

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

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Product: Hepatitis B Vaccine (Recombinant) HEPLISAV™

Subject: Review of toxicology data (toxicology studies with the ISS 1018 adjuvant alone)

Reviewer: Claudia Wrzesinski

Reference: BLA sections reviewed (toxicology studies with the ISS 1018 adjuvant alone): 4.2.3.1 Single-dose Toxicity
4.2.3.2 Repeat-dose Toxicity
4.2.3.3 Genotoxicity Study

Sponsor: Dynavax Technology Corporation

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EXECUTIVE SUMMARY (adjuvant alone):

The 1018 ISS adjuvant alone was evaluated in 3 genotoxicity studies, 2 safety escalation studies (rabbit and baboons) and 3 repeated toxicology studies (mice, rats, and cynomolgus monkeys). No significant toxicological findings were found which would prevent the use of this adjuvant at a dose of 0.05 mg/kg for 2 intramuscular (IM) administrations 2 months apart.

No genotoxicity was observed in the standard battery of tests composed of *in vitro* bacterial mutation, chromosome aberration and *in vivo* mouse erythrocyte micronucleus test. No adverse effects were identified in 2 safety-pharmacology studies with doses of 1018 ISS up to 1.6 mg/kg in rabbits and 25 mg in baboons.

In a repeat-dose toxicity study mice received a total of 3 IM doses (2 mg/kg/dose; clinical dose is 0.05 mg/kg) of the 1018 ISS adjuvant alone separated by 2 weeks. Animals experienced no mortality and exhibited no clinical signs of toxicity. The main findings included splenomegaly, lymphoid hyperplasia, mononuclear cell infiltrates in multiple tissues as well as mild anemia accompanied by a extramedullary hematopoiesis (EMH) in the spleen and liver; reversibility was observed at the end of the recovery phase. These findings are typical for oligonucleotide class effects.

Further, two repeated dose toxicity studies in rats and monkeys were performed with the 1018 ISS adjuvant alone. (b) (4) rats or cynomolgus monkey received subcutaneously (SC) either 0.5, 1.5, 12.5 mg/kg/week of 1018 ISS adjuvant or PBS weekly for 8 weeks. On an mg/kg dose, the proposed clinical dose of 0.05 mg/kg is approximately 10, 50 or 250-fold lower than that used in the toxicity studies. Treatment-related findings were more pronounced in rats than in monkeys most likely due to a broader expression of Toll-like receptor (TLR) 9 in rodents. In general, no mortality or clinical signs of systemic toxicity were observed in these studies. The main treatment-related findings were inflammatory changes at the injection-sites and in key target organs, including the liver, kidney, lymph nodes, and spleen. These findings were consistent with previously described class effects for oligonucleotides. Most of the observed changes were reversible, except for the proximal tubular degeneration in the kidneys of rats. Monkeys showed a treatment related modest increase in activated partial thromboplastin time, splenomegaly and hyperplasia of the Kupffer cells with blue granular pigment inclusions at the highest dose group level; animals at the lowest dose level only showed some injection-site inflammation and minimal mononuclear cell infiltration in the liver.

PRODUCT:

Hepatitis B Vaccine (Recombinant) HEPLISAV™

PROPOSED USE:

Immunization against infection caused by all known subtypes of hepatitis B virus in healthy adults 18 through 70 years of age

INTRODUCTION:

Dynavax is submitting an application for consideration of US licensure for the HEPLISAV™ vaccine, which consists of 20 mcg of recombinant yeast cell-derived hepatitis B virus surface antigen and 3000 mcg Dynavax's proprietary adjuvant, 1018 ISS. The proposed indication for this vaccine is the prevention of infection caused by all known subtypes of hepatitis B virus in adults age 18 through 70 years. The immunization schedule consists of 2 doses given 1 month apart. The aim of the Dynavax hepatitis B vaccine program is to develop a vaccine that provides a more rapid (2-dose vaccine regimen), and potent immune response to HBsAg than currently licensed vaccines.

1018 ISS adjuvant used in HEPLISAV is a synthetic 22-mer phosphorothioate oligodeoxynucleotide adjuvant containing a 22-mer oligonucleotide with the sequence: 5' TGA CTG TGA ACG TTC GAG ATG A 3' which activates Toll-like receptor 9 (TLR9). This TLR plays an important role in the pathogen recognition by distinguishing unmethylated CpG sequences and leads to an activation of the innate immune system. TLR9 receptors are transmembrane proteins which are distributed within the cytoplasm, as well as within the endoplasmic reticulum (ER) and are expressed in various immune cells including dendritic cells, B lymphocytes, monocytes and NK cells. The 1018 ISS adjuvant in HEPLISAV is thought to have the following effects: (1) activation of pDCs through TLR9, (2) conversion of pDCs into activated dendritic cells that present the processed HBsAg component of HEPLISAV to CD4⁺ T cells, and (3) promotion of Th1 T-cell differentiation through the production of IFN- α and IL-12. No TLR 9 has been approved as an adjuvant for vaccines, 1018 ISS is not approved in any product yet.

STUDIES SUBMITTED FOR SUPPORT OF THE ADJUVANT SAFETY:

Genotoxicity studies:

1. Study no. 01-GT1 “A Bacterial Mutation Test with 1018 ISS Adjuvant”
2. Study no. 01-GT2 “A Chromosome Aberration Test with 1018 ISS Adjuvant”
3. Study no. 04-413 “A Mouse Erythrocyte Micronucleus Test with 1018 ISS Adjuvant”

Safety pharmacology studies:

4. Study no. 98-0034 “Safety Evaluation of Escalating IV Doses of 1018 ISS Adjuvant in Rabbits, including Pyrogenicity”
5. Study no. 98-0033 “Safety Evaluation of Escalating IV Doses of 1018 ISS Adjuvant in Baboons”

Repeated dose toxicity studies:

6. Study no. 00-157 “A 8-Week Subcutaneous Toxicity Study of 1018 ISS Adjuvant in Cynomolgus Monkeys, with a 4-Week Recovery Period”
7. Study no. 00-158 “An 8-Week Subcutaneous Toxicity Study of 1018 ISS Adjuvant in Rats, with a 4-Week Recovery Period”
8. Study no. 00-141 “A Toxicity Study of 1018 ISS Adjuvant in Mice Administered as Three IM Injections in Weeks 0, 2, and 4, with a 3-Week Recovery Period”

SUMMARY OF GENOTOXICITY STUDIES SUBMITTED FOR SUPPORT OF THE ADJUVANT SAFETY:

Study no. 01-GT1: 1018 ISS bacterial mutation test

The 1018 ISS adjuvant was evaluated for genotoxicity in a bacterial mutation test designed to meet the requirements of OECD and other international regulatory agencies. The test used the amino acid-requiring strains of *Salmonella typhimurium* (strains (b) (4)) and *Escherichia coli* strain (b) (4) for the detection of point mutations. The test article was supplied as a formulation in (b) (4) for injection USP. Initially the test agent was tested at a maximum dose of 5000 µg/plate using the standard *pour plate* version of the bacterial mutation test in the presence and absence of a supplemented liver fraction (S9 mix) prepared from rats. Consequently, the test article was evaluated in a confirmatory independent test in which the bacteria were pre-incubated with appropriate concentrations of the test article for 30 minutes at 37°C prior to mixing with top agar and plating. The test article did not lead to an increase in the number of revertant colonies with any strain of bacteria in either the absence or presence of S9 mix. The test also included positive control in the absence of S9 (sodium azide, 9-aminoacridine, 2-nitroflurone, 4-nitroquinoline, N-oxide) and in the presence of S9 mix (2-amioanthracene, benzo[a]pyrene), untreated controls and sterility controls which confirmed the sensitivity of the test system as well as the activity of the S9 mix.

In conclusion, the test article 1018 ISS did not show any evidence or genotoxicity in this *in vitro* test for mutagenicity when tested up to the maximum concentration of 5 mg/plate.

Study no. 01-GT2: 1018 ISS chromosomal aberration test

1018 ISS was tested for evidence of genotoxicity in an *in vitro* chromosome aberration test in human lymphocyte culture designed to meet the requirements of OECD and other international regulatory agencies. In the human lymphocyte culture system human blood was mixed with culture medium, and lymphocytes were stimulated into division using phytohemagglutinin (PHA). After 48 hours of culture, the dividing lymphocytes were exposed to the test article 1018 ISS up to a maximum final concentration of 5000 µg/mL (as recommended by OECD) for either 4 and 24 hours in the absence or for 4 hours in the presence of a supplemented liver fraction (S9 mix) prepared from rats to simulate mammalian metabolism. Then, cells were arrested in the metaphase stage, fixed and stained.

At the four highest dose levels the test agent 1018 ISS did seem to cause a reduction in the relative mitotic index (RMI) up to 39% in the absence and 30% in the presence of S9, indicating slight toxicity. The four highest dose levels were used for the evaluation of the

chromosome aberrations. A total of 200 metaphases per treatment group were examined and the proportion of metaphases in the group treated with the test agent was compared to the concurrent solvent control. The test agent 1018 ISS did not cause any statistically significant increases in the incidence of cells with chromosome damage while the positive control agents caused highly significant increases in the proportion of cells with chromosome damage, confirming the sensitivity of the system and the effectiveness of the S9 mix. Further, the proportion of aberrant metaphases for all vehicle and test article treated cultures was within the laboratory historical control range.

In conclusion, the test article 1018 ISS did not show any evidence of genotoxicity in this *in vitro* test for induction of chromosome damage when tested up to the maximum concentration 5 mg/plate.

Study no. 04-413: 1018 ISS mammalian erythrocyte micronucleous Test

The assay consisted of two phases. The first phase assessed the toxicity of the test article (1018 ISS) and identified the dose levels for the definitive study. The second phase was the definitive micronucleus study which evaluated the potential of 1018 ISS to increase the incidence of micronucleated polychromatic erythrocytes in the bone marrow of male and female (b) (4) mice.

Test species: (b) (4) mice, 6 to 8 weeks old.

Supplier: (b) (4)

Test article: 1018 ISS oligonucleotide (lot: (b) (4)) formulated in a stock solution of (b) (4). On the day of treatment, dilutions of 1018 ISS stock solution with PBS were prepared.

Negative control: PBS

Positive control: Cyclophosphamide monohydrate dissolved in sterile distilled water at a concentration of 2.5 mg/mL.

Phase 1: 3 dose range-finding studies: pilot toxicity, toxicity, and supplemental toxicity

Pilot toxicity: Five male and five female mice received 2000 mg/kg 1018 ISS; two male mice received each either, 1, 10, 100 or 1000 mg/kg 1018 ISS. Mice were observed after dose administration and daily thereafter for 3 days for clinical signs of toxicity. Body weights were recorded before dose administration, 1 and 3 days after dose administration.

In the highest dose group mortality was observed in 1 out of 5 males and 2 out of 5 females. Lethargy and piloerection was observed in all groups. Additionally, hunched position, lacrimation, and partially closed eyes were observed in males receiving 1000 mg/kg and in males and females receiving 2000 mg/kg 1018 ISS. One male mouse receiving 1000 mg/kg and one female mouse receiving 2000 mg/kg showed irregular breathing; one female mouse was cool to the touch in the 2000 mg/kg group. Reduction in mean body weight up to 18% was observed in groups treated with the test agent.

Toxicity and supplemental toxicity: Five animals per group received a dose of 1000, 1300, 1600 or 1800 mg/kg 1018 ISS in the toxicity study and 100, 200, 400 or 800 mg/kg 1018 ISS in the supplemental toxicity study. Mice were observed after dose administration and daily thereafter for 3 days for clinical signs of toxicity. Body weights were recorded before dose administration as well as 1 and 3 days after dose administration.

Mice receiving 100 and 200 mg/kg did not show any mortality, in all other groups mortality was observed: 1 out of 5 males at 400 mg/kg, 2 out of 5 males at 800 mg/kg, 2 out of 5 males and 2 out of 5 females at 1000, 1300 and 1600 mg/kg, and 5 out of 5 males and 3 out of 5 females at 1800 mg/kg. Lethargy and piloerection were observed in all groups. Hunched position was seen in males and females treated with 1018 ISS at doses above 400 mg/kg, and crusty eyes was observed in males at 800 mg/kg, in females at 1000 mg/kg and in males and females at 1300, 1600 and 1800 mg/kg. Eyes partially closed were observed in males at 800 mg/kg and above, and females at doses at and above 1000 mg/kg. Lacrimation was observed in males at 800 mg/kg and 1300 mg/kg, and tremors were observed in males and females at 1300, 1600 and 1800 mg/kg. Reduction in mean body weight up to 22% was observed in groups treated with the test agent. The dose of 400 mg/kg was identified as the maximal tolerated dose and therefore this dose was used for the micronucleus test.

Phase 2: Definitive Micronucleus Study

Treatment	Number of mice/sex used for bone marrow collection after dose administration	
	24 h	48 h
Vehicle control (PBS)	5	5
Low dose 1018 ISS (100 mg/kg)	5	0
Mid dose 1018 ISS (200 mg/kg)	5	0
High dose 1018 ISS (400 mg/kg)	5	5
Positive control CP (50 mg/kg)	5	0

The test article was administered intraperitoneal (IP) at a dose volume of 20mL/kg as well as the controls. Twenty-four and 48 hours after dose administration five mice per group were sacrificed and the bone marrow smear from the femur was prepared and

stained with May-Gruenwald-Giemsa. Bone marrow cells, polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) were analyzed for the presence of micronuclei. The incidence of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes was determined for each mouse and treatment group. Statistical significance was determined using the Kastenbaum-Bowman Tables.

No mortality was observed in any group, piloerection and lethargy was observed in all animals receiving 1018 ISS. Reductions (up to 47%) in the ratio of polychromatic erythrocytes to total erythrocytes were observed in 1018 ISS-treated groups relative to the respective PBS controls. These reductions demonstrated bioavailability of 1018 ISS to the bone marrow and cytotoxicity. The number of micronucleated polychromatic erythrocytes per 10000 polychromatic erythrocytes in 1018 ISS-treated groups was not statistically increased relative to the respective PBS controls in either male or female mice, regardless of dose level or bone marrow collection time ($p > 0.05$, Kastenbaum-Bowman Tables).

In conclusion, the results of the assay indicate that under the conditions described in this report, a single IP administration of 1018 ISS at doses up to 400 mg/kg did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes, in either male or female ^{(b) (4)} mice. Therefore, 1018 ISS did not show any evidence of genotoxic activity and was found negative in the mouse micronucleus assay.

SUMMARY OF SAFETY PHARMACOLOGY STUDIES SUBMITTED FOR SUPPORT OF THE ADJUVANT SAFETY:

Study no. 98-0034: Safety evaluation of escalating IV doses of 1018 ISS adjuvant in rabbits including pyrogenicity, non-GLP

The sponsor submitted a non-GLP safety pharmacology study of the 1018 ISS adjuvant in (b) (4) rabbits. Eight naive (b) (4) rabbits (weighing approximately 2 Kg at the start of the study) were divided in 2 groups. Animals in the treatment group received 0.1 mg (= 0.05 mg/Kg), 0.5 mg (= 0.25 mg/Kg), and 1.6 mg (= 0.8 mg/Kg) of 1018 ISS given in 3 ml per dose by intravenous (IV) injection. Dose one (0.1 mg/Kg) was given at week 0, dose two (0.5 mg/Kg) at week 3 and dose 3 (0.8 mg/Kg) at week 6. Animals of the control group (four rabbits) received 3 ml PBS IV at week 0, 3 and 6. At the time of the first, second and third injections, body temperatures and vital signs (heart rate, systolic blood pressure, and ophthalmic examinations) were collected on rabbits at pre-dosing and at 1, 2, and 24 hours after dosing. In addition, food and water consumption was monitored throughout the study, and animals were weighed at weeks 0, 3, 6, and 9. No clinical relevant changes were observed in body temperature, heart rate, systolic blood pressure, ophthalmic examination or weights.

