charles river

FINAL REPORT

Test Facility Study No. 5002033

A 1-month (3 doses) Study of mRNA-1653 by Intramuscular Injection in Sprague Dawley Rat with a 2-Week Recovery Period

SPONSOR:
Moderna Therapeutics, Inc.
200 Technology Square, Third Floor
Cambridge, MA 02139
USA

TEST FACILITY:
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QUALITY ASSURANCE STATEMENT

Study Number: 5002033

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

		Dates Findings Submitted to:	
Date(s) of Audit	Phase(s) Audited	Study Director	Study Director Management
06-Apr-2017	Final Study Plan	10-Apr-2017	10-Apr-2017
10-Apr-2017	Study Schedule	10-Apr-2017	10-Apr-2017
19-Apr-2017	Addition of Study Plan to Provantis	19-Apr-2017	19-Apr-2017
19-Apr-2017	Dose Preparation	19-Apr-2017	19-Apr-2017
19-Apr-2017	Study Plan Amendment 1	19-Apr-2017	19-Apr-2017
21-Apr-2017	Draize Evaluation	28-Apr-2017	28-Apr-2017
05-May-2017	Study Plan Amendment 2	05-May-2017	05-May-2017
19-May-2017	Blood Collection	19-May-2017	19-May-2017
19-May-2017	Necropsy	19-May-2017	19-May-2017
24-May-2017	Tissue Trimming	24-May-2017	24-May-2017
28-Jun-2017	Study Plan Amendment 3	28-Jun-2017	28-Jun-2017
29-Jun-2017 - 10-Jul-2017	Data Review - Animal Care	11-Jul-2017	11-Jul-2017
29-Jun-2017 - 10-Jul-2017	Data Review - Formulations	11-Jul-2017	11-Jul-2017
29-Jun-2017 - 11-Jul-2017	Data Review - Technical Operations	11-Jul-2017	11-Jul-2017
29-Jun-2017 - 11-Jul-2017	Data Review - Technical Operations	11-Jul-2017	11-Jul-2017
29-Jun-2017 - 11-Jul-2017	Data Review - Shipping/Receiving	11-Jul-2017	11-Jul-2017
29-Jun-2017 - 11-Jul-2017	Data Review - Clinical Pathology	11-Jul-2017	11-Jul-2017
29-Jun-2017 - 11-Jul-2017	Data Review - Veterinary Services	11-Jul-2017	11-Jul-2017
29-Jun-2017 - 11-Jul-2017	Draft Report - Materials and Methods	11-Jul-2017	11-Jul-2017
29-Jun-2017 - 11-Jul-2017	Draft Phase Report - Ophthalmology	11-Jul-2017	11-Jul-2017
29-Jun-2017 - 11-Jul-2017	Report Preparation	11-Jul-2017	11-Jul-2017
04-Jul-2017 - 05-Jul-2017	Data Review - Analytical Chemistry	06-Jul-2017	06-Jul-2017
05-Jul-2017	Draft Phase Report - Dose Formulation Analysis	05-Jul-2017	05-Jul-2017
16-Aug-2017	Data Review - Bioanalysis & Immunology	17-Aug-2017	17-Aug-2017
16-Aug-2017	Final Phase Report - Immunology	17-Aug-2017	17-Aug-2017
06-Sep-2017 - 07-Sep-2017	Data Review - Necropsy	07-Sep-2017	07-Sep-2017
06-Sep-2017 - 07-Sep-2017	Data Review - Shipping/Receiving	07-Sep-2017	07-Sep-2017
06-Sep-2017 - 07-Sep-2017	Data Review - Histology	08-Sep-2017	08-Sep-2017
06-Sep-2017 - 07-Sep-2017	Report Preparation	07-Sep-2017	07-Sep-2017
07-Sep-2017	Draft Phase Report - Pathology	08-Sep-2017	08-Sep-2017

QUALITY ASSURANCE STATEMENT - Study Number: 5002033

QA INSPECTION DATES

Date(s) of Audit	Phase(s) Audited	Dates Findings Submitted to: Study Director	Study Director Management
25-Sep-2017	Study Plan Amendment 4	25-Sep-2017	25-Sep-2017
25-Sep-2017	Draft Report - Results	26-Sep-2017	26-Sep-2017
05-Oct-2017	Final Report	05-Oct-2017	05-Oct-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), Japan (MHLW), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Exceptions to GLPs included the following study elements:

- Characterization of the Test Item was performed by the Sponsor 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

1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Study Director [redacted]

Test Facility Management

1.2. Individual Scientists (IS) at Test Facility

Analytical Chemistry
(Concentration and
Particle size Analysis) [redacted]
CR MTL, Senneville, QC

Ophthalmology [redacted]
CR MTL, Senneville, QC

Biomarkers
(IL-1ß, IL-6, TNF-α,
IP-10, MIP-1-α, MCP-1) [redacted]
CR MTL, Senneville, QC

Immunology
(Purity Analysis) [redacted]
CR MTL, Senneville, QC

Pathology [redacted]
CR MTL, Senneville, QC

1.3. PIs at Sponsor-designated Test Site

ATA analysis [redacted]
SIGMOVIR BIOSYSTEMS INC., Rockville, MD

2. SUMMARY

The objectives of this study were to determine the potential toxicity of mRNA-1653, when given by intramuscular injection for 1 month (3 doses) to Sprague Dawley rat 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 Concentration (µg/mL)	No. of Animals: Main Study[a] Males	Main Study[a] Females	Recovery Study[b] Males	Recovery Study[b] Females
1	Reference Item	0	200	0	10	10	5	5
2	mRNA-1653	10	200	50	10	10	-	-
3	mRNA-1653	50	200	250	10	10	-	-
4	mRNA-1653	150	200	750	10	10	5	5

[a] = 10/sex/groups 1 to 4 necropsied 1 day following the last dose.
[b] = The remaining 5/sex/groups 1 to 4 necropsied 2 weeks following the last dose.

The following parameters and endpoints were evaluated in this study: clinical observations consisting of twice daily examinations for mortality/moribundity and weekly detailed examinations; local irritation assessment 24- and 72-hour postdose on dosing days, weekly on non- dosing weeks and recovery period; weekly body weights and food consumption measurements; ophthalmic examinations prior to dose initiation and during Week 4; body temperature on Days 1 and 29 predose, 6 and 24 hours postdose; clinical pathology assessment (hematology, coagulation, and clinical chemistry) at termination; cytokine analysis (IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α and MCP-1) on Days 1, 15 and 29 at 6 hours postdose and on Day 43; Anti-Therapeutic Antibody (ATA) analysis for neutralizing antibodies prior to dose initiation and at study termination; gross necropsy findings, organ weights, and histopathologic examinations.

There were no mRNA-1653-related ophthalmic changes.

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

mRNA-1653-treated main study and recovery animals had significant detectable antibody responses against hMPV/A2 and PIV/3 strain of virus.

The primary mRNA-1653-related findings were related to local inflammation. The injection site inflammation generally occurred with a dose-related increased incidence/severity at 10, 50 and 150 µg/dose. Very slight to severe edema was noted at the injection site, following dosing of males and females (peaking 24 hours postdose and generally decreasing by 72 hours postdose). Although sporadic in occurrence, very slight to mild, and (on rare occasions) moderate to severe erythema was noted at each dose occasion but was only considered mRNA-1653-related at 150 µg/dose. Additionally, swelling (soft or firm) and localized skin redness was noted at the injection site following the second and occasionally present upon third dose at 150 µg/dose level. Macroscopically, at the injection site, observations of firmness and swelling were correlated with microscopic changes noted as minimal to marked mixed cell inflammation at ≥ 10 µg/dose. Microscopic changes at the injection site consisted of mostly neutrophils, but also including macrophages and lymphocytes present in connective and subcutaneous tissues. Edema, necrotic

debris, hemorrhage and/or rare degenerated myofibers were also occasionally present. The popliteal, inguinal and iliac lymph nodes of animals dosed at ≥ 10 µg/dose exhibited increased incidence and severity of minimal to moderate mixed cell inflammation was which correlated macroscopically with enlargement. Minimal to mild mixed cell inflammation was also noted in the sciatic nerve (also present in surrounding connective tissue) of animals at ≥ 10 µg/dose. The lymph nodes and sciatic nerve inflammatory changes were regarded as secondary to the injection site inflammation. mRNA-1653-related microscopic findings were still noted in the popliteal lymph node, injection site and sciatic nerve of recovery animals. The popliteal lymph node mixed cell inflammation occurred with a decreased incidence and severity indicating partial recovery. The sciatic nerve and injection site was characterized by mononuclear cell infiltration present in lower numbers compared to the mixed cell inflammation observed in the main study animals indicating a partial recovery. Clinical signs (i.e. edema, soft swelling, and erythema) observed at the injection site including gross pathology findings (firm abnormal consistency, swelling and thick) and inguinal and iliac lymph nodes enlargement were not present in the recovery study animals indicating complete recovery of those findings.

mRNA-1653-related systemic changes indicative of inflammation were observed in animals given ≥ 10 µg/dose and included minimally to mildly increased hematopoiesis of the myeloid lineage in the bone marrow. This change was considered a reactive response to the pronounced inflammation observed at the injection site. Additional systemic findings included increases in absolute and/or relative spleen weights in males at ≥ 50 µg/dose and females at ≥ 10 µg/dose without correlating histopathology, and minimal to mild decreased cellularity of the splenic periarteriolar lymphoid sheath often associated with an increase in macrophages in both sexes at all dose levels tested. Clinical pathology changes suggestive of inflammation were also observed in males and/or females given mRNA-1653 at all doses (unless noted otherwise) and included: minimal to marked increases in neutrophil, eosinophil and large unstained cell counts with concomitant increases in white blood cell counts, minimal decreases in lymphocyte counts and platelet counts starting at 10 µg/dose, minimal increases in activated partial thromboplastin time and mild increases in fibrinogen, starting at 10 µg/dose, minimal increases in globulin, minimal decreases in albumin, with concomitant decreases in A/G ratio. Minimal increases in body temperature postdose and increases in MCP-1, IP-10 and MIP-1α at 150 µg/dose were suggestive of inflammation. At the end of the 2-week recovery period, all aforementioned organ weight and microscopic observations were considered fully reversed. Clinical pathology parameters returned to normal levels for most recovery animals and were considered fully recovered.

