that the local response to the adjuvant (ISS) component of the combination vaccine, 1018 ISS Adjuvant + HBsAg, was comparable to the adjuvant (Alum) component of the control (Engerix) commercial vaccine formulation. Minimal inflammation persisted in Group 5 at the recovery necropsy, consistent with partial resolution. Injection-site mineralization was also a treatment-related change that appeared to be associated with necrotic myofibers at the injection-sites. The incidence of injection-site mineralization was low in Group 1 and Group 2, but increased with the dose of 1018 ISS Adjuvant in Groups 3, 4 and 5 (3, 10, and 14 animals in Groups 3, 4, and 5, respectively) at the terminal necropsy. Hence, this change was regarded as 1018 ISS-related. Mineralization had partially resolved at the recovery necropsy of Group 5 animals.

Study deficiencies:

ISS dose relative to phase 3 clinical dose

lack of vehicle control

Clinical chemistry: lack of acute phase reactant measurement
lack of body temperature measurements

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

Conclusions

The results of this repeat-dose toxicity study show the combination of HBV and ISS produced expected toxicologic class effects in mice. Target organs were the hematopoietic system, spleen, liver and injection site. These target organs are consistent with expected oligonucleotide class effects in rodents. Immune stimulation is the most common and consistent finding associated with repeated administration of phosphorothioate oligonucleotides in rodents, but not monkeys. The increased splenic weight and the extramedullary hematopoiesis observed in spleens and livers of the combination HBV-ISS vaccine were consistent with the class effect. Also consistent with previous studies was the reversibility of the immunostimulatory response. Inflammation at the site of administration is also a class effect, and this effect was partially reversible. The mild anemia observed in this study has also been previously observed in rodents administered phosphorothioate oligonucleotides. This study resulted in no unexpected findings considering the known class effects of the adjuvant in mice receiving the HBV-ISS vaccine. While the adjuvant dose does not provide the direct toxicologic support (lack of NOAEL) intended by matching the clinical dose, on a mg/kg basis the highest adjuvant dose in the mouse toxicology study (2.5 mg/kg) was approximately 42-fold greater than the intended clinical dose of 0.06 mg/kg. Toxicology studies of the adjuvant alone were conducted in mice, rats, rabbits and nonhuman primates. Toxicities in these species included inflammation at the injection site and target organs including liver, kidney, spleen and lymph node (see accompanying review by Claudia Wrzesinski, Ph.D., DVM). Numerous clinical studies at the current intended and greater doses have been conducted with possible autoimmune adverse events (Wegener's granulomatous disease and thyroiditis) providing a basis to evaluate safety of this adjuvant. Clinical autoimmune adverse events cannot be adequately predicted in animal models.

Reproductive and developmental toxicology

Study title: Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Intramuscularly Administered HBsAg + 1018 ISS in Rats, Including a Postnatal Behavioral/Functional and Immunological Evaluation

Key study findings:

Study no.: Dynavax 05-463, (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: 12 Feb 2007

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and stability: HBsAg, ADW (b) (4) stability demonstrated through (b) (4) months at (b) (4) RH; 1018 ISS, (b) (4); stability through (b) (4) months at (b) (4) RH, and (b) (4)

Methods

Doses: Antigen dose (2.5 mcg HBsAg) . Adjuvant dose (3000 mcg 1018 ISS)

Species/strain: Rat (b) (4)

Number/sex/group:50

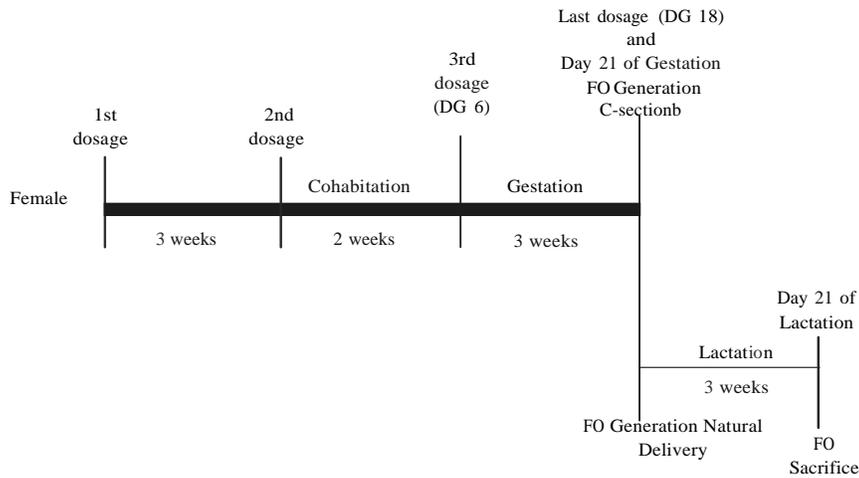
Route, formulation, volume: intramuscular (alternating thighs), PBS solution, 0.3 mL

Study design:

Dosage Group	Number of Rats	Treatment	HBsAg (mcg)	1018 ISS (mcg)	Dose Volume (mL)
I	50	PBS	0	0	IM (0.3)
II	50	HBsAg	2.5	0	IM (0.3)
III	50	HBsAg + 1018 ISS	2.5	1.5 mcg	IM (0.3)
IV	50	HBsAg + 1018 ISS	2.5	15 mcg (0.05)	IM (0.3)
V	50	HBsAg + 1018 ISS	2.5	300 mcg	IM (0.3)
VI	50	HBsAg + 1018 ISS	2.5	3000 mcg (10 mg/kg)	IM (0.3)
VII	50	1018 ISS	0	3000 mcg (10 mg/kg)	IM (0.3)

Female rats were given the test articles and/or the vehicle control article by intramuscular injection twice during the three-week pre-mating period (days 1 and 19 of study) and twice during the period of presumed gestation on days 6 and 18 (GD 6 and GD 18).

Developmental study design: based on sponsor submission



Study design: Natural delivery

F1 Generation Pups

Postnatal day 4	Preweaning →	Postnatal day 21	postweaning→	Postnatal day 72
				sacrifice

F1 Generation Rats

Postnatal day 90	cohabitation→	Postnatal day 111	gestation→	Day 21 gestation
		F1 male sacrifice		F1 generation c-section

Parameters and endpoints evaluated:

Body weights: days 1,2,4,5,11,14,19,21 of study and days 0,3,6,7,10,13,16 and 18 of presumed gestation, and postdosing days 19,21 and 25 of presumed gestation and days 1,4,7,10,14, 20 and 21 postpartum.

Food consumption: days 1,2,4,5,11,14,19,21 of study and days 0,3,6,7,10,13,16 and 18 of presumed gestation, and postdosing days 19,21 and 25 of presumed gestation and days 1,4,7,10,14, 20 and 21 postpartum

Mating Performance

Mating was evaluated daily during the cohabitation period and confirmed by observation of spermatozoa in a smear of the vaginal contents and/or a copulatory plug observed *in situ*.

Natural Delivery – F0 subset 2

Female rats were evaluated for: Duration of Gestation (day 0 of presumed gestation to the time the first pup is observed), abortions, premature deliveries, adverse clinical signs during parturition, litter size (defined as all pups delivered), and pup viability at birth.

Serum-Antibody Sample Collection – F0 Rats Subset 1

Samples were collected at the three time points, one or two days prior to the initiation of dosage (baseline bleed), at completion of the premating period [on day 21 of study (2 days after the second injection)] and on day 21 of presumed gestation [GD 21 bleeds (3 days after the fourth injection)], blood samples (at least 1.0 mL each) from 10 female rats per group assigned to the FO Subset 1 portion of the study. Blood was collected from the lateral tail vein.

After maternal blood sample collection on day 21 of presumed gestation, rats were sacrificed, and fetal blood were collected via decapitation and pooled per litter (FL) (FL GD 21 pool bleeds).