Study no. 98-0033: Safety evaluation of escalating IV and SC doses of 1018 ISS adjuvant in baboons

The sponsor submitted a GLP safety pharmacology study of the 1018 ISS adjuvant (lot number: (b) (4)) in baboons (2-4 years of age) with additional measurements of *in vivo* cytokine response. Eight baboons in total, 5 male and 3 females, resulting in 2 animals per group were immunized with a total of 4 escalating doses of 1018 ISS (0.5 mg, 2 mg, 8 mg and 25 mg) or PBS either SC or IV with a 3 week interval between each immunization.

The serum cytokine responses were measured at time points 0, 1 hour, and 6 hours postimmunization. Clinical observation was performed twice daily, and food consumption was estimated once daily, vital signs (body weight, body temperature, respiratory rate, heart rate, blood pressure, and injection site appearances) were determined at the time just before each dosing, 1 hour after dosing and 6 hours after dosing; at the same time points direct ophthalmic examinations were performed.

One animal receiving the 1018 ISS adjuvant showed a minimal swelling at the injection site, no other treatment-related effects were observed in any of the monitored parameters during the 5-month period of the study. Serum cytokine levels (IL-12, IL-6, IL-8, IFN- γ , and TNF- α) of animals treated with 1018 ISS compared to animals treated with PBS did not show any significant changes in any animal at any time point post treatment.

**SUMMARY OF REPEATED DOSE TOXICITY STUDIES
SUBMITTED FOR SUPPORT OF THE ADJUVANT SAFETY:**

**Study no. 00-157: 8-Week Toxicity Study of 1018 ISS Administered by
Subcutaneous Injection in Cynomolgus Monkeys**

Performing laboratory: (b) (4)

Study initiation date: February 22, 2001

Final Report date: February 28, 2002

Test article batch/lot: 1018 ISS: (b) (4)

Animal species and strain: Cynomolgus monkeys

Breeder/supplier: (b) (4)

Number of animal per group and sex: 2 to 3 males and females (see below)

Age: 2-3 years

Body weight range: Males: 2.06 to 2.86 kg, females: 2.01 to 2.99 kg

Route and site of administration: Subcutaneous

Volume of injection: 0.25 mL/kg based on most recent body weight

Frequency of administration and study duration: Once per week for 8 consecutive weeks

Dose: 0, 0.5, 2.5 or 12.5 mg/kg/week 1018 ISS

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study by the sponsor. The test article was within the specification and was assumed to be stable for the study duration.

Means of administration: Needle and syringe

Report status: Final

Experimental design:

Group	Treatment	Number of Animals (#/sex/group)	
		Treatment phase	Recovery phase
1	0 mg/kg/week	3	2
2	0.5 mg/kg/week	3	-
3	2.5 mg/kg/week	3	-
4	12.5 mg/kg/week	3	2

Randomization procedure: Animals were weighed prior to treatment and randomized by sex into treatment groups using a standard by weight block randomization procedure.

Statistical analysis plan: The sexes were analyzed separately. No statistical analyses were required for the recovery groups due to the small number of animals in each group.

Group Pair-wise comparison (body weights, hematology excluding leukocyte count, clinical chemistry, organ weights):

For each specified endpoint and for all collection intervals, Levene's test was used to assess homogeneity of group variances. If Levene's test was not significant ($p < 0.01$), Dunnett's test (Dunnett, 1955) was used to compare each treatment group with the control group. If Levene's test was significant ($p < 0.01$), comparisons with the control group were made using Welch's t-test (Welch, 1937) with a Bonferroni correction. Results of all pair-wise comparisons were reported at the 0.05 and 0.01 level of significance. All endpoints were analyzed using two-tailed tests unless indicated otherwise.

Log Transformation (leukocyte counts):

Historical data indicate that leukocyte counts (total and differential) are not normally distributed; therefore, a log transformation was performed on these data. The transformed data were then analyzed as described in the Group Pair-wise Comparisons section.

Rank Transformation (urine analysis: urine volume, specific gravity, pH):

For each specified endpoint and for all collection intervals, a rank transformation was performed on these data. The transformed data were then analyzed using Dunnett's test (Dunnett, 1955) to compare each treatment group with the control group. All endpoints were analyzed using 2-tailed tests unless indicated otherwise.

Parameters	Frequency of Testing
Cageside observation ¹	Twice a day
Clinical observations ²	Daily
Body weight	3 days after arrival, prior to randomization, prior to dose initiation, and weekly thereafter, with the exception of Week 9
Food consumption	Daily
Physical examination	Pretest and prior to necropsy
Ophthalmologic exam	Prior to initiation of dosing, and all survivors were examined again at 3 days prior to the terminal necropsy and 1 day prior to the recovery necropsy
Electrocardiographic examination:	Prior to initiation of dosing and all survivors were examined again at 2 days prior to the terminal necropsy, and 2 days prior to the recovery necropsy
Clinical chemistry*	Pretest, 24 hours after the last dose, and at 1 and 4 weeks after the last dose.
Hematology*	Day 1 (predose and at 2 and 6 hours postdose), on the day of the week 8 dose (predose and at 2, 6, and 24 hours postdose), and 1 and 4 weeks after the last dose

¹ Cageside observations include mortality, morbidity, general health, injury and availability of food/water.

² Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Parameters	Frequency of Testing
Coagulation*	Pre-dose, and at 2, 6, and 24 hours postdose on both day 1 and the day of the week 8 dose.
Urinalysis	At the terminal and recovery sacrifices (urine was collected directly from the bladder)
Complement split product (Bb) analysis	Day 1 (pre-dose and at 2 and 6 hours post-dose), on the day of the week 8 dose (pre-dose and at 2, 6, and 24 hours post-dose), and 1 and 4 weeks after the last dose.
Necropsy	Terminal necropsy: 1 day after the last dose for half of the animals and 2 days after the last dose for the rest of the animals Recovery necropsy: 4 weeks after the last dose
Tissues for histopathology	Terminal and recovery necropsy

*(femoral artery/vein)

Postmortem procedures: The following tissues were collected at necropsy.

Organ/Tissue	Collected	Not collected
Adrenal glands	*!	
Aorta	!	
Bone (sternum, rib & femur)	!	
Bone marrow (sternum, rib & femur)	X	
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	*!	
Cervix	!	
Colon	!	
Duodenum	!	
Epididymides	*!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		!
Gall bladder	!	
Gross lesions (if any)	!	
Heart	*!	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	*!	
Lacrimal glands		!
Larynx		!
Liver	*!	

Organ/Tissue	Collected	Not collected
Lung (main-stem; bronchi)	!	
Lymph nodes (cervical)		!
Lymph nodes (mandibular)	!	
Lymph nodes (mesenteric)	!	
Regional lymph nodes	!	
Mammary glands (females only)	!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		!
Ovaries	*!	
Pancreas	!	
Pituitary gland	*!	
Prostate	!	
Rectum	!	
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	*!	
Stomach (squamous and glandular)		
Target organs	!	
Testes	*!	
Thymus	*!	
Thyroid (w/ parathyroid glands)	*!	
Tongue	!	
Trachea	!	
Ureters	!	
Uterus (w/ cervix)	!	
Urinary bladder	!	
Vagina	!	

Table of Histology: All tissues from the control and high-dose animals sacrificed on the day after the last dose were examined, from the low- and mid-dose groups target organs and any gross lesions or tissue masses were examined; after the recovery phase only target organs, any gross lesions or tissue masses were examined in the control and high-dose animals. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

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 WEEK (WK), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE, if ≥ 1.5 or ≤ 0.7)	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR B) HEPATOBILIARY	<p>Alanine aminotransferase (ALT) Pretest: F \uparrow 1.55 G2 (43.3 U/L) Pretest: F \uparrow 1.52 G3 (42.7 U/L) 8 WK : F \uparrow 2.25 G4 (79.8 U/L) <i>Recovery phase:</i> 1 WK M \downarrow 0.68 G4 (25.5 U/L) 4 WK M \downarrow 0.67 G4 (34.5 U/L) 1 WK F \uparrow 2.47 G4 (60.5 U/L) 4 WK F \uparrow 1.69 G4 (55.0 U/L)</p> <p>Aspartate aminotransferase (AST): <i>Recovery phase:</i> 1 WK M \downarrow 0.58 G4 (22.0 U/L) 4 WK M \downarrow 0.46 G4 (32.5 U/L)</p> <p>Sorbitol dehydrogenase: <i>Recovery phase:</i> 1 WK F \downarrow 0.49 G4 (8.25 U/L)</p> <p>Alkaline phosphatase (ALP): <i>Recovery phase:</i> 1 WK F \downarrow 0.58 G4 (263.0 U/L) 1 WK F \downarrow 0.63 G4 (290.5 U/L)</p>	<p>Glutamate dehydrogenase Total bile acids</p> <p>Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin</p>
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)		Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Creatine kinase Fasting triglycerides

Table of Clinical Chemistry Results

One female animal in the terminal necropsy group 4 showed an increase in ALT (199 U/L) and AST (134 U/L) and sorbitol dehydrogenase (24.2 U/L) on week 8 and also showed histopathologically a minimal chronic inflammation of the liver, mild hypertrophy with mild pigmentation of Kupffer cells and blue color pigment in liver. The 2 remaining female animals in this group showed mild hypertrophy with mild pigmentation of Kupffer cells and blue color pigment in liver, but without a chronic inflammation of the liver and no increase in liver enzymes.

Female animals showed an increase in AST after the recovery phase, while male animals showed a decrease after the recovery phase.

HEMATOLOGY MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY WEEK (WK), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE, if ≥ 1.5 or ≤ 0.7)	Not of NOTE
RED BLOOD CELLS	Reticulocytes: <i>Recovery phase:</i> 1 WK F ↓ 0.50 G4 (181.45x10 ³ /μL)	Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC)
WHITE BLOOD CELLS	Total leukocytes: 8 WK:24h F ↓ 0.49 G4 (6.76x10 ³ /μL)* Neutrophils count: 8 WK:24h M ↓ 0.38 G4 (1.7x10 ³ /μL)* 8 WK:24h M ↓ 0.50 G3 (2.853x10 ³ /μL)* 8 WK:24h F ↓ 0.39 G4 (2.214x10 ³ /μL)* <i>Recovery phase:</i> 1 WK M ↓ 0.54 G4 (1.845x10 ³ /μL) 4 WK M ↓ 0.59 G4 (1.570x10 ³ /μL) Lymphocyte count: SD1:0h M ↓ 0.60 G3 (6.5x10 ³ /μL) SD1:0h M ↓ 0.63 G4 (6.9x10 ³ /μL) 8 WK:2h M ↓ 0.60 G3 (4.8x10 ³ /μL) 8 WK:24h F ↓ 0.55 G2 (4.0x10 ³ /μL) Monocytes count: pretest: M ↓ 0.50 G3 (0.170x10 ³ /μL) SD1:0h M ↓ 0.60 G3 (0.340x10 ³ /μL)* SD1:0h M ↓ 0.63 G4 (0.380x10 ³ /μL)* 8 WK:0h M ↓ 0.44 G3 (0.217x10 ³ /μL)** 8 WK:0h M ↓ 0.60 G4 (0.316x10 ³ /μL)* 8 WK:2h M ↓ 0.57 G2 (0.310x10 ³ /μL) 8 WK:2h M ↓ 0.42 G3 (0.230x10 ³ /μL) 8 WK:2h M ↓ 0.71 G4 (0.384x10 ³ /μL) 8 WK:6h M ↓ 0.63 G3 (0.330x10 ³ /μL)	Large unstained cells (LUC)

HEMATOLOGY MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY WEEK (WK), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE, if ≥ 1.5 or ≤ 0.7)	Not of NOTE
	<p>8 WK:24h M ↓ 0.57 G2 (0.250x10³/ μL) 8 WK:24h M ↓ 0.42 G3 (0.193x10³/ μL)* 8 WK:24h M ↓ 0.71 G4 (0.218x10³/ μL) pretest: F ↓ 0.50 G3 (0.170x10³/ μL) SD1:0h F ↓ 0.56 G4 (0.356x10³/ μL) SD1:2h F ↓ 0.54 G2 (0.267x10³/ μL) SD1:2h F ↓ 0.60 G3 (0.297x10³/ μL) 8 WK:2h F ↓ 0.66 G3 (0.270x10³/ μL) 8 WK:24h F ↓ 0.62 G2 (0.217x10³/ μL) 8 WK:24h F ↓ 0.62 G3 (0.217x10³/ μL) 8 WK:24h F ↓ 0.69 G2 (0.246x10³/ μL) <i>Recovery phase:</i> 4 WK M ↓ 0.45 G4 (0.250x10³/μL)</p> <p>Eosinophils count: pretest: M ↑ 2.31 G2 (0.287x10³/ μL) pretest: M ↓ 0.34 G3 (0.043x10³/ μL) SD1:0h M ↑ 1.70 G2 (0.483x10³/ μL) SD1:0h M ↓ 0.40 G4 (0.114x10³/ μL) SD1:2h M ↑ 1.88 G2 (0.147x10³/ μL) SD1:2h M ↓ 0.42 G3 (0.033x10³/ μL) SD1:2h M ↓ 0.59 G4 (0.046x10³/ μL) SD1:6h M ↓ 0.44 G3 (0.053x10³/ μL) SD1:6h M ↓ 0.56 G4 (0.068x10³/ μL) 8 WK:2h M ↓ 0.50 G4 (0.094x10³/ μL) 8 WK:6h M ↑ 2.09 G2 (0.230x10³/ μL) 8 WK:6h M ↓ 0.67 G4 (0.074x10³/ μL) 8 WK:24h M ↓ 0.63 G3 (0.070x10³/ μL) 8 WK:24h M ↓ 0.59 G4 (0.065x10³/ μL) pretest: F ↓ 0.33 G2 (0.043x10³/ μL) pretest: F ↓ 0.41 G3 (0.053x10³/ μL) SD1:0h F ↓ 0.22 G2 (0.080x10³/ μL) SD1:0h F ↓ 0.42 G3 (0.150x10³/ μL) SD1:2h F ↓ 0.43 G2 (0.030x10³/ μL) 8 WK:0h F ↓ 0.50 G2 (0.125x10³/ μL) 8 WK:2h F ↓ 0.38 G2 (0.077x10³/ μL) 8 WK:2h F ↓ 0.69 G3 (0.140x10³/ μL) 8 WK:2h F ↓ 0.64 G4 (0.130x10³/ μL) 8 WK:6h F ↓ 0.44 G2 (0.060x10³/ μL) 8 WK:24h F ↓ 0.35 G2 (0.047x10³/ μL) <i>Recovery phase:</i> 1 WK M ↓ 0.54 G4 (0.105x10³/μL)</p> <p>Basophils count: SD1:0h M ↓ 0.40 G3 (0.030x10³/ μL) SD1:0h M ↓ 0.45G3 (0.040x10³/ μL) SD1:2h M ↓ 0.64 G2 (0.023x10³/ μL) SD1:2h M ↓ 0.64 G3 (0.023x10³/ μL) SD1:6h M ↑ 1.56 G2 (0.067x10³/ μL) 8 WK:0h M ↓ 0.62 G3 (0.037x10³/ μL)</p>	

HEMATOLOGY MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY WEEK (WK), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE, if ≥ 1.5 or ≤ 0.7)	Not of NOTE
	8 WK:2h M ↓ 0.55 G3 (0.037x10 ³ /μL) 8 WK:6h M ↓ 0.60 G3 (0.035x10 ³ /μL) 8 WK:24h M ↓ 0.52 G3 (0.020x10 ³ /μL) SD1:0h F ↓ 0.56 G2 (0.033x10 ³ /μL) SD1:6h F ↓ 0.66 G2 (0.037x10 ³ /μL) 8 WK:0h F ↓ 0.57 G2 (0.040x10 ³ /μL) 8 WK:6h F ↓ 0.36 G2 (0.030x10 ³ /μL) 8 WK:24h F ↓ 0.42 G2 (0.020x10 ³ /μL) 8 WK:24h F ↓ 0.46 G2 (0.022x10 ³ /μL) <i>Recovery phase:</i> 4 WK M ↓ 0.58 G4 (0.055x10 ³ /μL)	
CLOTTING POTENTIAL	Platelet count <i>Recovery phase:</i> 1 WK F ↓ 0.69 G4 (434.0 K/mm ³) 4 WK F ↓ 0.65 G4 (366.5 K/mm ³) Activated partial-thromboplastin time clotting time (APTT): SD1: 2h F ↑1.53 G4 (33.34 sec) 8WK F ↑1.65 G4 (38.93 sec) <i>Recovery phase:</i> 1 WK F ↓ 0.55 G4 (21.65 sec)	Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology: ND

Table of Hematology Results: *($P \leq 0.05$), **($P \leq 0.01$)

Total leukocyte and neutrophil counts were decreased in males and females at 12.5 mg/kg/week at the 8-weeks-24-hour interval. After a recovery phase of 1 and 4 weeks levels returned to baseline, except for 1 male animal in which the neutrophil level stayed low.