In the liver, a minimal to mild hepatocellular vacuolation was noted in Reference and Test Item-dosed animals. Increased incidence and severity were noted at 150 µg/dose and considered mRNA-1653. Liver weights (relative to body weights) were higher in a statistically significant manner in females given 150 µg/dose without any microscopic correlations.

When compared to controls, following each dose, a tendency towards dose-dependent lower mean body weight gains was noted in males given ≥ 10 µg/dose and in females given ≥ 50 µg/dose; these changes were only cumulative at 150 µg/dose and associated with a slightly reduced food consumption at that dose. The body weight and food consumption changes were generally comparable or rebounded during the 2-week recovery period.

In conclusion, administration of mRNA-1653 by intramuscular injection for 1 month (3 doses) was clinically well tolerated (no mortality, no major decreases in body weight/food consumption or deleterious changes in hematology, coagulation or clinical chemistry parameters) in rats up to

150 µg/dose. Starting at 10 µg/dose, generally dose-dependent changes in clinical signs at the injection site, clinical pathology parameters, cytokines, consistent with an inflammatory response at the injection site, were noted. Dose-related target organ effects were limited to the injection site, the bone marrow, the inguinal, popliteal and/or ileac lymph nodes, the connective tissue surrounding the sciatic nerve, the spleen and the liver of animals given mRNA-1653. At the end of the 2-week recovery period, all changes were fully recovered with exception of the injection site, popliteal lymph node, and the connective tissue surrounding the sciatic nerve which were considered to be partially recovered.

3. INTRODUCTION

The objectives of this study were to determine the potential toxicity of mRNA-1653, when given by intramuscular injection for 1 month (3 doses) to Sprague Dawley rat and to evaluate the potential reversibility of any findings.

The design of this study was 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*.
- ICH Harmonised Tripartite Guideline S3a. *Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies*.
- ICH Harmonised Tripartite Guideline S8. *Notes for Guidance on Immunotoxicity Testing of Human Pharmaceuticals*.
- Japanese Guidelines for Nonclinical Studies of Drugs Manual (1995). *Guidelines for Nonclinical Pharmacokinetic Studies*, and *Guidelines for General Pharmacology Studies*, and *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 05 Apr 2017, and dosing was initiated on 19 Apr 2017. The in-life phase of the study was completed on 01 Jun 2017. The experimental start date was 05 Apr 2017, and the experimental completion date will be the date the pathology report is signed. The study plan, the last amended study plan, and deviations are presented in Appendix 1.

4. MATERIALS AND METHODS

4.1. Test and Reference Items

4.1.1. Test Item

Identification:		mRNA-1653
	Supplier:	Moderna Therapeutics, Inc.
	Lot No.:	MTDP 17038
	Concentration:	2.2 mg/mL
	Expiration 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:	Off-white nanoparticle suspension
	Storage Conditions:	Kept in a freezer set to maintain -20°C

4.2. Reference Item

Identification: Phosphate-buffered Saline (PBS) pH 7.2

Supplier: [redacted]

Batch (Lot) Number: 1854892

Expiration Date: 30 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 and Reference Item. A Summary of Analysis was provided to the Test Facility and is presented in Appendix 2.

4.4. Reserve Samples

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

4.5. 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, on dry ice, to Moderna Therapeutics Cambridge MA.

4.6. Dose Formulation and Analysis

4.6.1. Preparation of Reference Item

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

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 stored in a refrigerator set to maintain 4°C until use. They were removed from the refrigerator and allowed to warm to room temperature for at least 30 minutes before dosing.

Any residual volumes were discarded. Details of the preparation and dispensing of the Reference Item have been retained in the Study Records.

4.6.2. Preparation of Test Item

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

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 stored in a refrigerator set to maintain 4°C. The dose formulations were

allowed to warm to room temperature for at least 30 minutes prior to dosing. Stock vials were used only once.

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.

4.6.3. 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	Concentration	Homogeneity	Sampling From
Day 1[b]	All groups	2 and 4[a] (see Appendix 1)	Preparation vessel
Day 29[b]	All groups	N/A	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 submitted as soon as possible following preparation.

All samples to be analyzed were transferred on ice packs to the analytical laboratory (CR MTL).

4.6.3.1. Analytical Method

Analyses were performed by IEX-HPLC using a validated analytical procedure (CR MTL Study No. 1801997).

4.6.3.2. Concentration Analysis

Duplicate sets of samples (0.5 mL) for each sampling time point were sent to the analytical laboratory; the remaining samples were retained at the Test Facility as backup samples. 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%. After acceptance of the analytical results, backup samples were discarded.

4.6.3.3. Homogeneity Analysis

Duplicate sets of samples (0.5 mL) for each sampling time point were sent to the analytical laboratory; the remaining samples were retained at the Test Facility as backup samples. Homogeneity results were considered acceptable if the relative standard deviation of the mean value at each sampling location was ≤ 5%. After acceptance of the analytical results, backup samples were discarded.

4.6.3.4. Stability Analysis

No stability analysis was 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.

4.7. Test System

4.7.1. Receipt

On 05 Apr 2017, 110 Crl:CD(SD) Sprague-Dawley rats were received from Charles River Canada Inc., St. Constant, QC. At the initiation of dosing, the animals were 8 weeks old.

4.7.2. 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 used in this study was considered to be the minimum required to properly characterize the effects of the Test Item. This study was designed such that it did 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.

4.7.3. Animal Identification

At study assignment, each animal was identified using a subcutaneously implanted electronic identification chip.

4.7.4. Environmental Acclimation

An acclimation period of 14 or 15 days was allowed between animal receipt and the start of dosing in order to accustom the animals to the laboratory environment.

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

Healthy 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 in at extremes of body weight range were not assigned to groups.

No animals were replaced during this study.

The alternate animals were released from the study on Day 2. The disposition of all animals was documented in the study records.

4.7.6. Husbandry

4.7.6.1. 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. The room in which the animals were kept was 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. Where possible, control group animals were housed on a separate rack from the Test Item treated animals.

4.7.6.2. Environmental Conditions

Target temperatures of 19°C to 25°C with a relative target humidity of 30% to 70% were maintained. A 12-hour light/12-hour dark cycle was maintained.

4.7.6.3. Food

PMI Nutrition International Certified Rodent Chow No. 5CR4 (14% protein) was provided ad libitum throughout the study, except during designated procedures.

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 were no known contaminants in the feed that could have interfered with the objectives of the study.

4.7.6.4. Water

Municipal tap water after treatment by reverse osmosis and ultraviolet irradiation was freely available to each animal via an automatic watering system.

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

It is considered that there were no known contaminants in the water that could have interfered with the outcome of the study.

4.7.6.5. 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.

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

4.8. Experimental Design

Text Table 3
Experimental Design

Group No.	Test Material	Dose Level (µg/dose)	Dose Volume (µL)	Dose Concentration (µg/mL)	Animal Numbers			
					Main Study[a]		Recovery Study[b]	
					Males	Females	Males	Females
1	Reference Item	0	200	0	1001-1010	1501-1510	1011-1015	1511-1515
2	mRNA-1653	10	200	50	2001-2010	2501-2510	-	-
3	mRNA-1653	50	200	250	3001-3010	3501-3510	-	-
4	mRNA-1653	150	200	750	4001-4010	4501-4510	4011-4015	4511-4515

[a] = 10/sex/groups 1 to 4 necropsied 1 day following the last dose.
[b] = The remaining 5/sex/groups 1 to 4 necropsied 2 weeks following the last dose.

4.8.1. Administration of Test Materials

The Test and Reference Items were administered to Groups 1 to 4 animals via intramuscular injection into the lateral compartment of the thigh on Days 1, 15, and 29. The dose volume for each animal was constant. The volume for each dose was administered using a syringe/needle within the demarcated area. The injection site was alternated on each dosing occasion.

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.

4.8.2. Justification of Route and Dose Levels

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

The doses selected were based upon tolerability data of various lipid nanoparticle formulations in rats, which were expected to drive toxicities observed. The mid and low dose levels were chosen based upon observed efficacy with this modified mRNA construct in a lipid nanoparticle formulation. Doses with this test material or similarly formulated material up to 1 mg/kg (sponsor internal data) were well-tolerated in rats.

4.9. In-life Procedures, Observations, and Measurements

The in-life procedures, observations, and measurements listed below were performed for main study and recovery animals.

4.9.1. 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. Animals were not removed from cage during observation, unless necessary for identification or confirmation of possible findings.

4.9.2. Clinical Observations

4.9.2.1. Detailed Clinical Observations

A detailed clinical observation was performed weekly during the dosing and recovery periods, and at least every two weeks during the predosing period. The animals were removed from the cage during observation.

4.9.3. Local Irritation Assessment

All animals had the dose injection site examined for signs of erythema/edema on days of dosing; at least 24 and 72 hours post-dose (end of each group) and weekly when there is no dosing and during recovery period. Examinations were also performed following Day 29 dosing. No assessment was performed on main animals at 72 hours postdose as animals were sent to necropsy on Day 30. Observations were scored according to the Local Irritation Assessment scoring table as follows:

Erythema (Redness)	Score	Edema (Swelling)	Score
No erythema	0	No edema	0
Very slight erythema	1	Very slight edema	1
Mild erythema	2	Slight edema	2
Moderate to severe erythema	3	Moderate edema	3
Severe erythema (beet redness to slight eschar formation, injury in depth)	4	Severe edema	4
Notable dermal lesion (maximized)	M		

Any other abnormalities were recorded as they were observed.

4.9.4. Body Weights

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

4.9.5. Food Consumption

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

4.10. Ophthalmic Examinations

All animals were subjected to funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations once prestudy and again during Week 4 of dosing. The mydriatic used was 1% tropicamide.

