charles river

FINAL REPORT

Test Facility Study No. 5002231

A 1 Month (3 doses) Intramuscular Injection Vaccine Study of mRNA-1706 in Sprague-Dawley Rats With a 2-Week Recovery Period

SPONSOR:
Moderna Therapeutics, Inc.
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USA

TEST FACILITY:
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QUALITY ASSURANCE STATEMENT

Study Number: 5002231

This Study has been audited by Quality Assurance in accordance with the applicable Good Laboratory Practice regulations. Reports were submitted in accordance with SOPs as follows:

QA INSPECTION DATES

Date(s) of Audit	Phase(s) Audited	Dates Findings Submitted to: Study Director	Study Director Management
22-Mar-2017	Final Study Plan	23-Mar-2017	23-Mar-2017
28-Mar-2017	Study Plan Amendment 1	28-Mar-2017	28-Mar-2017
03-May-2017	Addition of Study Plan to Provantis	03-May-2017	03-May-2017
03-May-2017 - 04-May-2017	Dose Preparation	04-May-2017	04-May-2017
03-May-2017	Study Plan Amendment 2	03-May-2017	03-May-2017
04-May-2017	Dose Administration	04-May-2017	04-May-2017
04-May-2017	Body Temperature	04-May-2017	04-May-2017
01-Jun-2017	Necropsy	10-Aug-2017	10-Aug-2017
13-Jun-2017	Immunology	20-Jul-2017	20-Jul-2017
13-Jun-2017	Procedural Statement	20-Jul-2017	20-Jul-2017
20-Jun-2017	Study Plan Amendment 3	20-Jun-2017	20-Jun-2017
20-Jun-2017	Study Plan Amendment 4	20-Jun-2017	20-Jun-2017
27-Jun-2017	Study Plan Amendment 5	27-Jun-2017	27-Jun-2017
14-Jul-2017	Data Review - Bioanalysis & Immunology	17-Jul-2017	17-Jul-2017
14-Jul-2017	Final Phase Report - Immunology	17-Jul-2017	17-Jul-2017
02-Aug-2017 - 10-Aug-2017	Data Review - Animal Care	16-Aug-2017	16-Aug-2017
02-Aug-2017 - 10-Aug-2017	Draft Phase Report - Ophthalmology	16-Aug-2017	16-Aug-2017
02-Aug-2017 - 10-Aug-2017	Draft Report - Materials and Methods	16-Aug-2017	16-Aug-2017
02-Aug-2017 - 10-Aug-2017	Report Preparation	16-Aug-2017	16-Aug-2017
02-Aug-2017 - 10-Aug-2017	Data Review - Technical Operations	16-Aug-2017	16-Aug-2017
02-Aug-2017 - 10-Aug-2017	Data Review - Formulations	16-Aug-2017	16-Aug-2017
02-Aug-2017 - 10-Aug-2017	Data Review - Clinical Pathology	16-Aug-2017	16-Aug-2017
02-Aug-2017 - 10-Aug-2017	Data Review - Technical Operations	16-Aug-2017	16-Aug-2017
02-Aug-2017 - 10-Aug-2017	Data Review - Veterinary Services	16-Aug-2017	16-Aug-2017
02-Aug-2017 - 10-Aug-2017	Data Review - Shipping/Receiving	16-Aug-2017	16-Aug-2017
13-Aug-2017 15-Aug-2017	Data Review - Analytical Chemistry	16-Aug-2017	16-Aug-2017
13-Aug-2017 15-Aug-2017	Final Phase Report - Dose Formulation Analysis	16-Aug-2017	16-Aug-2017
28-Aug-2017	Data Review - Necropsy	29-Aug-2017	29-Aug-2017
28-Aug-2017 - 29-Aug-2017	Data Review - Histology	29-Aug-2017	29-Aug-2017

QUALITY ASSURANCE STATEMENT - Study Number: 5002231

QA INSPECTION DATES

Date(s) of Audit	Phase(s) Audited	Dates Findings Submitted to: Study Director	Study Director Management
28-Aug-2017	Data Review - Shipping/Receiving	29-Aug-2017	29-Aug-2017
29-Aug-2017	Report Preparation	29-Aug-2017	29-Aug-2017
29-Aug-2017	Draft Phase Report - Pathology	29-Aug-2017	29-Aug-2017
21-Sep-2017	Study Plan Amendment 6	21-Sep-2017	21-Sep-2017
25-Sep-2017	Study Plan Amendment 7	25-Sep-2017	25-Sep-2017
03-Nov-2017 - 06-Nov-2017	Final Report	07-Nov-2017	07-Nov-2017

In addition to the above-mentioned audits, process-based and/or routine facility inspections were also conducted during the course of this study. Inspection findings, if any, specific to this study were reported by Quality Assurance to the Study Director and Management and listed as a Phase Audit on this Quality Assurance Statement.

The Final Report has been reviewed to assure that it accurately describes the materials and methods, and that the reported results accurately reflect the raw data.

[redacted]
Quality Assurance Auditor

[redacted]
Date

COMPLIANCE STATEMENT

The study was performed in accordance with the OECD Principles of Good Laboratory Practice and as accepted by Regulatory Authorities throughout the European Union, United States of America (FDA), Japan (MHLW), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Any portion of this study conducted in the USA was performed in accordance with the U.S. Department of Health and Human Services, Food and Drug Administration. United States Code of Federal Regulations, Title 21, Part 58: Good Laboratory Practice for Nonclinical Laboratory Studies and as accepted by Regulatory Authorities throughout the European Union (OECD Principles of Good Laboratory Practice) and Japan (MHLW).

Exceptions from the above regulations are listed below.

- Characterization of the Test Item was performed by the Sponsor subcontractor according to established SOPs, controls, and approved test methodologies to ensure integrity and validity of the results generated; these analyses were not conducted in compliance with the GLP or GMP regulations.
- Analysis of cytokines and anti-therapeutic antibody were conducted using scientifically qualified methods and in accordance with all applicable analytical procedures.
- Pathology peer review

This study was conducted in accordance with the procedures described herein. All deviations authorized/acknowledged by the Study Director are documented in the Study Records. The report represents an accurate and complete record of the results obtained.

There were no deviations from the above regulations that affected the overall integrity of the study or the interpretation of the study results and conclusions.

[redacted] Date: [redacted]

Study Director [redacted]

1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Study Director

Test Facility Management

[redacted]

1.2. Individual Scientists (IS) at Test Facility

Ophthalmology

[redacted]
Consultant Ophthalmologist
Senneville, QC, Canada

Pathology

[redacted]
Charles River Laboratories Montreal ULC
Senneville, QC, Canada

Analytical Chemistry
(Concentration and Particle
Size Analysis)

[redacted]
Charles River Laboratories Montreal ULC
Senneville Site (CR MTL), QC, Canada

Immunology
(Purity Analysis)

[redacted]
Charles River Laboratories Montreal ULC
Senneville, QC, Canada

Biomarkers
(IL-1ß, IL-6, TNF-α,
IP-10, MIP-1-α, MCP-1)

[redacted]
Charles River Laboratories Montreal ULC
Senneville, QC, Canada

1.3. PIs at Sponsor or Sponsor-designated Test Site(s)

Anti-Drug
Antibody Analysis (ADA)

[redacted]
Integrated BioTherapeutics, Inc.
Rockville, MD, USA

2. SUMMARY

The objective of this study were to determine the potential toxicity of mRNA-1706, when given by intramuscular injection for 1 month (3 doses) to rats and to evaluate the potential reversibility of any findings following a 2-week recovery period.

The study design was as follows:

Text Table 1
Experimental Design

Group No.	Test Material	Dose Level (µg/dose)	Dose Volume (µL/dose)	Dose Concentration (mg/mL)	No. of Animals			
					Main Study		Recovery Study	
					Males	Females	Males	Females
1	Reference Item	0	200	0	10	10	5	5
2	mRNA-1706	10	200	0.05	10	10	-	-
3	mRNA-1706	50	200	0.25	10	10	-	-
4	mRNA-1706	100	200	0.5	10	10	5	5

The following parameters and endpoints were evaluated in this study: clinical observations consisting of twice daily examinations for mortality/moribundity and weekly detailed examinations; detailed examination of the injection sites; weekly body weights and food consumption measurements; ophthalmic examinations; body temperature; clinical pathology assessment (hematology, coagulation, clinical chemistry) at termination; cytokine analysis (IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α and MCP-1); Anti-Drug Antibody (ATA – antibody titer) analysis prior to dosing initiation and at termination; gross necropsy findings, organ weights, and histopathologic examinations.

On Day 30, following the last dose injection, all animals (with the exception of animal no.2001) were positive for antibodies against ZIKV lysate, showing a dose responsive increase. On Day 43, following the recovery period, animals dosed at 100 µg/dose showed higher response when compared to Day 30.

There were no mRNA-1706-related changes in food consumption and ophthalmology.

There were no unscheduled deaths during the course of this study.

The primary mRNA-1706-related findings were observed at the site of injection of animals given ≥ 10 µg/dose. A generally dose responsive increase in incidence and/or severity of swelling was noted at the injection site, following dose administration of both males and females. Extension of the injection site swelling to hindlimb and inguinal areas was also noted in animals given 100 µg/dose. In addition, skin redness at the injection site, was noted with a higher incidence throughout the dosing period for animals given ≥ 50 µg/dose. Macroscopically, the injection sites were characterized by firmness, swelling and occasionally by the presence of a clot in the thigh muscle used for the injection. These macroscopic findings were observed in males and females at all dose levels with no clear dose relationship and correlated histologically with inflammation and/or hemorrhage. In addition, enlargement of the draining lymph nodes (inguinal, popliteal and iliac) was observed in males and females at all dose levels with no evidence of dose relationship and correlated histologically with mixed cell infiltration. Clinical signs (i.e. swelling and redness of the skin) observed at the injection site and gross pathology findings as well as microscopic findings observed at the inguinal, iliac and popliteal lymph nodes

were no longer observed in recovery animals, indicating a complete to recovery for these changes and partial recovery for the findings at injection site.

mRNA-1706-related systemic changes associated with inflammation were also observed in animals given ≥ 10 µg/dose and included minimally increased cellularity of the myeloid lineage in the bone marrow. This change was likely a reactive response to the pronounced inflammation observed at the injection site. Other systemic findings included minimal to mild decreased lymphoid cellularity of the splenic periarteriolar lymphoid sheathand the paracortex of the mesenteric lymph node. In the liver, minimal to mild hypertrophy of the Kupffer cell was noted with occasional centrilobular degeneration characterized by the presence of mixed inflammatory cells in the sinusoid accompagnied by single cell necrosis or degeneration of hepatocytes. Clinical pathology changes suggestive of inflammation were also observed in males and/or females given mRNA-1706 at all doses (unless noted otherwise) and included: minimal to moderate increases in neutrophil, monocyte, eosinophil and large unstained cell counts with concomitant increases in white blood cell counts, minimal to moderate decreases in lymphocyte counts and platelet counts (females at ≥50 µg/dose), minimal increases in activated partial thromboplastin time and mild increases in fibrinogen, minimal increases in globulin, minimal decreases in albumin, with concomitant decreases in A/G ratio. Increase in body temperature postdose (≥ 50 µg/dose), along with elevations of cytokine levels IP-10, MIP-1α and MCP-1, were all suggestive of inflammation. All mRNA-1706-related systemic changes returned close to control values and, as such, were considered fully recovered.

Additional findings related to mRNA-1706 were observed in the adrenal glands of animals given ≥ 50 µg/dose and consisted of dose-related to minimal cortical hypertrophy and correlated with increased adrenal weights. This finding showed significance at 100 µg/dose. Single cell necrosis was also observed in the thymus. These findings were all fully recovered following the 2-week recovery period.

When compared to controls, following each dose, a tendency towards lower mean body weight gains was noted in males and females given ≥ 10 µg/dose; these changes sometimes reached statistical significance in both genders. From Day -1 to 28, the changes were down to 0.60X controls in males and 0.78X controls in females. The body weight changes were generally comparable or rebounded during the 2-week recovery period.

In conclusion, administration of mRNA-1706 by intramuscular injection for 1 month (3 doses) was clinically well tolerated (no mortality, no major decreases in body weight and no changes in food consumption or deleterious changes in hematology, coagulation or clinical chemistry parameters) in rats up to 100 µg/dose. Starting at 10 µg/dose, generally dose-dependent changes in clinical signs at the injection site, clinical pathology parameters and cytokines were consistent with an inflammatory response at the injection site. Dose-related target organ effects were limited to the injection site, the bone marrow, the inguinal, mesenteric, iliac and popliteal lymph nodes, adrenal gland, liver, spleen and thymus of animals given mRNA-1706. At the end of the 2-week recovery period, all changes were fully recovered.

3. INTRODUCTION

The objectives of this study were to determine the potential toxicity of mRNA-1706, when given by intramuscular injection for 1 month (3 doses) to rats and to evaluate the potential reversibility of any findings following a 2-week recovery period.

The design of this study is based on the study objectives, the overall product development strategy for the Test Item, and the following study design guidelines:

- OECD Guideline 407. *Repeated Dose 28-day Oral Toxicity Study in Rodents*.
- Committee for Medicinal Products for Human Use (CHMP). *Note for Guidance on Repeated Dose Toxicity*. CPMP/SWP/1042/99corr.
- ICH Harmonised Tripartite Guideline M3 (R2). *Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals*.
- Japanese Guidelines for Nonclinical Studies of Drugs Manual (1995). *Guidelines for Nonclinical Pharmacokinetic Studies*, *Guidelines for Toxicity Studies of Drugs (Chapter 3, Repeated Dose Toxicity Studies)*
- Appendix to Director General Notification, No. 12-Nousan-8147, 24 Nov 2000, Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries of Japan (JMAFF).

The Study Director signed the study plan on 15 Mar 2017, and dosing was initiated on 03 May 2017 (males) and 04 May 2017 (females). The in-life phase of the study was completed on 02 Jun 2017 (main animals) and 15 Jun 2017 (recovery animals); the last date of necropsy. The experimental start date was 16 Mar 2017, and the experimental completion date is 31 Oct 2017. The study plan, study plan amendments, and deviations are presented in Appendix 1.