On study day 1, 2 hours after the first administration of the 1018 ISS adjuvant Activated partial-thromboplastin time clotting time (APTT) was increased by 53% in female animals receiving 12.5 mg/kg/week (group 4), 6 hours post administration APTT was increased by 34%. Six hours after the 8th administration of the adjuvant animals receiving 12.5 mg/kg/week showed an increase in the APPT of 65% (females) and 23% (males), 24 hours after the 8th administration APTT were high in all groups, including the control group. No increase in APTT was observed after the recovery phase.

All treatment groups showed an occasional decrease in monocyte, basophil and eosinophil counts as compared to the control group. However, this was seen before and after the administration of the adjuvant and was not related to the dose. Therefore these changes were not clearly treatment-related and might be incidental.

Systemic toxicity:

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

SEX	MALES				FEMALES			
GROUPS	1	2	3	4	1	2	3	4
NUMBER OF ANIMALS	3/2	3/0	3/0	3/2	3/2	3/0	3/0	3/2
BODY WEIGHT (in kg)	2.57/2.64	2.57	2.34	2.40/2.60	2.31/2.29	2.61	2.60	2.20/2.43
BRAIN	69.79/59.33	68.97	67.69	64.49/66.36	58.74/61.79	64.68	62.56	61.55/56.55
ADRENALS	0.41/0.52	0.55	0.47	0.51/0.48	0.41/0.44	0.52	0.46	0.54/0.42
EPIDIDYMIDES	0.70/0.53	0.76	0.53	0.55/0.48				
HEART	7.69/9.77	9.65	9.60	9.60/9.55	8.19/7.16	9.74	10.86**	8.24/9.46
KIDNEYS	11.98/12.72	12.72	12.68	13.15/15.95	11.57/13.12	12.49	11.65	12.20/12.72
LIVER	48.07/50.80	56.33	47.55	53.39/58.09	49.73/46.34	54.12	57.54	52.48/58.40
PITUITARY	35/44	42	39	39/41	37/42	55	46	42/44
SPLEEN	3.67/3.66	3.52	2.83	5.38/4.09	3.69/3.85	3.56	5.40	6.16/4.50
TESTES	0.78/1.24	1.32	0.78	1.38/1.43				
THYROID and PARATHYROID	0.29/0.41	0.23	0.29	0.52/0.41	0.30/0.27	0.32	0.35	0.34/0.25
THYMUS	5.16/6.17	5.60	5.01	3.66/6.44	4.43/4.60	4.55	2.64	4.55/2.85
OVARIES					0.16/0.16	0.22	0.28	0.22/0.33
UTERUS					NC	NC	NC	NC

Table of organ weight. Absolute organ weights are expressed as mean (grams), body weights are expressed as mean in kg. *different from controls at ≤ 0.05 ; **different from controls at $P \leq 0.01$. Entries in the table for group 1 and 4 are expressed both as organ weight from the animals taken at terminal necropsy and recovery necropsy (terminal necropsy organ weight / recovery necropsy organ weight), Group 2 and 3 only terminal organ weights were provided. NC = Not collected. *different from controls at $P \leq 0.05$

The average median heart weight for female animals in group 3 was statistically significant increased. In this group two animals showed a higher organ weight of their hearts of 11.46 and 11.35 g while other animal's heart weights were between 8 to 10 g. Only a limited histopathology evaluation was performed for these animals and therefore the histopathology of the heart was not evaluated. However, no changes in clinical chemistry of clinical signs were observed for these animals.

At the terminal necropsy, 1 male animal in group 4 showed a significantly enlarged spleen of 10.71 g, in histopathology the spleen showed lymphoid hyperplasia and mild congestion. Groups 3 and 4 of female animals exhibited increases in the spleen organ weight. In all cases microscopic evaluation showed minimal to moderate lymphoid hyperplasia. These changes can be explained by the immune stimulation due to the adjuvant.

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High variance in the organ weight of the testes as well as thymuses was observed leading to differences in the mean organ weight between different groups, no histopathologic findings (apart from immaturity for testes) were described for these organs.

Gross Pathology:***Terminal Sacrifice:***

Group	Findings
3M	Red mild discoloration at the injection site (3M: 1/3, 4M: 1/3, 3F: 1/3, 4F: 3/3)
1M, 2M, 3M, 4M	Adhesion in the lung (mild: 1M: 1/3, 2M: 1/3, 3M: 2/3; moderate: 4M: 1/3)
2F, 3F	Mild tan discoloration in the lung (2F: 1/3, 3F: 1/3)
1F	Mild black focus in the lung (1F: 1/3)
3F	Mild tan focus in the lung (3F: 1/3)
4M	Mild white focus in the spleen (4M: 1/3)
3F	Moderate cyst in the brain (3F: 1/3)
3F	Mild irregular surface in the brain (3F: 1/3)
4F	Moderate ovary cyst (4F: 1/3)
3F	Mild enlarged ovary (3F: 1/3)
3F	Mild red focus on the ovary (3F: 1/3)
3F	Moderate granular surface on the spleen (3F: 1/3)
4F	Mild white foci on the spleen (4F: 1/3)
4F	Absent thyroid gland (4F: 1/3)

Gross pathology findings at the terminal sacrifice, frequency of the finding (number of animals with the finding/total number of animals in the group) is stated in brackets

Recovery sacrifice:

Groups	Findings
4M	Mild red focus on the cecum (4M: 1/2)
4M	Minimal nodule on the skin (4M: 1/2)

Gross pathology findings at the recovery sacrifice, frequency of the finding (number of animals with the finding/total number of animals in the group) is stated in brackets

Microscopic finding:***Terminal sacrifice:***

Groups	Findings
1M	Altered eosinophilic (minimal) foci in the adrenal gland cortex (1M: 1/3)
4F	Lymphocytic infiltration (minimal) in the adrenal gland medulla (4F: 1/3)
3F	Lymphocytic infiltration (minimal) in the brain (3F: 1/3)
1M, 4M, 1F, 4F	Lymphocytic infiltration (minimal) in the esophagus (1M: 1/3, 4M: 1/3, 1F: 1/3, 4F: 1/3)
1M, 4M, 1F, 4F	Mononuclear cell infiltration (minimal) in the heart (1M: 3/3, 4M: 2/3, 1F: 1/3, 4F: 1/3)
3F	Chronic moderate inflammation at the injection site: (3F: 1/3)

Groups	Findings
2M, 3M, 4M, 1F, 2F, 3F, 4F	Acute inflammation at the injection site: (minimal: 2M: 2/3, 3M: 1/3, 2F: 1/3, 3F: 2/3; mild: 2M: 1/3, 3M: 2/3, 2F: 1/3, 4F: 1/3; moderate: 4M: 2/3, 4F: 2/3)
3M, 4M, 3F, 4F	Congestion at the injection site (minimal: 2M: 1/3, 3M: 1/3, 3F: 1/3; mild: 4M: 1/3, 4F: 3/3)
1M, 2M, 3M, 4M, 2F, 3F, 4F	Chronic active inflammation at the injection site (minimal: 1M: 2/3; mild: 2M: 2/3, 3M: 1/3, 4M: 2/3, 2F: 1/3, 3F: 1/3, 4F: 1/3; moderate: 2M: 1/3, 3M: 1/3, 4M: 1/3, 2F: 2/3, 3F: 1/3, 4F: 2/3)
1M, 4M, 1F, 4F	Chronic interstitial inflammation (minimal) in the kidney (1M: 3/3, 4M: 3/3, 1F: 3/3, 4F: 3/3)
1M, 4M, 1F	Tubular mineralization (minimal) in the kidney (1M: 2/3, 4M: 1/3, 1F: 1/3)
4M	Granulomatous inflammation in the cecum (minimal) (4M: 1/3)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Chronic inflammation in the liver (minimal: 1M: 3/3, 2M: 3/3, 3M: 3/3, 4M: 2/3, 1F: 3/3, 2F: 3/3, 3F: 2/3, 4F: 3/3, mild: 4M: 1/3, 3F: 1/3)
4M, 4F	Hypertrophy of the liver (minimal: 4M: 1/3, 4F: 2/3; mild: 4M: 2/3, 4F: 1/3)
4M, 4F	Pigment in the liver (minimal: 4M: 1/3, 4F: 2/3; mild: 4M: 2/3, 4F: 1/3)
1M, 3M, 4M, 1F, 3F	Chronic inflammation in the lung (minimal: 1M: 1/3, 3M: 1/3, 4M: 1/3, 1F: 3/3, 3F: 1/3)
2M, 3M, 4M, 2F, 3F	Adhesion in the lung (minimal: 2M: 1/3, 3M: 2/3, 4M: 1/3, 2F: 1/3, 3F: 1/3; mild: 2M: 1/3, 3M: 1/3, 4M: 1/3)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Histocytes in the lung (minimal: 1M: 2/3, 2M: 1/3, 3M: 2/3, 4M: 3/3, 1F: 3/3, 2F: 1/3, 3F: 2/3, 4F: 3/3)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Pigment, lung mite in the lung (minimal: 1M: 3/3, 2M: 1/3, 3M: 2/3, 4M: 3/3, 1F: 3/3, 2F: 1/3, 3F: 2/3, 4F: 3/3)
1M, 4M, 1F, 4F	Lymphoid hyperplasia in the mandibular lymph node (minimal: 1M: 1/3, 4M: 1/3, 1F: 1/3, 4F: 3/3)
3M	Chronic active inflammation in the mandibular lymph node (mild: 3M: 1/3)
2M	Medullary plasmocytosis in the mandibular lymph node (minimal: 2M: 1/3)
1M, 2F, 3F	Hemosiderin pigment in the mesenteric lymph node (minimal: 1M: 1/3, 2F: 1/3, 3F: 1/3; mild: 3F: 1/3)
4F	Ovary cyst (moderate: 4F: 1/3)
1F, 3F	Mineralization in the ovary (minimal: 1F: 2/3, 3F: 2/3)
3F	Corpus luteum present (mild: 3F: 1/3)
1M	Acute inflammation in the pancreas (minimal: 1M: 1/3)
4F	Lymphocytic infiltration in the pancreas (minimal: 4F: 1/3)
1F	Ectopic tissue in the pancreas (mild: 1F: 1/3)
1F	Mineralization in the pituitary gland (minimal: 1F: 1/3)

Groups	Findings
1M, 4M, 1F	Lymphocyte infiltration in the mandibular salivary gland (minimal: 1M: 2/3, 4M: 3/3, 1F: 1/3)
4M	Lymphocytic infiltration in the seminal vesicle (minimal: 4M: 1/3)
1M	Lymphocytic infiltration in the skeletal muscle (minimal: 1M: 1/3)
4M	Chronic inflammation in the skeletal muscle (mild: 4M: 1/3)
4M	Chronic myositis in the skeletal muscle (minimal: 4M: 1/3)
4M, 1F, 4F	Congestion in the spleen (minimal: 1F: 1/3, 4F: 1/3; mild: 4M: 1/3, 4F: 1/3)
1M, 2M, 4M, 1F, 2F, 3F, 4F	Lymphoid hyperplasia in the spleen (minimal: 1M: 1/3, 2M: 1/3, 4M: 3/3, 1F: 3/3, 3F: 1/3; 4F: 2/3; mild: 2F:1/3; moderate 3F: 1/3)
1M	Cyst in the thyroglossal duct of the thyroid (minimal: 1M: 1/3)
1M, 4M	Lymphocytic infiltration in the thyroid gland (minimal: 1M:1/3, 4M: 1/3)
1M	Lymphocytic infiltration in the tongue (minimal: 1M; 1/3)
1M, 4M, 1F, 4F	Lymphocystic infiltration of the urinary bladder (minimal: 1M: 3/3, 4M: 2/3; 1F: 1/3; 4F: 2/3; mild: 1F: 1/3)

Microscopic findings at the terminal sacrifice, severity of the finding as well as frequency (number of animals with the finding/total number of animals in the group) are stated in brackets.

Recovery sacrifice:

Groups	Findings
4M, 4F	Chronic active inflammation at the injection site (minimal: 4M: 1/2, mild: 4M: 1/2; 4F: 2/2)
4M	Granulomatous inflammation at the cecum (mild: 4M: 1/2)
1M, 4M, 1F, 4F	Chronic inflammation in the liver (minimal: 1M: 2/2, 4M: 2/2, 1F: 2/2, 4F: 1/2; mild: 4F: 1/2)
4M	Pigment in the liver (minimal: 4M: 1/2; 4F: 2/2)
1F	Adhesion of the liver (minimal: 1F: 1/2)
1M, 4M	Lymphoid hyperplasia in the mandibular lymph node (minimal: 1M: 1/2, 4M: 1/2)
1M, 4M	Granulomatous inflammation in the mesenteric lymph node (minimal: 1M: 1/2)
4M	Lymphoid hyperplasia in the mandibular lymph node (minimal: 4M: 1/2)
1M, 4M, 4F	Hemodiserin Pigment in the mesenteric lymph node (minimal: 1M: 1/2, 4M: 1/2, mild: 4M: 1/2)
1F	Adhesion at the spleen (minimal: 1F: 1/2)
4F	Lymphoid hyperplasia in the spleen (minimal: 4F: 1/2)

Microscopic findings at the recovery sacrifice, severity of the finding as well as frequency (number of animals with the finding/total number of animals in the group) are stated in brackets

At the terminal necropsy all animals in the high dose group (group 4) showed minimal to mild hypertrophy of the liver with a hypertrophy of the Kupffer cells and pigment in these cells. Blue pigment was also found in the liver. The hypertrophy of the liver and

Kupffer cells was reversible and was not observed after the recovery phase. Traces of pigments were still found in the liver of male animals after the recovery phase. Neither hypertrophy of the liver/Kupffer cells nor pigments in the liver were seen in the lower dose groups.

One male animal in the highest dose group showed myositis of the skeletal muscle accompanied by chronic inflammation at the terminal necropsy.

Local toxicity:

After the recovery, the histopathologic evaluation showed a minimal to moderate acute and minimal to moderate chronic active inflammation at the injection site with the highest severity in animals receiving the highest dose of 1018 ISS (group 4). After recovery phase only animals receiving the highest dose of 1018 ISS showed a minimal to mild chronic active inflammation at the injection site.

Complement Split Product:

Blood samples were collected at pre-dose, 2 and 6 hours post dose on day 1, at pre-dose, 2, 6 and 24 hours on the day of the 8-week injection and at 1 and 4 weeks after the last dose.

Bb fragment: Measured by the (b) (4) that cross-reacts with Bb from other species, including cynomolgus monkeys.

C5a anaphlatoxin: Measured by a (b) (4) method developed for measuring human C5a.