4.11. Body Temperature

Body temperature was recorded via subcutaneous implanted transponder on Day 1 and Day 29 at predose, 6 and 24 hours post dose (end of each group). When body temperature was significantly above normal range (36.0°C to 38.0°C) the temperature was monitored daily until return to normal.

4.12. Laboratory Evaluations

4.12.1. Clinical Pathology

4.12.1.1. 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

X = Sample collected.
[a] Samples collected from those animals scheduled for euthanasia on Day 30.

4.12.1.2. Hematology

Blood samples were analyzed for the parameters specified in Text Table 5.

Text Table 5
Hematology Parameters

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

A blood smear was prepared from each hematology sample. Blood smears were labeled, stained, and stored.

4.12.1.3. Coagulation

Blood samples 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

4.12.1.4. Clinical Chemistry

Blood samples 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

4.12.2. Bone Marrow Smear Evaluation

Bone marrow smears were collected and prepared as described Section 4.14.9.

4.12.3. Cytokines Analysis

Blood was collected from the jugular vein of all recovery animals. After collection, blood samples for serum was 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 setting			2400x g, 10 minutes, set at 4°C	1200x g, 10 minutes, set at 4°C
Timepoints			**Sample Type**	
Day	**Hours**	**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	N/A	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; N/A = not applicable
* The assay validation of IFN-α did not work appropriately and serum samples analysis was not conducted.

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 qualified multiplex Luminex method (non-GLP). 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.

Any residual/retained samples were discarded prior to report finalization, following Study Director approval.

4.13. Anti-Therapeutic Antibody (ATA) 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 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). 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 SIGMOVIR BIOSYSTEMS INC., Rockville, MD.

The samples were analyzed for rat anti-HMPV antibodies using a qualified neutralizing antibody assay method.

Any residual/retained samples were discarded following issuance of the Final Report.

4.14. 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
[a] See Tissue Collection and Preservation table for listing of tissues.

4.14.1. Unscheduled Deaths

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

4.14.2. Scheduled Euthanasia

Main study and recovery animals surviving until scheduled euthanasia had a terminal body weight recorded, samples for clinical pathology, anti-therapeutic antibodies and cytokines were collected (as appropriate), and were euthanized by exsanguination by incision from the abdominal aorta following isoflurane anesthesia. When possible, 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.

4.14.3. 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.

4.14.4. 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 to body weight ratio (using the terminal body weight) and organ to brain weight ratios 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	Thymus
Heart	Uterus
Kidney[a]	

[a] Paired organ weight.

4.14.5. 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

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

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

4.14.6. 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.

4.14.7. Histopathology

Histopathological evaluation was performed by a board-certified veterinary pathologist.

4.14.8. Peer Review

An on-site pathology peer review was conducted by [redacted] from Moderna Therapeutics Cambridge, MA.

4.14.9. Bone Marrow Smear Analysis

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

5. CONSTRUCTED VARIABLES

Body Weight Gains	calculated between at least each 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

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 were reported whenever possible. 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 Gains	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
SRS (CR MTL in-house application built with SAS and SAS system for Windows)	1.4	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 CO_2, as appropriate
Johnson Controls Metasys	MVE 7.0 / 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
Dynamics (Wyatt)	7.1.9.3	Data acquisition for particle size analysis for the Test Item using DLS
Fragment Analyzer CE System	1.1.0.11	Test Item purity data acquisition
Bio Plex Manager (Bio-Rad)	Version 6.1	Cytokine data collection

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. At least one year after issue of the draft report, the Sponsor will be contacted.

Electronic data generated by the Test Facility were archived as noted above, except 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 raw data and the final report (original) related to the ATA analysis will be retained at the Sponsor designated Test Site.

9. RESULTS

9.1. Dose Formulation Analyses

(Appendix 3)

Dose formulation concentration results were within specification. Homogeneity testing showed that the formulation technique used produced homogeneous preparations.

9.2. End of Use Bulk Test Item Analysis

(Appendix 3 and Appendix 16)

The bulk Test Item analysis demonstrated that the Test Item was suitable for use during the study period; the concentration, purity and particle size results obtained were consistent with the Summary of Analysis.

9.3. Mortality

(Appendix 4)

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

9.4. Clinical Observations

(Table 1 and Appendix 5)

Following the second and/or third (last) dosing occasion, a dose-related (in severity; from slight to severe) firm swelling was noted on the injection site. Soft swelling (slight to moderate in severity) was also noted at the injection site of individual females given ≥ 50 µg/dose, following the second dose. In addition, skin redness at the injection site was noted in individual males and females given 150 µg/dose, following the last dose.

During the recovery period, firm swelling (slight) was still noted on Day 39 and redness, still noted for a few animals on Day 43, as such, these findings were considered partially reversed. Soft swelling was not observed during the 2-week recovery period.

9.5. Local Irritation Assessment

(Appendix 6)

Very slight to severe edema was noted at the injection site following dosing of males and females at ≥ 10 µg/dose. The incidence and severity of these findings were dose-dependent. The apex of severity was noted 24 hours postdose and generally decreased 72 hours postdose.

Sporadic, generally very slight to mild erythema, and on rare occasions, moderate to severe erythema, noted at the injection site, was considered mRNA-1653-related only at 150 µg/dose and occurred at a higher incidence and severity 72 hours postdose.

With the exception of one male, edema and erythema were no longer observed at the end of the recovery period, and as such, they were considered completely reversed.

9.6. Body Weights and Body Weight Gains

(Figure 1, Figure 2, Table 2, Table 3, Appendix 7, and Appendix 8)

When compared to controls, following each dose, a tendency towards dose-dependent lower mean body weight gains was noted in males given ≥ 10 µg/dose and in females given ≥ 50 µg/dose; these changes sometimes reached statistical significance. The changes were only cumulative at 150 µg/dose; from Days -1 to 28, when compared to controls, the body weight changes were 0.85X for males and 0.79X, for females. The body weight changes were generally comparable or rebounded during the 2-week recovery period.

9.7. Food Consumption

(Table 4 and Appendix 9)

The weekly food consumption appeared slightly noticeably lower in animals given mRNA-1653, and, more consistently at 150 µg/dose. The food consumption changes were generally comparable or rebounded during the 2-week recovery period.

9.8. Ophthalmic Examinations

(Appendix 14)

There were no mRNA-1653-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.

9.9. Body Temperature

(Table 5 and Appendix 10)

When compared to controls, the mean body temperature appeared minimally increased in males and females given ≥ 10 µg/dose, 6 and/or 24 hours post Day 1 and Day 29 doses. These generally statistically-significant changes were considered mRNA-1653-related.

9.10. Hematology

(Table 6 and Appendix 11)

mRNA-1653-related hematology changes were noted for males and females starting at 10 µg/dose and included increases in neutrophil (NEUT), eosinophil (EOS) and/or large unstained cell (LUC) counts (with concomitant increases in white blood cell [WBC] counts) and decreases in lymphocyte (LYMPH), platelet (PLT) and reticulocyte (RETIC) counts. These changes are illustrated in Text Table 14.

Text Table 14
Hematology Changes

Dose (µg/dose)	10		50		150	
Parameter	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
WBC						
Day 30	1.4	**1.6**	**1.9**	**1.7**	**2.0**	**1.6**
Day 43					1.0	1.0
NEUT						
Day 30	**5.7**	7.8	**11.2**	**13.0**	**12.6**	**14.0**
Day 43					1.1	1.2
LYMPH						
Day 30	0.8	-	**0.7**	**0.7**	**0.7**	**0.6**
Day 43					1.0	0.9
EOS						
Day 30	**2.8**	**3.2**	**3.4**	**4.6**	**4.3**	**3.9**
Day 43					1.0	1.2
LUC						
Day 30	**3.0**	1.7	**2.4**	1.3	1.7	1.2
Day 43					1.1	0.8
PLT						
Day 30	-	0.9	-	**0.9**	**-**	**0.7**
Day 43					-	1.0
RETIC						
Day 30	**0.8**	-	**0.7**	**-**	**0.7**	-
Day 43					**1.3**	-

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 2.0X and 1.7X controls, respectively) were noted in males and females given ≥ 10 µg/dose, mainly due to minimal to marked increases in NEUT, LUC (up to 12.6X and 3.0X controls for males and 14.0X and 1.7X controls for females) and/or EOS (up to 4.3X controls for males and up to 4.6X controls for females). Minimal decreases in LYMPH counts were noted for males at ≥ 10 µg/dose and females at ≥ 50 µg/dose (down to 0.7X and 0.6X controls, respectively).

Minimal decreases in PLT were noted in females at ≥ 10 µg/dose (down to 0.7X controls).

Minimal decreases in RETIC were noted in males at ≥ 10 µg/dose (down to 0.7X controls).

Of the above changes noted following the dosing period, near to full recovery of the findings were noted following the 2-week 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-1653-related.

9.11. Coagulation

(Table 7 and Appendix 12)

mRNA-1653-related increases in activated partial thromboplastin time (APTT) and in fibrinogen (FIB) were noted in males and females starting at 10 µg/dose. The changes are illustrated in Text Table 15.

Text Table 15
Coagulation Changes

Dose (µg/dose)	10		50		150	
Parameter	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
APTT						
Day 30	-	1.1	**1.1**	**1.1**	**1.2**	**1.3**
Day 43					1.0	1.1
FIB						
Day 30	**2.0**	**1.6**	**2.1**	**2.1**	**2.3**	**2.2**
Day 43					0.9	1.1

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.

Minimal increases in APTT were noted for males given ≥ 50 µg/dose and females given ≥ 10 µg/dose (up to 1.2X controls for males and 1.3X controls for females). Mild increases in FIB were noted for males and females given ≥ 10 µg/dose (up to 2.3X controls for males and up to 2.2X controls for females. At the end of the 2-week recovery period, changes were near to 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-1653-related.

9.12. Clinical Chemistry

(Table 8 and Appendix 13)

mRNA-1653-related decreases in albumin (ALB) and increases in globulin (GLOB) were noted for males and females; these changes were reflected by overall decrease in A/G ratio. The changes are illustrated in Text Table 16.