4. MATERIALS AND METHODS

4.1. Test Item

Identification:	mRNA-1706
Batch (Lot) No.:	MTDP17036
Concentration:	2.1 mg/mL
Retest Date:	An end-of-use analysis of the bulk Test Item was performed to demonstrate the stability of the Test Item during the dosing period.
Physical Description:	White to off-white lipid nanoparticle dispersion
Storage Conditions:	Kept in a freezer set to maintain -20°C
Supplier:	Moderna Therapeutics, Inc.

4.2. Reference Item

Identification:	Phosphate-buffered Saline (PBS) pH 7.2
Supplier:	Life Technologies

Lot Number: 1854892

Expiration Date: Dec 2018

Physical Description: Liquid

Storage Conditions: Kept in a controlled temperature area set to maintain 21°C

4.3. Test and Reference Item Characterization

The Sponsor provided to the Test Facility documentation of the identity, strength, purity, composition, and stability for the Test Item. A Summary of Analysis was provided to the Test Facility and is presented in Appendix 2.

4.4. Analysis of Test Item

A sample (2 vials) of the Test Item was taken on the completion of the dosing period. Analysis of bulk Test Item for concentration, particle size and purity was performed.

The first vial was transferred on ice pack to the analytical laboratory at the Test Facility for concentration and particle size analysis.

The second vial was transferred on ice pack to the molecular biology laboratory at the Test Facility for purity analysis.

Purity and Particle size analysis was performed by IEX- HPLC, Differential Light Scattering (DLS) and capillary electrophoresis (CE) using validated analytical procedures.

4.5. Reserve Samples

For each batch (lot) of Test and Reference Item components, a reserve sample (1 mL or 1 vial) was collected and maintained under the appropriate storage conditions by the Test Facility.

4.6. Test and Reference Item Inventory and Disposition

Records of the receipt, distribution, and storage of Test and Reference Items were maintained. With the exception of reserve samples, all unused Sponsor-supplied bulk Test Item was returned to the Sponsor.

4.7. Dose Formulation and Analysis

Dose formulation preparations were performed under a laminar flow hood using clean procedures.

4.7.1. Preparation of Reference Item

The Reference Item, Phosphate Buffered Saline pH 7.2, was dispensed on days of dosing (i.e. Days 1, 15 and 29) for administration to Group 1 control animals and as required to dilute the bulk Test Item for administration to Groups 2 to 4 animals. The aliquots were transferred directly to room temperature.

Details of the preparation and dispensing of the Reference Item have been retained in the Study Records.

4.7.2. Preparation of Test Item

Test Item dosing formulations were diluted with Phosphate Buffered Saline, pH 7.2, as necessary for administration. The dosing formulations were prepared on each days of dosing (i.e. Days 1, 15 and 29) and were transferred directly to room temperature.

Any residual volumes of formulated Test Item were stored in a refrigerator set at 4°C and were discarded prior to finalisation following approval by the Study Director.

Details of the preparation and dispensing of the Test Item have been retained in the Study Records.

4.7.3. Sample Collection and Analysis

Dose formulation samples were collected for analysis as indicated in Text Table 2.

Text Table 2
Dose Formulation Sample Collection Schedule

Interval	Homogeneity	Concentration	Sampling From
Day 1[b]	2 to 4[a]	All groups	Preparation vessel
Day 29[b]	N/A	All groups	Preparation vessel

N/A = not applicable.

[a] The homogeneity results obtained from the top, middle and bottom preparations were averaged and utilized as the concentration results.

[b] Samples were collected on the first preparation of the study and on the last preparation of the study.

Samples to be analyzed were transferred on ice pack as soon as possible following preparation to the analytical laboratory (CR MTL).

4.7.3.1. Analytical Method

Analyses were performed by HPLC using a validated analytical procedure (Validation Study Number 1801737).

4.7.3.2. Concentration and Homogeneity Analysis

Duplicate sets of samples (0.5 mL) for each sampling time point were sent to the analytical laboratory; triplicate set of samples (0.5 mL) were retained at the Test Facility as backup samples. Samples were collected from the top, middle and bottom of the preparation vessel for each concentration except for Group 1 and on Day 29 (the last preparation of the study) where only concentration analysis was required; the formulation was then only sampled from the middle.

Concentration results were considered acceptable if mean sample concentration results were within or equal to ± 15% of theoretical concentration. Each individual sample concentration result was considered acceptable if it was within or equal to ± 20%.

Homogeneity results were considered acceptable if the relative standard deviation (RSD) of the mean value at each sampling location was ≤ 5%.

After acceptance of the analytical results, backup samples were discarded.

4.7.3.3. Stability Analysis

There was no stability analysis performed for concentration used on this study, however, end of use stability analysis on the bulk test item was performed at the end of the dosing period.

4.8. Test System

4.8.1. Receipt

On 16 Mar 2017, one hundred and twenty-one Crl:CD(SD) Sprague-Dawley rats (61 males and 60 females) were received from Charles River Canada Inc., St. Constant, QC, Canada. At the dosing onset, animals were 13 weeks old and males weighed between 375 and 520 g and females weighed between 221 and 295 g.

4.8.2. Justification for Test System and Number of Animals

The Sprague Dawley rat was chosen as the animal model for this study as it is an accepted rodent species for preclinical toxicity testing by regulatory agencies.

The total number of animals to be used in this study was considered to be the minimum required to properly characterize the effects of the Test Item. This study has been designed such that it does not require an unnecessary number of animals to accomplish its objectives.

At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. Acceptable models which do not use live animals currently do not exist.

4.8.3. Animal Identification

Each animal was identified using a subcutaneously implanted electronic identification chip.

4.8.4. Environmental Acclimation

A minimum acclimation period of 48 days was allowed between animal receipt and the start of dosing in order to accustom the animals to the laboratory environment

4.8.5. Selection, Assignment, Replacement, and Disposition of Animals

Animals were assigned to groups by a stratified randomization scheme designed to achieve similar group mean body weights. Males and females were randomized separately. Animals at extremes of body weight range were not assigned to groups.

No animal were replaced before or after the initiation of dosing.

All rats remaining unassigned to groups after Day 2 were released from the study and their disposition documented in the study records.

4.8.6. Husbandry

4.8.6.1. Housing

Animals were group housed (up to 3 animals of the same sex and same dosing group together) in polycarbonate cages containing appropriate bedding equipped with an automatic watering valve.

These housing conditions were maintained throughout the study. The rooms in which the animals were kept were documented in the study records.

Animals were separated during designated procedures/activities. Each cage was clearly labeled with a color-coded cage card indicating study, group, animal number(s), and sex. Cages were arranged on the racks in group order. Control group animals were housed on a separate rack from the Test Item-treated animals.

4.8.6.2. Environmental Conditions

Target temperatures of 19°C to 25°C with a relative target humidity of 30% to 70% were generally maintained. A 12-hour light/12-hour dark cycle was maintained (refer to Appendix 1 for one exception), except when interrupted for designated procedures.

4.8.6.3. Food

PMI Nutrition International Certified Rodent Chow No. 5CR4 (14% protein) was provided ad libitum throughout the study, except during designated procedures (refer to Appendix 1 for one exception). Wet pellets were provided to all animals of Group 4 as warranted by clinical signs.

The feed was analyzed by the supplier for nutritional components and environmental contaminants. Results of the analysis are provided by the supplier and are on file at the Test Facility.

It is considered that there are no known contaminants in the feed that would interfere with the objectives of the study.

4.8.6.4. Water

Municipal tap water after treatment by reverse osmosis and ultraviolet irradiation was freely available to each animal via an automatic watering system (except during designated procedures) (refer to Appendix 1 for one exception). Water bottles were provided, when required.

Periodic analysis of the water is performed, and results of these analyses are on file at the Test Facility.

It is considered that there are no known contaminants in the water that could interfere with the outcome of the study.

4.8.6.5. Animal Enrichment

Animals were socially housed for psychological/environmental enrichment and were provided with items such as a hiding device and a chewing object, except when interrupted by study procedures/activities.

4.8.6.6. Veterinary Care

Veterinary care was available throughout the course of the study, and animals were examined by the veterinary staff as warranted by clinical signs or other changes.

Hydrotherapy was provided to Animal No. 4513 from Day 2 to 4, due to swollen at injection site. This treatment was considered necessary for the well-being of the animal.

All veterinary examinations and recommended therapeutic treatments were documented in the study records.

4.9. Experimental Design

Text Table 3
Experimental Design

Group No.	Test Material	Dose Level (µg/dose)	Dose Volume (µL/dose)	Dose Concentration (mg/mL)	Animals No.			
					Main Study		Recovery Study	
					Males	Females	Males	Females
1	Reference Item	0	200	0	1001-1010	1501-1510	1011-1015	1511-1515
2	mRNA-1706	10	200	0.05	2001-2010	2501-2510	-	-
3	mRNA-1706	50	200	0.25	3001-3010	3501-3510	-	-
4	mRNA-1706	100	200	0.5	4001-4010	4501-4510	4011-4015	4511-4515

4.9.1. Administration of Test Materials

The Test and Reference Items were administered to the appropriate animals from Groups 1 to 4 via intramuscular injection into the lateral compartment of the thigh alternating legs on Days 1, 15 and 29 (site 1= left thigh, site 2 = right thigh). The volume for each dose was administered using a syringe/needle within the demarcated area. The first day of dosing was designated as Day 1.

The injection area was marked as frequently as required to allow appropriate visualization of administration sites. Hair have been clipped or shaved as required to improve visualization of the injection sites. The injection site was documented in the raw data for each dose administered.

4.9.2. Justification of Route and Dose Levels

The intramuscular route of exposure was selected because this is the intended route of human exposure.

The dose levels for this toxicology study were chosen to approximate a substantial multiple of the anticipated clinical starting dose and top clinical dose. The highest dose tested was expected to represent the intended maximum human clinical dose and volume and were administered by the clinical route (intramuscular). At this dose level, minimal systemic toxicity was expected, but it was possible mild to moderate injection site reaction (redness, swelling) and potentially elevation of systemic cytokine/acute phase markers may have been observed. The mid- and low-dose were selected to evaluate the dose-dependent effect of this compound.

4.10. In-life Procedures, Observations, and Measurements

4.10.1. Mortality/Moribundity Checks

Throughout the study, animals were observed for general health/mortality and moribundity twice daily, once in the morning and once in the afternoon (refer to Appendix 1 for exceptions). Animals were not removed from cage during observation, unless necessary for identification or confirmation of possible findings.

4.10.2. Clinical Observations

4.10.2.1. Detailed Clinical Observations

The animals were removed from the cage, and a detailed clinical observation was performed every two weeks during the predosing period and weekly during the dosing and recovery periods, starting Day -1.

In addition detailed examination of the injection sites was performed at predose, 24, 48 and 72 hours post each dose (refer to Appendix 1 for exceptions). Following Day 29 dosing, no assessment was performed on main study animals at 72 hours post dose as animals were sent to necropsy on Day 30. Any clinical signs at the injection site such as swelling, redness, vocalization upon palpation, warm to the touch, etc. were recorded as observed.

4.10.3. Body Weights

Animals were weighed individually every two weeks during the predosing period and weekly during the dosing and recovery periods, starting Day -1. A fasted weight was recorded on the day of necropsy.

4.10.4. Food Consumption

Food consumption was quantitatively measured weekly during the dosing and recovery periods, starting on Day -7.

4.10.5. Ophthalmic Examinations

Animals had funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations once prior to the dosing initiation (all animals) and on Day 23 for males and Day 22 for females of the dosing period. As there were no Test Item-related ophthalmoscopic findings at the end of the dosing period, examinations were not performed on recovery animals. The mydriatic used was 1% tropicamide.

4.10.6. Body Temperature

Rectal body temperature was recorded on un-sedated animals on Day 1 and on Day 29 at predose, 6 and 24 hours post dose (end of each group). Additional body temperature measurement was performed on all Group 4 female animals (Day 1) and on Animals Nos. 4511 to 4513 (Day 29) at 48 hours postdose since the 24 hours postdose values were significantly above normal range.

4.11. Laboratory Evaluations

4.11.1. Clinical Pathology

4.11.1.1. Sample Collection

Blood was collected from the abdominal aorta following isoflurane anesthesia. After collection, samples were transferred to the appropriate laboratory for processing.

Animals were fasted overnight before blood sampling (for clinical chemistry). Samples were collected according to Text Table 4.

Text Table 4
Samples for Clinical Pathology Evaluation

Group Nos.	Time Point	Hematology	Coagulation	Clinical Chemistry
1 to 4[a]	Day 30	X	X	X
1 and 4	Day 43	X	X	X

[a] Samples were collected from animals sent to euthanasia on Day 30.

4.11.1.2. Hematology

Blood samples (target volume of 0.5 mL collected in a tube containing EDTA as anticoagulant) were analyzed for the parameters specified in Text Table 5.

Text Table 5
Hematology Parameters

Red blood cell count	White blood cell count
Hemoglobin concentration	Neutrophil count (absolute)
Hematocrit	Lymphocyte count (absolute)
Mean corpuscular volume	Monocyte count (absolute)
Red Blood Cell Distribution Width	Eosinophil count (absolute)
Mean corpuscular hemoglobin concentration	Basophil count (absolute)
Mean corpuscular hemoglobin	Large unstained cells (absolute)
Reticulocyte count (absolute)	Other cells (as appropriate)
Platelet count	

A blood smear was prepared from each hematology sample. Blood smears were labeled, stained, and stored. Some blood smears were read to investigate results.

4.11.1.3. Coagulation

Blood samples (target volume of 0.9 mL collected in a 1.0 mL tube containing citrate as anticoagulant) were processed for plasma, and plasma was analyzed for the parameters listed in Text Table 6.

Text Table 6
Coagulation Parameters

Activated partial thromboplastin time	Prothrombin time
Fibrinogen	Sample Quality

4.11.1.4. Clinical Chemistry

Blood samples (target volume of 0.7 mL collected in a serum separator tube) were processed for serum, and the serum was analyzed for the parameters specified in Text Table 7.