Mean values of the Bb split product from group 2 and 3 were not different from the control group. Animals of group 4 showed a significant increase in the Bb split product 2 and 6 hours post dose on day 1 as well as 2 and 6 hours on the day of the 8-week injection, at these time points the levels of the Bb split product rose above the normal range. The highest levels with up to 2.6 time the pre-dose levels were measured 2 hours after the administration; afterwards levels decreased. Twenty-four hours after the last dose the mean levels had fallen under the limit of the normal range again, but were still slightly higher than in the control animals. After the recovery phase the control group and group 4 did not show any difference in the level of split product Bb. These results present significant evidence of complement activation. In animals with the highest Bb levels the C5a levels were also evaluated and no increase in the C5a level was detected.

Test article related effects
<ul style="list-style-type: none">• Pigment in liver• Hypertrophy of liver and Kupffer cells

Test article related effects
<ul style="list-style-type: none"> • Increase in split Bb products • Slight increase in APTT • Splenomegaly with lymphoid hyperplasia

Assessment:

No treatment-related, mortality, nor any toxicologically relevant changes were found in clinical signs, body weight (gain), food consumption, urinalysis, ophthalmoscopic parameters or electrocardiographic parameters. The study design did not include some specific endpoints for the evaluation of immunostimulatory agents, like body temperature, a specific method of injection site assessment and evaluation of acute phase reactants; body weight was only determined weekly. The toxicology study was performed at many fold higher doses of 1018 ISS on a mass basis compared to that intended for clinical use and exceeded the number of doses administered. The animals received either 0.5, 1.5, 12.5 mg/kg/week of 1018 ISS adjuvant or PBS weekly for 8 weeks. On mg/kg dose, the proposed clinical dose of 0.05 mg/kg is approximately 10, 50 or 250-fold lower than that used in the toxicity study. Most of the observed changes in this toxicology study fall into previously described class effects induced by oligonucleotides.

Polyanionic effect:

Oligonucleotides have been described to interact with cationic sites on various blood proteins due to their polyanionic structure resulting in the inhibition of the coagulation cascade and the activation of the alternative complement pathway. The inhibition of the coagulation cascade through the administered 1018 ISS adjuvant was reflected in a slight increase in APTT levels in male and female animals of the highest dose group receiving 12.5 mg/kg/dose after the treatment phase which was resolved after the recovery phase. The activation of the alternative complement pathway was seen in the modest increase in complement split product Bb, in many of the animals receiving 12.5 mg/kg/week 1018 ISS adjuvant. However, the elevation in Bb was not accompanied by any increase in C5a. Therefore, the alternative pathway activation was not associated with accumulation of biologically active split products that would pose a risk.

Accumulation in target organs:

Circulating oligonucleotides are quickly cleared by uptake in various tissues and accumulate primarily in their target organs including liver, kidney and spleen. Animals in the highest dose group showed blue granular pigment in the liver and Kupffer cells which was suspected to reflect deposition of the 1018 ISS oligonucleotide and was associated with a reversible minimal to mild hypertrophy of the liver and Kupffer cells. Traces of pigments were still found after the recovery phase in the liver of male animals. Neither hypertrophy of the liver/Kupffer cells nor pigments in the liver were seen in the lower dose groups.

Immune stimulation:

The immune stimulative effects of the adjuvant were reflected in changes at the injection sites, the mandibular lymph nodes as well as the spleen. These changes were generally dose-dependent and completely or partially resolved after the recovery phase. At the terminal necropsy, 1 male animal in group 4 showed a significantly enlarged spleen weighing 10.71g, in the histopathology evaluation the spleen showed lymphoid hyperplasia mild congestion. Group 3 and 4 of female animals showed increases in the spleen organ weight, in all cases microscopic evaluation showed minimal to moderate lymphoid hyperplasia. Injection site changes showed an acute and chronic active inflammation after the treatment phase which manifested as a chronic active inflammation after the recovery phase.

Many oligonucleotids have also been described to induce cytopenias (e.g. peripherical anemia, neutropenia). In this submitted non-human primate study the leukocyte and neutrophil counts were modestly decreased in males and female animals receiving 12.5 mg/kg/week after the treatment phase and returned to baseline after the recovery phase. All treatment groups also show occasionally a decrease in monocyte, basophil and eosinophil counts compared to the control group; however, this was seen before and after the administration of the adjuvant and was not related to the dose, therefore these changes are not clearly treatment-related and might be incidental.

Study no. 00-158: 8-Week Toxicity Study of 1018 ISS Administered by Subcutaneous Injection in Rats

Performing laboratory: (b) (4)
Study initiation date: 02.01.2001
Final Report date: 02.21.2002
Test article batch/lot: 1018 ISS oligonucleotide: (b) (4)
Animal species and strain: (b) (4) rats
Breeder/supplier: (b) (4)
Number of animal per group and sex: 10
Age: 9 weeks
Body weight range: Males: 268 to 307 grams, females: 187 to 213 grams
Route and site of administration: Subcutaneous
Volume of injection: 0.25 mL/kg and were based on the most recent body weights
Frequency of administration and study duration: Once per week for 8 weeks, with a 4 week recovery period
Dose: 0, 0.5, 2.5, 12.5 mg/kg/week
Stability: Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study. The test article was within the specification and was assumed to be stable for the study duration.
Means of administration: Needle and syringe
Report status: Final
Experimental design:

Group	Treatment 1018ISS (mg/kg/week)	Number of Animals (#/sex/group)	
		Treatment phase (1 day after last dose)	Recovery phase (4 weeks after last dose)
1	0	10	5
2	0.5	10	
3	2.5	10	
4	12.5	10	5

Randomization procedure: Animals were weighed prior to treatment and randomized by sex into treatment groups using a standard by weight by block randomization procedure.

Statistical analysis plan:

For each specified endpoint and for all collection intervals, Levene's test (Milliken and Johnson, 1992) was used to assess homogeneity of group variances. If Levene's test was not significant ($p < 0.01$), Dunnett's test was used to compare each treatment group with the control group. If Levene's test was significant ($p < 0.01$), comparisons with the control group were made using Welch's t-test with a Bonferroni correction. Results of all pair-

wise comparisons were reported at the 0.05 and 0.01 significance levels. For body weights, food consumption, hematology (except leukocytes counts), clinical chemistry and organ weights group pair-wise comparisons were used. Since historical data indicate that leukocyte counts (total and differential) were not normally distributed a log transformation was performed on these data.

Parameters	Frequency of Testing
Cageside observation ³	Twice a day
Clinical observations ⁴	Once daily
Body weight	Within 3 days after arrival, prior to randomization, prior to the initial dose, and weekly thereafter
Food consumption	Weekly
Body temperature	NC
Ophthalmologic exam	NC
Clinical chemistry*	At the terminal and recovery sacrifices
Hematology*	At the terminal and recovery sacrifices
Coagulation*	NC
Immunological response	NC
Plasma test article concentration**	Predose, and at 1 and 4 hours postdose on day 1 and on the day of dosing in Week 8.
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	NC
Necropsy	Terminal necropsy: 1 day after the last dose; Recovery necropsy: 4 weeks after the last dose
Tissues for histopathology	Terminal necropsy: 1 day after the last dose; Recovery necropsy: 4 weeks after the last dose

*(cardiac puncture), **(orbital sinus), (NC = not collected)

Postmortem procedures: The following tissues were collected at necropsy.

Organ/Tissue	Collected	Not collected
Adrenal glands	*!	
Aorta	!	
Bone (sternum & femur)	!	
Bone marrow (sternum & femur)	!	
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	*!	

³ Cageside observations include mortality, morbidity, injury, and availability of food and water.

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
Cervix	!	
Colon	!	
Duodenum	!	
Epididymides	*!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		!
Gross lesions (if any)	!	
Harderian gland	!	
Heart	*!	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	*!	
Lacrimal glands	!	
Larynx		!
Liver	*!	
Lung (main-stem; bronchi)	*!	
Lymph nodes (axillary)	!	
Lymph nodes (iliac)	!	
Lymph nodes (mandibular)	!	
Lymph nodes (mesenteric)	!	
Lymph nodes (renal)	!	
Lymph nodes (sciatic)	!	
Mammary glands		!
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		!
Ovaries	*!	
Pancreas	!	
Peyer's patch (if applicable)		!
Pituitary gland	*!	
Prostate	!	
Rectum	!	
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	*!	

Organ/Tissue	Collected	Not collected
Stomach (squamous and glandular)	!	
Testes	*!	
Thymus	*!	
Thyroid (w/parathyroid glands)	*!	
Tongue	!	
Trachea	!	
Ureters		!
Uterus (w/cervix)	*!	
Urinary bladder	!	
Vagina	!	

Table of Histology: All dose groups (10 animals per group) were evaluated at the terminal necropsy, at the recovery phase high dose and control group animals (5 animals per group) were examined. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Results:

Morbidity and mortality: No treatment-related animal was observed. One female animal in group 3 was sacrificed on day 23, this animal showed no body weight gain and malocclusion, with a lower incisor stuck in the roof of the mouth and a protruding and swollen left eye. The conditions leading to the sacrifice of this animal were unrelated to treatment with 1018 ISS.

CLINICAL CHEMISTRY MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY PHASE, SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE, if ≥ 1.5 or ≤ 0.7)	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose

CLINICAL CHEMISTRY MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY PHASE, SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE, if ≥ 1.5 or ≤ 0.7)	NOT OF NOTE
LIVER FUNCTION: A) HEPATOCELLULAR B) HEPATOBILIARY	<p>Alanine aminotransferase (ALT or SGPT): TN: M \uparrow 1.52 G2 (80.8 U/L) RN: F \uparrow 3.02 G4 (103.2 U/L)*</p> <p>Aspartate aminotransferase (AST or SGOT): TN: M \uparrow 1.21 G2 (145.2 U/L) TN: M \uparrow 1.34 G3 (161.6 U/L) TN: M \uparrow 1.38 G4 (166.3 U/L) TN: F \uparrow 1.47 G2 (125.6 U/L) TN: F \uparrow 1.42 G3 (121.7 U/L) TN: F \uparrow 2.23 G4 (191.5 U/L)** RN: F \uparrow 2.50 G4 (203.0 U/L)*</p> <p>Sorbitol dehydrogenase: RN: F \uparrow 1.72 G4 (36.96 U/L)*</p> <p>Total bilirubin: RN: M \downarrow 0.67 G4 (0.20 mg/dL)</p>	<p>Glutamate dehydrogenase Total bile acids</p> <p>Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids</p>
ACUTE PHASE REACTANTS		Fibrinogen: ND
KIDNEY FUNCTION	<p>Blood urea nitrogen: TN: M \uparrow 1.50 G4 (21.2 mg/dL)* TN: F \uparrow 1.73 G4 (22.5 mg/dL)*</p>	Creatinine
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	<p>Total protein: TN: F \downarrow 0.44 G4 (4.71 g/dL)*</p> <p>Albumin: TN: F \downarrow 0.60 G4 (2.25 g/dL)**</p> <p>Globulin: TN: F \downarrow 0.68 G4 (2.46 g/dL)*</p> <p>Total cholesterol: RN: F \uparrow 1.75 G4 (121.2 mg/dL)*</p>	<p>A/G Ratio Cholinesterase: ND Creatine kinase: ND Fasting triglycerides: ND</p>

Table of Clinical Chemistry Results: TN: terminal necropsy, RN: recovery necropsy, * (ND = not determined) *different from controls at $P \leq 0.05$, **different from controls at $P \leq 0.01$

After the treatment phase male and female animals showed a slight dose-dependent statistically significant increase in aspartate aminotransferase with the highest (2 fold) increase for female animals in group 4. Further, female animals of group 4 showed statistically significant increased alanine aminotransferase (70%) and sorbitol dehydrogenase (SDH by 41%). These increases in liver enzymes were still observed after the recovery phase for female animals. Histopathologically, these changes were associated with chronic inflammation in the liver and hyperplasia of the Kupffer cells.

After the treatment phase male and female animals receiving 2.5 and 12.5 mg/kg/week also showed a slight, statistical significant dose-related decrease in total protein, albumin and globulin levels with the highest severity in female animals receiving 12 mg/kg/week. These changes could be a reflection of the toxicities observed in the liver. The levels of total protein, albumin and globulin returned to normal levels after the recovery phase.

Urea nitrogen (BUN) was 50% and 73% higher than control levels in male and female animals, respectively at a dose of 12.5 mg/kg/week after the treatment phase, with resolution following the recovery period. The increase in BUN may reflect the observed histopathological changes (chronic interstitial inflammation and tubular degeneration) in the kidneys.

HEMATOLOGY MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY PHASE, SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE, if ≥ 1.5 or ≤ 0.7)	NOT OF NOTE
RED BLOOD CELLS	<p>Total Erythrocyte Count (RBC): TN: M ↓ 0.86 G3 ($7.212 \times 10^6/\mu\text{L}$)** TN: M ↓ 0.69 G4 ($5.798 \times 10^6/\mu\text{L}$)** TN: F ↓ 0.85 G3 ($6.691 \times 10^6/\mu\text{L}$)** TN: F ↓ 0.65 G4 ($5.118 \times 10^6/\mu\text{L}$)**</p> <p>Hemoglobin Conc. (Hb): TN: M ↓ 0.83 G3 (12.30 g/dL)** TN: M ↓ 0.71 G4 (10.61 g/dL)** TN: F ↓ 0.79 G3 (11.74 g/dL)** TN: F ↓ 0.66 G4 (9.76 g/dL)**</p> <p>Hematocrit (Hct): TN: M: G1: 47%, G2: 47%, G3: 41%**, G4: 35%** TN: F: G1: 46%, G2: 47% G3: 38%**, G4: 32%**</p>	Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Reticulocytes
WHITE BLOOD CELLS	<p>Total leukocytes (WBC): TN: M ↑ 1.44 G3 ($19.37 \times 10^3/\mu\text{L}$)* TN: M ↑ 1.84 G4 ($19.37 \times 10^3/\mu\text{L}$)** TN: F ↑ 1.91 G3 ($18.97 \times 10^3/\mu\text{L}$)** TN: F ↑ 2.48 G4 ($24.68 \times 10^3/\mu\text{L}$)** RN: M ↑ 2.31 G4 ($22.68 \times 10^3/\mu\text{L}$) RN: F ↑ 1.83 G4 ($17.06 \times 10^3/\mu\text{L}$)</p> <p>Neutrophil count: TN: M ↑ 1.95 G3 ($3.910 \times 10^3/\mu\text{L}$)** TN: F ↑ 2.05 G3 ($16.751 \times 10^3/\mu\text{L}$)** TN: F ↑ 2.64 G4 ($21.817 \times 10^3/\mu\text{L}$)** RN: M ↑ 1.72 G4 ($2.046 \times 10^3/\mu\text{L}$)</p> <p>Lymphocyte count: TN: M ↑ 1.89 G4 ($20.109 \times 10^3/\mu\text{L}$)** TN: F ↑ 1.89 G4 ($20.109 \times 10^3/\mu\text{L}$)** RN: M ↑ 2.44 G4 ($19.396 \times 10^3/\mu\text{L}$)* RN: F ↑ 1.90 G4 ($14.028 \times 10^3/\mu\text{L}$)**</p> <p>Monocyte count: TN: M ↑ 1.95 G3 ($0.491 \times 10^3/\mu\text{L}$)** TN: M ↑ 2.29 G4 ($0.757 \times 10^3/\mu\text{L}$)** TN: F ↑ 2.32 G3 ($0.374 \times 10^3/\mu\text{L}$)** TN: F ↑ 2.00 G4 ($0.322 \times 10^3/\mu\text{L}$)** RN: M ↑ 1.78 G4 ($0.490 \times 10^3/\mu\text{L}$)</p> <p>Basophil count: TN: M ↑ 2.99 G4 ($0.290 \times 10^3/\mu\text{L}$)** TN: F ↑ 3.07 G3 ($0.129 \times 10^3/\mu\text{L}$)**</p>	Eosinophils count

HEMATOLOGY MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY PHASE, SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE, if ≥ 1.5 or ≤ 0.7)	NOT OF NOTE
	TN: F \uparrow 1.43 G4 (0.060x10 ³ / μ L)** RN: F \downarrow 0.25 G4 (0.112x10 ³ / μ L)** Large unstained cells (LUC): RN: M \uparrow 3.34 G4 (0.3901x10 ³ / μ L)** RN: F \uparrow 3.11 G4 (0.1771x10 ³ / μ L)**	
CLOTTING POTENTIAL	Platelet count: TN: M \downarrow 0.64 G3 (686.6x10 ³ / μ L)** TN: M \downarrow 0.25 G4 (264.36x10 ³ / μ L)** TN: F \downarrow 0.49 G3 (571.0x10 ³ / μ L)** TN: F \downarrow 0.16 G4 (189.0x10 ³ / μ L)** Activated partial-thromboplastin time clotting time: TN: M: G1: 26 sec, G2: 29 sec, G3: 27 sec, G4: 33 sec TN: F: G1: 22 sec, G2: 21 sec, G3: 25 sec, G4: 33 sec	Prothrombin time Mean platelet volume Fibrinogen: ND
OTHERS		Bone marrow cytology: ND

Table of Hematology Results. TN: terminal necropsy, RN: recovery necropsy, * (ND = not determined) *different from controls at $P \leq 0.05$, **different from controls at $P \leq 0.01$

Male and female animals receiving 2.5 or 12.5 mg/kg/dose 1018 ISS showed a dose-dependent decrease in erythrocytic parameters and platelet counts and an increase in total leukocyte, lymphocyte, neutrophil, monocyte, and basophil counts and activated partial thromboplastin times (APTT). Changes in erythrocytic parameters, platelet counts and APTT times were resolved after the recovery phase, the increase in total leukocytes, lymphocytes, neutrophils and monocytes was still seen after the recovery phase.