Serum-Cytokine Sample Collection – F0 Rats Subset 2 (Part A)

Samples were collected at three time points, one or two days prior to the treatment (designated cytokine bleed CB baseline), and within 6 to 8 hours (\pm 30 minutes) post-treatment of injection on the first day of dosing (designated CB Day 1), and on the last day of treatment (designated CB GD 18), blood samples (at least 1.0 mL each) from 10 female rats/group assigned to the FO Subset 2 portion (Part A) of the study. Blood was collected from the lateral tail vein.

Serum-Antibody Sample Collection – F0 Rats Subset 2 (Part B)

Samples were collected at three time points, one or two days prior to the initiation of dosage (baseline bleed), at completion of the pre-mating period [on day 21 of study (2 days after the second injection)] and on day 21 of lactation [LD 21 bleeds (approximately 24 days after the fourth injection)], from 10 female rats per group assigned to the FO Subset 2 portion (Part B) of the study. Blood was collected from the lateral tail vein.

Hematology and Clinical Chemistry

Samples were collected on the day of scheduled sacrifice (FO Subset 1 and FO Subset 2 females on GD 21 and day 21 postpartum, respectively), at least 5 mL from 10 rats (fasted) per group assigned to hematological and clinical biochemical evaluations.

Erythrocyte Count (RBC)
Hematocrit (HCT)
Hemoglobin (HGB)
Mean Corpuscular Hemoglobin (MCH)
Mean Corpuscular Hemoglobin
Concentration (MCHC)
Mean Corpuscular Volume (MCV)
Leukocyte Count, Total (WBC)
Leukocyte Count, Differential
Platelet Count (PLAT)
Mean Platelet Volume (MPV)
Cell Morphology

Coagulation parameters:

Prothrombin time (PT)
Activated Partial Thromboplastin Time (APTT)
Fibrinogen

Clinical Chemistry

Total Protein (TP)
Triglycerides (TRI)
Albumin (A)
Globulin (G)
Albumin/Globulin Ratio (A/G)
Glucose (GLU)
Cholesterol (CHOL)
Total Bilirubin (TBILI)

Urea Nitrogen (BUN)
Gamma-Glutamyl Transferase
Creatinine (CREAT)
Alanine Aminotransferase (ALT)
Aspartate Aminotransferase (AST)
Alkaline Phosphatase (ALK)
Calcium (CA)
Phosphorus (PROS)
Sodium (NA)
Potassium (K)
Chloride (CL)

METHOD OF SACRIFICE – F0 GENERATION

Rats were sacrificed by carbon dioxide asphyxiation. On day 21 of presumed gestation, fetuses were sacrificed via decapitation. Pups were sacrificed by an intraperitoneal injection of sodium pentobarbital (pups :14 approximately days of age) or by carbon dioxide asphyxiation (pups approximately 15 days of age).

NECROPSY – F0 GENERATION

Gross lesions were retained in neutral-buffered 10% formalin for possible future evaluation. Representative photographs of gross lesions were taken. See Attachment 4 for tissues to be weighed and retained for possible histological evaluation. All other tissues were discarded.

Scheduled Sacrifice – F0 Females Subset 1

On day 21 of presumed gestation, all female rats in Subset 1 were sacrificed after blood sample collection (F0 GD 21 bleeds). Females were subjected to C-section for complete set of uterine and fetal examinations. The rats were examined for number and distribution of corpora lutea, implantation sites [placentae that appear abnormal (size, color or shape) were noted in the raw data], live and dead fetuses (a live fetus is defined as one that responds to stimuli; a dead fetus is defined as a term fetus that does not respond to stimuli and that is not markedly autolyzed; dead fetuses demonstrating marked to extreme autolysis are considered to be late resorptions), and early and late resorptions (a conceptus is defined as a late resorption if it is grossly evident that organogenesis has occurred; if this is not the case, the conceptus is defined as an early resorption). Each fetus were weighed and examined for sex and gross external alterations.

Representative photographs of fetal alterations were taken. Fetuses were tagged with identification noting study number, litter number, uterine distribution and fixative, and retained for possible future evaluation. Approximately one-half of the fetuses in each litter were retained in Bouin's solution; the remaining fetuses were retained in alcohol. Uteri of apparently nonpregnant rats were examined while being pressed between glass plates to confirm the absence of implantation sites.

Scheduled Sacrifice – F0 Females Subset 2

After completion of the 21-day postpartum period, female rats were sacrificed after blood collection (LD 21 bleeds), and a gross necropsy of the thoracic, abdominal and

pelvic viscera were performed. The number and distribution of implantation sites were recorded.

Rats that did not deliver a litter were sacrificed on day 25 of presumed gestation and examined for gross lesions. Uteri were examined while being pressed between glass plates to confirm the absence of implantation sites.

Dams with No Surviving Pups – F0 Females Subset 2

Dams with no surviving pups were sacrificed after the last pup is found dead, missing or presumed cannibalized. A gross necropsy of the thoracic, abdominal and pelvic viscera were performed.

Rats Found Dead or Unscheduled Sacrifice

Rats that die or are sacrificed before scheduled termination were examined for the cause of death or condition on the day the observation was made. The rats were examined for gross lesions. Pregnancy status and uterine contents of female rats were recorded. Aborted fetuses, conceptuses *in utero* and/or delivered pups were examined to the extent possible, using the same methods described for term fetuses. Uteri of apparently nonpregnant rats were examined while being pressed between glass plates to confirm the absence of implantation sites. See Attachment 4 for tissues to be weighed and retained for possible histological evaluation.

TESTS, ANALYSES AND MEASUREMENTS - F1 GENERATION

Viability

Prewaning period: Litters were observed for dead pups at least twice daily. The pups in each litter were counted once daily.

Postweaning period: Litters were observed for dead pups at least twice daily

Clinical Observations and/or General Appearance

Prewaning period: once daily

postweaning period: at least once weekly and at sacrifice

Body Weights

Prewaning period: days 1 (birth), 4, 7, 14 and 21 postpartum

Postweaning period: weekly

Days of CPS injection (positive control rats only): daily

Presumed gestation period: days 0, 7, 10, 14, 17 and 21 (F1 Subset 2 female rats only).

At sacrifice: terminal sacrifice weight

Food Consumption Values

Prewaning period: not recorded.

Postweaning period: weekly except during cohabitation.

Presumed gestation period: days 0, 7, 10, 14, 17 and 21 (F1 Subset 2 female rats only)

F1 Generation Serum-Antibody Sample Collection

Blood samples were collected from four F₁ generation male and/or female pups from five litters per dose group on day 4 postpartum (PND 4 pool bleeds). Samples were collected via cardiac puncture after sacrifice and were pooled by litter. Blood samples (as much as possible) were collected from two F₁ generation pups (male or female) from each of five litters per dosage group on day 21 (PND 21 bleeds) postpartum. Samples were collected via cardiac puncture after sacrifice.

Postweaning Developmental Observations - F₁ Subset 2

(25 rats/sex/group)

Sexual maturation: Female rats were evaluated for the age of vaginal patency, beginning on day 28 postpartum. Male rats were evaluated for the age of preputial separation, beginning on day 39 postpartum.

Motor activity: Motor activity were evaluated on days 22 and 61 (± 2 days) postpartum. One male and one female rat (when possible) from each litter (for a total of 25 rats /sex /group) were examined throughout the two testing periods.

Passive avoidance testing: Beginning at 24 ± 1 day postpartum, one male rat and one female rat from each litter, where possible (for a total of 25 rats /sex /group), were evaluated in a passive avoidance test for learning, short-term retention and long-term retention. Each rat is tested twice. The test sessions are separated by a one-week interval, and the criterion is the same for both days of testing.