Text Table 7
Clinical Chemistry Parameters

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin
Gamma-glutamyltransferase	Albumin/globulin ratio
Creatine Kinase	Glucose
Total bilirubin	Cholesterol
Urea nitrogen	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride
Sample Quality	

4.11.2. Cytokine Analysis

Blood was collected from the jugular vein of all recovery animals. After collection, blood samples for serum were allowed to clot at ambient room temperature and blood samples for plasma were transferred on wet ice to the appropriate laboratory for processing.

Text Table 8
Sample Collection Schedule

Target Blood Volume (mL)			0.5	0.5
Anticoagulant			**None (SST)**	**EDTA**
Centrifugation settings			2400x g, 10 minutes, set at 4°C	1200x g, 10 minutes, set at 4°C
Timepoints			**Sample Type**	
Day	**Hrs**	**No. of Males/ Females**	IFN-α*	IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α, MCP-1
1	6	5/5	X	X
15	6	5/5	X	X
29	6	5/5	X	X
43	NAp	5/5	X	X
Matrix			Serum	Plasma
Volume per aliquot (µL)			All volume	All volume
Number of aliquot(s)			1	1
Storage condition (set to maintain)			-80°C	-80°C
Responsible Lab (processing)			CR-SHB	CR-SHB

X = Sample collected, NAp = Not applicable
* The assay validation of IFN-α did not work appropriately and serum samples analysis was not conducted.

The number of aliquots and volumes were targets that may have been adjusted based on sample volume availability.

The samples for IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α and MCP-1 were analyzed by the Biomarkers department at CR MTL. Analysis for IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α and MCP-1 was conducted using a multiplex Luminex method. The procedures followed during the

course of this study along with the assays acceptance criteria were detailed in the appropriate analytical procedure. Samples were analyzed in duplicate.

A Biomarkers interpretative report (IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α and MCP-1) is included as an appendix to the Final Report.

4.11.3. Anti-Drug Antibody (ADA) Sample Collection, Processing and Analysis.

Before initiation of dosing and at study termination (on Day 30 for main animals and on Day 43 for recovery animals), blood was collected from all animals by jugular venipuncture and/or via the abdominal aorta while under isoflurane anesthesia (terminal samples).

Samples were mixed gently and allowed to clot at room temperature until centrifugation which was carried out as soon as practical (not exceeding 60 minutes after collection, refer to Appendix 1 for exceptions). The samples were centrifuged for 10 minutes in a refrigerated centrifuge (set to maintain 4°C) at 1200 g. The resultant serum was separated, transferred to uniquely labeled clear polypropylene tubes, frozen immediately over dry ice and transferred to a freezer set to maintain -80°C. Samples were shipped on dry ice to Integrated BioTherapeutics, Inc., Rockville, MD, USA

The samples were analyzed for rat anti-ZIKV antibodies using a qualified ELISA method.

Residual/retained samples were discarded prior issuance of the Final Report.

An Anti-therapeutic Antibody Report is included as an appendix to the Final Report

4.12. Terminal Procedures

Terminal procedures are summarized in Text Table 9.

Text Table 9
Terminal Procedures

Group No.	No. of Animals		Scheduled Euthanasia Day	Necropsy Procedures			Histology[a]	Histopathology[a]
	M	F		Necropsy	Tissue Collection	Organ Weights		
1	10	10	30	X	X	X	Full Tissue	Full Tissue
2	10	10					Full Tissue	Gross Lesions Target Tissues
3	10	10					Full Tissue	Gross Lesions Target Tissues
4	10	10					Full Tissue	Full Tissue
1	5	5	43	X	X	X	Full Tissue	Gross Lesions Target Tissues
4	5	5					Full Tissue	Gross Lesions Target Tissues

X = Procedure conducted; - = Not applicable.
[a] See Tissue Collection and Preservation table for listing of tissues.

4.12.1. Unscheduled Deaths

No animals died during the course of the study.

4.12.2. Scheduled Euthanasia

Main study and recovery animals surviving until scheduled euthanasia had a terminal body weight recorded, samples for clinical pathology, anti-drug antibodies and cytokines collected (as appropriate), and were euthanized by exsanguination by incision from the abdominal aorta following isoflurane anesthesia. The animals were euthanized rotating across dose groups such that similar numbers of animals from each group, including controls, were necropsied throughout the day. Animals were fasted overnight before their scheduled necropsy.

4.12.3. Necropsy

Main study and recovery animals were subjected to a complete necropsy examination, which included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

Necropsy procedures were performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology. A veterinary pathologist, or other suitably qualified person, was available.

4.12.4. Organ Weights

The organs identified in Text Table 10 were weighed at necropsy for all scheduled euthanasia animals. Paired organs were weighed together. In the event of gross abnormalities, in addition to the combined weight, the weight of each organ of a pair may be taken and entered as a tissue comment. Organ weight as a percent of body weight (using the terminal body weight) and organ weight as a percent of brain weight were calculated.

Text Table 10
Organs Weighed at Necropsy

Brain	Liver
Epididymis[a]	Lung
Gland, adrenal[a]	Ovary[a]
Gland, pituitary	Spleen
Gland, prostate	Testis[a]
Gland, thyroid[a]	Thymus
Heart	Uterus
Kidney[a]	

[a] Paired organ weight.

4.12.5. Tissue Collection and Preservation

Representative samples of the tissues identified in Text Table 11 were collected from all animals and preserved in 10% neutral buffered formalin, unless otherwise indicated.

Text Table 11
Tissue Collection and Preservation

Site, Injection	Large intestine, cecum
Animal identification	Large intestine, colon
Artery, aorta	Large intestine, rectum
Body cavity, nasal	Larynx
Bone marrow smear	Liver
Bone marrow	Lung
Bone, femur	Lymph node, mandibular
Bone, sternum	Lymph node, mesenteric
Brain	Lymph node, inguinal
Cervix	Lymph node, popliteal
Epididymis	Small intestine, duodenum
Esophagus	Small intestine, ileum
Eye[a]	Small intestine, jejunum
Gland, adrenal	Muscle, skeletal
Gland, harderian	Nerve, optic[a]
Gland, mammary	Nerve, sciatic
Gland, parathyroid	Ovary
Gland, pituitary	Pancreas
Gland, prostate	Skin
Gland, salivary	Spinal cord
Gland, seminal vesicle	Spleen
Gland, thyroid	Stomach
Gross lesions/masses	Testis[b]
Gut-associated lymphoid tissue	Thymus
Heart	Tongue
Kidney	Trachea
	Urinary bladder
	Uterus
	Vagina

[a] Preserved in Davidson's fixative.
[b] Preserved in Modified Davidson's fixative.

4.12.6. Histology

Tissues identified in Text Table 11 (except animal identification and bone marrow smears) were embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.

4.12.7. Histopathology

Histopathological evaluation was performed by a board-certified veterinary pathologist. The following target tissues identified by the study pathologist during microscopic evaluation were communicated to the study Director, evaluated and reported: gland adrenal, liver, spleen, injection site, lymph nodes (inguinal, popliteal and mesenteric), thymus and galt-associated lymphoid tissue.

4.12.8. Peer Review

A pathology peer review was conducted by [redacted] from Moderna Therapeutics, Cambridge, MA, USA.

4.12.9. Bone Marrow Smear Analysis

Two bone marrow smears were prepared from each euthanized animal, air dried, stained with Wright's Giemsa stain, and not coverslipped. Bone marrow smears were not evaluated.

5. CONSTRUCTED VARIABLES

Body Weight Gains	calculated between each scheduled interval as well as between the beginning and end of each phase
Organ Weight relative to Body Weight	calculated against the Terminal body weight for scheduled intervals
Organ Weight relative to Brain Weight	calculated against the brain weight for scheduled intervals

All results presented in the tables of the report are calculated using non-rounded values as per the raw data rounding procedure and may not be exactly reproduced from the individual data presented.

6. STATISTICAL ANALYSIS

All statistical tests were conducted at the 5% significance level. All pairwise comparisons were conducted using two sided tests and were reported at the 0.1%, 1%, and 5% levels.

Numerical data collected on scheduled occasions for the listed variables were analyzed as indicated according to sex and occasion. Descriptive statistics number, mean and standard deviation (or %CV or SE when deemed appropriate) were reported whenever possible. Values may also have been expressed as a percentage of predose or control values when deemed appropriate. Inferential statistics were performed according to the matrix below when possible, but excluded semi-quantitative data, and any group with less than 3 observations.

Text Table 12
Statistical Matrix

Variables for Inferential Analysis	Statistical Method Parametric/ Non-Parametric
Body Weight	X
Body Temperature	X
Hematology Variables	X
Coagulation Variables	X
Clinical Chemistry Variables	X
Cytokines	X
Organ Weights	X
Body Weight Changes	X
Organ Weight relative to Body Weight	X
Organ Weight relative to Brain Weight	X

The following pairwise comparisons were made:

Group 2 vs. Group 1

Group 3 vs. Group 1

Group 4 vs. Group 1

6.1. Parametric/Non-Parametric

Levene's test was used to assess the homogeneity of group variances.

Datasets with at least 3 groups were compared using an overall one-way ANOVA *F*-test if Levene's test was not significant or the Kruskal-Wallis test if it was. If the overall *F*-test or Kruskal-Wallis test was found to be significant, then the above pairwise comparisons were conducted using Dunnett's or Dunn's test, respectively.

Datasets with 2 groups (the designated control group and 1 other group) were compared using a *t*-test if Levene's test was not significant or Wilcoxon Rank-Sum test if it was.

7. COMPUTERIZED SYSTEMS

Critical computerized systems used in the study are listed below or presented in the appropriate Phase Report. All computerized systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data.

Text Table 13
Critical Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
Provantis	8	In-life; clinical pathology; postmortem
Dispense	8	Test Material receipt, accountability and/or formulation activities
In-house reporting software Nevis 2012 (using SAS)	Nevis 2 (SAS 9.2)	Statistical analyses of numerical in-life, clinical pathology and postmortem data
Mesa Laboratories AmegaView CMS	v3.0 Build 1208.8	Continuous Monitoring System. Monitoring of standalone fridges, freezers, incubators, and selected laboratories to measure temperature, relative humidity, and CO2, as appropriate
Johnson Controls Metasys	MVE 7.0 and 4.0	Building Automation System. Control of HVAC and other building systems, as well as temperature/humidity control and trending in selected laboratories and animal rooms
Empower 3 (Waters Corporation)	Build 3471 SR1	Data acquisition for dose formulation analysis, including regression analysis and measurement of concentration and recovery of dose formulations using HPLC and measurement of purity.
BioPlex Manager	6.1	Cytokines (IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α, MCP-1) data collection
Watson LIMS	7.4.2 SP1	Cytokines (IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α, MCP-1) data regression
Dynamics (Wyatt)	7.1.9.3.	Data acquisition for particle size analysis of the test item using DLS
Fragment Analyzer CE System	1.1.0.11	Data acquisition
Excel	2007	Data analyses and tabulation
SRS (PCS-MTL in-house application built with SAS and SAS system for Windows)	1.4	Statistical analyses of cytokines

8. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, documentation, study plan, samples, specimens, and final reports from this study were archived at the Test Facility by no later than the date of final report issue unless otherwise specified in the study plan. At least one year after issue of the draft report, the Sponsor will be contacted to determine disposition of materials associated with the study.

Electronic data generated by the Test Facility were archived as noted above, except that the data collected using Provantis 8 and reporting files stored on SDMS, which were archived at the Charles River Laboratories facility location in Wilmington, MA.

All records, retained samples and specimens, and reports generated from phases or segments performed by Sponsor-designated subcontractors were returned to the Test Facility for archiving. Archival location and duration are detailed in the applicable PI report(s) or details regarding the retention of the materials were provided to the Study Director for inclusion in the Final Report.

9. RESULTS

9.1. Dose Formulation Analyses

(Appendix 3)

The dose formulations were within specification. Homogeneity testing showed that the formulation technique used produced homogeneous preparations.

In addition, the end of use bulk Test Item analysis demonstrated that the test item was suitable for use during the study period.

9.2. Mortality

(Appendix 4)

There were no unscheduled deaths during the course of this study.

9.3. Clinical Observations

(Table 1 and Appendix 5)

Following each dosing, warm to the touch was observed for females given 100 µg/dose. This observation was also noted following the second dose administration for males given 100 µg/dose and for females given 50 µg/dose. Those observations correlate with higher body temperature observed (Refer to Section 9.7)

During the dosing period, some females given 100 µg/dose were also observed with prominent backbone, thinness, hunched posture and suspected dehydration. Those observations were not observed during the recovery period.

Following each dosing occasions, a dose-related swelling (soft to firm) was noted on the injection site and extended to hindlimb and inguinal areas for some animals given 100 µg/dose. In addition, skin redness at the injection site, was noted with a higher incidence throughout the dosing period for animals given ≥ 50 µg/dose. Given the absence of swelling and redness at the injection site at the end of the recovery period, these clinical observations were considered fully reversed.

9.4. Body Weights and Body Weight Gains

(Figure 1, Figure 2, Table 2, Table 3, Appendix 6, and Appendix 7)

When compared to controls, following each dose, a tendency towards lower mean body weight gains was noted in males and females given ≥ 10 µg/dose; these changes sometimes reached statistical significance in both genders. From Day -1 to 28, the changes were down to 0.60X controls in males and 0.78X controls in females. The body weight changes were generally comparable or rebounded during the 2-week recovery period.

9.5. Food Consumption

(Table 4 and Appendix 8)

Given the variability in weekly food consumption results, the occasional weekly changes, with no clear dose-relationship, were considered not mRNA-1706-related.

9.6. Ophthalmic Examinations

(Appendix 13)

There were no mRNA-1706-related ocular changes observed during the course of the study. The findings noted were age-related or incidental in origin and to be expected in this population of animals.

9.7. Body Temperature

(Table 5 and Appendix 9)

In general body temperatures were within normal ranges of 36-38°C. When compared to control animals and pre-dose body temperature measurements, the mean body temperature appeared minimally increased in males and females given ≥ 50 µg/dose at 6 and/or 24 hours post Day 1 and Day 29 doses. These statistically-significant changes were considered mRNA-1706-related.

9.8. Hematology

(Table 6 and Appendix 10)

mRNA-1706-related hematology changes were noted for males and females starting at 10 µg/dose and included increases in neutrophil (NEUT), monocytes (MONO), eosinophil (EOS) and/or large unstained cell (LUC) counts (with concomitant increases in white blood cell [WBC] counts) and decreases in lymphocyte (LYMPH), reticulocytes (RETIC) and platelet (PLT) counts. These changes are illustrated in Text Table 14.