Text Table 16
Clinical Chemistry Changes

Dose (µg/dose)	10		50		150	
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	**0.9**
GLOB						
Day 30	**1.3**	**1.2**	**1.4**	**1.2**	**1.4**	**1.3**
Day 43					1.0	**1.2**
A/G						
Day 30	**0.7**	**0.8**	**0.6**	**0.7**	**0.6**	**0.7**
Day 43					**0.9**	**0.9**

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 1.4X controls for each parameter) and affected the A/G ratio (down to 0.6X controls in males and down to 0.7X controls in females). At the end of the 2-week recovery period, changes were near to fully recovered.

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-1653-related.

9.13. Cytokines

(Appendix 15)

When compared to controls, mRNA-1653-related increases in MCP-1 and IP-10 were observed at 150 µg/dose on Days 1, 15 and 29, 6 hours postdose. The magnitude of increases observed were higher for IP-10, ranging from 5.2 to 10.4X for males, and from 7.0 to 17.5X for females, and were apparent for all animals at all dosing timepoints. The increases in MCP-1 were observed for all animals at almost all dosing timepoints, but the magnitude of increases was lower, ranging from 1.2 to 3X for males and from 1.2 to 6.4X for females. Following the 2-week recovery period (i.e. Day 43), the level of both MCP-1 and IP-10 were back to the normal range values for all animals.

mRNA-1653-related slight increases in MIP-1α were observed on Days 1 and 15, 6 hours postdose, for some animals, at a similar incidence and magnitude in both genders given 150 µg/dose. Such increases were not observed on Day 29, 6 hours postdose and at the recovery timepoint (i.e. Day 43). These increases were considered to be related due to similar incidence, magnitude and pattern observed in the mRNA-1653 dosed group.

No mRNA-1653 changes were observed in IL-1β, TNF-α and IL-6.

9.14. Anti-Therapeutic Antibody (ATA)

(Appendix 17)

The Day 30 samples from mRNA-1653-treated Main Study animals had detectable antibody responses against hMPV/A2 and PIV/3 strain of virus. The Day 43 samples from Recovery Study animals previously given 150 µg/dose had higher antibody titers compared to Day 30 titers, indicative of the booster effect on Day 30.

9.15. Gross Pathology

(Appendix 18)

9.15.1. Terminal Necropsy (Day 30)

Gross pathology findings related to mRNA-1653 were seen in the injection site and the popliteal lymph node and are summarized in Text Table 17.

Text Table 17
Summary of Gross Pathology Findings – Terminal Necropsy (Day 30)

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (µg/dose)	**0**	**10**	**50**	**150**	**0**	**10**	**50**	**150**
No. Animals Examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Injection Site (No. Examined)	10	10	10	10	10	10	10	10
Abnormal consistency; firm	0	8	10	9	0	6	10	10
Swelling	0	4	6	10	0	1	6	9
Thick	0	0	0	3	0	0	1	1
Popliteal Lymph Node (No. Examined)	10	10	10	10	10	10	10	10
Enlargement	0	3	4	1	0	0	1	3
Lymph Node, Inguinal (No. Examined)	10	10	10	10	10	10	10	10
Enlargement	0	1	1	3	0	0	0	3
Lymph Node[a] (No. Examined)	0	1	1	3	0	0	2	4
Enlargement	0	1	1	3	0	0	2	4

a: Iliac lymph node

At the injection site, firm abnormal consistency, swelling and/or thick was observed in both sexes at 10, 50 and/or 150 µg/dose. Swelling occurred with a dose-related increased incidence. These injection site changes correlated microscopically with mixed cell inflammation.

In the lymph nodes (popliteal, inguinal and iliac), enlargement occurred in both sexes at 10, 50 and/or 150 µg/dose. Enlargement correlated microscopically with perinodal mixed cell inflammation.

Other gross findings observed were considered incidental, of the nature observed in this strain and age of rats, and/or were of similar incidence in Reference and Test Item-treated animals and, therefore, were considered not mRNA-1653-related.

9.15.2. Recovery Necropsy (Day 43)

(Appendix 18)

Following the 2-week recovery period, the mRNA-1653-related popliteal lymph node enlargement observed at the terminal necropsy was still present in females at 150 µg/dose and are summarized in Text Table 18. Other mRNA-1653-related gross findings observed at the terminal necropsy at the injection site (firm abnormal consistency, swelling and thick) and in the inguinal and iliac lymph nodes (enlargement) were not observed in recovery animals.

Text Table 18
Summary of Gross Pathology Findings – Recovery Necropsy (Day 43)

	Males		Females	
Group	**1**	**4**	**1**	**4**
Dose (µg/dose)	**0**	**150**	**0**	**150**
No. Animals Examined	**5**	**5**	**5**	**5**
Popliteal Lymph Node (No. Examined)	5	5	5	5
Enlargement	0	0	0	2

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 Test Item-treated animals and, therefore, were considered not mRNA-1653-related.

9.16. Organ Weights

(Appendix 18)

9.16.1. Terminal Necropsy (Day 30)

The organ weight changes related to mRNA-1653 were increases in liver and spleen weights, and are summarized in Text Table 19.

Text Table 19
Summary of Organ Weight Data – Terminal Necropsy (Day 30)

	Males			Females		
Group	**2**	**3**	**4**	**2**	**3**	**4**
Dose (µg/dose)	**10**	**50**	**150**	**10**	**50**	**150**
No. Animals per Group	**10**	**10**	**10**	**10**	**10**	**10**
Terminal Body Weight	-6.0	-5.2	**-10.4**	-1.0	-4.2	-6.0
Liver (No. Weighed)[a]	10	10	10	10	10	10
Absolute value	-	-	-	0.47	-1.69	2.72
% of body weight	-	-	-	1.71	2.40	**9.34**
% of brain weight	-	-	-	-2.08	-0.97	2.68
Spleen (No. Weighed)	10	10	10	10	10	10
Absolute value	3.65	15.17	14.79	**24.86**	**31.14**	**21.37**
% of body weight	10.27	**21.80**	**27.58**	**26.40**	**37.21**	**28.66**
% of brain weight	1.96	15.35	15.47	**21.44**	**32.18**	**21.17**

[a] All values expressed as percent difference of control (Group 1) means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used.
- = Not Test Item-related

Liver weights (relative to body weights) were higher in a statistically significant manner in females given150 µg/dose. The higher liver weights had no microscopic correlations.

Statistically-significant higher (absolute and/or relative to body and/or brain weights) spleen weights occurred in males given ≥ 50 µg/dose and females given ≥ 10 µg/dose. These spleen weight changes had no microscopic correlation.

There were other isolated organ weight values that were statistically different from their respective controls. 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 secondary to the lower terminal body weight, and therefore, not mRNA-1653-related.

9.16.2. Recovery Necropsy (Day 43)

Following the 2-week recovery period, mRNA-1653-related higher liver weight changes observed for Main Study animals were still present in females at 150 µg/dose; these changes had no microscopic correlations. These liver weight changes are summarized in Text Table 20. The higher spleen weights observed for Main Study animals were not present in recovery animals.

Text Table 20
Summary of Organ Weight Data – Recovery Necropsy (Day 43)

	Males	Females
Group	**4**	**4**
Dose (µg/dose)	**150**	**150**
No. Animals per Group	**5**	**5**
Terminal Body Weight	-2.0	-4.4
Liver (No. Weighed)[a]	5	5
Absolute value	-	8.62
% of body weight	-	**13.45**
% of brain weight	-	6.93

[a] All values expressed as percent difference of control (Group 1) means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – P ≤ 0.05; refer to data tables for actual significance levels and tests used.
- = Not Test Item-related

There were other isolated organ weight values that were statistically different from their respective controls. 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 secondary to the lower terminal body weight, and therefore, not mRNA-1653-related.

9.17. Histopathology

(Appendix 18)

9.17.1. Terminal Necropsy (Day 30)

mRNA-1653-related microscopic changes were noted at the injection site, liver, bone marrow, spleen, lymph nodes (popliteal, inguinal and iliac) and sciatic nerve which are summarized in Text Table 21.

Text Table 21
Summary of Microscopic Findings – Terminal Necropsy (Day 30)

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (µg/dose)	**0**	**10**	**50**	**150**	**0**	**10**	**50**	**150**
No. Animals Examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Injection Site (No. Examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell	(1)[a]	(10)	(10)	(10)	(3)	(9)	(10)	(10)
Minimal	1	1	-	-	2	1	-	-
Mild	-	1	1	-	1	4	-	-
Moderate	-	8	5	5	-	4	6	5
Marked	-	-	4	5	-	-	4	5
Liver (No. Examined)	10	10	10	10	10	10	10	10
Vacuolation, hepatocellular	(1)	(1)	(2)	(7)	(2)	(1)	(2)	(9)
Minimal	1	1	2	3	2	1	2	5
Mild	-	-	-	4	-	-	-	4
Bone Marrow (No. Examined)	10	10	10	10	10	10	10	10
Increased hematopoiesis: myeloid	0	10	10	10	0	10	10	10
Minimal	-	10	7	3	-	10	8	5
Mild	-	-	3	7	-	-	2	5
Popliteal Lymph Node (No. Examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell	(0)	(9)	(8)	(10)	(0)	(10)	(9)	(9)
Minimal	-	1	3	2	-	3	1	4
Mild	-	8	5	7	-	7	6	3
Moderate	-	-	-	1	-	-	2	2
Inguinal Lymph Node (No. Examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell	(0)	(2)	(1)	(4)	(0)	(0)	(0)	(6)
Minimal	-	2	1	3	-	-	-	2
Mild	-	-	-	1	-	-	-	4
Lymph Node[b] (No. Examined)	0	1	1	3	0	0	2	4
Inflammation, mixed cell	(0)	(1)	(1)	(2)	(0)	(0)	(2)	(4)
Minimal	-	1	1	1	-	-	1	-
Mild	-	-	-	1	-	-	1	4
Sciatic Nerve (No. Examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell	(0)	(9)	(10)	(10)	(0)	(10)	(10)	(7)
Minimal	-	8	9	9	-	9	9	6
Mild	-	1	1	1	-	1	1	1
Spleen (No. Examined)	10	10	10	10	10	10	10	10
Decreased cellularity; periarteriolar lymphoid sheath	(0)	(2)	(4)	(7)	(0)	(1)	(2)	(7)
Minimal	-	2	4	3	-	1	2	6
Mild	-	-	-	4	-	-	-	1
Increased macrophages; periarteriolar lymphoid sheath	(0)	(0)	(3)	(5)	(0)	(0)	(2)	(7)
Minimal	-	-	3	5	-	-	2	7

[a] Numbers in parentheses represent the number of animals with the finding.
[b] Iliac lymph node

At the injection site, there was a minimal to marked mixed cell inflammation in both sexes given Reference and Test items. The exacerbation of the mixed cell inflammation was considered mRNA-1653-related at ≥ 10 µg/dose, based on the increased incidence and severity compared to controls. The change occurred with an apparent dose-related increase in severity and was characterized by an infiltration of mostly neutrophils but also macrophages and lymphocytes in the intramuscular connective tissue and subcutis; edema, necrotic debris, hemorrhage and/or rare

degenerated myofibers were also present. The injection site mixed cell inflammation correlated macroscopically with firm abnormal consistency, swelling and/or thick.