Systemic toxicity:

In groups 3 and 4 clinical findings included scabbed areas, abrasions, hair absent, and/or hair sparse at or around the injection site, which generally appeared after at least 3 weekly doses and was still observed in the high dose group (other dose groups were not evaluated) after a 4 week recovery dose. The incidence rate and persistence was dose-dependent.

Male animals receiving 12.5 mg/kg/week showed a slight decrease in body weight gain and the total body weight gain was only 86% of the control group. During the recovery period, male group 4 animals showed a slightly higher body weight gain. Female animals showed no difference in body weight gain. Male and female animals in the high dose group showed an approximately 10% reduced weekly food consumption during the treatment period.

Organ Weight:

SEX	MALES				FEMALES			
GROUPS	1	2	3	4	1	2	3	4
NUMBER OF ANIMALS	10/5	10	10	10/5	10/5	10	10	10/5
BODY WEIGHT (terminal)	426/464	444	427	400/451	242/269	245	245	243/264
BRAIN	2.01/2.05	2.00	2.03	1.95/1.95	1.52/1.90	1.83	1.82	1.84/1.83
ADRENALS	0.069/0.063	0.064	0.068	0.074/0.036	0.073/0.080	0.072	0.074	0.077/0.073
EPIDIDYMIDES	1.26/3.13	1.29	1.25	1.25/1.34				
HEART	1.56/1.76	1.63	1.57	1.44/1.82	1.02/1.09	0.97	1.01	0.98/1.19
KIDNEYS	3.62/3.78	3.76	3.72	3.71/4.20	2.08/2.14	2.08	2.12	2.26/2.37
LIVER	13.14/13.82	14.27	25.08*	35.35*/ 21.24*	7.67/8.39	7.96	16.93*	25.82*/ 13.25*
LUNG	1.85/1.89	1.87	1.86	1.91/1.78	1.39/1.35	1.33	1.42	1.53/
PITUITARY	14/15	14	15	15/17	0.017/0.019	0.018	0.018	0.018/0.022
SPLEEN	0.78/0.76	0.8	1.52*	3.48*/2.08*	0.51/0.52	0.55	0.95*	2.60*/1.10*
PROSTATE	0.58/0.19	0.51	0.56	0.55/0.68				
TESTES	3.45/7.83	3.53	3.26	3.37/3.63				
THYROID and PARATHYROID	0.032/0.029	0.031	0.030	0.031/0.032	0.027/0.028	0.025	0.026	0.025/0.029
THYMUS	0.36/0.33	0.38	0.34	0.34/0.35	0.30/0.31	0.34	0.36	0.32/0.33
OVARIES					0.12/0.141	0.133	0.145	0.152/0.153
UTERUS					0.79/0.89	0.56	0.72	1.00/0.90

Table of organ weight. Absolute weights are expressed as mean (grams). Entries in the table are expressed both as organ weight from the animals taken at 1 day after the last administration (terminal necropsy) and 4 weeks after the last administration (recovery necropsy) [organ weight terminal necropsy / organ weight recovery necropsy]. *different from controls at $P \leq 0.01$

Absolute organ weights were statistically increased for the liver and spleen in both sexes for group 3 and 4 at the terminal and recovery sacrifice; they were considered treatment related. These changes were associated with histopathological findings, chronic inflammation in the liver and hyperplasia of the Kupffer cells as well as hyperplasia and increased extramedullary hematopoiesis in the spleen.

Gross Pathology:***Terminal necropsy:***

Group	Findings
3M	Small (mild) epididymidis (3M: 1/10)
2M	Mild opacity of cornea (2M: 1/10)
3F	Eye absent (3F: 1/10)
2F	Mass at the eye (3F: 1/10)
4M, 2F	Mild red discoloration at the eye (4M: 1/10, 2F: 1/10)
3M, 4M, 3F, 4F	Minimal to moderate scab at the injection site (3M: 3/10, 4M: 4/10, 3F: 1/10, 4F: 4/10)
4F	Mild tan discoloration at the injection site (4F: 1/10)
3M	Moderate abrasion at the injection site (3M: 1/10)
2M, 3M, 4M	Mild to moderate pelvic dilatation of the kidney (2M: 1/10, 3M: 3/10, 4M: 1/10)
3M, 4M, 4F	Mild to moderate enlarged liver (3M: 4/10, 4M: 7/10, 4F: 6/10)
3M, 4M, 3F	Generally mild enlarged lymph nodes (3M: 1/10, 4M: 3/10, 4F: 4/10)
3M, 4M	Mild to moderate enlarge iliac lymph node (3M: 2/10, 4M: 1/10)
3M, 4M, 3F, 4F	Mild to moderate enlarge mandibular lymph node (3M: 1/10, 4M: 5/10, 3F: 1/10, 4F:5/10)
3M, 4M, 3F, 4F	Mild enlarge mesenteric lymph node (3M: 1/10, 4M: 4/10, 3F: 1/10, 4F:5/10)
3M, 4F	Mild to moderate enlarged axillary lymph node (3M: 1/10, 4F: 3/10)
3M, 4F	Mild to moderate enlarged renal lymph node (3M: 1/10, 4F: 1/10)
2M, 3M, 4M	Mild skin scab (2M: 1/10, 3M: 1/10, 4M: 1/10)
4F	Minimal alopecia (4F: 1/10)
3M, 4M, 3F, 4F	Minimal to severe enlarged spleen (3M: 5/10, 4M: 10/10, 3F: 2/10, 4F:10/10)
3M	Small testis (3M: 1/10, mild)
2F, 4F	Mild to moderate fluid distended uterus (2F: 1/10, 4F: 3/10)
3M	Moderate calculi in the urinary bladder (3M: 1/10)

Gross pathology findings at the terminal sacrifice, frequency of the finding (number of animals with the finding/total number of animals in the group) is stated in brackets

Recovery necropsy:

Group	Findings
4M	Mild pelvic dilatation of the kidney (4M: 1/5)
4F	Mild cyst in the kidney (4F: 1/5)
4M	Mild enlarged iliac lymph node (4M: 1/5)
4M	Mild enlarged mandibular lymph node (4M: 1/5)
4M, 4F	Mild enlarged mesenteric lymph node (4M: 1/5, 4F: 1/5)
4M	Mild enlarged spleen (4M: 1/5)
4F	Mild scab at the skin (4F: 2/5)
4F	Mild to moderate fluid distended uterus (4F: 1/5)
4F	Mild thickened uterus (4F: 1/5)
4M	Moderate calculi in the urinary bladder (3M: 1/5)

Gross pathology findings at the recovery sacrifice, frequency of the finding (number of animals with the finding/total number of animals in the group) is stated in brackets

After the treatment phase animals receiving 2.5 and 12.5 mg/kg/week showed enlargement of the spleen and various lymph nodes as well as pathological changes at the injection site. After the recovery phase these same findings were less frequent suggesting reversibility.

Microscopic finding:**Terminal necropsy:**

Groups	Findings
4M	Minimal lymphocytic infiltration in the adrenal cortex (4M: 1/10)
4M, 4F	Minimal coagulative necrosis in the adrenal cortex (4M: 1/10, 4F: 1/10)
4M, 4F	Minimal lymphocytic infiltration in the adrenal medulla (4M: 2/10, 4F: 1/10)
2M, 3M, 4M, 3F, 4F	Minimal to moderate hyperplasia in the bone marrow of the femur (minimal: 2M: 5/10, 3F: 10/10, mild: 2M: 2/10, 3M: 8/10, 4F: 4/10, moderate: 3M: 2/10, 4M: 5/10, 4F: 6/10)
2M, 3M, 4M, 3F, 4F	Minimal to severe hyperplasia in the bone marrow of the sternum (minimal: 2M: 5/10, 3F: 1/10, mild: 2M: 2/10, 3M: 8/10, 3M: 1/10, 4F: 4/10, moderate: 3M: 2/10, 4M: 5/10, 4F: 6/10, severe: 4M: 5/10)
4M	Minimal degeneration of the nerve fiber in the brain (4M: 1/10)
1M, 3M, 4M	Minimal lymphocytic infiltration in the epididymides (1M: 7/10, 3M: 1/10, 4M: 10/10)
3M, 4M	Minimal luminal cellular debris in the epididymides (3M: 1/10, 4M: 1/10)
3M	Moderate hypospermia in the epididymides (3M: 1/10)
3F	Moderate to severe hemorrhage at the eye (3F: 3/10)

Groups	Findings
1M, 2F	Minimal to mild pigmented macrophages in the eye (1M: 1/10, 2F: 2/10)
2M	Minimal chronic active inflammation in the eye (2M: 1/10)
3F	Severe suppurative inflammation in the eye (3F: 1/10)
1M, 1F	Minimal pigmented macrophages in the optic nerve (1M: 1/10, 1F: 1/10)
1M, 4M, 1F, 4F	Minimal to mild chronic active inflammation in the Haderian gland (1M: 4/10, 4M: 2/10, 1F: 2/10, 4F: 6/10)
1M, 4M, 1F, 4F	Cardiomyopathy (minimal: 1M: 9/10, 4M: 9/10, 1F: 6/10, 4F: 10/10, mild: 1M: 1/10)
3M	Minimal acute inflammation at the injection site (3M: 1/10)
3M, 4M, 3F, 4F	Minimal to moderate epidermal exudate at the left injection site (minimal: 2M: 1/10, 3F: 2/10, 4F: 2/10, mild: 3M: 4/10, 4M: 2/10, moderate: 3M: 1/10)
3M, 4M, 3F, 4F	Minimal to moderate epithelial hyperplasia at the left injection site (minimal: 4M: 1/10, 3F: 2/10, 4F: 2/10, mild: 3M: 1/10, 4M: 3/10, 3F: 1/10, 4F: 2/10, moderate: 3M: 1/10)
1M, 2M, 3M, 4M, 2F, 3F, 4F	Minimal to severe chronic active inflammation at the left injection site (minimal: 1M: 1/10, 2M: 3/10, 3M: 2/10, 4M: 4/10, 3F: 4/10, 4F: 2/10, mild: 2M: 1/10, 3M: 5/10, 4M: 1/10, 2F: 2/10, 3F: 4/10, 4F: 4/10, moderate: 2M: 1/10, 4M: 3/10, 3F: 1/10, 4F: 3/10, severe: 3M: 1/10, 4M: 2/10, 4F: 1/10)
4M, 4F	Mild ulcer at the left injection site (3M: 2/10, 4F: 1/10)
1M, 3M	Minimal to mild acute inflammation at the injection site (1M: 1/10, 3M: 1/10)
3M, 4M, 2F, 3F, 4F	Minimal to moderate epidermal exudate at the right injection site (minimal: 3M: 1/10, 4F: 1/10, mild: 3M: 1/10, 4M: 1/10, 2F: 1/10, 4F: 1/10, moderate: 3M: 1/10)
3M, 4M, 3F, 4F	Minimal to moderate epithelial hyperplasia at the right injection site (minimal: 3M: 1/10, 4F: 1/10, mild: 3M: 2/10, 4M: 1/10, 2F: 1/10, 4F: 2/10, moderate: 3M: 1/10, 4M: 1/10)
1M, 2M, 3M, 4M, 2F, 3F, 4F	Minimal to severe chronic active inflammation at the right injection site (minimal: 1M: 2/10, 2M: 4/10, 3M: 3/10, 4M: 3/10, 2F: 3/10, 3F: 2/10, 4F: 2/10, mild: 3M: 4/10, 2F: 3/10, 3F: 2/10, 4F: 2/10, moderate: 2M: 2/10, 3M: 2/10, 4M: 5/10, 2F: 2/10, 3F: 2/10, 4F: 2/10, severe: 3M: 1/10, 4F: 3/10)
3M, 2F, 4F	Minimal to mild ulcer at the right injection site (minimal: 2F: 1/10, mild: 3M: 1/10, 4F: 1/10)
2M, 3M, 4M, 3F, 4F	Minimal to moderate tubular degeneration (minimal: 2M: 3/10, 3M: 4/10, 3F: 1/10, 4F: 1/10, mild: 3M: 1/10, 4M: 5/10, 4F: 7/10, moderate: 4M: 5/10, 4F: 2/10)
4M	Mild hydronephrosis (4M: 1/10)
1M, 3M	Minimal to mild epithelial hyperplasia in the kidney (1M: 1/10, 3M: 1/10)
2M, 3M, 4M, 3F,	Minimal to moderate chronic interstitial inflammation in the kidney