Text Table 14
Hematology Changes

Dose (µg/dose)	10		50		100	
Parameter	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
WBC						
Day 30	**2.1**	**2.5**	**2.3**	**1.8**	**1.8**	0.90
Day 43					1.1	1.1
NEUT						
Day 30	**10.0**	**9.7**	**13.0**	**7.9**	**11.3**	4.0
Day 43					1.7	1.6
MONO						
Day 30	**2.0**	**1.8**	**2.1**	1.3	1.5	0.85
Day 43					1.5	1.2
LYMPH						
Day 30	0.78	0.97	**0.49**	**0.48**	**0.26**	**0.24**
Day 43					1.0	1.1
EOS						
Day 30	**3.0**	**6.5**	**2.3**	**3.8**	1.1	1.6
Day 43					0.86	0.96
LUC						
Day 30	**4.7**	**3.0**	**5.1**	**2.4**	3.0	1.3
Day 43					1.5	1.2
PLT						
Day 30	-	1.01	-	0.96	-	**0.80**
Day 43					-	1.1
RETIC						
Day 30	0.87	0.90	**0.80**	0.98	**0.72**	0.82
Day 43					**1.2**	1.2

Changes are expressed as X Fold from mean Group 1 (control) value.
–: indicates results were considered not to be meaningfully different from mean control value.
Bolded values were statistically significant.
Shaded boxes indicate no collection at this timepoint for corresponding groups.

Mild to moderate increases in WBC counts (up to 2.3X and 2.5X controls, respectively) were noted in males and females given ≥ 10 µg/dose, mainly due to minimal to moderate increases in NEUT, MONO, LUC, and EOS (up to 13.0X, 2.1X, 5.1X and 3.0X controls for males and 9.7X, 1.8X, 3.0X and 6.5X controls for females). Minimal to moderate dose-related decreases in LYMPH and RETIC counts were noted for males and females at ≥ 10 µg/dose (down to 0.26X and 0.24X controls, respectively). Increased observed in WBC, NEUT, MONO, LUC and EOS in animals given ≤ 50 µg/dose were followed by a decreased in the animals given 100 µg/dose. At the end of the recovery period, values for NEUT, MONO, LUC, were almost back to normal and LYMPH and RETIC values return to normal values.

Minimal dose-related decreases in PLT were noted in females given ≥ 50 µg/dose (down to 0.80X controls) with full recovery at the end of the recovery period.

Any other differences in hematology parameters, including those attaining statistical significance, were judged to be due to individual or biological variation or lacked true dose relationship and therefore were considered not mRNA-1706-related.

9.9. Coagulation

(Table 7 and Appendix 11)

mRNA-1706-related increases in activated partial thromboplastin time (APTT) and in fibrinogen (FIB) were noted in males and females given ≥ 10 µg/dose. The changes are illustrated in Text Table 15.

Text Table 15
Coagulation Changes

Dose (µg/dose)	10		50		100	
Parameter	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
APTT						
Day 30	**1.2**	**1.2**	**1.2**	**1.4**	**1.3**	**1.4**
Day 43					1.0	1.0
FIB						
Day 30	**2.3**	**2.4**	**2.5**	**2.6**	**2.3**	**2.4**
Day 43					**0.9**	1.0

Changes are expressed as X Fold from mean (Group 1) control value.
Bolded values were statistically significant.
Shaded boxes indicate no collection at this timepoint for corresponding groups.

Minimal increases in APTT were noted for males and females given ≥ 10 µg/dose (up to 1.3X controls for males and 1.4X controls for females). Mild increases in FIB were noted for males and females given ≥ 10 µg/dose (up to 2.5X controls for males and 2.6X controls in females). At the end of the 2-week recovery period, changes were fully recovered.

Any other differences in the coagulation parameters were judged to be due to individual or biological variability or lacked true dose relationship and therefore were considered not mRNA-1706-related.

9.10. Clinical Chemistry

(Table 8 and Appendix 12)

mRNA-1706-related decreases in albumin (ALB) and increases in globulin (GLOB) were noted for males and females reflected by overall decrease in A/G ratio. Dose-related decrease in glucose concentration (GLUC) and increase in aspartate aminotransferase were also observed. The changes are illustrated in Text Table 16.

Text Table 16
Clinical Chemistry Changes

Dose (µg/dose)	10		50		100	
Parameter	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
ALB						
Day 30	**0.9**	**0.9**	**0.9**	0.9	**0.9**	**0.9**
Day 43					1.0	1.0
GLOB						
Day 30	**1.2**	**1.1**	**1.2**	1.1	**1.2**	1.0
Day 43					0.9	1.0
A/G						
Day 30	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**
Day 43					1.1	1.0
GLUC						
Day 30	**0.8**	0.9	**0.8**	**0.8**	**0.7**	**0.7**
Day 43					1.0	**1.3**

Changes are expressed as X Fold from mean Group 1 (control) value.
Bolded values were statistically significant.
Shaded boxes indicate no collection at this timepoint for corresponding groups.

Minimal decreases in ALB and minimal increases in GLOB were noted for males and females given ≥ 10 µg/dose (0.9X controls and up to 1.2X controls for each parameter) and affected the A/G ratio (down to 0.8X controls, for both genders). At the end of the 2-week recovery period, changes were fully recovered.

Minimal decreased in GLUC were noted for males and females given ≥ 10 µg/dose (down to 0.7X controls, for both genders). At the end of the recovery period, changes were fully recovered.

Increase in AST was observed for females given 100 µg/dose but is mainly related to the increase in AST level in female no.4501 and is not considered mRNA-1706-related.

Any other differences in the clinical chemistry parameters, including those attaining statistical significance, were judged to be due to individual or biological variability or lacked true dose relationship and therefore were considered not mRNA-1706-related.

9.11. Cytokines Analysis

(Appendix 14 and Appendix 15)

When compared to control, statistically-significant higher concentrations of IP-10 were observed in both genders given 100 µg/dose, high incidence and magnitude of change observed for males and females of higher dose group (100 µg/dose) were considered to be mRNA-1706-related.

Higher concentrations of MIP-1α and MCP-1 were also observed in both genders given 100 µg/dose. Concentrations were comparable to control levels on Day 43.

No mRNA-1706 changes were observed for IL-1β, TNF-α and IL-6.

All the mRNA-1706-related increases observed tent to be reversible.

9.12. Anti-Drug Antibody (ADA)

(Appendix 16)

The Day 30 samples from mRNA-1706-treated Main Study animals had detectable antibody responses against ZIKV lysate. The Day 43 samples from Recovery Study animals previously given 100 µg/dose had higher response when compared to Day 30 results.

9.13. Gross Pathology

(Appendix 17)

9.13.1. Terminal Euthanasia Animals (Day 30)

mRNA-1706-related gross pathology findings were observed in lymph nodes (iliac, inguinal, popliteal) and at the injection site and their incidences are summarized in Tox Table 17.

Text Table 17
Summary of Gross Pathology Findings – Scheduled Euthanasia (Day 30)

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (µg/dose)	**0**	**10**	**50**	**100**	**0**	**10**	**50**	**100**
No. Animals Examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Site, Injections (No. Examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Abnormal consistency; firm	0	8	9	9	0	4	9	9
Swelling	0	1	3	2	0	5	6	7
Material accumulation; clot	0	0	2	0	0	0	0	0
Lymph node[a] (No. Examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Enlargement	0	0	0	4	0	1	3	4
Lymph node, inguinal (No. Examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Enlargement	0	0	1	0	0	0	0	1
Lymph node, popliteal (No. Examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Enlargement	1	3	0	1	0	3	0	0

[a] Iliac left as documented in individual animals.

Macroscopic findings attributed to the administration of mRNA-1706 at the injection site were characterized by firmness, swelling and occasionally by the presence of a clot in the thigh muscle used for the injection. These macroscopic findings were observed in males and females at all dose levels with no clear dose relationship and correlated histologically with inflammation and/or hemorrhage.

In addition, enlargement of the draining lymph nodes (inguinal, popliteal and iliac) was observed in males and females at all dose levels with no evidence of dose relationship and correlated histologically with mixed cell infiltration.

Other gross findings observed were considered incidental, of the nature commonly observed in this strain and age of rats, and/or were of similar incidence in reference and treated animals and, therefore, were considered unrelated to administration of mRNA-1706.

9.13.2. Recovery Euthanasia Animals (Day 43)

mRNA-1706-related gross findings noted at the terminal euthanasia in the injection site and draining lymph nodes were not observed at the end of the recovery period (Day 43). Other gross findings observed were considered incidental, of the nature commonly observed in this strain and age of rats, and/or were of similar incidence in reference and treated animals and, therefore, were considered unrelated to administration of mRNA-1706.

9.14. Organ Weights

(Appendix 17)

9.14.1. Terminal Euthanasia Animals (Day 30)

mRNA-1706-related organ weight changes were observed in the adrenal glands and are summarized in Text Table 18.

Text Table 18
Summary of Organ Weight Data – Scheduled Euthanasia (Day 30)

	Males			Females		
Group	**2**	**3**	**4**	**2**	**3**	**4**
Dose (µg/dose)	**10**	**50**	**100**	**10**	**50**	**100**
No. Animals per Group	**10**	**10**	**10**	**10**	**10**	**10**
Gland, adrenal (No. Weighed)[a]	(10)	(10)	(10)	(10)	(10)	(10)
Absolute value	7	2	14	4	**13**	**21**
% of body weight	**15**	5	**27**	6	11	**23**
% of brain weight	7	-2	11	3	13	**24**

[a] All values expressed as percent difference of reference group means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from reference group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used.

The group means for absolute and relative (to body and brain) weight of the adrenal were significantly higher in females given mRNA-1706 at 100 µg/dose compared to the concurrent reference. There was also a trend toward higher group means for absolute and relative (to body and brain) weight in males given mRNA-1706 at 100 µg/dose and females at 50 µg/dose but these values were statistically significant only in for absolute (females) or relative to body (males) weights. These increases of adrenal weights correlated histologically with cortical hypertrophy.

No other mRNA-1706-related organ weight changes were noted. There were other isolated organ weight values that were statistically different from their respective references. There were, however, no patterns, trends, or correlating data to suggest these values were toxicologically relevant. Thus, other organ weight differences observed were considered incidental and/or related to difference of terminal body weight and unrelated to administration of mRNA-1706.

9.14.2. Recovery Euthanasia Animals (Day 43)

mRNA-1706-related organ weight changes noted in the adrenal glands at the terminal euthanasia were not observed at the end of the recovery period (Day 43). There were isolated organ weight values that were statistically different from their respective references. There were, however, no patterns, trends or correlating data to suggest these values were toxicologically relevant. Thus,

the organ weight differences observed were considered incidental and/or related to difference of terminal body weight and unrelated to administration of mRNA-1706.

9.15. Histopathology

(Appendix 17)

9.15.1. Terminal Euthanasia Animals (Day 30)

mRNA-1706-related microscopic findings were observed at the injection site, in the draining lymph nodes (inguinal, popliteal, iliac), bone marrow, adrenal glands, liver, mesenteric lymph node, thymus and spleen and their incidence and severity are summarized in Text Table 19.

Text Table 19
Summary of Microscopic Findings – Scheduled Euthanasia (Day 30)

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (µg/dose)	**0**	**10**	**50**	**100**	**0**	**10**	**50**	**100**
No. Animals Examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Bone Marrow (No. Examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Increased cellularity; myeloid	(0)[a]	(5)	(6)	(7)	(0)	(3)	(5)	(8)
Minimal	0	5	6	7	0	3	5	8
Gland, Adrenal (No. Examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hypertrophy; cortical	(0)[a]	(0)	(5)	(10)	(0)	(0)	(4)	(9)
Minimal	0	0	5	10	0	0	4	9
Liver (No. Examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hypertrophy; Kupffer cell	(0)	(2)	(1)	(2)	(0)	(2)	(2)	(5)
Minimal	0	2	1	2	0	2	2	4
Mild	0	0	0	0	0	0	0	1
Degeneration/necrosis; centrilobular	(0)	(1)	(0)	(0)	(0)	(0)	(1)	(3)
Minimal	0	1	0	0	0	0	1	2
Mild	0	0	0	0	0	0	0	1
Lymph node; inguinal (No. Examined)	(10)	(9)	(8)	(10)	(10)	(10)	(10)	(10)
Infiltration, mixed cell	(0)	(0)	(1)	(1)	(0)	(0)	(1)	(5)
Minimal	0	0	1	1	0	0	1	4
Mild	0	0	0	0	0	0	0	1
Lymph node; popliteal (No. Examined)	(10)	(9)	(9)	(10)	(10)	(10)	(10)	(10)
Infiltration, mixed cell	(0)	(0)	(1)	(3)	(0)	(3)	(5)	(5)
Minimal	0	0	1	2	0	3	5	4
Mild	0	0	0	1	0	0	0	1
Lymph node[b] (No. Examined)	(0)	(0)	(0)	(4)	(0)	(1)	(3)	(4)
Infiltration, mixed cell	-	-	-	(4)	-	(1)	(3)	(4)
Minimal	-	-	-	1	-	1	1	0
Mild	-	-	-	2	-	0	2	3
Moderate	-	-	-	1	-	0	0	1
Lymph node; mesenteric (No. Examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Decreased cellularity; lymphoid, paracortex	(0)	(3)	(4)	(4)	(0)	(2)	(4)	(8)
Minimal	0	3	4	4	0	2	4	8

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (µg/dose)	**0**	**10**	**50**	**100**	**0**	**10**	**50**	**100**
No. Animals Examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Site, Injection (No. Examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation	(0)	(10)	(10)	(9)	(2)	(10)	(10)	(10)
Minimal	0	0	0	0	2	1	0	0
Mild	0	5	1	2	0	3	1	0
Moderate	0	5	6	4	0	6	3	2
Marked	0	0	3	3	0	0	6	8
Hemorrhage	(1)	(1)	(5)	(3)	(0)	(1)	(0)	(0)
Minimal	1	1	0	3	0	1	0	0
Mild	0	0	3	0	0	0	0	0
Moderate	0	0	2	0	0	0	0	0
Spleen (No. Examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Decreased cellularity; lymphoid, periarteriolar lymphoid sheath	(0)	(9)	(8)	(10)	(0)	(7)	(8)	(10)
Minimal	0	7	4	8	0	6	5	5
Mild	0	2	4	2	0	1	3	5
Single cell necrosis; lymphoid	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(6)
Minimal	0	0	0	1	0	0	0	6
Thymus (No. Examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Single cell necrosis; lymphoid	(0)	(0)	(3)	(3)	(0)	(0)	(0)	(8)
Minimal	0	0	3	3	0	0	0	6
Mild	0	0	0	0	0	0	0	2
Decreased cellularity; lymphoid	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Minimal	0	0	0	1	0	0	0	0

[a] Numbers in parentheses represent the number of animals with the finding.
[b] Iliac lymph node, examined only if gross abnormality observed.