Statistical methods

Healthy female rats were assigned to dosage groups (N = 50 rats per group) based on computer-generated (weight-ordered) randomization procedures. FO female rats were assigned to two subgroups: the first group (FO Rats Subset 1) will consist of the first 25 rats in each dosage group and were Caesarean-sectioned on presumed gestation day 21. The second group (FO Rats Subset 2) will consist of the remaining rats in each dosage group and were allowed to deliver naturally and rear offspring to weaning. A table of random units or a computer-generated randomization were used to select 20 female rats per dosage group for serum-antibody sample collection: at three time points from 10 females in Subset 1 (baseline, day 21 of study, and GD21), and 10 females in Subset 2 (baseline, day 21 of study, and LD 21). In addition a table of random units or a computer-generated randomization were used to select 10 female rats per dosage group from Subset 2 for serum-cytokine sample collection: at baseline, and 6 to 8 hours after the first (day 1) and last (GD 18) treatment. Day 1 of study were 21 days before the cohabitation period. Female rats were cohabited with breeder male rats, one male rat per female rat. The cohabitation period will consist of a maximum of 14 days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* were considered to be at day 0 of presumed gestation and assigned to individual housing. Female rats not mated within the first seven days of cohabitation were assigned alternate male rats that have mated and will remain in cohabitation for a maximum of seven additional days. Female rats not mated after completion of the 14-day cohabitation period were considered to be at day 0 of presumed gestation on the last day of cohabitation and assigned to individual housing. Beginning no later than day 20 of presumed gestation, FO

generation female rats assigned to subset 2 were individually housed in nesting boxes. Each dam and delivered litter were housed in a common nesting box during the postpartum period.

All F1 generation rats were weaned at the same age, based on observed growth and viability of the pups, on either day 21 postpartum or, if necessary, on day 28 postpartum.

Postweaning Developmental Observations - F1 Subset 2 (25 rats/sex/group)

Sexual Maturation: Female rats were evaluated for the age of vaginal patency, beginning on day 28 postpartum. Male rats were evaluated for the age of preputial separation, beginning on day 39 postpartum.

Motor Activity: Motor activity were evaluated on days 22 and 61 (± 2 days) postpartum. One male and one female rat (when possible) from each litter (for a total of 25 rats /sex /group) were examined throughout the two testing periods.

For Passive Avoidance Testing: Beginning at 24 ± 1 day postpartum, one male rat and one female rat from each litter, where possible (for a total of 25 rats /sex /group), were evaluated in a passive avoidance test for learning, short-term retention and long-term retention. Each rat is tested twice. The test sessions are separated by a one-week interval, and the criterion is the same for both days of testing. Dosage groups are compared for the following dependent measures: The number of trials to the criterion in the first session-- this measure were used to compare groups for overall learning performance.

Watermaze Testing

Beginning at 70 ± 2 days postpartum, one male rat and one female rat from each litter (for a total of 25 rats /sex /group) were evaluated in a water- filled M-maze for overt coordination, swimming ability, learning and memory. Each rat is tested twice. The test sessions are separated by a one-week interval, and the correct goal and the criterion are the same for both test sessions.

Reproductive Capacity

At approximately 90 days of age, the F1 generation rats (Subset 2; 25 per sex per group) were assigned to cohabitation, one male rat per female rat, based on computer generated random units or random unit tables, with the exclusion of sibling matings. The cohabitation period will consist of a maximum of 21 days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* will be considered to be at day 0 of presumed gestation and assigned to individual housing. Female rats that do not mate within the first 14 days of cohabitation were assigned alternate male rats from the same dosage group that has mated. Female rats not mated after completion of the 21-day cohabitation period were considered to be at day 0 of presumed gestation on the last day of cohabitation and assigned to individual housing. Female rats were Caesarean-sectioned on day 21 of presumed gestation. The rats were examined for number and distribution of corpora lutea, implantation sites [placentae that appear abnormal (size, color or shape) were noted in the raw data], live and dead fetuses (a live fetus is defined as one that responds to stimuli; a dead fetus is defined as a term fetus that does not respond to stimuli and that is not markedly autolyzed; dead fetuses demonstrating marked to extreme autolysis are considered to be late resorptions),

and early and late resorptions (a conceptus is defined as a late resorption if it is grossly evident that organogenesis has occurred; if this is not the case, the conceptus is defined as an early resorption). Each fetus was weighed and examined for sex and gross external alterations. Representative photographs of fetal alterations were taken. Fetuses were tagged with identification noting study number, litter number, uterine distribution and fixative, and retained for possible future evaluation. Approximately one-half of the fetuses in each litter were retained in Bouin's solution; the remaining fetuses were retained in alcohol.

Immunological Evaluation - Positive Control Article and/or Immunological Material Administration (F1 Subset 1)

All F1 generation rats in Subset 1 (10 rats per sex/group in Groups II to VII; 20/sex in Group I) were administered 0.5 mL of sheep red blood cells (sRBCs - immunological material) via intravenous administration once four days before euthanasia (at approximately PND 68).

Ten male and ten female rats in Group I were administered 50 mg/kg (10 mg/mL) cyclophosphamide (CPS -positive control article) via intraperitoneal injection for four consecutive days before euthanasia (at approximately PND 68, PND 69, PND 70, and on PND 71). This injection of cyclophosphamide were administered after the administration of the sRBCs (at approximately PND 68).

Necropsy and sacrifice F1 rats

Scheduled Sacrifice - Male and Female Rats (F1 Subset 1)

Male and female rats (10 rats per sex from Groups II through VII, and 20 rats per sex from the control group [including 10 rats per sex treated with the positive control agent for immunotoxicity evaluation]) were sacrificed on postnatal day 72. A gross necropsy of the thoracic, abdominal and pelvic viscera were performed

Scheduled Sacrifice - Male Rats (F1 Subset 2)

Male rats (Subset 2; up to 25 males per group) were sacrificed after completion of the 21-day cohabitation period. A gross necropsy of the thoracic, abdominal and pelvic viscera were performed. Testes and epididymides of male rats were excised and paired organ weights were recorded. The epididymides were retained in neutral buffered 10% formalin. The testes were fixed in Bouin's solution for 48 to 96 hours and then retained in neutral-buffered 10% formalin.

Scheduled Sacrifice - Female Rats (F1 Subset 2)

On day 21 of presumed gestation, female rats (F1 Subset 2; up to 25 per group) were sacrificed, and a gross necropsy of the thoracic, abdominal and pelvic viscera were performed. Female rats without a confirmed mating date were sacrificed on an estimated day 21 of presumed gestation. Uteri of apparently nonpregnant rats were examined while being pressed between glass plates to confirm the absence of implantation sites. Uteri and ovaries of apparently nonpregnant rats were retained in neutral buffered 10% formalin and discarded at the end of the study.

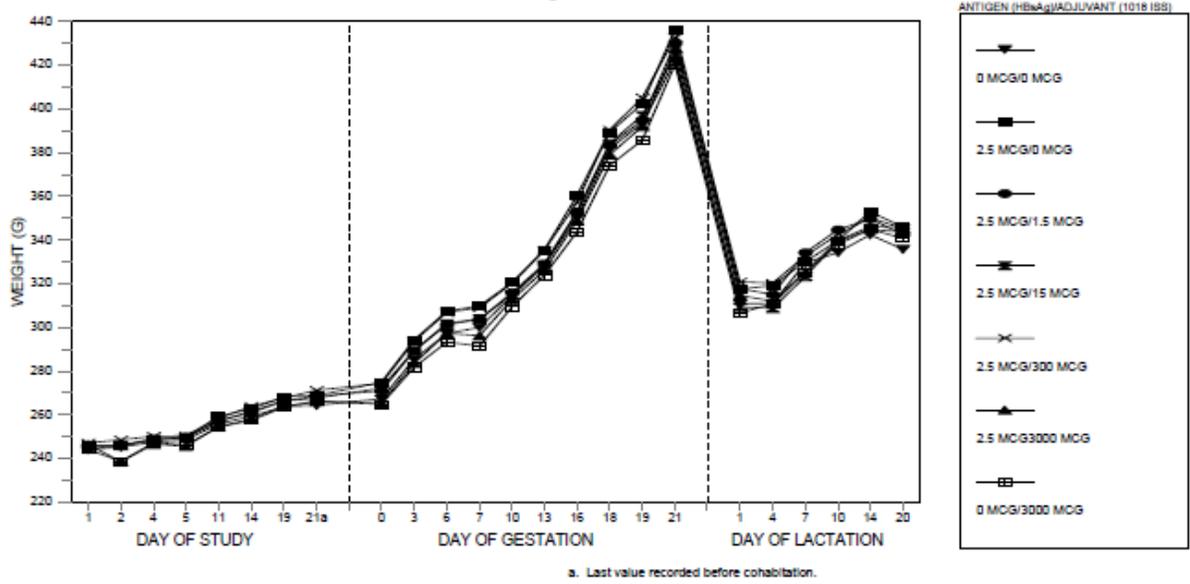
Rats Found Dead or Unscheduled Sacrifice (F1 Subset 2)