Groups	Findings
4F	(minimal: 2M: 4/10, 3M: 5/10, 3F: 1/10, 4F: 4/10, mild: 4M: 10/10, 4F: 5/10, moderate: 4F: 1/10)
1F, 2F, 3F, 4F	Minimal to mild tubular mineralization (minimal: 1F: 4/10, 2F: 3/10, 3F: 1/10, 4F: 1/10, mild: 4F: 1/10)
1M, 2M, 3M, 4M, 1F, 2F, 3F	Progressive chronic nephropathy (1M: 9/10, 2M: 6/10, 3M: 4/10, 4M: 10/10, 1F: 10/10, 2F: 9/10, 3F: 8/10)
3M, 4M, 4F	Minimal to mild green pigment in the kidney (minimal: 3M: 1/10, 4M: 2/10, 4F: 2/10, mild: 4M: 8/10, 4F: 8/10)
1M, 2M	Minimal to mild chronic pyelitis (minimal: 2M: 1/10, mild: 1M: 1/10)
1M, 4M	Mild to moderate pyelonephritis (mild: 1M: 1/10, moderate: 4M: 1/10)
1M, 1F, 4F	Minimal lymphocytic infiltration in the lacrimal gland (1M: 1/10, 1F: 1/10, 4F: 1/10)
3M, 4F, 3F, 4F	Minimal to moderate atrophy of the liver (minimal: 3M: 8/10, 4M: 6/10, 3M: 2/10, 4M: 1/10, mild: 2/10, 4M: 3/10, 3F: 1/10, 4F: 5/10, moderate: 4M: 1/10)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Minimal to severe chronic inflammation of the liver (minimal: 1M: 9/10, 2M: 9/10, 3M: 6/10, 1F: 8/10, 2F: 5/10, 3F: 1/10, mild: 1M: 1/10, 2M: 1/10, 3M: 4/10, 4M: 4/10, 1F: 2/10, 2F: 5/10, 3F: 9/10, 4F: 3/10, moderate: 4M: 4/10, 4F: 6/10, severe: 4F: 1/10)
3M, 4M, 3F, 4F	Minimal to moderate congestion in the liver (minimal: 3M: 6/10, 4M: 6/10, 3F: 2/10, 4F: 2/10, mild: 3M: 3/10, 4M: 1/10, 3M: 6/10, 4F: 5/10, moderate: 3F: 1/10)
3M, 4M, 3F, 4F	Mild to severe hyperplasia of the Kupffer cells (mild: 3M: 5/10, 3F: 10/10, 4F: 9/10, moderate: 3F: 5/10, 4M: 6/10, 4F: 1/10, severe: 4M: 4/10)
4F	Minimal hyperplasia of the bile duct (4F: 2/10)
1M, 4M, 1F, 4F	Minimal histiocytosis in the lung (1M: 10/10, 4M: 1/10, 1F: 10/10, 4F: 10/10)
3M, 3F, 4F	Mild to moderate hyperplasia of the axillary lymph node (mild: 3M: 1/10, 3F: 1/10, moderate: 4F: 3/10)
3M	Mild medullar plasmacytosis in the axillary lymph node (3M: 1/10)
3M, 4M	Mild to moderate hyperplasia of the iliac lymph node (mild: 3M: 2/10, moderate: 4M: 1/10)
3M, 4M	Mild medullar plasmacytosis in the iliac lymph node (mild: 3M: 2/10, 4M: 1/10)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Minimal to severe lymphoid hyperplasia in the mandibular lymph node (minimal: 1M: 2/10, 2M: 3/10, 4M: 1/10, 1F: 3/10, 2F: 2/10, 4F: 2/10, mild: 1M: 4/10, 2M: 3/10, 3M: 2/10, 4M: 2/10, 1F: 2/10, 2F: 3/10, 3F: 5/10, 4F: 3/10, moderate: 3M: 1/10, 4M: 7/10, 3F: 1/10, 4F: 5/10, severe: 3M: 1/10)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Minimal to moderate medullary plasmacytosis in the mandibular lymph node (minimal: 1M: 5/10, 2M: 3/10, 4M: 2/10, 1F: 7/10, 2F: 3/10, 3F: 3/10, 4F: 3/10, mild: 1M: 4/10, 2M: 4/10, 3M: 1/10, 4M: 4/10, 3F: 4/10, moderate: 2M: 1/10, 3M: 1/10, 4M: 4/10, 3F: 3/10,

Groups	Findings
	4F: 8/10)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Minimal to moderate lymphoid hyperplasia in the mesenteric lymph node (minimal: 1M: 1/10, 2M: 3/10, 3M: 2/10, 4M: 3/10, 1F: 2/10, 3F: 1/10, 4F: 2/10, mild: 1M: 3/10, 2M: 2/10, 3M: 2/10, 4M: 4/10, 3F: 4/10, moderate: 4M: 3/10, 3F: 3/10, 4F: 8/10)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Minimal to moderate medullary plasmacytosis in the mesenteric lymph node (minimal: 1M: 3/10, 3M: 2/10, 4M: 5/10, 1F: 3/10, 3F: 2/10, 4F: 3/10, mild: 4M: 2/10, 3F: 2/10, 4F: 5/10, moderate: 2M: 1/10, 3M: 1/10, 4M: 4/10)
3M, 4F	Moderate hyperplasia at the renal lymph node (3M: 1/10, 4F: 1/10)
4F	Minimal mineralization in the ovary (4F: 1/10)
1M, 4M	Minimal lymphocytic infiltration of the prostate gland (1M: 2/10, 4M: 1/10)
1M, 4M	Mild to moderate chronic active inflammation of the prostate gland (1M: 2/10, 4M: 2/10)
4M	Minimal lymphocytic infiltration of the seminal vesicle (1M: 2/10, 4M: 2/10)
2M, 3M, 4M, 4F	Minimal to mild hyperplasia of the skin (minimal: 2M: 1/10, 4F: 1/10, mild: 3M: 1/10, 4M: 1/10)
2M, 3M, 4M, 4F	Minimal to moderate chronic active inflammation of the skin (minimal: 4M: 1/10, 4F: 1/10, mild: 2M: 1/10, 3M: 1/10, 4M: 1/10, moderate: 4M: 1/10)
4M	Minimal hemorrhage at the skin (4M: 1/10)
2M, 3M, 4M	Mild edema of the skin (2M: 1/10, 3M: 1/10, 4M: 1/10)
2M, 3M, 4M, 4F	Minimal to moderate exudate of the skin (minimal: 3M: 1/10, mild: 2M: 1/10, moderate: 4M: 1/10)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Minimal to severe increased extramedullary hematopoieses in the spleen (minimal: 1M: 7/10, 2M: 5/10, mild: 2M: 5/10, 3M: 3/10, 3F: 7/10, moderate: 3M: 7/10, 4M: 1/10, 3F: 2/10, 4F: 2/10, severe: 2M: 1/10, 4M: 8/10)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Minimal to severe increased lymphoid hyperplasia in the spleen (minimal: 1M: 1/10, 2M: 4/10, 3M: 1/10, 4F: 1/10, mild: 2M: 1/10, 3M: 6/10, 4M: 4/10, 3F: 5/10, 4F: 1/10, moderate: 3M: 3/10, 4M: 1/10, 3F: 4/10, 4F: 8/10, severe: 4M: 5/10)
2M, 3M, 4M, 3F, 4F	Minimal to severe decreased hemosiderin pigment in the spleen (minimal: 2M: 2/10, 3M: 2/10, 3F: 2/10, mild: 3M: 6/10, 3F: 6/10, 4F: 1/10, moderate: 3M: 2/10, 4M: 8/10, 3F: 1/10, 4F: 8/10, severe: 4M: 2/10, 4F: 3/10)
4M	Minimal epithelia cyst in the nonglandular stomach (4M: 1/10)
1M, 4M	Minimal lymphocytic infiltration in the testis (1M: 1/10, 4M: 1/10)
4M	Minimal degeneration of the seminiferous tubules in the testis (4M: 1/10)
3M	Moderate immaturity of the testis (3M: 1/10)
4M	Minimal to mild lymphocytic infiltration in the urinary bladder (4M: 2/10)

Groups	Findings
1M, 3M, 4M	Minimal and moderate epithelia hyperplasia of the urinary bladder (minimal: 1M: 1/10, moderate: 3M: 1/10, 4M: 1/10)
1M, 3M, 4M	Moderate chronic active inflammation of the urinary bladder (1M: 1/10, 3M: 1/10, 4M: 1/10)

Microscopic findings at the terminal sacrifice, severity of the finding as well as frequency (number of animals with the finding/total number of animals in the group) are stated in brackets

Recovery necropsy:

Groups	Findings
1M	Minimal lymphocytic infiltration in the adrenal gland (1M: 1/5)
4M, 4F	Minimal hyperplasia in the bone marrow (femur) (4M, 1/5, 4F: 2/5)
4M, 4F	Minimal hyperplasia in the bone marrow (sternum) (4M, 2/5, 4F: 2/5)
1F, 4F	Minimal lymphocytic infiltration in the Haderian gland (1F: 2/5, 4F: 1/5)
1M, 4M, 1F, 4F	Minimal cardiomyopathy (1M: 5/5, 4M: 5/5, 1F: 4/5, 4F: 4/5)
1M, 4M, 4F	Minimal and moderate chronic active inflammation at the left injection site (minimal: 1M: 1/5, 4M: 3/5, 4F: 1/5, moderate: 4F: 1/5)
4F	Minimal and moderate epidermal exudate at the right injection site (4F: 2/5)
4F	Minimal and mild epithelial hyperplasia at the right injection site (4F: 1/5)
1M, 4M, 4F	Minimal and mild chronic active inflammation at the right injection site (minimal: 1M: 1/5, 4M: 1/5, 4F: 4/5, mild: 4M: 2/5)
4F	Minimal cyst in the kidney (4F: 1/5)
4M, 4F	Minimal to moderate tubular degeneration in the kidney (minimal: 4M: 1/5, 4F: 3/5, mild: 4M: 2/5, 4F: 2/5, moderate: 4M: 1/5)
4M	Moderate hydronephrosis (4M: 1/5)
4M, 4F	Chronic interstitial inflammation in the kidney (minimal: 4M: 1/5, 4F: 3/5, mild: 4M: 2/5, 4F: 2/5, moderate: 4M: 1/5)
1M, 4M, 1F	Minimal to mild nephropathy (1M: 5/5, 4M: 1/5, 1F: 3/5)
1F	Minimal pelvic mineralization in the kidney (1F: 1/5)
1F, 4F	Minimal tubular mineralization in the kidney (1F: 2/5, 4F: 2/5)
4M, 4F	Minimal green pigment in the kidney (4M: 5/5, 4F: 5/5)
4M	Moderate chronic pyelitis (4M: 1/5)
4M	Minimal lymphocytic infiltration in the lacrimal gland (4M: 1/5)
1M, 4M, 1F, 4F	Minimal to moderate chronic inflammation in the liver (minimal: 1M: 4/5, 4M: 2/5, 1F: 5/5, 4F: 1/5, mild: 1M: 1/5, 4M: 1/5, 4F: 4/5, moderate: 4M: 2/5)
4M	Minimal mixed altered foci in the liver (4M: 1/5)
4M	Mild congestion in the liver (4M: 1/5)
4M	Minimal hyperplasia in the bile duct (4M: 1/5)

Groups	Findings
4M, 4F	Minimal to moderate hyperplasia of the Kupffer cells (minimal: 4M: 2/5, 4F: 2/5, mild: 4M: 1/5, 4F: 2/5, moderate: 4M: 2/5)
4M, 4F	Mild green pigment in the liver (4M: 2/5, 4F: 5/5)
4M, 4F	Minimal to mild green pigment in the liver (minimal: 4M: 3/5, 4F: 5/5, mild: 4M: 2/5)
1M, 4M, 1F, 4F	Minimal histiocytosis in the lung (1M: 5/5, 4M: 5/5, 1F: 5/5, 4F: 5/5)
4M	Mild lymphoid hyperplasia in the iliac lymph node, (4M: 1/5)
1M, 4M, 4F	Minimal to moderate hyperplasia of the mandibular lymph node (minimal: 4M: 1/5, 4F: 2/5, mild: 1M: 1/5, 4M: 2/5, 4F: 3/5, moderate: 4M: 2/5)
1M, 4M, 4F	Minimal to moderate medullary plasmacytosis in the mandibular lymph node (minimal: 1M: 2/5, 4M: 1/5, 4F: 2/5, mild: 1M: 2/5, 4M: 3/5, 4F: 3/5, moderate: 4M: 2/5)
4M, 1F, 4F	Minimal to moderate lymphoid hyperplasia in the mesenteric lymph node, (minimal: 4M: 4/5, 1F: 2/5, 4F: 3/5, mild: 4M: 1/5, moderate: 4M: 1/5)
4M	Minimal mineralization in the ovary (4M: 1/5)
1M	Minimal lymphocytic infiltration the pancreas (1M: 1/5)
1F	Minimal chronic inflammation in the pancreas (1F: 1/5)
1M	Mild chronic active inflammation in the prostate (1M: 1/5)
1M	Minimal lymphocytic infiltration in the prostate (1M: 1/5)
4F	Minimal lymphocytic infiltration in the skeletal muscle (biceps femoris) (4F: 1/5)
4M, 4F	Minimal increased extramedullary hematopoiesis in the spleen (4M: 3/5, 4F: 2/5)
4M, 4F	Minimal to moderate hyperplasia in the spleen (minimal: 4F: 1/5, mild: 4F: 3/5, moderate: 4M: 5/5, 4F: 1/5)
4M, 4F	Minimal to mild decreased hemosiderin pigment in the spleen (minimal: 4M: 3/5, 4F: 5/5, mild: 4M: 2/5)
4M, 1F	Minimal lymphocytic infiltration in the urinary bladder (4M: 2/5, 1F: 1/5)
4M	Moderate epithelial hyperplasia in the urinary bladder (4M: 1/5)
4M	Minimal chronic inflammation in the urinary bladder (4M: 1/5)

Microscopic findings at the recovery sacrifice, severity of the finding as well as frequency (number of animals with the finding/total number of animals in the group) are stated in brackets

Treatment-related findings were found in both sexes at the terminal necropsy in the bone marrow (femur and sternum), injection sites (left and right), kidneys, liver, lymph nodes (especially mandibular and mesenteric), and spleen.

The bone marrow of the sternum and the femur of animals receiving the 1018 ISS adjuvant showed minimal to severe hyperplasia. The incidence as well as the severity was dose dependent. The erythroid cell population and megacaryocytes seemed to be the primary cause of the bone marrow hyperplasia, no increase in the myeloid cell population

was detected. After the recovery phase only animals receiving the highest dose of 1018 ISS still showed minimal hyperplasia indicating reversibility.

Male animals of all groups receiving the adjuvant as well as female animals receiving 2.5 and 12.5 mg/kg/week showed minimal to moderate tubular degeneration as well as minimal to moderate chronic interstitial inflammation in the kidney and/or green pigmentation in a dose dependent manner after the treatment phase. Pigmentation was not anymore seen after the recovery phase. However, male and female animals receiving 12.5 mg/kg/week 1018 ISS adjuvant still showed minimal to moderate tubular degeneration and minimal to moderate chronic interstitial inflammation in the kidney. The incidence and severity of changes were similar to those noted at the terminal sacrifice, indicating that those changes were not reversed after the recovery phase. Additionally, control animals showed a low degree of chronic progressive nephropathy, animals receiving the adjuvant demonstrated the same pathological changes at a similar incidence rate. This finding is considered to be a normal background finding in rats of the age and strain used in this study and is characterized microscopically by increasing numbers of tubules with degenerative and inflammatory changes, which increase in severity with progressing age. The treatment-related renal effects seem to not accelerate the progression of this chronic process.

Male and female animals receiving 2.5 and 12.5 mg/kg/week of the 1018 ISS adjuvant showed treatment-related congestion in the liver (minimal to moderate) as well as dose-dependent liver atrophy (minimal to moderate) and Kupffer cell hyperplasia (mild to severe). The cytoplasm of these Kupffer cells was markedly distended (hypertrophic) such that the hepatic sinuses in the central vein region were nearly concealed. The affected Kupffer cells at 12.5 mg/kg/week extended throughout the lobule, with the most extensive involvement in the central vein region. In the most severely involved livers, the Kupffer cells in the central vein region displaced the hepatocytes and, to a significant extent, resulted in hepatic atrophy in cells extending into the mid-zone of the hepatic lobules. Further animals of all groups showed a chronic inflammation (minimal to severe) of the liver, the severity and frequency of this observation was dose dependent. The chronic inflammation in the liver was primarily characterized as a mixed mononuclear cell infiltrate in the periportal region, the incidence of chronic inflammation was clearly increased in both sexes at 2.5 and 12.5 mg/kg/week, corresponding to the increased Kupffer cell activity. After the recovery phase animals receiving high dose 1018 ISS still showed minimal to moderate chronic inflammation in the liver and hyperplasia of the Kupffer cells. However, a considerable recovery in the severity of these findings was apparent. Kupffer cell hyperplasia was markedly decreased and hepatocellular regeneration occurred.

Animals receiving 2.5 and 12.5 mg/kg/week of the 1018 ISS adjuvant showed increased extramedullary hematopoiesis, lymphoid hyperplasia, and decreased hemosiderin pigment in the spleen, these findings were dose-dependent. The increased extramedullary hematopoiesis was primarily due to the erythroid cellular elements and numerous megakaryocytes, these changes were similar to those seen in the bone marrow sections. However, a significant degree of myeloid hyperplasia was not observed in the spleen. These changes in the spleen and bone marrow correlated with anemia and

thrombocytopenia. It seems that erythropoiesis was occurring at such a rapid rate that it depleted the splenic reserves of iron. After the recovery phase extramedullary erythropoiesis and megakaryocytosis were observed at a lesser degree and a (b) (4) indicated a substantial increase in the amount of iron stainable material.