At the injection site, the microscopic findings attributed to the administration of mRNA-1706 were minimal to marked inflammation and minimal to moderate hemorrhage. The inflammation was observed in males and females at all dose levels with no dose relationship between 50 µg/dose and 100 µg/dose but with lower severity of the findings at 10 µg/dose. It was characterized locally by extensive infiltration of mixed inflammatory cells, mainly granulocytes, with associated edema and fibrin exudates and correlated macroscopically with firmness of the thigh (abnormal consistency; firm). It was accompanied by mild to moderate hemorrhage (correlating macroscopically with material accumulation; clot) in some males at 50 µg/dose. This inflammatory process was not otherwise associated with degeneration of the muscle. Similar incidence and severity of minimal degeneration/necrosis of myofiber was observed in reference and mRNA-1706-dosed animals and was considered related to the experimental procedures (intramuscular injection).

In the inguinal, popliteal and iliac lymph nodes (considered as draining injection site), there was minimal to moderate mixed cell infiltration in males and/or females administered mRNA-1706 at all dose levels. The inflammatory infiltrate was composed of clusters of degenerated granulocytes in the lymph node sinuses or in the adjacent adipose/connective tissue.

A dose related minimal increase of myeloid cellularity was observed in the bone marrow of males and females at all dose levels.

In the adrenal glands, a dose related minimal cortical hypertrophy was present in males and females administered mRNA-1706 at ≥ 50 µg/dose and correlated with the increased adrenal weights.

In the liver, a low incidence of minimal to mild hypertrophy of the Kupffer cell was observed in males and females at all dose levels with no clear dose relationship. The hypertrophied Kupffer cells sometimes contained finely granular brownish pigment. In one male at 10 µg/dose, one female at 50 µg/dose and three females at 100 µg/dose, there was minimal to mild degeneration in the centrilobular region characterized by presence of mixed inflammatory cell in the sinusoid with single cell necrosis or degeneration of hepatocytes.

Microscopic findings similar in nature were observed in the spleen and mesenteric lymph nodes at all dose levels as well as in thymus at ≥ 50 µg/dose. They consisted of minimal to mild decreased lymphoid cellularity and/or single cell necrosis of lymphocyte. The decreased lymphoid cellularity was present in the periarteriolar sheath of the spleen, in the cortex of the thymus and in the paracortex of the mesenteric lymph node. In these areas, the lymphoid cells were less densely populated with prominent dendric cells. Single cell lymphoid necrosis was observed in the depleted region in these organs, mainly at 100 µg/dose.

After evaluation of all dose group animals, the GALT was no longer considered as a target organ.

Other microscopic findings observed were considered incidental, of the nature commonly observed in this strain and age of rats, and/or were of similar incidence and severity in reference and treated animals and, therefore, were considered unrelated to administration of mRNA-1706.

9.15.2. Recovery Euthanasia Animals (Day 43)

Microscopic findings noted in the adrenal glands, bone marrow, lymph nodes (mesenteric, popliteal, inguinal), thymus, spleen and liver at the terminal euthanasia were not observed at the end of the recovery period (Day 43) and therefore, considered completely recovered.

At the injection site, the inflammation and hemorrhage present at the end of dosing were no longer observed but minimal mononuclear cell infiltration was noted in males and females at 100 µg/dose as well as in one control female. This microscopic finding was interpreted to result from the healing process from previous inflammation at the injection site. The incidence and severity are summarized in Text Table 20.

Text Table 20
Summary of Microscopic Findings – Scheduled Euthanasia (Day 43)

	Males		females	
Group	**1**	**4**	**1**	**4**
Dose (µg/dose)	**0**	**100**	**0**	**100**
No. Animals Examined	**5**	**5**	**5**	**5**
Site, injections (No. Examined)	(5)	(5)	(5)	(5)
Infiltration; mononuclear cell	(0)[a]	(5)	(1)	(2)
Minimal	0	5	1	2

[a] Numbers in parentheses represent the number of animals with the finding.

Other microscopic findings observed were considered incidental, of the nature commonly observed in this strain and age of rats, and/or were of similar incidence and severity in reference and treated animals and, therefore, were considered unrelated to administration of mRNA-1706.

10. CONCLUSION

In conclusion, administration of mRNA-1706 by intramuscular injection for 1 month (3 doses) was clinically well tolerated (no mortality, major decreases in body weight and no changes in food consumption or deleterious changes in hematology, coagulation or clinical chemistry parameters) in rats up to 100 µg/dose. Starting at 10 µg/dose, generally dose-dependent changes in clinical signs at the injection site, clinical pathology parameters and cytokines were consistent with an inflammatory response at the injection site. Dose-related target organ effects were limited to the injection site, the bone marrow, the inguinal, mesenteric, iliac and popliteal lymph nodes, adrenal gland, liver, spleen and thymus of animals given mRNA-1706. At the end of the 2-week recovery period, all changes were partially or fully recovered.

Figure 1

Summary of Body Weights - Males

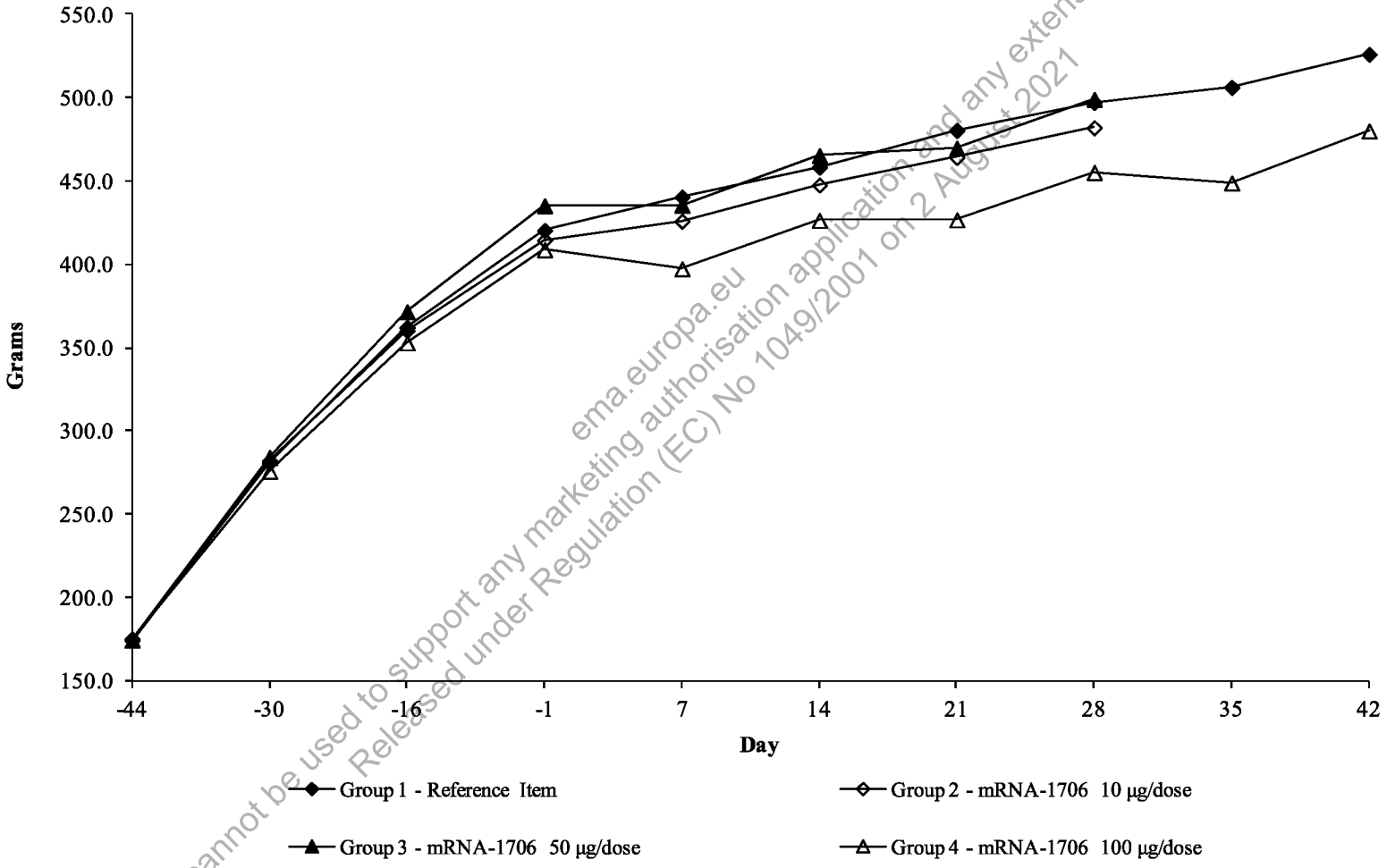

Figure 2

Summary of Body Weights - Females

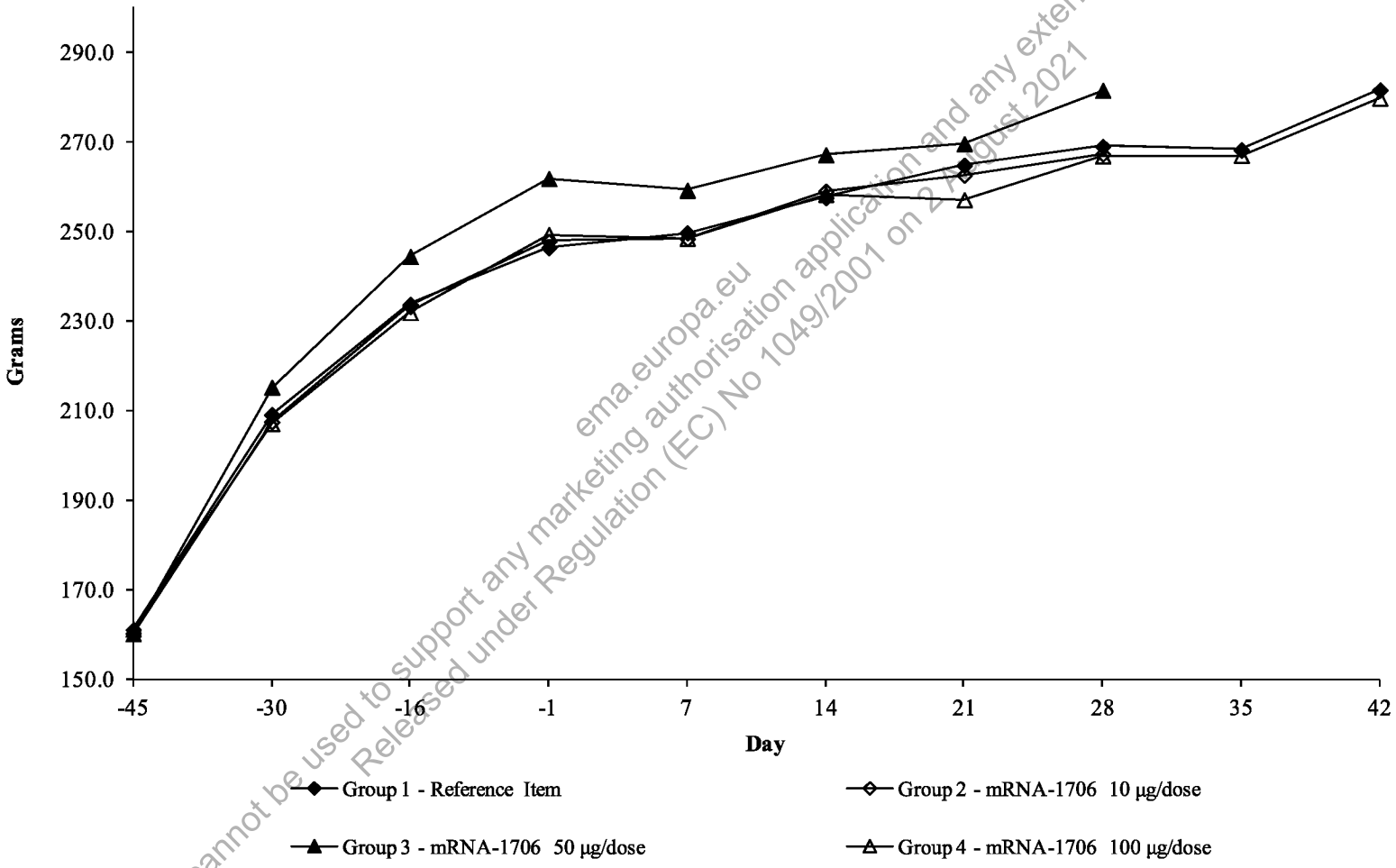

Table 1

Summary of Clinical Observations

5002231

Day numbers relative to Start Date

Sex: Male

	0 ug/dose	10 ug/dose	50 ug/dose	100 ug/dose
Abnormal Gait				
Number of Observations	.	.	.	1
Number of Animals	.	.	.	1
Days from - to	.	.	.	30 30
Hyperreactive				
Number of Observations	8	.	.	1
Number of Animals	2	.	.	1
Days from - to	-9 30	.	.	30 30
Vocalization Increased				
Number of Observations	7	.	1	1
Number of Animals	2	.	1	1
Days from - to	-1 30	.	2 2	30 30
Hunched Posture				
Number of Observations	.	.	.	1
Number of Animals	.	.	.	1
Days from - to	.	.	.	30 30
Limited Usage				
Number of Observations	.	.	.	1
Number of Animals	.	.	.	1
Days from - to	.	.	.	30 30
Swollen Soft				
Number of Observations	7	18	16	14
Number of Animals	7	10	10	6
Days from - to	16 16	16 30	16 30	2 32
Swollen Firm				
Number of Observations	.	15	49	95
Number of Animals	.	10	10	15
Days from - to	.	4 18	-37 30	2 30