Rats that die or are sacrificed before scheduled termination were examined for the cause of death or condition on the day the observation is made. The rats were examined for gross lesions. Testes and epididymides of male rats were excised and paired organ weights were recorded. The epididymides were retained in neutral buffered 10% formalin. The testes were fixed in Bouin's solution for 48 to 96 hours and then retained in neutral-buffered 10% formalin. Pregnancy status and uterine contents of female rats were recorded. Aborted fetuses, conceptuses *in utero* and/or delivered pups were examined to the extent possible, using the same methods described for term fetuses. Uteri of apparently nonpregnant (mated) rats were examined while being pressed between glass plates to confirm the absence of implantation sites and retained in neutral-buffered 10% formalin.

Results

MATERNAL BODY WEIGHTS - F0 GENERATION FEMALE RATS

Figure 1



From the sponsor submission

Summary Tables Reproductive and Developmental Toxicology

Reproductive and Developmental Toxicity: Mating, Reproductive Parameters: F₀

Maternal Dose 1018 ISS Adjuvant / HBsAg (mcg)	0 / 0	0 / 2.5	1.5 / 2.5	15 / 2.5	300 / 2.5	3000 / 2.5	3000 / 0
1018 ISS Adjuvant Dose Level (mg/kg)	0	0	0.005	0.05	1.0	10	10
Control/Test Article(s)	PBS (Control)	HBsAg	1018 ISS Adjuvant ± HBsAg	1018 ISS Adjuvant ± HBsAg	1018 ISS Adjuvant ± HBsAg	1018 ISS Adjuvant ± HBsAg	1018 ISS Adjuvant
F₀ Females (Dams/Does):							
Number Evaluated	50	50	50	50	50	50	50
Number Pregnant	48	4	4	4	46	50	4
Number Died or Sacrificed Moribund	0	1 ^a	0	0	0	3 ^b	2 ^b
Number Delivered and Sacrificed	0	1 ^c	2 ^c	0	0	1 ^c	0
Clinical Observations: Injection Sites							
Pre-cohabitation Period: Scab(s)	(+) 3	(+) 1	(+) 2	(-) 0	(+) 1	(++) 10**	(++) 7*
Pre-cohabitation Period: Abrasion	(+) 1	(+) 1	(-) 0	(-) 0	(-) 0	(+) 3	(+) 1
Gestation Period: Scab(s)	(+) 2	(+) 2	(+) 2	(-) 0	(+) 2	(+++) 22**	(+++) 19**
Gestation Period: Abrasion	(-) 0	(+) 1	(-) 0	(-) 0	(-) 0	(++) 7**	(++) 4**
Lactation Period: Scab(s)	(+) 1	(-) 0	(-) 0	(-) 0	(+) 1	(++) 7**	(++) 8**
Lactation Period: Abrasion	(-) 0	(-) 0	(-) 0	(-) 0	(-) 0	(+) 1	(++) 3**

* Significantly different from the control group value ($p \leq 0.05$); ** Significantly different from the control group ($p \leq 0.01$); + = Mild; ++ = Moderate; +++ = Severe; DG = day of (presumed) gestation; DS = day of study.

^a The early sacrifice of one dam (Animal No. 4493) in the HBsAg-alone group on Day 2 of lactation (DL 2) due to clinical signs was not considered related to HBsAg because there were no clinical signs in any other animals from that group or any clinical signs in animals from other groups that were attributed to HBsAg.

^b Two dams (Animal Nos. 4728 and 4734) in the 3000 mcg/dose 1018 ISS-alone group were found dead, and two dams (Nos. 4692 and 4699) in the 2.5/3000 mcg/dose HBsAg/1018 ISS group were sacrificed during delivery. In addition, one dam (No. 4683) in the 2.5/3000 mcg/dose HBsAg/1018 ISS group was sacrificed due to adverse clinical signs on Day 14 of gestation (DG 14). These findings were possibly related to the 3000 mcg doses of the 1018 ISS.

^c The early sacrifice following delivery in two dams (Nos. 4461 and 4505) with mis-timed pregnancies and two dams (Nos. 4517 and 4671) that had no-confirmed mating date but delivered litters were unrelated to the test articles because these events were due to technical errors.

Table from sponsor submission

Reproductive and Developmental Toxicity: Maternal Effects

Maternal Dose 1018 ISS Adjuvant / HBsAg (mcg)	0 / 0	0 / 2.5	1.5 / 2.5	15 / 2.5	300 / 2.5	3000 / 2.5	3000 / 0
1018 ISS Adjuvant Dose Level (mg/kg)	0	0	0.005	0.05	1.0	10	10
<u>Control/Test Article(s)</u>	<u>PBS</u> <u>(Control)</u>	<u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant</u>
F₀ Females (Dams/Does): (Continued)							
Mean Pre-cohabitation Body Weight (DS 21) (%) ^d	264.3 g	+1.7	+1.6	+1.2	+2.6	+0.6	+0.8
Mean Gestation Body Weight (DG 21) (%) ^d	422.1 g	+3.4	+1.8	+0.6	+2.5	+1.3	-0.4
Mean Pre-cohabitation Feed Consumption (%) ^d							
DS 1-2	17.6 g	+1.1	+1.7	+2.3	+4.0	-39.8**	-41.5**
DS 4-5	19.8 g	+2.0	+4.0	+0.5	-3.0	-8.1*	-10.1*
DS 19-21	18.6 g	+6.4	+8.6*	+5.4	+9.1**	-12.4**	-14.0**
Mean Gestation Feed Consumption (DG 0-21) (%) ^d							
DG 0-3	22.4 g	+4.9	+1.8	+2.7	+4.5	-4.5	-8.0**
DG 6-7	23.7 g	+4.2	+2.5	+0.0	+4.6	-30.4**	-36.7**
DG 7-10	24.6 g	-0.8	-3.7	+0.0	-1.2	-13.4**	-13.8**
DG 10-13	25.4 g	+0.8	+0.4	+0.4	+2.0	-6.7*	-7.9**
DG 13-16	24.7 g	+0.8	+0.4	+0.8	+1.6	+9.3**	+4.8
DG 18-19	26.4 g	-3.8	-0.4	+1.5	+0.0	-14.4**	-14.8**
DG 6-19	25.4 g	+0.8	-0.8	+0.0	+0.8	-4.7	-7.1**
DG 6-21	24.9 g	+0.8	+0.0	+0.4	+0.8	-3.6	-6.8**
DG 0-21	24.3 g	+2.0	+0.4	+1.2	+1.6	-2.9	-6.6**
Mean Number Days Prior to Mating	2.7	3.1	2.4	2.7	2.6	2.6	2.4
Number of Females Sperm-Positive	49	48	48	49	48	49	50
Number of Pregnant Females/Number Rats in Cohabitation	48/50	48/50	49/50	49/50	46/50	50/50	47/50

* Significantly different from the control group value ($p \leq 0.05$); ** Significantly different from the control group ($p \leq 0.01$); DS = day of study; DG = day of (presumed) gestation.

^d For controls, group means are shown (in grams). For treated groups, percent difference from control means is shown. Statistical significance is based on actual data (not on the percent differences).