Further, lymphoid hyperplasia and medullary plasmacytosis was seen in the mandibular and mesenteric lymph nodes in all animals, but with an increased severity and frequency in animals receiving 2.5 or 12.5 mg/kg/week of the 1018 ISS adjuvant. After the recovery phase a notable improvement in the severity of both lymphoid hyperplasia and medullary plasmacytosis was observed.

Local toxicity:

Treatment-related changes at the injection sites were noted in a dose-dependent manner in all treated groups of both sexes after the terminal necropsy. Predominantly a minimal to severe dose-dependent chronic active inflammation was observed at the injection site. In some animals the inflammation extended in the epidermis with an exudate on the surface and epithelial hyperplasia. Single animals showed an ulcer at the injection site. After the recovery phase a chronic active inflammation was still seen at the injection site, but with a considerable recovery in severity.

Test article related effects

- Reduced body weight gain and food consumption
- Dose related decrease in platelet count, erythrocyte count, hemoglobin, HKT, modest increase in APTT
- Minimal to severe hyperplasia in bone marrow, probably reflecting compensatory response to peripheral cytopenia
- Congestion, atrophy and chronic inflammation in the liver
- Kupffer cell hyperplasia in the liver
- Tubular degeneration, pigmentation and chronic interstitial inflammation in the kidney
- Lymphoid hyperplasia and medullary plasmacytosis in the mandibular and mesenteric lymph nodes
- Increased extramedullary hematopoiesis, lymphoid hyperplasia, and decreased hemosiderin pigment spleen

Assessment:

The study design did not include some specific endpoints for the evaluation of immunostimulatory agents, such as body temperature, a specific method of injection site assessment, and evaluation of acute phase reactants; body weight was only determined weekly. The toxicology study was performed at many fold higher doses of 1018 ISS on a mass basis compared to that intended for clinical use and exceeded the number of doses administered. The animals received either 0.5, 1.5, 12.5 mg/kg/week of 1018 ISS adjuvant or PBS weekly for 8 weeks. On mg/kg dose, the proposed clinical dose of 0.05 mg/kg is approximately 10, 50 or 250-fold lower than that used in the toxicity study. Most of the observed changes in this toxicology study fall into previously described oligonucleotides induced class effects.

Polyanionic effect:

Oligonucleotides have been described to interact with cationic sites on various blood proteins due to their polyanionic structure resulting in the inhibition of the coagulation cascade and the activation of the alternative complement pathway. The inhibition of the coagulation cascade through the administered 1018 ISS adjuvant was reflected in an increase in the APTT in animals receiving 2.5 or 12.5 mg/kg/dose 1018 ISS. Further these animals showed a dose-dependent decrease in erythrocytic parameters and platelet counts - an observation which has been described for various oligonucleotides. Histopathological changes of increased extramedullary hematopoiesis, lymphoid hyperplasia, and decreased hemosiderin pigment in the spleen as well as hyperplasia in the bone marrow reflected a compensatory response to the peripheral anemia. All these changes were resolved after the recovery phase.

Changes in erythrocytic parameters, platelet counts and APTT times were resolved after the recovery phase; the increase in total leukocytes, lymphocytes, neutrophils and monocytes was still seen after the recovery phase.

Accumulation in the target organs:

Oligonucleotides are quickly absorbed by the tissues and cleared from the blood circulation, main target organs include the kidney, liver, spleen and lymph nodes. Absorbed oligonucleotides can be seen as pigments in these organs. Rats receiving 2.5 or 12.5 mg/kg/week of the 1018 ISS adjuvant showed pigmentation of the liver and kidney reflecting the accumulation of the adjuvant in its target organs. The liver further showed treatment-related congestion (minimal to moderate) as well as dose-dependent atrophy (minimal to moderate), chronic inflammation (minimal to severe) and Kupffer cell hyperplasia (mild to severe). Although animals receiving 12.5 mg/kg/week of the 1018 ISS adjuvant still showed chronic inflammation in the liver and hyperplasia of the Kupffer cells after the recovery phase, a considerable recovery in the severity of these

findings was apparent. These changes in the liver were also associated with an increase in absolute organ weight as well as a slight dose-dependent increase in aspartate aminotransferase, alanine aminotransferase and sorbitol dehydrogenase after the treatment phase.

The accumulation of the 1018 ISS adjuvant in the kidneys lead in male animals of all groups as well as female animals receiving 2.5 and 12.5 mg/kg/week to minimal to moderate tubular degeneration and minimal to moderate chronic interstitial inflammation in a dose dependent manner. The incidence and severity of these changes were similar after the recovery and terminal sacrifice, indicating that those changes were not reversible. These histopathological changes were also reflected in an increase of the urea nitrogen (BUN) after the treatment phase in animals receiving 12.5 mg/kg/week 1018 ISS adjuvant with resolution following the recovery period. These findings are of concern, especially since no reversibility has been shown for the chronic interstitial inflammation and the tubular degeneration. However, the frequency and the severity of these changes occurred in a dose dependent manner. At the lowest dose given to the rats, 3 out of 10 or 4 out of 10 male animals showed only minimal tubular degeneration or interstitial inflammation, respectively. Even the lowest given dose to the rats of 0.5 mg/kg/week was 10 times higher than the proposed human dose of 0.05 mg/kg/week. Further the human dose was given weekly for 8 weeks, while the anticipated human dose will be given two times with an interval of two months in-between. Rats are known to have a relatively long tubular system compared to primates and might be more prone to tubular toxicities than primates. Most importantly, in the submitted non-human primate study (study 0-157) doses up to 12.5 mg/kg/week of the 1018 ISS adjuvant were given and no adverse findings in the kidney were observed. Non-human primates show comparable class effects for this adjuvant and are the most relevant animal model for this adjuvant.

Immune stimulation:

Inflammatory reaction at the injection site, lymphoid hyperplasia in the spleen, mandibular and mesenteric lymph nodes are results of the immune stimulatory class effect of the 1018 ISS adjuvant and show significant recovery after the recovery phase.

Further, an acute phase response with activation and hyperplasia of the Kupffer cells and secondary effects on hepatocytes is seen in animals receiving 2.5 or 12.5 mg/kg/week of the 1018 adjuvant. This finding is a described class effect of oligonucleotides.

Study no. 00-141: 5-Week Toxicity Study of Immunostimulatory Phosphorothioate Oligonucleotides with a 3-Week Recovery in Mice Given Intramuscular Injection every other Week

Performing laboratory: (b) (4)

Study initiation date: 07/14/2001

Final Report date: 08/10/2000

Test article batch/lot: 1018 ISS a stock solution of 1 mg/mL oligonucleotide in PBS: Dynavax Lot No (b) (4), Batch (b) (4) PBS: Lot No. (b) (4)

Animal species and strain: (b) (4) mice

Breeder/supplier: (b) (4)

Number of animal per group and sex: Terminal sacrifice: 10; recovery sacrifice: 5

Age: 11 to 12 weeks

Body weight range: 15.5 to 25.2 g

Route and site of administration: Intramuscular, right thigh muscle

Volume of injection: 0.05 mL

Frequency of administration and study duration: 3 administrations, 2 weeks apart (day 1, 15, 29)

Dose: 50 µg 1018 ISS

Stability: The sponsor submitted a certificate of analysis; the test agent was within the specification. Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study.

Means of administration: Syringe and needle

Report status: Final

Experimental design:

Group	Treatment	Number of Animals (#/sex/group)	
		Treatment phase	Recovery phase
1	PBS	10	5
2	50 µg 1018 ISS	10	5

Randomization procedure: Using the stratified randomization scheme animals were assigned to groups with similar group mean body weights.

Statistical analysis plan:

Group means and standard deviations were calculated for all numerical data. All statistical comparisons were performed at the 0.05 level of significance using SAS version (b) (4) or higher. Continuous normal data, such as body weight, organ weight, organ weight ratios, and clinical pathology parameters were analyzed. For body weight, change per day from the pretreatment body weight to the weekly body weight for each termination week was analyzed using a three-way Analysis of Variance (ANOVA) with

dose, sex, and time as factors. For organ weight, organ weight ratios, and clinical pathology data, termination days were analyzed separately using a two-way ANOVA with dose and sex as factors.

Parameters	Frequency of Testing
Cageside observation ⁵	Daily
Body weight	Weekly
Food consumption	Weekly
Body temperature	NC
Ophthalmologic exam	NC
Clinical chemistry*	6 and 56 days after final dose
Hematology*	6 and 56 days after final dose
Coagulation*	6 and 56 days after final dose
Immunological response	NC
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	NC
Necropsy	6 and 56 days after final dose
Tissues for histopathology	6 and 56 days after final dose

*(cardiac puncture), 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 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. (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	!*	
Aorta	!	
Bone (femur)	!	
Bone marrow (femur)	!	
Brain (cerebrum, cerebellum, medulla/pons, and olfactory bulb)	!*	
Cecum	!	
Cervix	!	
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		!

⁵ Cageside observations include mortality, morbidity, general health and signs of toxicity.

Organ/Tissue	Collected	Not collected
Gall bladder	!	
Gross lesions (if any)	!	
Heart	!*	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!*	
Lacrimal glands		!
Larynx		!
Liver	!*	
Lung (main-stem; bronchi)	!	
Lymph nodes (mandibular)		!
Lymph nodes (mesenteric)	!	
Mammary glands	!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		!
Ovaries	!*	
Pancreas	!	
Peyer's patch (if applicable)		!
Pituitary gland	!	
Prostate	!	
Rectum	!	
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Seminal Vesicles	!	
Skeletal muscle (thigh)	!	
Skin	!	
Spinal cord (thoracic)	!	
Spleen	!*	
Stomach (squamous and glandular)	!	
Testes	!*	
Thymus	!*	
Thyroid (w/ parathyroid glands)	!	
Tongue	!	
Trachea	!	
Ureters		!

Organ/Tissue	Collected	Not collected
Uterus (w/ cervix)	!	
Urinary bladder	!	
Vagina	!	

Table of Histology: Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Results:

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

CLINICAL MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE, if ≥ 1.5 or ≤ 0.7)	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 Sorbitol dehydrogenase Total bile acids
B) HEPATOBILIARY	Alkaline phosphatase (ALP): SD35: F \downarrow 0.60 G2 (96.6 IU/L)	Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		Fibrinogen: ND
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Triglycerides: SD35: F \downarrow 0.50 G2 (95.6 mg/dL) Creatine kinase: SD35: F \downarrow 0.38 G2 (116.6 IU/L)	Albumin Globulin Total cholesterol Cholinesterase: ND Total protein

Table of Clinical Chemistry Results. ND = not determined

Female animals treated with 1018 ISS showed a slight reversible decrease in alkaline phosphatase, triglycerides and creatine kinase after the terminal sacrifice. The values were within the normal values reported for laboratory mice; no difference were observed at the end of the recovery phase.

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 ≥ 1.5 or ≤ 0.7)	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	Monocyte count: SD35: F \uparrow 2.23 G2 (0.76/nL) SD35: M \uparrow 1.80 G2 (0.18/nL) Total leukocytes (WBC): SD56: M \uparrow 1.86 G2 (6.47/nL) Lymphocyte count: SD56: M \uparrow 1.94 G2 (4.53/nL)	Basophils, eosinophils count Neutrophil count Large unstained cells (LUC)
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen: ND
OTHERS		Bone marrow cytology: ND

Table of Hematology Results. ND = not determined

The administration of 1018 ISS lead to a slight increase in monocyte counts at day 35 (6 days after the last administration) as well as to a slight increase in total leukocytes and lymphocyte counts in male animals after the recovery phase. This increase reflects the activation of the immune system by the test agent.

Systemic toxicity:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight or food consumption was seen.

The mean absolute weight of the spleen in female mice treated with 1018 ISS was significantly increased to approximately 340% of the control group mean organ weight after the treatment phase (SD35). Male animals showed an increase of 80% in the mean absolute weight of the spleen in mice treated with 1018 ISS compared to control mice.

This effect was mostly reversible. On day 56 male animals did not show an increase in the mean absolute weight of the spleen, while the mean spleen weight of female animals given ISS was still 140% of the control group. Further, an increase of 32% in the absolute mean liver weight of 1018 ISS treated females was seen after the treatment phase.

This observation correlated with the histopathologically observed splenomegaly in female mice and extramedullary hematopoiesis which was seen in male and female mice with greater severity in female animals.

After the treatment phase, male and female animals receiving 1018 ISS showed a minimal to moderate extramedullary hematopoiesis in the liver (minimal: male: 8/10, mild: male: 1/10, female: 10/10) and the spleen (minimal: male: 4/10, mild: male: 4/10, female: 9/10, moderate: female: 1/10) with a higher severity in female animals. After the recovery phase, only female animals (4/5) showed a minimal extramedullary hematopoiesis in the liver, but not in the spleen. In gross pathology, a minimal to mild splenomegaly was noted for 1018 ISS treated female animals after the treatment phase.

These histopathology findings were associated with a mild, but statistically significant reduction in RBC (10%) HGB (8%) and HCT (15%) in female animals treated with 1018 ISS compared to animals of the control group after the treatment phase. Male animals treated with 1018 ISS only showed a 6% reduction in HGB compared to control animals. No differences were noted after the recovery phase.

Organ Weight:

SEX	MALES				FEMALES			
	1 D35	2 D35	1 D56	2 D56	1 D35	2 D35	1 D56	2 D56
GROUPS								
NUMBER OF ANIMALS								
BODY WEIGHT (terminal)	26.72	27.12	27.96	27.58	20.58	20.64	21.56	22.08
BRAIN	0.43875	0.43282	0.43670	0.41458	0.43057	0.42532	0.44610	0.43532
ADRENALS	0.00779	0.00724	0.00820	0.00630	0.00959	0.01130	0.01100	0.01104
HEART	0.14134	0.14365	0.14094	0.25334	0.11320	0.10936	0.10828	0.11136
KIDNEYS	0.49601	0.52737	0.51670	0.5373	0.29887	0.32445	0.31308	0.34142
LIVER	1.49633	1.56330	1.38448	1.41693	1.113951	1.47627*	1.06324	1.16794
SPLEEN	0.10278	0.18708*	0.10256	0.10774	0.09417	0.32184*	0.08986	0.12604
TESTES	0.20125	0.20238	0.20068	0.18718				
THYMUS	0.04734	0.04500	0.03664	0.04914	0.05247	0.05254	0.04478	0.04490
OVARIES					0.01685	0.02102	0.02034	0.02158

Table of organ weight. Absolute weights are expressed as mean (grams). *different from controls at $P \leq 0.05$.

The mean absolute weight of the spleen in female mice treated with 1018 ISS was significantly increased to approximately 340% of the control group mean after the treatment phase (SD35). Male animals showed an increase of 80% in the mean absolute

weight of the spleen in mice treated with 1018 ISS compared to control mice. This effect was mostly reversible; on day 56 male animals did not show an increase in the mean absolute weight of the spleen, while the mean spleen weight of female animals given 1018 ISS was still 140% of the control group. Further, an increase of 32% in the absolute mean liver weight of 1018 ISS treated females was seen after the treatment phase.