Table 1

Summary of Clinical Observations

5002231

Day numbers relative to Start Date

Sex: Male

	0 ug/dose	10 ug/dose	50 ug/dose	100 ug/dose
Warm to Touch				
Number of Observations	.	.	.	7
Number of Animals	.	.	.	6
Days from - to	.	.	.	15 17
Skin, Black				
Number of Observations	.	.	.	2
Number of Animals	.	.	.	1
Days from - to	.	.	.	-37 -23
Skin, Red				
Number of Observations	7	7	13	30
Number of Animals	5	4	9	13
Days from - to	2 14	3 17	-37 17	-48 30
Skin, Dry				
Number of Observations	.	2	.	.
Number of Animals	.	1	.	.
Days from - to	.	7 14	.	.
Skin, Scab				
Number of Observations	14	26	8	27
Number of Animals	5	10	7	11
Days from - to	-37 21	-37 30	-37 30	-48 30
Fur, Staining, Red				
Number of Observations	2	8	10	6
Number of Animals	2	4	5	4
Days from - to	14 28	-23 30	-9 30	-9 30
Fur, Thin Cover				
Number of Observations	11	28	21	24
Number of Animals	4	8	8	11
Days from - to	-37 14	-37 30	-37 30	-37 30

Table 1

Summary of Clinical Observations

5002231

Day numbers relative to Start Date

Sex: Male

	0 ug/dose	10 ug/dose	50 ug/dose	100 ug/dose
Pinna Partly Missing				
Number of Observations	40	.	27	51
Number of Animals	2	.	2	3
Days from - to	-37 43	.	-37 30	-37 30
Tail, Missing				
Number of Observations	.	.	.	19
Number of Animals	.	.	.	1
Days from - to	.	.	.	-48 30

Table 1

Summary of Clinical Observations

5002231

Day numbers relative to Start Date

Sex: Female

	0 ug/dose	10 ug/dose	50 ug/dose	100 ug/dose
Backbone Prominent				
Number of Observations	.	.	.	3
Number of Animals	.	.	.	3
Days from - to	.	.	.	2 30
Dehydrated Suspected				
Number of Observations	.	.	.	7
Number of Animals	.	.	.	6
Days from - to	.	.	.	2 30
Hunched Posture				
Number of Observations	.	.	.	5
Number of Animals	.	.	.	5
Days from - to	.	.	.	2 30
Limited Usage				
Number of Observations	.	.	1	20
Number of Animals	.	.	1	10
Days from - to	.	.	2 2	2 17
Swollen Soft				
Number of Observations	.	32	27	13
Number of Animals	.	10	10	6
Days from - to	.	16 30	16 30	3 32
Swollen Firm				
Number of Observations	.	13	39	104
Number of Animals	.	7	10	15
Days from - to	.	2 30	2 30	2 35
Thin				
Number of Observations	.	.	.	3
Number of Animals	.	.	.	3
Days from - to	.	.	.	2 30

Table 1

Summary of Clinical Observations

5002231

Day numbers relative to Start Date

Sex: Female

	0 ug/dose	10 ug/dose	50 ug/dose	100 ug/dose
Warm to Touch				
Number of Observations	.	.	7	49
Number of Animals	.	.	6	14
Days from - to	.	.	16 17	2 31
Skin, Red				
Number of Observations	4	7	18	50
Number of Animals	3	4	8	14
Days from - to	-37 28	7 29	3 30	-1 32
Skin, Dry				
Number of Observations	.	4	.	.
Number of Animals	.	2	.	.
Days from - to	.	28 30	.	.
Skin, Scab				
Number of Observations	16	2	12	9
Number of Animals	6	2	5	3
Days from - to	-37 43	7 30	-1 30	2 43
Fur, Erected				
Number of Observations	.	.	.	2
Number of Animals	.	.	.	2
Days from - to	.	.	.	2 30
Fur, Staining, Red				
Number of Observations	25	13	3	19
Number of Animals	6	6	2	5
Days from - to	7 43	14 30	28 30	7 43
Fur, Thin Cover				
Number of Observations	30	11	28	37
Number of Animals	7	3	7	7
Days from - to	-9 43	-1 30	-1 30	-37 43

Table 1

Summary of Clinical Observations

5002231

Day numbers relative to Start Date

Sex: Female

	0 ug/dose	10 ug/dose	50 ug/dose	100 ug/dose
Tail, Bent				
Number of Observations	.	.	.	7
Number of Animals	.	.	.	1
Days from - to	.	.	.	-37 3
Pinna Partly Missing				
Number of Observations	.	.	.	4
Number of Animals	.	.	.	1
Days from - to	.	.	.	21 30
Teeth, Clear				
Number of Observations	4	3	.	.
Number of Animals	2	1	.	.
Days from - to	-23 -9	-23 -1	.	.

Table 2

Summary of Body Weights (g)

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		Day -44	-30	-16	-1	7	14	21	28
1M	Mean	174.3	281.1	362.2	420.5	440.5	458.5	480.5	497.3
	SD	10.0	16.8	26.7	34.2	36.1	37.2	38.4	43.3
	N	15	15	15	15	15	15	15	15
2M	Mean	175.2	282.3	360.4	414.6	426.0	447.8	464.3	482.1
	SD	7.6	12.6	20.7	24.3	25.3	27.4	29.3	30.6
	N	10	10	10	10	10	10	10	10
	%Diff G1	0.5	0.4	-0.5	-1.4	-3.3	-2.3	-3.4	-3.1
3M	Mean	174.4	284.2	371.6	435.3	435.6	465.4	469.8	499.2
	SD	7.1	13.7	23.7	38.4	41.3	42.6	46.2	48.6
	N	10	10	10	10	10	10	10	10
	%Diff G1	0.1	1.1	2.6	3.5	-1.1	1.5	-2.2	0.4
4M	Mean	174.5	275.9	353.0	408.9	397.4b	426.5a	426.8c	455.3a
	SD	10.5	16.0	20.0	23.1	24.6	25.0	27.7	27.3
	N	15	15	15	15	15	15	15	15
	%Diff G1	0.1	-1.9	-2.5	-2.8	-9.8	-7.0	-11.2	-8.4

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunnett)

Table 2

Summary of Body Weights (g)

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		Day 35	Day 42
1M	Mean	506.4	526.2
	SD	22.6	24.1
	N	5	5
2M	Mean	--	--
	SD	--	--
	N	--	--
	%Diff G1	--	--
3M	Mean	--	--
	SD	--	--
	N	--	--
	%Diff G1	--	--
4M	Mean	449.0b	480.2a
	SD	16.4	19.3
	N	5	5
	%Diff G1	-11.3	-8.7

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (T-test)

Table 2

Summary of Body Weights (g)

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		Day -45	-30	-16	-1	7	14	21	28
1F	Mean	161.1	209.1	233.7	246.4	249.7	257.6	264.8	269.0
	SD	8.0	12.7	12.4	14.3	17.3	17.3	18.7	16.3
	N	15	15	15	15	15	15	15	15
2F	Mean	160.3	207.5	233.3	247.8	248.4	259.0	262.6	267.3
	SD	4.5	11.8	9.7	10.1	9.9	12.8	10.0	8.5
	N	10	10	10	10	10	10	10	10
	%Diff G1	-0.5	-0.8	-0.2	0.6	-0.5	0.5	-0.8	-0.6
3F	Mean	160.2	215.2	244.4	261.8	259.2	267.1	269.6	281.5
	SD	4.9	8.1	14.4	17.8	16.5	18.6	19.6	17.2
	N	10	10	10	10	10	10	10	10
	%Diff G1	-0.5	2.9	4.6	6.3	3.8	3.7	1.8	4.6
4F	Mean	161.2	207.1	231.9	249.3	248.5	258.3	257.1	266.9
	SD	7.7	9.9	12.8	15.4	17.2	19.5	19.4	19.7
	N	15	15	15	15	15	15	15	15
	%Diff G1	0.1	-1.0	-0.7	1.2	-0.5	0.3	-2.9	-0.8

Table 2

Summary of Body Weights (g)

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		Day 35	Day 42
1F	Mean	268.2	281.6
	SD	15.0	13.2
	N	5	5
2F	Mean	--	--
	SD	--	--
	N	--	--
	%Diff G1	--	--
3F	Mean	--	--
	SD	--	--
	N	--	--
	%Diff G1	--	--
4F	Mean	267.0	279.8
	SD	11.6	13.4
	N	5	5
	%Diff G1	-0.4	-0.6

Table 3

Summary of Body Weight Gains (g)

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		Day							
		Change -44 - -30	Change -30 - -16	Change -16 - -1	Change -1 - 7	Change 7 - 14	Change 14 - 21	Change 21 - 28	Change -1 - 28
1M	Mean	106.9	81.1	58.3	19.9	18.0	22.1	16.8	76.8
	SD	10.5	14.3	9.9	5.2	6.5	4.4	6.1	14.4
	N	15	15	15	15	15	15	15	15
2M	Mean	107.1	78.1	54.2	11.4e	21.8	16.5d	17.8	67.5
	SD	8.2	10.1	7.1	4.2	5.0	2.6	5.6	11.5
	N	10	10	10	10	10	10	10	10
3M	Mean	109.8	87.4	63.7	0.3f	29.8c	4.4f	29.4f	63.9d
	SD	10.5	12.8	15.1	5.7	3.1	5.4	5.4	12.4
	N	10	10	10	10	10	10	10	10
4M	Mean	101.4	77.1	55.9	-11.5f	29.1c	0.3f	28.5f	46.4f
	SD	7.1	7.5	6.6	6.2	3.2	4.8	6.1	8.3
	N	15	15	15	15	15	15	15	15

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)
d=p≤0.05,e=p≤0.01,f=p≤0.001 (Dunnett)

Table 3

Summary of Body Weight Gains (g)

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		Day Change 28 - 35	Change 35 - 42	Change 28 - 42
1M	Mean	16.2	19.8	36.0
	SD	4.9	3.1	4.0
	N	5	5	5
2M	Mean	--	--	--
	SD	--	--	--
	N	--	--	--
3M	Mean	--	--	--
	SD	--	--	--
	N	--	--	--
4M	Mean	1.4b	31.2c	32.6
	SD	7.2	3.6	8.0
	N	5	5	5

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (T-test)

Table 3

Summary of Body Weight Gains (g)

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		Day							
		Change -45 - -30	Change -30 - -16	Change -16 - -1	Change -1 - 7	Change 7 - 14	Change 14 - 21	Change 21 - 28	Change -1 - 28
1F	Mean	48.1	24.5	12.7	3.3	7.9	7.2	4.2	22.6
	SD	12.5	5.8	6.9	7.8	5.2	7.2	6.7	6.3
	N	15	15	15	15	15	15	15	15
2F	Mean	47.2	25.8	14.5	0.6	10.6	3.6	4.7	19.5
	SD	8.6	7.0	4.9	4.6	9.1	4.9	5.5	6.9
	N	10	10	10	10	10	10	10	10
3F	Mean	55.0	29.2	17.4	-2.6	7.9	2.5	11.9b	19.7
	SD	7.5	8.2	6.6	6.6	5.2	7.8	5.3	5.7
	N	10	10	10	10	10	10	10	10
4F	Mean	45.9	24.9	17.3	-0.7	9.8	-1.3b	9.9a	17.7
	SD	7.9	5.8	5.1	7.1	5.1	6.5	4.4	11.1
	N	15	15	15	15	15	15	15	15

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunnett)

Table 3

Summary of Body Weight Gains (g)

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		Day Change 28 - 35	Change 35 - 42	Change 28 - 42
1F	Mean	-0.4	13.4	13.0
	SD	5.0	3.0	4.0
	N	5	5	5
2F	Mean	--	--	--
	SD	--	--	--
	N	--	--	--
3F	Mean	--	--	--
	SD	--	--	--
	N	--	--	--
4F	Mean	-3.4	12.8	9.4
	SD	6.4	3.5	6.8
	N	5	5	5

Table 4

Summary of Food Consumption (g/animal/day)

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		Day (From/To)						
		-7/1	1/8	8/15	15/22	22/29	29/36	36/42
1M	Mean	29.87	28.84	30.93	30.12	29.99	27.32	29.28
	SD	1.79	1.92	1.84	1.97	1.85	0.16	0.16
	N	15	15	15	15	15	5	5
2M	Mean	28.16	26.11	29.07	27.32	28.37	--	--
	SD	1.64	1.17	1.09	1.17	1.14	--	--
	N	10	10	10	10	10	--	--
	%Diff G1	-5.71	-9.47	-6.00	-9.30	-5.39	--	--
3M	Mean	29.98	25.44	30.06	25.52	30.30	--	--
	SD	2.34	2.19	2.08	2.13	2.06	--	--
	N	10	10	10	10	10	--	--
	%Diff G1	0.38	-11.79	-2.80	-15.27	1.04	--	--
4M	Mean	28.69	22.42	33.55	29.14	33.25	27.72	34.80
	SD	1.60	1.49	2.01	2.47	1.78	1.75	1.37
	N	15	15	15	15	15	5	5
	%Diff G1	-3.93	-22.26	8.49	-3.25	10.89	1.46	18.85

Table 4

Summary of Food Consumption (g/animal/day)

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		Day (From/To)						
		-7/1	1/8	8/15	15/22	22/29	29/36	36/42
1F	Mean	18.75	18.25	19.02	19.27	19.21	17.06	19.84
	SD	0.89	1.61	1.07	1.31	1.22	0.22	0.88
	N	15	15	15	15	15	5	5
2F	Mean	19.09	17.21	19.03	17.78	18.57	--	--
	SD	0.66	0.68	1.64	0.70	0.90	--	--
	N	10	10	10	10	10	--	--
	%Diff G1	1.80	-5.72	0.05	-7.72	-3.35	--	--
3F	Mean	19.36	16.94	19.33	17.49	19.47	--	--
	SD	1.02	0.93	1.23	0.81	0.57	--	--
	N	10	10	10	10	10	--	--
	%Diff G1	3.23	-7.20	1.63	-9.22	1.34	--	--
4F	Mean	19.75	18.44	25.15	21.81	24.74	21.76	26.24
	SD	1.59	2.62	1.89	2.31	1.92	0.33	0.05
	N	15	15	15	15	15	5	5
	%Diff G1	5.30	1.02	32.21	13.22	28.76	27.55	32.26