Table from sponsor submission

Reproductive and Developmental Toxicity: Maternal Effects

Maternal Dose 1018 ISS Adjuvant / HBsAg (mcg)	0 / 0	0 / 2.5	1.5 / 2.5	15 / 2.5	300 / 2.5	3000 / 2.5	3000 / 0
1018 ISS Adjuvant Dose Level (mg/kg)	0	0	0.005	0.05	1.0	10	10
<u>Control/Test Article(s)</u>	<u>PBS</u> <u>(Control)</u>	<u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant</u>
Dams Assigned to Caesarean-Sectioning:							
Mean Number Corpora Lutea	16.2	17.0	15.8	16.4	16.2	16.4	17.1
Mean Number Implantations	15.3	16.0	14.8	15.1	15.4	16.2	15.6
Mean % Preimplantation Loss	4.8	4.6	6.4	8.8	4.6	1.7	9.2
Necropsy Observations							
Lymph Node(s): Large	0	0	2	0	1	2	7**
Liver: Large	0	0	0	0	0	14**	13**
Spleen: Large	0	0	0	0	0	19**	19**
Mean Absolute Organ Weights (%) [°]							
Liver	14.3 g	-0.3	-1.9	-1.7	-0.6	+69.6**	+76.4**
Kidneys Paired	2.28 g	+2.6	+1.8	-1.8	+0.0	+10.1**	+9.6**
Adrenals Paired	0.088 g	+0.0	-4.5	-2.3	+5.7	+13.6*	+11.4
Spleen	0.68 g	+2.9	+2.9	-1.5	+7.4	+151.5**	+144.1**

* Significantly different from the control group value ($p \leq 0.05$); ** Significantly different from the control group ($p \leq 0.01$).

[°] For controls, group means are shown (in grams). For treated groups, percent difference from control means is shown. Statistical significance is based on actual data (not on the percent differences).

Table from sponsor submission

Reproductive and Developmental Toxicity: F₁ Caesarean Reproductive Parameters, Fetal Skeletal and Soft Tissue Exam

Maternal Dose 1018 ISS Adjuvant / HBsAg (mcg)	0 / 0	0 / 2.5	1.5 / 2.5	15 / 2.5	300 / 2.5	3000 / 2.5	3000 / 0
1018 ISS Adjuvant Dose Level (mg/kg)	0	0	0.005	0.05	1.0	10	10
<u>Control/Test Article(s)</u>	<u>PBS</u> <u>(Control)</u>	<u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant</u>
F₁ Litters of Dams Assigned to Caesarean-Sectioning:							
Number Litters Evaluated	24	22	23	24	23	24	23
Mean Number Live Fetuses	14.6	15.2	14.0	14.4	14.9	14.4	14.9
Mean Number Resorptions	0.7	0.8	0.9	0.7	0.5	1.8	0.7
Number of Dead Fetuses	0	0	0	0	0	0	0
Mean % Resorbed Conceptuses per Litter	4.3	4.8	5.9	4.2	3.7	7.2	4.8
Mean Fetal Body Weight (g)	4.95	4.99	4.98	5.21	5.06	5.11	5.21
Fetal Sex Ratios (% males)	52.7	48.8	51.5	48.7	45.4	47.4	49.7
Fetal Anomalies:							
Gross External	NF	NF	NF	NF	NF	NF	NF
Soft Tissue Anomalies	NF	NF	NF	NF	NF	NF	NF
Skeletal Anomalies							
Cervical Rib at 7th Cervical Vertebra							
Number Litters (%)	2 (8.3)	1 (4.5)	0 (0.0)	2 (8.3)	0 (0.0)	8 (34.8)**	3 (13.0)
Number Fetuses (%)	2 (1.1)	1 (0.6)	0 (0.0)	2 (1.1)	0 (0.0)	10 (5.6)**	4 (2.3)
Ribs: Short							
Number Litters (%)	0 (0.0)	0 (0.0)	4 (17.4)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Number Fetuses (%)	0 (0.0)	0 (0.0)	5 (3.0)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total Affected Fetuses (Litters)	26 (18)	11** (6**)	15** (11**)	10** (7**)	7** (5**)	19* (5)	12** (8**)

* Significantly different from the control group value ($p \leq 0.05$); ** Significantly different from the control group ($p \leq 0.01$); NF = no findings.

Table from sponsor submission

Reproductive and Developmental Toxicity: Natural Delivery: Reproductive, Delivery Parameters

Maternal Dose 1018 ISS Adjuvant / HBsAg (mcg)	0 / 0	0 / 2.5	1.5 / 2.5	15 / 2.5	300 / 2.5	3000 / 2.5	3000 / 0
1018 ISS Adjuvant Dose Level (mg/kg)	0	0	0.005	0.05	1.0	10	10
<u>Control/Test Article(s)</u>	<u>PBS (Control)</u>	<u>HBsAg</u>	<u>1018 ISS Adjuvant + HBsAg</u>	<u>1018 ISS Adjuvant</u>			
Dams Assigned to Natural Delivery:							
Mean Lactation Body Weight (DL 20) (%) ^f	335.8 g	+3.0	+2.0	+2.4	+2.8	+2.9	+1.5
Mean Lactation Food Consumption (DL 1-14) (%) ^f	45.7 g	-3.1	+1.8	+2.0	-0.4	+5.7	+3.7
Mean Duration of Gestation (days)	22.6	22.7	22.9	22.7	22.7	22.8	22.9
Abnormal Parturition	NF	NF	NF	NF	NF	NF	NF
Necropsy Observations							
Spleen: Large	0	0	0	0	0	3**	5**
Mean Absolute Organ Weights (%) ^f							
Liver	15.27 g	-1.7	+6.6	+2.2	+2.1	+30.4**	+24.2**
Spleen	0.60 g	+3.3	+3.3	+3.3	+8.3	+96.7**	+75.0**
F₁ Litters (Pre-weaning):							
Number Litters Evaluated	24	25	24	24	23	22	22
Mean Number of Implantations	14.7	15.6	16.1	15.6	15.4	16.2	15.5
Mean Number Pups per Litter	13.7	14.1	14.1	14.4	14.1	14.7	13.8
Mean Number Liveborn Pups per Litter	13.3	14.0	13.7	14.2	14.0	14.1	13.0
Mean Number of Stillborn Pups per Litter	0.4	0.2	0.4	0.2	0.1	0.6	0.8
Postnatal Survival to Day 4 (Viability Index) (%)	99.0	94.8**	97.6	97.1**	95.0**	96.1**	99.0
Postnatal Survival to Weaning (Lactation Index) (%)	96.3	97.4	99.0**	99.0**	95.1	99.3**	99.6**
Number of Total Litters Losses	0	1	0	0	1	0	0
Mean Change in Pup Body Weights/Litter (%) ^{f, g}	32.8 g	-4.3	+0.0	-0.6	-3.6	-8.2	-2.7
Mean Pup Sex Ratios (Day 1) (% males)	48.8	47.5	47.6	50.1	50.7	47.2	50.4
Pup Clinical Signs	NF	NF	NF	NF	NF	NF	NF
Pup Necropsy Observations	NF	NF	NF	NF	NF	NF	NF

** Significantly different from the control group ($p \leq 0.01$); DL = day of lactation (postpartum); NF = no findings.

^f For controls, group means are shown in grams. For treated groups, the percent difference from control values is shown. Statistical significance is based on actual data (not on the percent differences).

From birth to weaning (Days 1 to 21). Values are the difference between the mean body weights on Day 1 and Day 21 postpartum.