In the lymph nodes (popliteal, inguinal and/or iliac), minimal to moderate perinodal mixed cell inflammation was seen in both sexes at ≥ 10 µg/dose. Mixed cell inflammation occurred generally with an increased incidence and severity in the popliteal lymph node. The lymph node inflammation correlated macroscopically with enlargement. Minimal to mild mixed cell inflammation was also noted in the sciatic nerve of animals at ≥ 10 µg/dose in connective tissue surrounding the nerve. The lymph nodes and sciatic nerve inflammatory changes were regarded as secondary to the injection site inflammation.

In the liver, there was minimal to mild hepatocellular vacuolation in both sexes given the Reference and Test Items. The increased incidence and severity observed at 150 µg/dose was considered mRNA-1653-related; it consisted of the presence of intracytoplasmic microvesicles with enlarged hepatocytes.

In the bone marrow, there was minimal to mild increased hematopoiesis of the myeloid lineage in both sexes at ≥ 10 µg/dose; this change was likely a reactive response to the inflammation observed at the injection site.

In the spleen, there was minimal to mild decreased cellularity of the periarteriolar lymphoid sheath often associated with an increase in macrophages in both sexes at 10, 50 and/or 150 µg/dose.

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 in Reference and Test Item-treated animals and, therefore, were considered not mRNA-1653-related.

9.17.2. Recovery Necropsy (Day 43)

Following the 2-week recovery period, mRNA-1653-related microscopic changes were mixed cell inflammation around the popliteal lymph node and mononuclear cell infiltration around the sciatic nerve and at the injection site. The popliteal lymph node mixed cell inflammation occurred with a decreased incidence and/or severity indicating partial recovery. The sciatic nerve and injection site mononuclear cell infiltration had a low number of cells compared to the mixed cell inflammation observed in Main Study animals, indicating partial recovery. The incidence of these microscopic findings is presented in Text Table 22. Other mRNA-1653-related microscopic findings observed in Main Study animals in the liver (hepatocellular vacuolation), inguinal and iliac lymph nodes (mixed cell inflammation), bone marrow (increased hematopoiesis, myeloid) and spleen (decreased cellularity and increased macrophages in the periarteriolar lymphoid sheath) were not present following the 2-week recovery period, indicating reversibility.

Text Table 22
Summary of Microscopic Findings – Recovery Necropsy (Day 43)

	Males		Females	
Group	**1**	**4**	**1**	**4**
Dose (µg/dose)	**0**	**150**	**0**	**150**
No. Animals Examined	**5**	**5**	**5**	**5**
Injection Site (No. Examined)	5	5	5	5
Infiltration, mononuclear cell	(0)[a]	(5)	(0)	(5)
Minimal	-	4	-	5
Mild	-	1	-	-
Popliteal Lymph Node (No. Examined)	5	5	5	5
Inflammation, mixed cell	(0)	(3)	(0)	(5)
Minimal	-	3	-	5
Nerve, sciatic (No. Examined)	5	5	5	5
Infiltration, mononuclear cell	(0)	(5)	(0)	(4)
Minimal	-	5	-	4

[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 in Reference and Test Item-treated animals and, therefore, were considered not mRNA-1653-related.

10. CONCLUSION

In conclusion, administration of mRNA-1653 by intramuscular injection for 1 month (3 doses) was clinically well tolerated (no mortality, no major decreases in body weight/food consumption or deleterious changes in hematology, coagulation or clinical chemistry parameters) in rats up to 150 µg/dose. Starting at 10 µg/dose, generally dose-dependent changes in clinical signs at the injection site, clinical pathology parameters, cytokines, consistent with an inflammatory response at the injection site, were noted. Dose-related target organ effects were limited to the injection site, the bone marrow, the inguinal, popliteal and/or ileac lymph nodes, the connective tissue surrounding the sciatic nerve, the spleen and the liver of animals given mRNA-1653. At the end of the 2-week recovery period, all changes were fully recovered with exception of the injection site, popliteal lymph node, and the connective tissue surrounding the sciatic nerve which were considered to be partially recovered.