Table 5

Summary of Body Temperature Values

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Parameter: Body Temp
°C

Group / Sex		Day 1 (pr)	Day 1 (p)	Day 2	Day 29 (pr)	Day 29 (p)	Day 30
1M	Mean	37.36	37.50	36.52	36.28	37.06	36.65
	SD	0.38	0.53	0.11	0.78	0.44	0.40
	N	15	15	15	15	15	15
2M	Mean	36.97	37.97d	36.75	36.94	37.92f	37.60
	SD	0.49	0.47	0.81	0.87	0.39	0.66
	N	10	10	10	10	10	10
	%Diff G1	-1.04	1.25	0.63	1.82	2.32	2.58
3M	Mean	36.97	38.30f	37.54b	36.40	38.54f	38.70c
	SD	0.34	0.61	0.46	0.60	0.33	0.52
	N	10	10	10	10	10	10
	%Diff G1	-1.04	2.13	2.79	0.33	3.99	5.58
4M	Mean	37.09	38.73f	37.77c	36.81	38.66f	38.86c
	SD	0.50	0.25	0.69	0.51	0.41	0.34
	N	15	15	15	15	15	15
	%Diff G1	-0.71	3.29	3.43	1.45	4.32	6.02

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)
d=p≤0.05,e=p≤0.01,f=p≤0.001 (Dunnett)

Table 5

Summary of Body Temperature Values

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Parameter: Body Temp
°C

Group / Sex		Day 1 (pr)	Day 1 (p)	Day 2	Day 3	Day 29 (pr)	Day 29 (p)	Day 30
1F	Mean	38.58	37.83	37.03	--	38.19	37.01	37.39
	SD	0.44	0.76	0.49	--	0.81	0.87	0.65
	N	15	15	15	--	15	15	15
2F	Mean	38.51	38.36	37.72e	--	38.55	38.06a	38.25f
	SD	0.33	0.65	0.59	--	0.91	0.90	0.44
	N	10	10	10	--	10	10	10
	%Diff G1	-0.18	1.41	1.87	--	0.95	2.83	2.31
3F	Mean	37.88a	38.56	38.54f	--	37.85	38.16a	38.97f
	SD	0.51	0.63	0.38	--	1.04	0.42	0.39
	N	10	10	10	--	10	10	10
	%Diff G1	-1.81	1.94	4.09	--	-0.88	3.10	4.24
4F	Mean	38.06	39.15c	39.30f	37.85	38.04	38.69c	39.21f
	SD	0.64	0.37	0.53	0.45	0.89	0.50	0.41
	N	15	15	15	15	15	15	15
	%Diff G1	-1.35	3.51	6.14	--	-0.38	4.52	4.89

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)
d=p≤0.05,e=p≤0.01,f=p≤0.001 (Dunnett)

Table 5

Summary of Body Temperature Values

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Parameter: Body Temp
°C

Group / Sex		Day 31
1F	Mean	--
	SD	--
	N	--
2F	Mean	--
	SD	--
	N	--
	%Diff G1	--
3F	Mean	--
	SD	--
	N	--
	%Diff G1	--
4F	Mean	37.40
	SD	0.44
	N	3
	%Diff G1	--

Table 6

Summary of Hematology Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		WBC 10^3/uL	NEUT 10^3/uL	LYMPH 10^3/uL	MONO 10^3/uL	EOS 10^3/uL	BASO 10^3/uL	LUC 10^3/uL
1M	Mean	7.084	0.991	5.814	0.149	0.084	0.008	0.041
	SD	2.122	0.239	1.935	0.040	0.036	0.007	0.021
	N	9	9	9	9	9	9	9
2M	Mean	15.186f	9.890a	4.528	0.305e	0.248f	0.017a	0.194c
	SD	1.821	1.833	0.641	0.106	0.067	0.008	0.108
	N	10	10	10	10	10	10	10
	%Diff G1	114.357	897.870	-22.125	104.851	193.684	118.571	371.892
3M	Mean	16.464f	12.879c	2.844b	0.320e	0.192d	0.016	0.208c
	SD	2.122	1.496	0.881	0.159	0.126	0.010	0.067
	N	10	10	10	10	10	10	10
	%Diff G1	132.396	1199.451	-51.087	114.925	127.368	105.714	405.946
4M	Mean	13.105f	11.159c	1.523c	0.217	0.092	0.008	0.122
	SD	2.661	2.391	0.610	0.085	0.080	0.004	0.037
	N	10	10	10	10	10	10	9
	%Diff G1	84.983	1025.908	-73.807	45.746	8.947	2.857	197.297

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)
d=p≤0.05,e=p≤0.01,f=p≤0.001 (Dunnett)

Table 6

Summary of Hematology Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		RBC 10^6/uL	HGB g/dL	HCT %	MCV fL(um3)	MCH pg	MCHC g/dL	RDW %
1M	Mean	8.488	14.28	42.51	50.13	16.82	33.57	13.30
	SD	0.360	0.63	1.92	1.65	0.50	0.46	0.72
	N	9	9	9	9	9	9	9
2M	Mean	8.329	14.29	42.57	51.16	17.20	33.61	13.89
	SD	0.620	0.93	2.83	1.39	0.57	0.79	0.45
	N	10	10	10	10	10	10	10
	%Diff G1	-1.871	0.09	0.14	2.05	2.25	0.13	4.44
3M	Mean	8.638	14.80	44.18	51.20	17.18	33.55	14.56c
	SD	0.922	1.30	4.72	1.64	0.59	0.78	0.78
	N	10	10	10	10	10	10	10
	%Diff G1	1.770	3.66	3.93	2.13	2.13	-0.05	9.47
4M	Mean	8.600	15.06	44.74	52.03	17.50	33.62	14.44b
	SD	0.294	0.42	1.28	1.07	0.50	0.53	0.58
	N	10	10	10	10	10	10	10
	%Diff G1	1.322	5.48	5.24	3.78	4.03	0.16	8.57

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunnett)

Table 6

Summary of Hematology Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		PLT 10^3/uL	RETIC 10^9/L
1M	Mean	1168.0	235.01
	SD	104.9	27.53
	N	9	9
2M	Mean	1122.6	204.71
	SD	188.8	36.08
	N	10	10
	%Diff G1	-3.9	-12.89
3M	Mean	1033.2	188.54b
	SD	248.2	39.83
	N	10	10
	%Diff G1	-11.5	-19.77
4M	Mean	1100.6	169.55c
	SD	153.2	21.72
	N	10	10
	%Diff G1	-5.8	-27.85

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunnett)

Table 6

Summary of Hematology Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		WBC 10^3/uL	NEUT 10^3/uL	LYMPH 10^3/uL	MONO 10^3/uL	EOS 10^3/uL	BASO 10^3/uL	LUC 10^3/uL
1F	Mean	4.257	0.715	3.373	0.084	0.049	0.004	0.035
	SD	1.366	0.490	0.903	0.052	0.009	0.005	0.029
	N	10	10	10	10	10	10	10
2F	Mean	10.775f	6.904c	3.288	0.147d	0.318c	0.010	0.104e
	SD	2.577	1.569	1.224	0.058	0.134	0.008	0.043
	N	10	10	10	10	10	10	10
	%Diff G1	153.113	865.594	-2.520	75.000	548.980	150.000	197.143
3F	Mean	7.632e	5.640c	1.611a	0.107	0.185b	0.005	0.083d
	SD	2.775	2.417	0.818	0.051	0.115	0.005	0.053
	N	10	10	10	10	10	10	10
	%Diff G1	79.281	688.811	-52.238	27.381	277.551	25.000	137.143
4F	Mean	3.844	2.829	0.820c	0.071	0.080	0.000	0.046
	SD	2.201	1.908	0.333	0.049	0.047	0.000	0.040
	N	10	10	10	10	10	10	9
	%Diff G1	-9.702	295.664	-75.689	-15.476	63.265	-100.000	30.159

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)
d=p≤0.05,e=p≤0.01,f=p≤0.001 (Dunnett)

Table 6

Summary of Hematology Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		RBC 10^6/uL	HGB g/dL	HCT %	MCV fL(um3)	MCH pg	MCHC g/dL	RDW %
1F	Mean	7.536	13.44	38.98	51.73	17.81	34.45	11.51
	SD	0.278	0.45	0.96	1.30	0.60	0.56	0.49
	N	10	10	10	10	10	10	10
2F	Mean	7.702	13.64	39.61	51.49	17.72	34.42	12.16a
	SD	0.399	0.52	1.35	1.32	0.41	0.27	0.30
	N	10	10	10	10	10	10	10
	%Diff G1	2.203	1.49	1.62	-0.46	-0.51	-0.09	5.65
3F	Mean	8.107b	14.38b	41.90b	51.72	17.75	34.34	12.77c
	SD	0.466	0.71	1.94	1.50	0.56	0.38	0.41
	N	10	10	10	10	10	10	10
	%Diff G1	7.577	6.99	7.49	-0.02	-0.34	-0.32	10.95
4F	Mean	8.294c	14.87c	43.50c	52.47	17.95	34.18	13.01c
	SD	0.430	0.74	2.41	0.85	0.27	0.48	0.69
	N	10	10	10	10	10	10	10
	%Diff G1	10.058	10.64	11.60	1.43	0.79	-0.78	13.03

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunnett)

Table 6

Summary of Hematology Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		PLT 10^3/uL	RETIC 10^9/L
1F	Mean	1123.4	170.35
	SD	83.9	41.20
	N	10	10
2F	Mean	1132.7	152.85
	SD	211.3	26.33
	N	10	10
	%Diff G1	0.8	-10.27
3F	Mean	1081.1	166.54
	SD	178.1	23.41
	N	10	10
	%Diff G1	-3.8	-2.24
4F	Mean	902.5b	139.73
	SD	56.0	26.78
	N	10	10
	%Diff G1	-19.7	-17.97

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunnett)

Table 6

Summary of Hematology Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		WBC 10^3/uL	NEUT 10^3/uL	LYMPH 10^3/uL	MONO 10^3/uL	EOS 10^3/uL	BASO 10^3/uL	LUC 10^3/uL
1M	Mean	7.672	1.184	6.160	0.162	0.086	0.012	0.064
	SD	2.094	0.394	1.925	0.053	0.021	0.004	0.011
	N	5	5	5	5	5	5	5
4M	Mean	8.658	1.966	6.276	0.238	0.074	0.008	0.094
	SD	0.939	0.945	0.607	0.055	0.017	0.004	0.032
	N	5	5	5	5	5	5	5
	%Diff G1	12.852	66.047	1.883	46.914	-13.953	-33.333	46.875

Table 6

Summary of Hematology Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		RBC 10^6/uL	HGB g/dL	HCT %	MCV fL(um3)	MCH pg	MCHC g/dL	RDW %
1M	Mean	8.070	13.90	41.98	52.02	17.22	33.12	12.96
	SD	0.099	0.12	0.43	0.66	0.24	0.31	0.53
	N	5	5	5	5	5	5	5
4M	Mean	7.782	13.66	41.48	53.36	17.56	32.88	15.04b
	SD	0.402	0.47	1.63	1.57	0.59	0.30	0.79
	N	5	5	5	5	5	5	5
	%Diff G1	-3.569	-1.73	-1.19	2.58	1.97	-0.72	16.05

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (T-test)

Table 6

Summary of Hematology Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		PLT 10^3/uL	RETIC 10^9/L
1M	Mean	1221.6	224.28
	SD	104.7	33.57
	N	5	5
4M	Mean	1251.6	272.36a
	SD	132.3	22.30
	N	5	5
	%Diff G1	2.5	21.44

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (T-test)

Table 6

Summary of Hematology Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		WBC 10^3/uL	NEUT 10^3/uL	LYMPH 10^3/uL	MONO 10^3/uL	EOS 10^3/uL	BASO 10^3/uL	LUC 10^3/uL
1F	Mean	4.772	0.754	3.786	0.130	0.050	0.002	0.054
	SD	1.455	0.221	1.206	0.070	0.010	0.004	0.028
	N	5	5	5	5	5	5	5
4F	Mean	5.442	1.186	3.988	0.152	0.048	0.006	0.064
	SD	1.855	0.552	1.327	0.083	0.015	0.005	0.029
	N	5	5	5	5	5	5	5
	%Diff G1	14.040	57.294	5.335	16.923	-4.000	200.000	18.519

Table 6

Summary of Hematology Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		RBC 10^6/uL	HGB g/dL	HCT %	MCV fL(um3)	MCH pg	MCHC g/dL	RDW %
1F	Mean	7.172	12.82	37.82	52.74	17.86	33.90	11.52
	SD	0.329	0.60	1.81	0.34	0.34	0.83	0.19
	N	5	5	5	5	5	5	5
4F	Mean	6.762	12.24	36.60	54.18	18.08	33.44	13.12c
	SD	0.291	0.40	1.03	1.54	0.39	0.29	0.31
	N	5	5	5	5	5	5	5
	%Diff G1	-5.717	-4.52	-3.23	2.73	1.23	-1.36	13.89

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (T-test)

Table 6

Summary of Hematology Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		PLT 10^3/uL	RETIC 10^9/L
1F	Mean	1111.0	170.62
	SD	102.5	21.23
	N	5	5
4F	Mean	1174.8	198.28
	SD	116.2	31.31
	N	5	5
	%Diff G1	5.7	16.21

Table 7

Summary of Coagulation Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		PT sec	APTT sec	FIB mg/dL
1M	Mean	17.20	15.07	280.2
	SD	0.78	0.55	21.9
	N	10	10	10
2M	Mean	16.06e	17.44f	656.2b
	SD	0.74	0.80	94.8
	N	10	10	10
	%Diff G1	-6.63	15.73	134.2
3M	Mean	16.44	18.15f	686.5c
	SD	0.79	1.36	87.6
	N	10	10	10
	%Diff G1	-4.42	20.44	145.0
4M	Mean	16.53	19.06f	652.8c
	SD	0.88	0.96	54.9
	N	10	10	10
	%Diff G1	-3.90	26.48	133.0