Table from sponsor submission

Reproductive and Developmental Toxicity

Natural Delivery: F₁ Developmental Parameters

Maternal Dose 1018 ISS Adjuvant / HBsAg (mcg)	0 / 0	0 / 2.5	1.5 / 2.5	15 / 2.5	300 / 2.5	3000 / 2.5	3000 / 0
1018 ISS Adjuvant Dose Level (mg/kg)	0	0	0.005	0.05	1.0	10	10
<u>Control/Test Article(s)</u>	<u>PBS</u> <u>(Control)</u>	<u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant</u>
F₁ Pups Immune Evaluation Subset:							
Spleen Weights	NF	NF	NF	NF	NF	NF	NF
Immunotoxicity Evaluations ^h	NF	NF	NF	NF	NF	NF	NF
F₁ Males Behavioral Subset (Post-weaning):							
Number Evaluated Post-weaning	25	26	25	25	25	25	25
Number Died or Sacrificed Moribund	0	0	1 ⁱ	0	0	0	1 ⁱ
Clinical Observations	NF	NF	NF	NF	NF	NF	NF
Necropsy Observations	NF	NF	NF	NF	NF	NF	NF
Mean Body Weight Change (%) ^{j, k}	480.8 g	-2.4	-1.7	-2.2	+0.0	-8.3	-4.7
Mean Absolute Feed Consumption (PNDs 8-113) (%) ^k	29.5 g	-1.7	-1.4	-3.0	+0.3	-4.1	-5.1
Mean Age of Preputial Separation (days)	47.1	46.1	45.9	46.4	46.2	47.0	47.1
Motor Activity	NF	NF	NF	NF	NF	NF	NF
Passive Avoidance	NF	NF	NF	NF	NF	NF	NF
Watermaze Performance	NF	NF	NF	NF	NF	NF	NF
Mean Number Days Prior to Mating	3.6	2.7	3.0	2.2*	3.9	3.1	3.6
Number of Males that Mated	25	24	25	25	24	23	23
Number of Fertile Males (Fertility Index)	22/25	24/24	21/25	24/25	23/24	20/23	21/23
Organ Weights	NF	NF	NF	NF	NF	NF	NF

* Significantly different from the control group ($p \leq 0.05$); NF = no findings; PND = postnatal days.

^h Immune evaluation included T-cell-dependent IgM splenocyte antibody responses to sheep red blood cells.

ⁱ Two male rats assigned to behavioral evaluations were euthanized prior to scheduled sacrifice. One male rat in the 2.5/1.5 mcg HBsAg/1018 ISS Adjuvant maternal dosage group was sacrificed due to a damaged eye on PND 92, and one male rat in the 3000 mcg 1018 ISS Adjuvant alone maternal dosage group was sacrificed due to adverse clinical observations on

PND 28. None of these events were considered related to the test articles administered to the F₀ females.

^j From weaning to mating (Day 1 to Precohabitation, when rats were 92 to 103 days of age).

^k For controls, group means are shown. For treated groups, percent difference from control is shown.

Table from sponsor submission

Reproductive and Developmental Toxicity

Natural Delivery: F₁ Developmental Parameters, Mating and Reproductive Performance

Maternal Dose 1018 ISS Adjuvant / HBsAg (mcg)	0 / 0	0 / 2.5	1.5 / 2.5	15 / 2.5	300 / 2.5	3000 / 2.5	3000 / 0
1018 ISS Adjuvant Dose Level (mg/kg)	0	0	0.005	0.05	1.0	10	10
<u>Control/Test Article(s)</u>	<u>PBS</u> <u>(Control)</u>	<u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant</u>
F₁ Females Behavioral Subset (Post-weaning):							
Number Evaluated Post-weaning	25	25	25	25	25	25	25
Number Died or Sacrificed Moribund	0	1 ¹	0	0	0	0	0
Clinical Observations:	NF	NF	NF	NF	NF	NF	NF
Necropsy Observations	NF	NF	NF	NF	NF	NF	NF
Organ Weights	NF	NF	NF	NF	NF	NF	NF
Mean Premating Body Weight Change (%) ^{m, n}	240.1 g	-0.6	+1.2	+2.4	+4.1	+0.4	-1.0
Mean Gestation Body Weight Change (DG 0-21) (%) ⁿ	181.4 g	+2.0	-7.3	-1.6	+2.2	-2.0	-1.8
Mean Absolute Feed Consumption (PNDs 8-71) (%) ⁿ	21.1 g	-3.8	+0.5	-2.8	+1.4	-0.5	-5.2
Mean Absolute Gestation Feed Consumption (DG 0-21) (%) ⁿ	28.0 g	-4.3	-3.9	-1.8	-2.9	-5.4	-6.1
Mean Age of Vaginal Patency (days)	31.7	31.8	31.8	32.2	32.8	32.4	32.1
Motor Activity	NF	NF	NF	NF	NF	NF	NF
Passive Avoidance	NF	NF	NF	NF	NF	NF	NF
Watermaze Performance	NF	NF	NF	NF	NF	NF	NF
Mean Number Days Prior to Mating	3.6	2.7	2.5	2.2*	3.1	3.2	3.2
Number of Females Sperm Positive	25	25	24	25	23	25	24
Number of Pregnant Females (Fertility Index)	22/25	25/25	21/25	24/25	24/25	22/25	22/25
Mean Number Corpora Lutea	16.5	17.6	16.0	16.7	17.6	16.3	15.8
Mean Number Implantations	15.9	16.7	14.3	15.7	16.8	15.3	15.0

* Significantly different from the control group ($p \leq 0.05$); DG = day of (presumed) gestation; PND = postnatal day; NF = no findings.

¹ One female rat descendant from the 2.5 mcg HBsAg-alone maternal dosage group delivered early and was sacrificed on DG19. This was not considered related to the test articles administered to the F₀ females.

^m From weaning to mating (Day 1 to Precohabitation, when rats were 92 to 103 days of age).

ⁿ For controls, group means are shown. For treated groups, percent difference from control is shown.
Table from sponsor submission

Reproductive and Developmental Toxicity

Natural Delivery: F₁ Developmental Parameters: Mating and Reproductive Performance

Maternal Dose 1018 ISS Adjuvant / HBsAg (mcg)	0 / 0	0 / 2.5	1.5 / 2.5	15 / 2.5	300 / 2.5	3000 / 2.5	3000 / 0
1018 ISS Adjuvant Dose Level (mg/kg)	0	0	0.005	0.05	1.0	10	10
<u>Control/Test Article(s)</u>	<u>PBS</u> <u>(Control)</u>	<u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant +</u> <u>HBsAg</u>	<u>1018 ISS</u> <u>Adjuvant</u>
F₂ Litters:							
Mean Number Live Conceptuses per Litter	15.1	16.1	13.7	14.7	16.1	14.8	14.4
Mean Number Resorptions	0.7	0.6	0.7	1.0	0.7	0.4	0.6
Number of Litters with Dead Conceptuses	0	0	0	0	0	0	0
Mean % Resorbed Conceptuses per Litter	4.5	4.3	5.0	6.5	3.9	3.0	3.7
Mean Fetal Body Weights (%) ^o	4.88 g	+3.1	+5.5	+2.4	+2.2	+4.5	+4.1
Mean Fetal Sex Ratios (% males) per Litter	49.0	47.5	55.5	47.7	48.1	48.5	45.7
Fetal Anomalies	NF	NF	NF	NF	NF	NF	NF

NF = no findings

^o For controls, group means are shown. For treated groups, percent difference from control is shown.

Table modified from tables provided by sponsor.

Results

Two dams in the 3000 mcg/dose 1018 ISS-alone Group VII were found dead near the end of the gestation period (on DG 20 or 22), and three dams in the 2.5/3000 mcg/dose HBsAg/1018 ISS Group VI were sacrificed on DG 14 or during delivery due to adverse clinical signs. Although these events occurred in groups administered the high-dose of 3000 mcg/dose 1018 ISS, the relationship to treatment is uncertain because there were dissimilar clinical signs, litter status (i.e., numbers of live/dead fetuses or pups), gross findings, and histological findings in these 5 females, and no deaths or problems in delivery occurred in the other four antigen-treated groups (i.e., Groups II to V).