Figure 1

Summary of Body Weights - Males

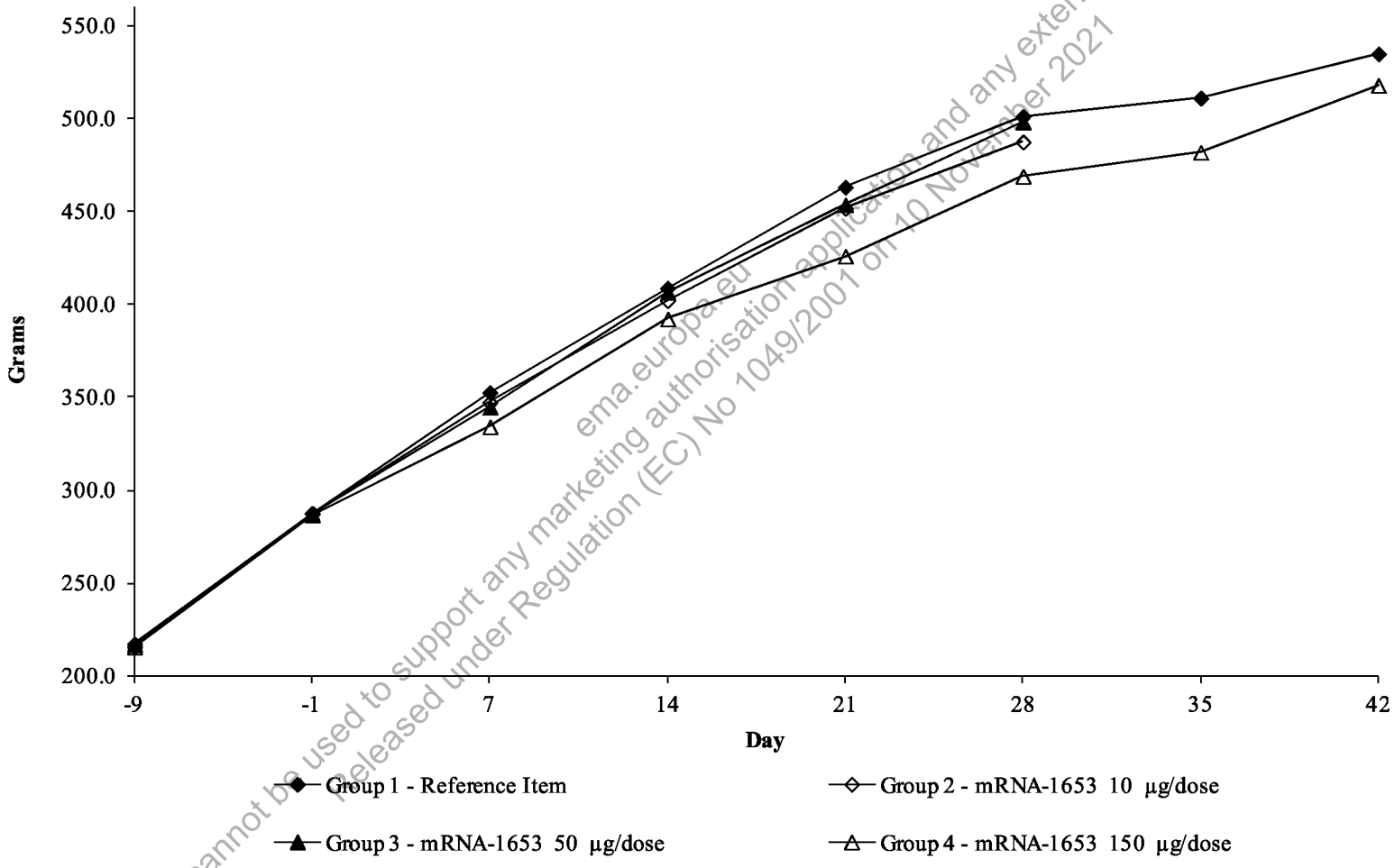

Figure 2

Summary of Body Weights - Females

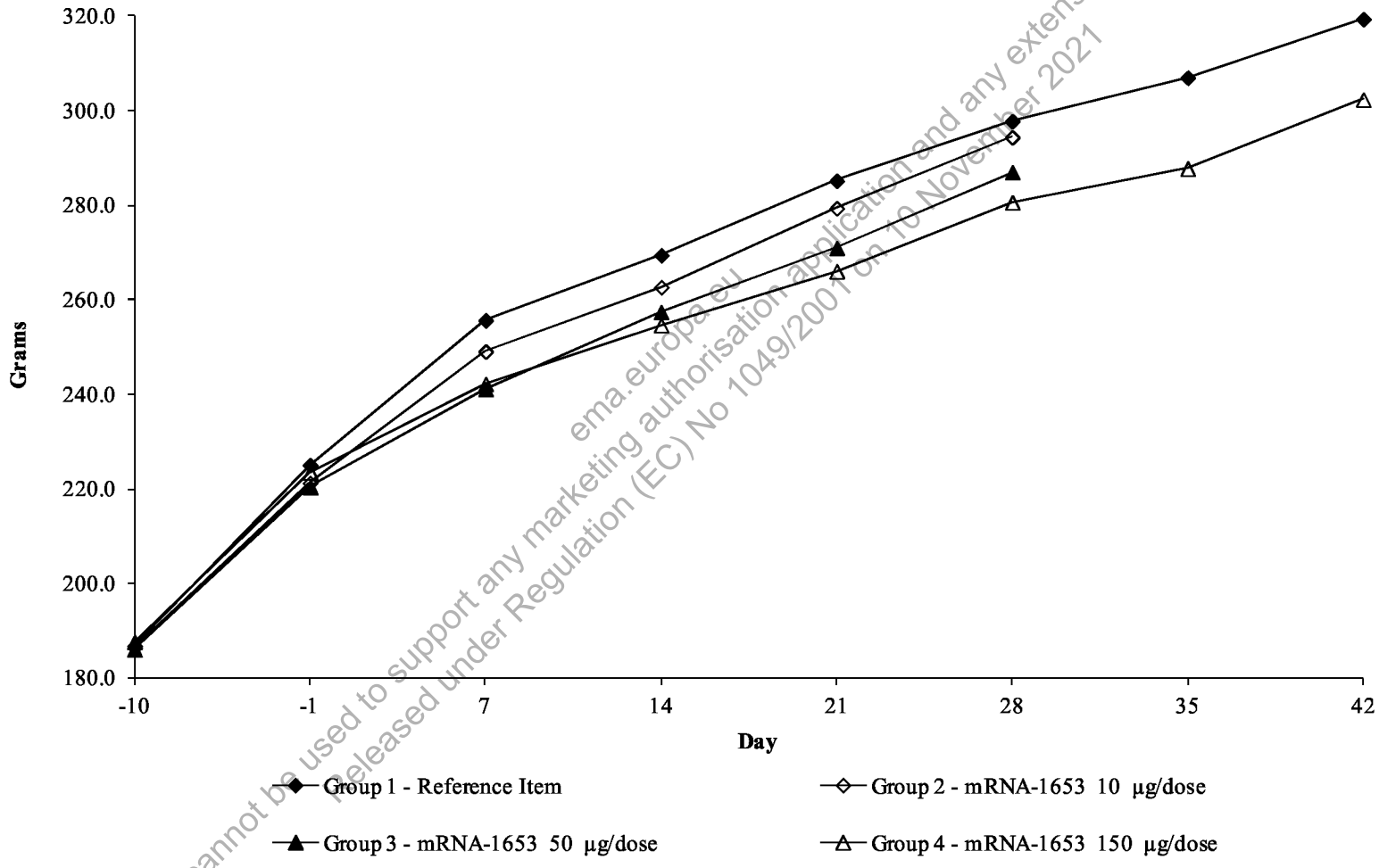

Table 1

Summary of Clinical Observations

5002033

Day numbers relative to Start Date

Sex: Male

	0 ug/dose	10 ug/dose	50 ug/dose	150 ug/dose
Hunched Posture				
Number of Observations	.	.	.	2
Number of Animals	.	.	.	2
Days from - to	.	.	.	30 30
Swollen Firm				
Number of Observations	2	10	10	19
Number of Animals	1	10	10	15
Days from - to	32 39	30 30	30 30	30 39
Skin, Red				
Number of Observations	.	.	.	9
Number of Animals	.	.	.	5
Days from - to	.	.	.	30 43
Skin, Lesion				
Number of Observations	.	1	.	.
Number of Animals	.	1	.	.
Days from - to	.	-4 -4	.	.
Skin, Lesion w/ Discharge				
Number of Observations	.	1	.	.
Number of Animals	.	1	.	.
Days from - to	.	2 2	.	.
Skin, Scab				
Number of Observations	9	1	3	9
Number of Animals	4	1	2	6
Days from - to	-4 30	4 4	-4 30	11 30
Fur, Erected				
Number of Observations	.	.	.	2
Number of Animals	.	.	.	2
Days from - to	.	.	.	30 30

Table 1

Summary of Clinical Observations

5002033

Day numbers relative to Start Date

Sex: Male

	0 ug/dose	10 ug/dose	50 ug/dose	150 ug/dose
Fur, Staining, Red				
Number of Observations	3	5	4	2
Number of Animals	1	3	4	2
Days from - to	-4 11	-4 30	30 30	30 30
Fur, Thin Cover				
Number of Observations	10	6	3	6
Number of Animals	2	2	2	3
Days from - to	-4 39	-4 11	25 30	11 39
Malocclusion				
Number of Observations	.	3	.	.
Number of Animals	.	1	.	.
Days from - to	.	18 30	.	.
Tail, Bent				
Number of Observations	.	3	.	.
Number of Animals	.	1	.	.
Days from - to	.	18 30	.	.

Table 1

Summary of Clinical Observations

5002033

Day numbers relative to Start Date

Sex: Female

	0 ug/dose	10 ug/dose	50 ug/dose	150 ug/dose
Abnormal Gait				
Number of Observations	.	.	6	.
Number of Animals	.	.	1	.
Days from - to	.	.	16 25	.
Activity Decreased				
Number of Observations	.	.	.	1
Number of Animals	.	.	.	1
Days from - to	.	.	.	2 2
Teeth Grinding				
Number of Observations	.	.	5	.
Number of Animals	.	.	1	.
Days from - to	.	.	17 25	.
Uncoordinated				
Number of Observations	.	.	1	.
Number of Animals	.	.	1	.
Days from - to	.	.	16 16	.
Hunched Posture				
Number of Observations	.	.	.	1
Number of Animals	.	.	.	1
Days from - to	.	.	.	2 2
Limited Usage				
Number of Observations	.	.	5	.
Number of Animals	.	.	1	.
Days from - to	.	.	16 25	.
Post Puncture Swelling				
Number of Observations	2	.	.	.
Number of Animals	1	.	.	.
Days from - to	18 25	.	.	.

Table 1

Summary of Clinical Observations

5002033

Day numbers relative to Start Date

Sex: Female

	0 ug/dose	10 ug/dose	50 ug/dose	150 ug/dose
Swollen Soft				
Number of Observations	.	.	8	3
Number of Animals	.	.	3	3
Days from - to	.	.	16 25	18 18
Swollen Firm				
Number of Observations	.	10	17	31
Number of Animals	.	10	10	15
Days from - to	.	30 30	16 30	18 39
Skin, Brown				
Number of Observations	.	.	3	.
Number of Animals	.	.	1	.
Days from - to	.	.	16 18	.
Skin, Red				
Number of Observations	11	3	7	12
Number of Animals	3	3	2	8
Days from - to	4 39	4 25	16 30	18 39
Skin, Lesion				
Number of Observations	.	.	2	.
Number of Animals	.	.	1	.
Days from - to	.	.	17 18	.
Skin, Scab				
Number of Observations	5	.	8	4
Number of Animals	3	.	2	4
Days from - to	-4 30	.	16 30	18 30
Fur, Erected				
Number of Observations	.	.	.	1
Number of Animals	.	.	.	1
Days from - to	.	.	.	2 2

Table 1

Summary of Clinical Observations

5002033

Day numbers relative to Start Date

Sex: Female

	0 ug/dose		10 ug/dose		50 ug/dose		150 ug/dose	
Fur, Staining, Red								
Number of Observations	9		2		4		2	
Number of Animals	5		1		2		1	
Days from - to	-4	39	25	30	16	30	25	30
Fur, Thin Cover								
Number of Observations	6		.		4		8	
Number of Animals	3		.		3		5	
Days from - to	4	30	.		4	30	18	39
Skin Staining								
Number of Observations	1		.		.		.	
Number of Animals	1		.		.		.	
Days from - to	-4	-4	.		.		.	
Teeth, Clear								
Number of Observations	.		1		.		2	
Number of Animals	.		1		.		2	
Days from - to	.		30	30	.		30	30

Table 2

Summary of Body Weights (g)

Group 1 - Reference Item
Group 2 - mRNA-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		Day -9	-1	7	14	21	28	35	42
1M	Mean	215.67	287.67	352.67	408.87	463.20	501.33	511.20	535.00
	SD	11.00	15.31	21.25	27.77	33.85	38.08	30.95	35.16
	N	15	15	15	15	15	15	5	5
2M	Mean	217.30	287.50	347.30	402.20	451.90	487.60	--	--
	SD	7.35	9.25	15.34	21.45	29.12	36.44	--	--
	N	10	10	10	10	10	10	--	--
	%Diff G1	0.76	-0.06	-1.52	-1.63	-2.44	-2.74	--	--
3M	Mean	217.00	287.10	344.70	406.50	453.70	498.10	--	--
	SD	7.99	13.62	23.13	32.54	42.48	48.17	--	--
	N	10	10	10	10	10	10	--	--
	%Diff G1	0.62	-0.20	-2.26	-0.58	-2.05	-0.64	--	--
4M	Mean	215.67	286.73	334.27	392.33	426.07b	469.07	482.00	518.00
	SD	10.61	12.75	15.66	17.55	18.80	21.58	29.57	33.32
	N	15	15	15	15	15	15	5	5
	%Diff G1	0.00	-0.32	-5.22	-4.04	-8.02	-6.44	-5.71	-3.18