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)
d=p≤0.05,e=p≤0.01,f=p≤0.001 (Dunnett)

Table 7

Summary of Coagulation Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		PT sec	APTT sec	FIB mg/dL
1F	Mean	17.86	14.94	188.1
	SD	0.59	0.65	16.0
	N	10	10	10
2F	Mean	17.22	18.64f	454.1b
	SD	0.54	0.86	38.7
	N	9	9	9
	%Diff G1	-3.57	24.80	141.4
3F	Mean	18.11	20.31f	483.6c
	SD	1.20	1.71	37.5
	N	10	10	10
	%Diff G1	1.40	35.94	157.1
4F	Mean	19.73	20.77f	446.1c
	SD	2.13	1.60	92.1
	N	10	10	10
	%Diff G1	10.47	39.02	137.2

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)
d=p≤0.05,e=p≤0.01,f=p≤0.001 (Dunnett)

Table 7

Summary of Coagulation Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		PT sec	APTT sec	FIB mg/dL
1M	Mean	17.08	14.14	273.8
	SD	0.86	0.47	13.7
	N	5	5	5
4M	Mean	17.96	14.60	249.8a
	SD	0.34	0.66	15.4
	N	5	5	5
	%Diff G1	5.15	3.25	-8.8

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (T-test)

Table 7

Summary of Coagulation Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		PT sec	APTT sec	FIB mg/dL
1F	Mean	17.34	15.34	160.8
	SD	0.23	0.85	17.0
	N	5	5	5
4F	Mean	17.80	14.82	162.4
	SD	0.73	1.20	20.5
	N	5	5	5
	%Diff G1	2.65	-3.39	1.0

Table 8

Summary of Clinical Chemistry Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		AST U/L	ALT U/L	ALP U/L	GGT U/L	CK U/L	TBIL mg/dL	UREAN mg/dL
1M	Mean	88.8	39.4	96.2	2.0	356.0	0.065	16.5
	SD	23.9	5.9	22.7	0.0	216.2	0.015	3.2
	N	10	10	10	10	9	10	10
2M	Mean	96.0	37.1	88.8	2.0	499.3	0.077	18.6
	SD	23.5	3.9	16.4	0.0	301.4	0.030	2.9
	N	10	10	10	10	10	10	10
	%Diff G1	8.1	-5.8	-7.7	0.0	40.3	18.462	12.7
3M	Mean	107.1	42.8	120.6	2.0	567.9	0.089	16.9
	SD	33.3	8.9	28.3	0.0	438.7	0.016	3.2
	N	10	10	10	10	10	10	10
	%Diff G1	20.6	8.6	25.4	0.0	59.5	36.923	2.4
4M	Mean	116.7	42.4	130.1b	2.0	618.7	0.078	15.9
	SD	32.9	7.0	23.9	0.0	527.7	0.021	2.6
	N	10	10	10	10	9	10	10
	%Diff G1	31.4	7.6	35.2	0.0	73.8	20.000	-3.6

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunnett)

Table 8

Summary of Clinical Chemistry Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		CREAT mg/dL	GLUC mg/dL	CHOL mg/dL	TRIG mg/dL	TPROT g/dL	ALB g/dL	GLOB g/dL
1M	Mean	0.38	203.9	67.6	72.4	6.17	3.80	2.37
	SD	0.04	25.0	11.5	26.1	0.23	0.15	0.28
	N	10	10	10	10	10	10	10
2M	Mean	0.42	170.0e	79.5	49.1	6.26	3.48f	2.78e
	SD	0.04	26.4	20.2	12.7	0.26	0.10	0.21
	N	10	10	10	10	10	10	10
	%Diff G1	10.53	-16.6	17.6	-32.2	1.46	-8.42	17.30
3M	Mean	0.45a	163.3f	82.5	69.4	6.30	3.47f	2.83f
	SD	0.05	20.2	18.8	35.8	0.33	0.18	0.28
	N	10	10	10	10	10	10	10
	%Diff G1	18.42	-19.9	22.0	-4.1	2.11	-8.68	19.41
4M	Mean	0.45a	148.0f	78.9	58.2	6.18	3.43f	2.75e
	SD	0.05	16.9	21.6	18.1	0.23	0.08	0.25
	N	10	10	10	10	10	10	10
	%Diff G1	18.42	-27.4	16.7	-19.6	0.16	-9.74	16.03

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)
d=p≤0.05,e=p≤0.01,f=p≤0.001 (Dunnett)

Table 8

Summary of Clinical Chemistry Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		A/G ratio	CA mg/dL	PHOS mg/dL	NA mmol/L	K mmol/L	CL mmol/L
1M	Mean	1.63	10.58	6.59	141.2	5.13	100.8
	SD	0.23	0.44	0.45	1.2	0.29	1.4
	N	10	10	10	10	10	10
2M	Mean	1.27b	10.73	7.27d	140.4	5.23	99.6
	SD	0.11	0.31	0.53	0.8	0.31	1.2
	N	10	10	10	10	10	10
	%Diff G1	-22.09	1.42	10.32	-0.6	1.95	-1.2
3M	Mean	1.24b	10.65	7.61e	140.3	5.61e	99.7
	SD	0.13	0.43	0.64	1.9	0.27	2.4
	N	10	10	10	10	10	10
	%Diff G1	-23.93	0.66	15.48	-0.6	9.36	-1.1
4M	Mean	1.26b	10.37	7.52e	140.9	5.57e	100.8
	SD	0.13	0.44	0.75	1.8	0.31	2.0
	N	10	10	10	10	10	10
	%Diff G1	-22.70	-1.98	14.11	-0.2	8.58	0.0

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)
d=p≤0.05,e=p≤0.01,f=p≤0.001 (Dunnett)

Table 8

Summary of Clinical Chemistry Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		AST U/L	ALT U/L	ALP U/L	GGT U/L	CK U/L	TBIL mg/dL	UREAN mg/dL
1F	Mean	138.4	59.6	54.5	2.0	333.1	0.056	14.2
	SD	157.7	78.6	12.3	0.0	241.9	0.024	2.4
	N	10	10	10	10	10	10	10
2F	Mean	95.2	37.0	67.5	2.0	397.4	0.065	20.3c
	SD	16.7	8.1	14.2	0.0	251.7	0.021	3.7
	N	10	10	10	10	10	10	10
	%Diff G1	-31.2	-37.9	23.9	0.0	19.3	16.071	43.0
3F	Mean	152.6	50.9	73.0a	2.0	465.7	0.085a	15.5
	SD	129.1	21.9	16.1	0.0	429.0	0.030	2.9
	N	10	10	10	10	10	10	10
	%Diff G1	10.3	-14.6	33.9	0.0	39.8	51.786	9.2
4F	Mean	214.0	61.4	88.6c	2.0	258.5	0.082	15.7
	SD	206.5	31.3	14.2	0.0	154.7	0.021	4.0
	N	10	10	10	10	10	10	10
	%Diff G1	54.6	3.0	62.6	0.0	-22.4	46.429	10.6

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunnett)

Table 8

Summary of Clinical Chemistry Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		CREAT mg/dL	GLUC mg/dL	CHOL mg/dL	TRIG mg/dL	TPROT g/dL	ALB g/dL	GLOB g/dL
1F	Mean	0.43	188.1	74.1	46.4	6.61	4.48	2.13
	SD	0.05	29.2	15.7	15.1	0.34	0.27	0.25
	N	10	10	10	10	10	10	10
2F	Mean	0.45	173.2	78.9	48.4	6.45	4.05b	2.40d
	SD	0.05	21.8	11.3	17.8	0.22	0.05	0.21
	N	10	10	10	10	10	10	10
	%Diff G1	4.65	-7.9	6.5	4.3	-2.42	-9.60	12.68
3F	Mean	0.45	145.4f	79.9	55.9	6.49	4.14	2.35
	SD	0.05	20.6	20.8	18.2	0.53	0.37	0.20
	N	10	10	10	10	10	10	10
	%Diff G1	4.65	-22.7	7.8	20.5	-1.82	-7.59	10.33
4F	Mean	0.44	135.3f	64.6	49.5	6.00e	3.82c	2.18
	SD	0.05	16.8	24.2	25.7	0.44	0.36	0.22
	N	10	10	10	10	10	10	10
	%Diff G1	2.33	-28.1	-12.8	6.7	-9.23	-14.73	2.35

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)
d=p≤0.05,e=p≤0.01,f=p≤0.001 (Dunnett)

Table 8

Summary of Clinical Chemistry Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1706 10 µg/dose
Group 3 - mRNA-1706 50 µg/dose
Group 4 - mRNA-1706 100 µg/dose

Group / Sex		A/G ratio	CA mg/dL	PHOS mg/dL	NA mmol/L	K mmol/L	CL mmol/L
1F	Mean	2.14	10.77	6.31	142.5	4.49	103.5
	SD	0.33	0.37	0.91	1.5	0.24	2.0
	N	10	10	10	10	10	10
2F	Mean	1.68c	10.86	6.37	141.0d	4.90	101.4
	SD	0.16	0.15	0.76	0.9	0.31	1.6
	N	10	10	10	10	10	10
	%Diff G1	-21.50	0.84	0.95	-1.1	9.13	-2.0
3F	Mean	1.77a	10.80	7.76e	141.9	4.82	101.2
	SD	0.11	0.38	0.92	1.2	0.48	1.8
	N	10	10	10	10	10	10
	%Diff G1	-17.29	0.28	22.98	-0.4	7.35	-2.2
4F	Mean	1.77a	10.32e	7.47e	140.0f	4.64	101.9
	SD	0.23	0.30	0.72	1.2	0.31	2.5
	N	10	10	10	10	10	10
	%Diff G1	-17.29	-4.18	18.38	-1.8	3.34	-1.5

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)
d=p≤0.05,e=p≤0.01,f=p≤0.001 (Dunnett)

Table 8

Summary of Clinical Chemistry Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		AST U/L	ALT U/L	ALP U/L	GGT U/L	CK U/L	TBIL mg/dL	UREAN mg/dL
1M	Mean	91.0	43.4	88.4	2.0	445.6	0.058	14.4
	SD	22.2	6.6	17.5	0.0	297.8	0.013	1.9
	N	5	5	5	5	5	5	5
4M	Mean	84.4	37.4	90.6	2.0	355.2	0.058	13.8
	SD	22.6	6.2	5.2	0.0	298.5	0.004	1.8
	N	5	5	5	5	5	5	5
	%Diff G1	-7.3	-13.8	2.5	0.0	-20.3	0.000	-4.2

Table 8

Summary of Clinical Chemistry Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		CREAT mg/dL	GLUC mg/dL	CHOL mg/dL	TRIG mg/dL	TPROT g/dL	ALB g/dL	GLOB g/dL
1M	Mean	0.32	219.0	91.8	73.0	6.12	3.72	2.40
	SD	0.04	42.4	19.1	14.3	0.23	0.18	0.12
	N	5	5	5	5	5	5	5
4M	Mean	0.36	221.0	65.4a	59.4	5.98	3.76	2.22
	SD	0.05	70.0	13.3	7.5	0.22	0.11	0.20
	N	5	5	5	5	5	5	5
	%Diff G1	12.50	0.9	-28.8	-18.6	-2.29	1.08	-7.50

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (T-test)

Table 8

Summary of Clinical Chemistry Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		A/G ratio	CA mg/dL	PHOS mg/dL	NA mmol/L	K mmol/L	CL mmol/L
1M	Mean	1.56	10.66	7.24	139.2	5.04	101.2
	SD	0.11	0.13	0.56	1.6	0.30	1.5
	N	5	5	5	5	5	5
4M	Mean	1.70	10.44	7.72	139.2	5.28	101.6
	SD	0.20	0.25	0.15	1.9	0.47	1.7
	N	5	5	5	5	5	5
	%Diff G1	8.97	-2.06	6.63	0.0	4.76	0.4

Table 8

Summary of Clinical Chemistry Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		AST U/L	ALT U/L	ALP U/L	GGT U/L	CK U/L	TBIL mg/dL	UREAN mg/dL
1F	Mean	120.2	47.8	73.6	2.0	709.0	0.050	17.2
	SD	15.4	5.0	23.4	0.0	273.7	0.012	2.2
	N	5	5	5	5	5	5	5
4F	Mean	83.8b	42.6	71.0	2.0	216.8b	0.036	14.8
	SD	17.1	8.4	9.6	0.0	113.5	0.026	0.8
	N	5	5	5	5	5	5	5
	%Diff G1	-30.3	-10.9	-3.5	0.0	-69.4	-28.000	-14.0

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (T-test)

Table 8

Summary of Clinical Chemistry Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		CREAT mg/dL	GLUC mg/dL	CHOL mg/dL	TRIG mg/dL	TPROT g/dL	ALB g/dL	GLOB g/dL
1F	Mean	0.46	173.0	72.8	54.2	6.50	4.50	2.00
	SD	0.09	26.6	20.5	14.3	0.36	0.29	0.16
	N	5	5	5	5	5	5	5
4F	Mean	0.36	217.0a	73.2	59.4	6.30	4.30	2.00
	SD	0.05	23.9	20.6	20.1	0.32	0.23	0.20
	N	5	5	5	5	5	5	5
	%Diff G1	-21.74	25.4	0.5	9.6	-3.08	-4.44	0.00

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (T-test)

Table 8

Summary of Clinical Chemistry Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1706 100 µg/dose

Group / Sex		A/G ratio	CA mg/dL	PHOS mg/dL	NA mmol/L	K mmol/L	CL mmol/L
1F	Mean	2.26	10.18	6.76	140.0	4.54	102.4
	SD	0.24	0.18	1.07	0.7	0.28	1.8
	N	5	5	5	5	5	5
4F	Mean	2.16	10.38	7.16	139.4	4.76	103.0
	SD	0.27	0.23	0.87	1.1	0.29	1.0
	N	5	5	5	5	5	5
	%Diff G1	-4.42	1.96	5.92	-0.4	4.85	0.6