However, deaths and early sacrifices of control rats near expected delivery are rare so this low incidence in each of the two groups given 3000 mcg of 1018 ISS can not be discounted and is therefore considered to be of uncertain relationship to treatment. Apart from the above morbidity and mortality, there were no clinical signs of systemic adverse effects of the antigen or adjuvant either alone or in combination. The number of rats that had local reactions (scabs and/or abrasions) at the injection sites was increased in the groups that received the highest dose (3000 mcg) of 1018 ISS, either alone or in combination with the HBsAg. These results may be related to the immunostimulatory properties of 1018 ISS, as dose-dependent injection site reactions are expected to occur with this adjuvant.

Body weights, body weight gains, and absolute and relative feed consumption values during the pre-mating (prior to cohabitation), gestation and lactation periods were largely unaffected by dosages of the test article as high as 2.5/3000 mcg HBsAg/1018 ISS. There were, however, transient reductions in body weight gains after the first and third doses and feed consumption on the days of dosage administration in the 2.5/3000 mcg HBsAg/1018 ISS and 0/3000 mcg HBsAg/1018 ISS dosage groups, and subsequent rebound increases in these parameters for the next few days post-dosing. Also, a possible more persistent decrease in food consumption during gestation was evident for the groups that received the highest dose (3000 mcg) of 1018 ISS, either alone or in combination with the HBsAg, but overall body weight gain was clearly not affected in these groups. All mating and fertility parameters for both Caesarean and natural delivery rats as well as Caesarean-sectioning and litter parameters, were unaffected by dosages of the test articles as high as 2.5/3000 mcg HBsAg/1018 ISS or HBsAg administered alone. Time to mating and pregnancy rates were comparable among the groups. There were no dead fetuses, and all placentae appeared normal across groups. Also, there was no effect on fetal development based on gross external, soft tissue and skeletal examinations.

In the natural delivery litters, the number of stillborn pups was increased in the 2.5/3000 mcg/dose HBsAg/1018 ISS and 3000 mcg/dose 1018 ISS-alone groups (13 and 17 stillborns, compared to 9 in the control group), but these marginal increases were not considered to be related to the treatments because they were due to only 3 to 4 additional litters each with only 1 stillborn and 1 additional litter in each with 3 to 4 stillborns. In addition, only the incidence in the 3000 mcg/dose 1018 ISS-alone group was outside the historical control range for this Testing Facility.

The number of pups found dead on postnatal days 2 to 4 (PND 2 to 4) was statistically higher than control in groups administered HBsAg alone (Group II) or in combination with 1018 ISS (Groups V and VI), and on PND 8 to 14 (Groups II and V). There were no such increases in the 1018 ISS-alone (Group VII). There was no evidence of a dose response relationship for pup deaths with regard to the dosage of the adjuvant, 1018 ISS (Groups II to V). The range of total pup deaths in the HBsAg-treated groups during PNDs 2 to 4 and PNDs 8 to 14 was not outside the historical control range for this testing facility. The total number of nonsurviving offspring

(i.e. stillborn plus all pre-weaning deaths) were comparable across all groups (23, 30, 21, 17, 33, 27, and 21 in the control (Groups II, III, IV, V, VI, and VII, respectively). These increases in pup mortality do not appear HBsAg treatment-related.

The following changes in hematologic parameters occurred in the F0 female groups that received the highest dose (3000 mcg) of 1018 ISS, either alone or in combination with the HBsAg: the average number of white blood cells (WBC) was increased; the average values that reflect circulating erythrocyte mass were reduced [i.e., red blood cells counts, hemoglobin (HGB) and hematocrit (HCT)]; mean platelet counts were reduced and mean platelet volume (MPV) was increased; the number of lymphocytes, monocytes and large unstained cells (LUC, large lymphocytes or monocytes) were increased (consistent with the immunostimulatory properties of 1018 ISS); the number of eosinophils was reduced (possibly an artifact of the shifts in other differential cell counts); activated prothrombin time (APTT) was increased; and the plasma concentration of fibrinogen was modestly reduced. The changes were attributed to the high dosage (3000 mcg) of 1018 ISS. These changes were not considered adverse or toxicologically important because of the small magnitude of change. Additionally, these changes are known class effects of phosphorothioate oligonucleotides when given repeatedly to rodents.

Changes in clinical chemistry parameters that were attributed to the 300 or 3000 mcg dosages of 1018 ISS included: decreases in total protein, albumin, calcium, globulin, triglycerides and potassium; and minor increases in glucose, aspartate aminotransferase (AST) and blood urea nitrogen (BUN). When observed at more than one 1018 ISS dose level, the above changes were generally dose-dependent. By the end of the lactation period (DL 21), the above changes in clinical pathology parameters were either absent or diminished, relative to DG 21, reflecting ongoing recovery after the last dosage administered on DG 18. These changes were not considered adverse or toxicologically important because of the small magnitude of change (the means were well within the range of normal values). These changes in serum chemistry parameters are known class effects of phosphorothioate oligonucleotides in rodents.

Treatment-related necropsy findings for the F0 generation dams included an increased incidence of enlarged liver and/or spleen in the groups that received the highest dose (3000 mcg) of 1018 ISS, either alone or in combination with the HBsAg. The number of rats with large lymph nodes (iliac, inguinal, mediastinal, mesenteric, renal) was significantly increased in the HBsAg/1018 ISS or in the 1018 ISS-alone dosage group. These findings were considered an effect of the highest (3000 mcg) dosage of 1018 ISS and are consistent with the expected immunostimulatory properties of this test article.

At the Caesarean-sectioning on DG 21, the absolute and or relative weights of the liver, kidneys, adrenals and spleen were increased for dams received the highest dose (3000 mcg) of 1018 ISS, either alone or in combination with the HBsAg. In the rats assigned to natural delivery and sacrificed on DL 21, only the weights of the liver and spleen were increased in these high dosage groups (3000 mcg 1018 ISS) and the magnitude of the increases was reduced relative to Caesarean-sectioned animals, trending toward reversal of the organ weight increases. These changes in organ weights are consistent with the known target organs of 1018 ISS.

In F0 female rats, IM injection with HBsAg in combination with 1018 ISS generated a strong antibody response to HBsAg that was measured prior to mating (DS 21), during gestation (DG 21) and sustained in the dams through the end of the lactation period (DL21). In rats, a 3000 mcg dose of 1018 ISS, which is approximately 200 times the human dose based on a body weight basis, induced IL-12p40 and IFN-g after a single injection. Although there was small decrease in

cytokine responses after the fourth injection (DG 18), which could be due to the rats becoming less sensitive to the immunostimulatory effects of 1018 ISS after repeated injections, the IL-12p40 levels were still greater than 28-fold above baseline. The 300 mcg 1018 ISS + HBsAg dose (approximately 20 times the human dose) induced only a 2-fold increase in IL-12p40 levels over baseline, and the 15 mcg 1018 ISS + HBsAg dose (a 1 X human dose) did not induce an increase in either IL-12p40 or IFN-g levels after the first or last injection.

F1 Generation Rats - Post-weaning

No treatment-related mortality/morbidity was observed in the F1 generation male or female rats, and there were no clear treatment-related effects on any of the parameters evaluated in the F1 generation, including clinical signs, body weight, feed consumption, necropsy observations, sexual maturation, behavioral and developmental parameters, mating, fertility, and Caesarean-sectioning and litter parameters in the F1 females assigned to reproductive evaluation.

There were no statistically significant effects on absolute or relative spleen weight in either sex for F1 generation animals assigned to humoral immune response evaluation (T-cell dependent IgM splenocyte antibody responses to sheep red blood cells) using the (b) (4) Assay. treatment of maternal animals (F0 generation) with 2.5 mcg HBsAg and various doses of 1018 ISS, or either test article alone, did not adversely affect the humoral immune response in F1 generation male animals. With the exception of the 2.5 mcg HBsAg alone and 2.5 mcg HBsAg + 300 mcg 1018 ISS (maternal treatment) groups, female F1 generation rats were unaffected in their ability to respond to the T cell-dependent antigen, sheep erythrocytes. A statistically significant decrease in the IgM antibody-forming cell (AFC) response was observed in F1 female offspring of dams that had received 2.5 mcg HBsAg alone or 2.5 mcg HBsAg + 300 mcg 1018 ISS. There were no statistically significant decreases in specific activity or total spleen activity for the other groups derived from maternal animals that had received 2.5 mcg HBsAg + 1.5, 15, or 3000 mcg 1018 ISS, or 3000 mcg 1018 ISS-alone. Hence, there was no indication of a dose-related effect. Accordingly, based on the weight of evidence, including the negative male animal data, treatment of F0 generation rats with the combination of HBsAg + 1018 ISS does not appear to induce suppression of T-cell dependent spleen IgM antibody responses to sheep red blood cells in F1 generation rats.