Gross Pathology:

Day 35

Group	Findings
1F, 2	Minimal white discoloration of the epicardium (1F: 1/10, 2F: 2/10)
2F	Minimal to mild increased size of the spleen (2F: minimal: 9/10, mild: 1/10)
2M	Green calculus 1mm attached to liver capsule (2M: 1/10)

Gross pathology findings at the terminal sacrifice, severity of the finding as well as frequency (number of animals with the finding/total number of animals in the group) are stated in brackets

Day 56

Group	Findings
2F, 2M	Minimal white discoloration of the heart (2M: 1/10, 2F: 2/10)
2M	Liver lobe (quadrate or left medial) with minimal yellow linear streaks (2M: 2/10)

Gross pathology findings at the recovery sacrifice, frequency of the finding (number of animals with the finding/total number of animals in the group) is stated in brackets

Microscopic finding:

Day 35

Groups	Findings
2M, 2F	Extramedullary hematopoiesis in the liver (minimal: 2M: 8/10, mild: 2M: 1/10, 2F: 10/10)
2M, 2F	Enhanced extramedullary hematopoiesis in the spleen (minimal: 2M: 4/10, mild: 2M: 4/10, 2F: 9/10, moderate: 2F: 1/10)
1M	Hepatocellular necrosis (1M: 1/10)
1M, 1F	Pleocellular infiltrate in the liver parenchyma (1M: 1/10, 1F: 1/10)
2M, 1F, 2F	Mineralization of the epicardium (2M: 1/10, 1F: 1/10, 2F: 3/10)
2M, 1F	Perivascular mononuclear cell infiltrate in the lung (2M: 1/10, 1F: 1/10)
1M, 1F, 2F	Mononuclear cell infiltrate in the pleura (1M: 2/10, 1F: 1/10, 2F: 1/10)
1M	Thyroid cyst (1M: 1/10)

Groups	Findings
2F	Mononuclear cell infiltrate in the interstitium of the parathyroid (2F: 1/10)
2M	Mononuclear cell infiltration at the perineurium of the sciatic nerve (2M: 1/10)
2M, 2F	Fibroplasia of the subcutis (2M: 2/10, 2F: 3/10)
2M, 2F	Mononuclear cell infiltrate of the subcutis (2M: 2/10, 2F: 3/10)
2M, 2F	Mononuclear cell infiltrate at the skeletal muscle (2M: 1/10, 2F: 3/10)
2F	Fibroplasia at the skeletal muscle (2F: 3/10)
1M	Mononuclear cell infiltrate at the skeletal muscle of the tongue (1M: 1/10)
1F	Pleocellular infiltration in the endometrium (1F: 1/10)
1F, 2F	Pleocellular infiltration of the cervical submucosa (1F: 4/10, 2F: 5/9)
1F	Pleocellular infiltration of the vaginal submucosa (1F: 1/10)
1F	Congestion of the anal glands (1F: 2/10)
1M, 2M, 1F, 2F	Regeneration of the skeletal muscle fibers at the injection site (1M: 3/10, 2M: 1/10, 1F: 2/10, 2F: 6/10)
2M, 1F	Pleocellular infiltration of the skeletal muscle at the injection site (2M: 3/10, 1F: 2/10)
1M, 2M, 2F	Mineralization of the skeletal muscle fibers at the injection site (1M: 2/10, 2M: 8/10, 2F: 6/10)
1M	Hemorrhage at the skeletal muscle (1M: 1/10)
2M, 1F	Degeneration of the skeletal muscle fibres at the injection site (1M: 1/10, 2F: 5/10)
1M	Infiltration of multinucleated giant cell in the muscle (1M: 1/10)
2M, 2F	Fibroplasia of the interstitium at the injection site (minimal: 2M: 2/10; mild: 2M: 5/10, 2F: 5/10; moderate: 2F: 5/10)
2M, 2F	Mononuclear cell infiltrate at skeletal muscle at the injection site (minimal: 2M: 4/10, 2F: 3/10; mild: 2M: 3/10, 2F: 7/10)

Microscopic findings at the terminal sacrifice, severity of the finding as well as frequency (number of animals with the finding/total number of animals in the group) are stated in brackets

Day 56

Groups	Findings
2F	Extramedullary hematopoiesis in the liver (minimal: 2F: 4/5)
2M, 2F	Fibroplasia of the interstitium at the injection site (minimal: 2M: 1/5, 2F: 1/5)
2M, 2F	Mononuclear cell infiltrate at skeletal muscle at the injection site (minimal: 2M: 4/5, 2F: 4/5)
1M, 2M	Hepatocellular necrosis (1M: 2/5, 2M: 1/5)
1M, 2M	Pleocellular infiltrate in the liver parenchyma (1M: 2/5, 2M: 1/5)
1M, 2M, 2F	Mineralization of the epicardium (1M: 1/5, 2M: 1/5, 2F: 3/5)
1M, 1F, 2F	Mononuclear cell infiltrate in the pleura (1M: 1/5, 1F: 2/5, 2F: 1/5)
2F	Foreign body (hair embolus) in the brain (2F: 1/5)
1M	Mononuclear cell infiltrate in the parathyroid (1M: 1/5)

Groups	Findings
2F	Mononuclear cell infiltrate at the skeletal muscle (2F: 1/5)
2F	Fibroplasia at the skeletal muscle (2F: 2/5)
1F, 2F	Pleocellular infiltration in the endometrium (1F: 1/5, 2F: 2/5)
1F, 2F	Pleocellular infiltration of the cervical submucosa (1F: 5/5, 2F: 4/5)
2F	Pleocellular infiltration of the cervical mucosa (2F: 1/5)
1F, 2F	Pleocellular infiltration of the vaginal submucosa (1F: 3/5, 2F: 2/5)
2F	Pleocellular infiltration of the vaginal mucosa (2F: 1/5)
1F	Congestion of the anal glands (1F: 1/5)
2F	Regeneration of the skeletal muscle fibers at the injection site (2F: 1/5)
2F	Mineralization of the skeletal muscle fibers at the injection site (2F: 2/5)
2M, 2F	Mononuclear cell infiltration of the skeletal muscle at the injection site (2M: 3/5, 3F: 4/5)
2F	Fibroplasia in the interstitium at the injection site (2F: 1/5)

Microscopic findings at the recovery sacrifice, severity of the finding as well as frequency (number of animals with the finding/total number of animals in the group) are stated in brackets

After the treatment phase, male and female animals receiving 1018 ISS showed a minimal to moderate extramedullary hematopoiesis in the liver (minimal: male: 8/10, mild: male: 1/10, female: 10/10) and spleen (minimal: male: 4/10, mild: male: 4/10, female: 9/10, moderate: female: 1/10) with a higher severity in female animals. After the recovery phase, only female animals (4/5) showed a minimal extramedullary hematopoieses in the liver, but not in the spleen. In gross pathology a minimal to mild splenomegaly was noted in 1018 ISS treated female animals after the treatment phase.

These histopathology findings were associated with a mild reduction in RBC (10%), HGB (8%) and HCT (15%) in female animals treated with 1018 ISS compared to animals of the control group after the treatment phase. Male animals treated with 1018 ISS only showed a 6% reduction in HGB compared to control animals. No differences were noted after the recovery phase.

Local toxicity:

At the injection site, regeneration of the skeletal muscle fibers as well as regeneration and mineralization of the skeletal muscle fibers was observed in all groups and therefore induced through the injection procedure. Five out of ten 1018 ISS treated female animals showed degeneration of the skeletal muscle at the injection site while only one animal in the male control animals and no animal in the female control group showed muscle degeneration. This finding was fully reversible and was not further observed after the recovery phase. Fibroplasia and mononuclear cell infiltrate were only observed in animals treated with the 1018 ISS adjuvant and therefore clearly test article related. After the treatment phase, 7 out of 10 males and all 10 females receiving 1018 ISS showed a proliferation of fibroblasts between the muscle bundles, the subcutis and the dermis accompanied by infiltrating mononuclear inflammatory cells, female animals showed a

higher severity than male animals. After the recovery phase only two female animals still showed fibroplasia at the interstitium of the injection site.

Test article related effects
<ul style="list-style-type: none">• Minimal to moderate extramedullary hematopoiesis in the liver and spleen• Minimal to mild splenomegaly• Reduction in RBC, HGB, and HCT• Fibroplasia and mononuclear cell infiltrate at the injection site

Assessment:

The study design did not include all specific endpoints for the evaluation of immunostimulatory agents, like body temperature, a specific method of injection site assessment and evaluation of acute phase reactants; body weight was only determined weekly. No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight or food consumption was seen. The observed adverse effects are previously described class effects of the oligonucleotides.

Polyanionic effect:

After the treatment phase animals receiving 1018 ISS showed a reduction in erythrocyte parameters and a minimal to moderate compensatory extramedullary hematopoiesis in the liver and spleen with a higher severity in female animals. In gross pathology a splenomegaly was noted for 1018 ISS treated female animals. This finding was further reflected in a statistically significant increase in mean absolute spleen (male and females) and liver (females) weight. These effects were largely reversible. Oligonucleotides have been described to produce peripheral anemia and thrombocytopenia, these effects are more pronounced in rodents compared to primates (including humans) most likely due to a broader expression of TLR9 receptors in rodents.

Accumulation in target organs:

A statistically significant increase in spleen (male and females) and liver (females) weight was observed in animals receiving the 1018 ISS adjuvant. The mean absolute weight of the spleen in female mice treated with 1018 ISS was significantly increased to approximately 340% of the control group mean after the treatment phase (SD35). Male

animals showed an increase of 80% in the mean absolute weight of the spleen in mice treated with 1018 ISS compared to control mice. This effect was mostly reversible.

Immune stimulation:

Locally, minimal to moderate fibroplasia with a minimal to mild mononuclear cell infiltration was observed in animals treated with the 1018 ISS adjuvant which was mostly resolved after the recovery phase.

OVERALL ASSESSMENT:

A series of toxicity studies were conducted to investigate the safety of the 1018 ISS adjuvant in mice, rats, rabbits, baboons and cynomolgus monkeys. No genotoxicity was observed in the standard battery of tests composed of *in vitro* bacterial mutation, chromosome aberration and *in vivo* mouse erythrocyte micronucleus test. No adverse effects were identified in two safety-pharmacology studies with doses of 1018 ISS up to 1.6 mg/kg in rabbits and 25 mg in baboons.

Three repeated dose toxicity studies were performed at many fold higher doses of 1018 ISS on a mass basis compared to that intended for clinical use and exceeded the number of doses administered (b) (4) rats or cynomolgus monkey received either 0.5, 1.5, 12.5 mg/kg/week of 1018 ISS adjuvant or PBS weekly for 8 weeks. On a mg/kg dose, the proposed clinical dose of 0.05 mg/kg is approximately 10, 50 or 250-fold lower than that used in the toxicity studies. The study design did not include an evaluation of body temperature, a specific method of injection site assessment and an evaluation of coagulation factors or acute phase reactants; body weight was only determined weekly. In general, most of the findings fell within the previously described class effects for oligonucleotides with changes observed at the site of injection and various target organs such as the liver, kidney, lymph node and spleen. Adverse effects were most commonly found in the high dose group of 12.5 mg/kg given weekly for 8 week which is a many fold higher dose given than the one given in the clinical trial (0.05mg/kg, twice).

Oligonucleotides have a polyanionic structure which leads to the interaction of the cationic site on blood proteins. Administration of oligonucleotides to monkeys can lead to the inhibition of factor H an endogenous inhibitor of the complement cascade, this results in the activation of the alternative complement system, release of the anaphylatoxin C5a and an anaphylactoid-like response. The performed repeated toxicology study in non human primates showed that the administration of 1018 ISS adjuvant can lead to an activation of the alternative complement pathway observed as a modest increase in complement split product Bb, in many animals receiving 12.5 mg/kg/week 1018 ISS adjuvant. However, the elevation in Bb was not accompanied by any increase in C5a. Therefore the alternative pathway activation was not associated with accumulation of biologically active split products that would pose a risk for an anaphylactoid-like response. The inhibition of the coagulation cascade through the administered 1018 ISS adjuvant was reflected in a slight increase in APTT levels in male and female animals receiving 12.5 mg/kg/dose weekly for 8 weeks after the treatment phase which was resolved after the recovery phase and was not observed in participants of the clinical trials.

Oligonucleotides have also been shown to produce peripheral anemia and thrombocytopenia; these effects are more pronounced in rodents compared to primates (including humans) most likely due to a broader expression of TLR9 receptors in rodents.

In the submitted non-human primate study the leukocyte and neutrophil counts were modestly decreased in male and female animals receiving 12.5 mg/kg/week after the treatment phase but returned to baseline after the recovery phase. Also, mice and rats in the repeated dose study showed a dose-dependent decrease in erythrocytic parameters and platelet counts which were resolved after the recovery phase. Additionally, these animals exhibited increased extramedullary hematopoiesis, lymphoid hyperplasia, and decreased hemosiderin pigment in the spleen as well as hyperplasia in the bone marrow, these changes reflected compensatory response to the peripheral anemia typical for rodents. All these changes were resolved after the recovery phase.

After the initial distribution of the oligonucleotides, the liver, kidney, lymph nodes and spleen are primary target organs of oligonucleotide uptake. Kidneys have been described as having the highest oligonucleotide concentration with preferential uptake in the proximal tubular cells especially in monkeys and rats. This uptake can lead to dose-dependent degenerative changes in the proximal tubular epithelium as well as to regenerative changes. In the submitted repeated dose toxicity study in rats, this kidney-accumulation of the oligonucleotides was observed and accompanied by tubular degeneration and chronic interstitial inflammation in a dose dependent manner. The incidence and severity of these changes were similar after the recovery and terminal sacrifice, indicating that those changes were not reversed after the recovery phase. These histopathological changes were also reflected in an increase of the urea nitrogen (BUN) after the treatment phase in animals receiving 12.5 mg/kg/week 1018 ISS adjuvant with resolution following the recovery period. Rats are known to have a relatively long tubular system compared to primates and are more prone to tubular toxicities than primates. Most importantly, at the same mg/kg/week doses no renal toxicity findings were reported in the non-human primate study. The pharmacokinetics of oligonucleotides has been described as very similar between humans and monkeys. Similar doses on an mg/kg basis of an oligonucleotide given by the same route of administration induced nearly identical plasma levels in monkeys and humans. Clearance, volume of distribution, initial distribution half-lives and terminal elimination half-lives are very similar between monkeys and humans. Therefore, the toxicity profiles in monkeys compared to rodents are more likely predictive of those in humans. The non human primate is considered to be the most relevant animal model for prediction of toxicological adverse events of this adjuvant in healthy adult humans and was considered to be safe given at the proposed dose. However, if the target clinical population would include individuals with kidney damage an evaluation of the risk for adjuvant induced kidney damage in this specific population is indicated.

The liver is reported to accumulate the second highest oligonucleotide concentration and contain the highest amount of oligonucleotides because of its relative organ size. In the submitted studies, rodents and non-human primates showed a dose dependent accumulation of blue granular pigment in the liver and Kupffer cells, this pigment accumulation is suspected to be a deposit of the 1018 ISS oligonucleotide. Further, the liver showed treatment-related dose-dependent chronic inflammation and hypertrophy of liver and Kupffer cells. In some cases these liver changes were also associated with an

increase in absolute organ weight as well as a slight dose-dependent increase in liver enzymes. In all submitted studies, a considerable recovery in the severity of these findings was apparent or complete recovery was achieved after the recovery phase.

The immune stimulative effects of the adjuvant were reflected in an acute and chronic inflammation at the injection sites, lymphoid hyperplasia in the lymph nodes, especially the mandibular and mesenteric lymph nodes, as well as the spleen with splenomegaly. These changes were generally dose-dependant and completely or partially resolved after the recovery phase. Further, an acute phase response with activation and hyperplasia of the Kupffer cells and secondary effects on hepatocytes can also be accounted to the preciously described immune stimulatory effects of oligonucleotides.

CONCLUSIONS:

In the BLA (125428) adequate nonclinical toxicology data regarding the 1018 ISS adjuvant have been presented for the safety of 1018 ISS adjuvant as used in the HEPLISAV vaccine. No issues regarding non-clinical toxicology have been identified that preclude approval of the BLA in healthy adults. However, clinical population with renal disease may be subject to greater risk based on the nonclinical findings that should be evaluated in the light of clinical findings.