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		Day -10	-1	7	14	21	28	35	42
1F	Mean	186.87	225.07	255.67	269.47	285.20	297.80	307.00	319.40
	SD	7.54	10.75	11.90	11.01	13.01	11.62	9.67	10.92
	N	15	15	15	15	15	15	5	5
2F	Mean	186.50	221.30	249.10	262.70	279.40	294.40	--	--
	SD	4.17	11.48	15.93	17.63	18.54	23.46	--	--
	N	10	10	10	10	10	10	--	--
	%Diff G1	-0.20	-1.67	-2.57	-2.51	-2.03	-1.14	--	--
3F	Mean	186.10	220.40	241.10a	257.40	271.00	287.00	--	--
	SD	4.65	6.62	9.49	12.95	16.97	21.90	--	--
	N	10	10	10	10	10	10	--	--
	%Diff G1	-0.41	-2.07	-5.70	-4.48	-4.98	-3.63	--	--
4F	Mean	187.67	223.53	242.20a	254.67a	266.07b	280.67	287.80	302.40
	SD	7.03	11.51	13.42	12.27	13.39	16.59	26.20	25.34
	N	15	15	15	15	15	15	5	5
	%Diff G1	0.43	-0.68	-5.27	-5.49	-6.71	-5.75	-6.25	-5.32

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		Day Change -9 - -1	Change -1 - 7	Change 7 - 14	Change 14 - 21	Change 21 - 28	Change -1 - 28	Change 28 - 35
1M	Mean	72.00	65.00	56.20	54.33	38.13	213.67	37.20
	SD	7.34	8.12	8.38	8.03	8.37	28.78	6.65
	N	15	15	15	15	15	15	5
2M	Mean	70.20	59.80	54.90	49.70	35.70	200.10	--
	SD	7.04	9.73	9.24	8.83	9.33	33.08	--
	N	10	10	10	10	10	10	--
3M	Mean	70.10	57.60	61.80	47.20	44.40	211.00	--
	SD	9.55	12.49	9.99	12.01	8.40	38.25	--
	N	10	10	10	10	10	10	--
4M	Mean	71.07	47.53c	58.07	33.73f	43.00	182.33a	21.40h
	SD	6.68	5.13	5.09	4.20	6.11	13.32	5.37
	N	15	15	15	15	15	15	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)
g=p≤0.05,h=p≤0.01,i=p≤0.001 (T-test)

Table 3

Summary of Body Weight Gains (g)

Group 1 - Reference Item
Group 2 - mRNA-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		Day Change 35 - 42	Day Change 28 - 42
1M	Mean	23.80	61.00
	SD	10.18	13.32
	N	5	5
2M	Mean	--	--
	SD	--	--
	N	--	--
3M	Mean	--	--
	SD	--	--
	N	--	--
4M	Mean	36.00a	57.40
	SD	5.87	9.42
	N	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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		Day Change -10 - -1	Change -1 - 7	Change 7 - 14	Change 14 - 21	Change 21 - 28	Change -1 - 28	Change 28 - 35
1F	Mean	38.20	30.60	13.80	15.73	12.60	72.73	8.80
	SD	6.39	2.82	4.41	5.06	5.18	5.31	5.26
	N	15	15	15	15	15	15	5
2F	Mean	34.80	27.80	13.60	16.70	15.00	73.10	--
	SD	9.58	7.91	3.69	3.20	7.80	15.99	--
	N	10	10	10	10	10	10	--
3F	Mean	34.30	20.70b	16.30	13.60	16.00	66.60	--
	SD	6.20	4.57	6.06	9.65	8.77	19.10	--
	N	10	10	10	10	10	10	--
4F	Mean	35.87	18.67c	12.47	11.40	14.60	57.13c	7.20
	SD	7.36	5.96	5.63	5.87	5.51	10.80	6.38
	N	15	15	15	15	15	15	5

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

Table 3

Summary of Body Weight Gains (g)

Group 1 - Reference Item
Group 2 - mRNA-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		Day Change 35 - 42	Day Change 28 - 42
1F	Mean	12.40	21.20
	SD	5.94	5.76
	N	5	5
2F	Mean	--	--
	SD	--	--
	N	--	--
3F	Mean	--	--
	SD	--	--
	N	--	--
4F	Mean	14.60	21.80
	SD	8.20	3.11
	N	5	5

Table 4

Summary of Food Consumption (g/animal/day)

Group 1 - Reference Item
Group 2 - mRNA-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		Day (From/To)						
		-7/1	1/8	8/15	15/22	22/29	29/36	36/42
1M	Mean	30.93	33.55	34.37	35.72	36.81	33.16	32.32
	SD	1.69	2.23	2.05	2.56	2.73	2.96	1.81
	N	15	15	15	15	15	5	5
2M	Mean	30.74	32.11	33.91	33.41	35.89	--	--
	SD	0.87	0.89	1.56	1.81	1.77	--	--
	N	10	10	10	10	10	--	--
	%Diff G1	-0.62	-4.28	-1.33	-6.47	-2.51	--	--
3M	Mean	30.77	31.75	35.60	34.48	37.64	--	--
	SD	1.81	2.09	3.11	2.73	2.93	--	--
	N	10	10	10	10	10	--	--
	%Diff G1	-0.53	-5.36	3.59	-3.47	2.25	--	--
4M	Mean	30.49	29.21	34.25	31.67	35.25	30.64	34.18
	SD	0.45	1.14	1.48	1.82	1.14	0.49	0.16
	N	15	15	15	15	15	5	5
	%Diff G1	-1.42	-12.94	-0.35	-11.33	-4.26	-7.60	5.75

Table 4

Summary of Food Consumption (g/animal/day)

Group 1 - Reference Item
Group 2 - mRNA-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		Day (From/To) -7/1	1/8	8/15	15/22	22/29	29/36	36/42
1F	Mean	21.63	23.08	25.52	22.99	23.63	22.66	22.42
	SD	1.01	1.21	4.80	0.93	1.48	0.77	0.71
	N	15	15	15	15	15	5	5
2F	Mean	21.20	22.60	23.23	23.00	23.70	--	--
	SD	1.69	1.19	1.62	1.21	1.91	--	--
	N	10	10	10	10	10	--	--
	%Diff G1	-1.97	-2.08	-8.97	0.03	0.28	--	--
3F	Mean	20.81	21.45	22.09	21.63	22.36	--	--
	SD	0.70	0.66	1.38	1.89	2.59	--	--
	N	10	10	10	10	10	--	--
	%Diff G1	-3.78	-7.06	-13.44	-5.93	-5.39	--	--
4F	Mean	22.31	20.99	23.07	21.03	23.42	23.38	24.22
	SD	0.61	0.58	1.21	0.41	1.12	0.38	1.53
	N	15	15	15	15	15	5	5
	%Diff G1	3.14	-9.07	-9.59	-8.52	-0.90	3.18	8.03

Table 5

Summary of Body Temperature Values (°C)

Group 1 - Reference Item
Group 2 - mRNA-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 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
1M	Mean	36.94	37.67	36.81	--	36.62	37.96	36.26
	SD	0.45	0.53	0.31	--	0.41	0.40	0.31
	N	15	15	15	--	15	15	15
2M	Mean	36.72	38.57c	37.32a	--	36.62	38.22	36.89a
	SD	0.53	0.58	0.45	--	0.21	0.44	0.59
	N	10	10	10	--	10	10	10
	%Diff G1	-0.60	2.38	1.39	--	0.00	0.68	1.74
3M	Mean	36.86	39.12c	38.29c	36.95z	36.79	38.13	36.97b
	SD	0.36	0.36	0.61	0.21	0.50	0.44	0.57
	N	10	10	10	2	10	10	10
	%Diff G1	-0.22	3.84	4.03	--	0.46	0.45	1.96
4M	Mean	36.90	39.13c	38.56c	36.90z	36.68	38.88c	37.90c
	SD	0.32	0.32	0.47	0.14	0.24	0.53	0.63
	N	15	15	15	2	15	15	15
	%Diff G1	-0.11	3.88	4.76	--	0.16	2.42	4.52

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunnett)
Group excluded from statistical analysis (N<3): z

Table 5

Summary of Body Temperature Values (°C)

Group 1 - Reference Item
Group 2 - mRNA-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 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	37.13	37.89	37.79	--	38.43	38.42	38.37
	SD	0.47	0.45	0.52	--	0.32	0.47	0.50
	N	15	15	15	--	15	15	15
2F	Mean	37.41	38.00	38.05	--	38.85	38.10	38.60
	SD	0.84	0.52	0.47	--	0.49	0.85	0.48
	N	10	10	10	--	10	10	10
	%Diff G1	0.75	0.30	0.70	--	1.10	-0.83	0.61
3F	Mean	37.38	38.95f	38.40d	--	38.60	38.80	38.53
	SD	0.33	0.59	0.31	--	0.42	0.56	0.78
	N	10	10	10	--	10	10	10
	%Diff G1	0.66	2.81	1.62	--	0.45	0.99	0.43
4F	Mean	37.36	39.38f	39.11f	37.32	38.56	39.25b	38.93d
	SD	0.49	0.35	0.65	0.60	0.47	0.55	0.32
	N	15	15	15	9	15	15	15
	%Diff G1	0.61	3.94	3.51	--	0.35	2.15	1.46

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 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	8.500	0.865	7.245	0.213	0.074	0.019	0.087
	SD	2.067	0.318	1.817	0.095	0.023	0.010	0.040
	N	10	10	10	10	10	10	10
2M	Mean	11.700	4.919f	6.074	0.222	0.207a	0.016	0.261f
	SD	2.139	1.585	1.473	0.081	0.143	0.008	0.106
	N	10	10	10	10	10	10	10
	%Diff G1	37.647	468.671	-16.163	4.225	179.730	-15.789	200.000
3M	Mean	15.415f	9.665f	5.054d	0.216	0.251c	0.020	0.210e
	SD	2.831	2.786	1.092	0.065	0.096	0.007	0.084
	N	10	10	10	10	10	10	10
	%Diff G1	81.353	1017.341	-30.242	1.408	239.189	5.263	141.379
4M	Mean	16.911f	10.926f	5.367d	0.156	0.321c	0.018	0.151
	SD	4.324	3.204	2.102	0.047	0.102	0.018	0.096
	N	10	10	10	10	10	10	8
	%Diff G1	98.953	1163.121	-25.921	-26.761	333.784	-5.263	73.851