Maternal-transferred antibody (anti-HBsAg IgG1 and IgG2a, transferred *in utero* and through milk during the lactation period) was measured in fetuses and pups. For the fetuses and pups, the levels of maternal-transferred antibody measured were proportional to the response seen in the F0 females up to the end of the weaning period. High levels of maternal-transferred antibodies were measured in the F1 generation prior to birth (fetuses), shortly after birth (PND 4), and at the end of the weaning period (PND 21), and could still be detected up to 2 months post-weaning (PND 65 to 78).

Discussion and Conclusion

The study was designed to assess the potential effects of HBsAg + 1018 ISS treatment of female rats on gestation, parturition, lactation and maternal behavior (from implantation through lactation and weaning) and on the development of the offspring of the treated female rats with emphasis on the evaluation of the functionality of the immune response in the F1 generation. In F0 female rats, IM injections of HBsAg in combination with 1018 ISS generated a strong antibody response to HBsAg that was sustained through the end of the lactation period. The 3000 mcg dose of 1018 ISS (approximately 200 times the human dose based on a body weight basis) induced IL-12p40 and IFN-g after a single injection. This cytokine response decreased after the

fourth injection on DG 18, but IL-12p40 levels were still greater than 28-fold above baseline. The 300 mcg 1018 ISS + HBsAg dose (approximately 20 times the human dose) induced only a 2-fold increase in IL-12p40 levels over baseline, and the 15 mcg 1018 ISS + HBsAg dose (a 1 X human dose) did not induce an increase in either IL-12p40 or IFN-g levels after the first or last injection.

Antibody (anti-HBsAg IgG1 and IgG2a) was transferred *in utero* and through milk during the lactation period as measured in fetuses and pups. The pharmacologic/immunostimulatory properties of 1018 ISS, were evident in the highest dose (3000 mcg) of 1018 ISS, either alone or in combination with the HBsAg as local injection site reactions (scabs and/or abrasions), changes in hematologic parameters, increased incidence of enlarged liver and/or spleen, increased number of rats with large lymph nodes, increased absolute and or relative weights of the liver, kidneys, adrenals and spleen (Caesarean-sectioned dams), increased weights of the liver and spleen (naturally delivery dams only).

Changes in serum chemistry parameters included a decreases in total protein, albumin, calcium, globulin, triglycerides and potassium; and minor increases in glucose, aspartate aminotransferase (AST) and blood urea nitrogen (BUN). , These are known class effects of phosphorothioate oligonucleotides in rodents and were slight as well as not apparent or diminished by the end of the lactation period.

There was a low incidence of mortality or morbidity (requiring sacrifice prior to or during delivery) in five F₀ generation dams in the 3000 mcg/dose 1018 ISS-alone and the 2.5/3000 mcg/dose HBsAg/1018 ISS groups. No deaths or problems in delivery occurred in the antigen-alone group indicating that these findings appear related to the 3000 mcg doses of the 1018 ISS.

Dosages of the test article as high as 2.5/3000 mcg HBsAg/1018 ISS had minimal or no effects on body weights, body weight gains, and absolute and relative feed consumption values, mating and fertility parameters for both Caesarean and natural delivery rats as well as Caesarean-sectioning and litter parameters. There was no effect on fetal development based on gross external, soft tissue and skeletal examinations. In the natural delivery section of this study, no treatment-related mortality was seen in the F₁ generation rats post-weaning, with no effects on observations including clinical signs, body weight, food consumption, observations at necropsy, sexual maturation, behavioral and developmental markers, mating, fertility and Caesarean-section/littering parameters in the F₁ females in reproductive evaluation. Treatment of the F₀ generation rats with Heplisav did not induce suppression of the IgM response by T cells in the sheep-red blood cell assay.

Maternal toxicity was observed at the 3000 mcg dose of 1018 ISS, alone or in combination with the 2.5 mcg HBsAg antigen as possible mortality and humane sacrifice of rats prior to and during delivery, and transient decreases in maternal body weight. A NOAEL for reproductive and developmental toxicity as well as F₁ generation was 2.5 mcg HBsAg alone, 300 mcg 1018 alone or 2.5/3000 mcg HBsAg/1018 ISS.

The sponsor seeks a Pregnancy labeling category based on lack of evidence of impaired fertility or harm to the fetus due to Heplisav. Four intramuscular doses of the components of the vaccine were administered (up to approximately a 200-fold excess relative to the human dose for 1018 ISS Adjuvant and approximately 25-fold excess relative to the human dose for HBsAg on a mg/kg basis). There were no adverse effects on maternal reproductive development, fetal development, the growth and development of the offspring or any of the other parameters

evaluated, even though the highest dose level produced an appreciable degree of maternal toxicity.

The results of this repeat-dose toxicity study indicate that the combination of 50 mcg (approximately 2 mg/kg) 1018 ISS Adjuvant + HBsAg produced findings typically observed in mice receiving oligonucleotide treatment. Organs affected by high doses of 1018 ISS Adjuvant (an approximate 43-fold greater than clinical dosage for HEPLISAV on a body weight basis) were the hematopoietic system, spleen, liver, and injection-site. Complete resolution of treatment-related effects was observed at the end of the 3-week recovery period at lower doses of 1018 ISS Adjuvant. All of the effects observed in this study were consistent with the known class effect of structurally similar oligonucleotides, or were expected effects of the vaccine components (i.e. injection-site reaction). Thus, this study resulted in no unexpected findings in mice administered a combination 1018 ISS Adjuvant + HBsAg vaccine. A NOAEL was not demonstrated, but no severe toxicity was observed, and most effects reflected the expected immunostimulatory properties of the vaccine.

The multi-generation reproductive toxicity study in rats, with 4 IM injections of the complete vaccine combination (3000 mcg 1018 ISS Adjuvant + 2.5 mcg HBsAg [approximately 200-fold clinical multiple for 1018 ISS Adjuvant and 25-fold clinical multiple for HBsAg on a body weight basis]) with extensive evaluation of fertility, mating behavior, gestation, embryo-fetal development, parturition, lactation, maternal behavior, development of the offspring [including a postnatal behavioral/functional and immunological evaluation] did not show detrimental effects on reproductive function of the maternal animals or on development of the offspring at dosage up to a level that produced appreciable maternal toxicity (3000 mcg 1018 ISS Adjuvant). The NOAEL for reproductive and developmental toxicity and the growth and development of the F₁ generation was the highest dose levels tested, ie, 2.5 mcg of HBsAg alone, 3000 mcg of 1018 ISS Adjuvant alone or 3000 mcg 1018 ISS Adjuvant + 2.5 mcg of HBsAg. The NOAEL for the vaccine combination (approximately 200-fold clinical multiple for 1018 ISS Adjuvant and 25-fold clinical multiple for HBsAg on a body weight basis) showed an acceptable safety margin relative to clinical dose for HEPLISAV. The sponsor's claim of a Pregnancy category B is supported by this nonclinical reproductive toxicity study.

Overall Conclusion:

The submission is acceptable with regards to nonclinical toxicology. The toxicity findings demonstrate the immunomodulatory activity of Heparisav, especially due to the adjuvant 1018 ISS) and its potential for toxicity in humans as indicated from findings in the completed clinical studies. The reproductive and developmental toxicity study has similar concerns (see pg 39-41) while supporting the claim of Pregnancy category B.

Concurrence: Martin D. Green