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		RBC 10^6/uL	HGB g/dL	HCT %	MCV fL(um3)	MCH pg	MCHC g/dL	RDW %
1M	Mean	7.821	14.40	42.89	54.90	18.41	33.54	12.42
	SD	0.357	0.44	1.26	1.77	0.61	0.60	0.30
	N	10	10	10	10	10	10	10
2M	Mean	7.496	13.84a	41.13	54.92	18.49	33.68	13.06b
	SD	0.309	0.38	1.35	2.06	0.75	0.39	0.54
	N	10	10	10	10	10	10	10
	%Diff G1	-4.155	-3.89	-4.10	0.04	0.43	0.42	5.15
3M	Mean	7.492	13.97	41.67	55.63	18.66	33.53	13.19b
	SD	0.299	0.54	1.78	1.39	0.52	0.47	0.37
	N	10	10	10	10	10	10	10
	%Diff G1	-4.207	-2.99	-2.84	1.33	1.36	-0.03	6.20
4M	Mean	7.731	14.15	41.98	54.37	18.32	33.69	13.73c
	SD	0.371	0.39	1.16	1.69	0.68	0.58	0.57
	N	10	10	10	10	10	10	10
	%Diff G1	-1.151	-1.74	-2.12	-0.97	-0.49	0.45	10.55

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		PLT 10^3/uL	RETIC 10^9/L
1M	Mean	1087.3	258.03
	SD	115.6	13.62
	N	10	10
2M	Mean	1105.2	213.03c
	SD	123.5	14.21
	N	10	10
	%Diff G1	1.6	-17.44
3M	Mean	1167.6	194.65c
	SD	104.9	27.14
	N	10	10
	%Diff G1	7.4	-24.56
4M	Mean	1071.0	170.99c
	SD	144.2	11.48
	N	10	10
	%Diff G1	-1.5	-33.73

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 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	6.188	0.487	5.393	0.149	0.071	0.006	0.083
	SD	1.534	0.157	1.605	0.045	0.013	0.005	0.029
	N	10	10	10	10	10	10	10
2F	Mean	9.770e	3.822	5.425	0.138	0.228b	0.015d	0.143
	SD	2.754	1.406	1.498	0.056	0.091	0.007	0.072
	N	10	10	10	10	10	10	10
	%Diff G1	57.886	684.805	0.593	-7.383	221.127	150.000	72.289
3F	Mean	10.586f	6.332c	3.710d	0.095	0.329c	0.009	0.105
	SD	2.822	1.993	1.095	0.043	0.188	0.010	0.065
	N	10	10	10	10	10	10	10
	%Diff G1	71.073	1200.205	-31.207	-36.242	363.380	50.000	26.506
4F	Mean	10.160e	6.586c	3.100e	0.092	0.276c	0.007	0.098
	SD	2.034	1.244	1.358	0.097	0.106	0.007	0.055
	N	10	10	10	10	10	10	10
	%Diff G1	64.189	1252.361	-42.518	-38.255	288.732	16.667	18.072

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		RBC 10^6/uL	HGB g/dL	HCT %	MCV fL(um3)	MCH pg	MCHC g/dL	RDW %
1F	Mean	7.372	13.56	40.04	54.37	18.41	33.88	11.10
	SD	0.257	0.58	1.38	0.70	0.37	0.41	0.50
	N	10	10	10	10	10	10	10
2F	Mean	7.111	13.10	38.46	54.16	18.41	34.03	11.65
	SD	0.435	0.64	1.79	1.96	0.61	0.60	0.52
	N	10	10	10	10	10	10	10
	%Diff G1	-3.540	-3.39	-3.95	-0.39	0.00	0.44	4.95
3F	Mean	7.291	13.65	39.50	54.22	18.72	34.53e	12.12b
	SD	0.170	0.41	0.99	1.34	0.46	0.37	0.50
	N	10	10	10	10	10	10	10
	%Diff G1	-1.099	0.66	-1.35	-0.28	1.68	1.92	9.19
4F	Mean	7.599	13.89	40.89	53.83	18.28	33.98	12.32c
	SD	0.192	0.43	1.55	1.95	0.51	0.45	0.18
	N	10	10	10	10	10	10	10
	%Diff G1	3.079	2.43	2.12	-0.99	-0.71	0.30	10.99

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		PLT 10^3/uL	RETIC 10^9/L
1F	Mean	1189.2	181.32
	SD	171.8	40.10
	N	10	10
2F	Mean	1117.4	192.08
	SD	152.9	21.21
	N	10	10
	%Diff G1	-6.0	5.93
3F	Mean	1034.5a	183.84
	SD	117.7	37.86
	N	10	10
	%Diff G1	-13.0	1.39
4F	Mean	876.3c	173.38
	SD	99.2	36.80
	N	10	10
	%Diff G1	-26.3	-4.38

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-1653 150 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	8.374	1.112	6.916	0.188	0.104	0.020	0.040
	SD	3.554	0.360	3.077	0.077	0.034	0.017	0.023
	N	5	5	5	5	5	5	5
4M	Mean	8.326	1.228	6.760	0.176	0.108	0.014	0.042
	SD	1.710	0.453	1.679	0.044	0.044	0.005	0.008
	N	5	5	5	5	5	5	5
	%Diff G1	-0.573	10.432	-2.256	-6.383	3.846	-30.000	5.000

Table 6

Summary of Hematology Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1653 150 µg/dose

Group / Sex		RBC 10^6/uL	HGB g/dL	HCT %	MCV fL(um3)	MCH pg	MCHC g/dL	RDW %
1M	Mean	7.882	14.26	42.10	53.44	18.06	33.78	12.54
	SD	0.242	0.36	0.86	1.01	0.54	0.43	0.51
	N	5	5	5	5	5	5	5
4M	Mean	7.456a	13.72	40.98	54.94a	18.38	33.46	14.66c
	SD	0.275	0.81	2.16	1.05	0.58	0.42	0.33
	N	5	5	5	5	5	5	5
	%Diff G1	-5.405	-3.79	-2.66	2.81	1.77	-0.95	16.91

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-1653 150 µg/dose

Group / Sex		PLT 10^3/uL	RETIC 10^9/L
1M	Mean	1184.8	218.66
	SD	24.6	30.40
	N	5	5
4M	Mean	1200.4	275.72a
	SD	123.8	26.93
	N	5	5
	%Diff G1	1.3	26.10

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-1653 150 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	7.124	1.506	5.302	0.188	0.066	0.006	0.054
	SD	2.283	0.857	2.208	0.046	0.021	0.009	0.034
	N	5	5	5	5	5	5	5
4F	Mean	6.972	1.732	4.906	0.200	0.078	0.008	0.044
	SD	1.668	0.959	1.486	0.101	0.039	0.004	0.019
	N	5	5	5	5	5	5	5
	%Diff G1	-2.134	15.007	-7.469	6.383	18.182	33.333	-18.519

Table 6

Summary of Hematology Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1653 150 µg/dose

Group / Sex		RBC 10^6/uL	HGB g/dL	HCT %	MCV fL(um3)	MCH pg	MCHC g/dL	RDW %
1F	Mean	7.026	13.02	37.70	53.66	18.56	34.56	11.12
	SD	0.242	0.44	1.37	1.96	0.62	0.62	0.47
	N	5	5	5	5	5	5	5
4F	Mean	6.980	12.50	36.64	52.56	17.92	34.08	12.98c
	SD	0.300	0.61	1.37	2.00	0.74	0.45	0.22
	N	5	5	5	5	5	5	5
	%Diff G1	-0.655	-3.99	-2.81	-2.05	-3.45	-1.39	16.73

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-1653 150 µg/dose

Group / Sex		PLT 10^3/uL	RETIC 10^9/L
1F	Mean	1204.2	159.92
	SD	169.6	18.25
	N	5	5
4F	Mean	1224.2	166.28
	SD	173.2	36.33
	N	5	5
	%Diff G1	1.7	3.98

Table 7

Summary of Coagulation Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		PT sec	APTT sec	FIB mg/dL
1M	Mean	17.45	16.21	302.8
	SD	0.93	0.81	20.5
	N	10	10	10
2M	Mean	16.64	16.84	613.6c
	SD	0.72	0.44	89.7
	N	10	10	10
	%Diff G1	-4.64	3.89	102.6
3M	Mean	17.02	18.37c	647.2c
	SD	0.61	1.04	38.8
	N	10	10	10
	%Diff G1	-2.46	13.33	113.7
4M	Mean	17.01	19.37c	697.8c
	SD	0.95	0.71	58.9
	N	10	10	10
	%Diff G1	-2.52	19.49	130.4

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

Table 7

Summary of Coagulation Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		PT sec	APTT sec	FIB mg/dL
1F	Mean	17.81	15.29	280.4
	SD	0.71	1.18	35.5
	N	10	10	10
2F	Mean	17.83	16.42	454.6c
	SD	0.66	1.09	88.9
	N	10	10	10
	%Diff G1	0.11	7.39	62.1
3F	Mean	17.88	17.11b	590.6c
	SD	0.58	0.72	138.0
	N	10	10	10
	%Diff G1	0.39	11.90	110.6
4F	Mean	18.39	19.23c	604.7c
	SD	1.07	1.14	66.3
	N	10	10	10
	%Diff G1	3.26	25.77	115.7

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

Table 7

Summary of Coagulation Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1653 150 µg/dose

Group / Sex		PT sec	APTT sec	FIB mg/dL
1M	Mean	17.96	15.74	323.2
	SD	0.74	0.72	46.8
	N	5	5	5
4M	Mean	17.82	15.68	301.0
	SD	0.45	0.80	27.9
	N	5	5	5
	%Diff G1	-0.78	-0.38	-6.9

Table 7

Summary of Coagulation Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1653 150 µg/dose

Group / Sex		PT sec	APTT sec	FIB mg/dL
1F	Mean	17.70	15.40	234.0
	SD	0.85	1.90	25.6
	N	5	5	5
4F	Mean	17.96	16.28	262.4
	SD	0.61	1.19	12.6
	N	5	5	5
	%Diff G1	1.47	5.71	12.1

Table 8

Summary of Clinical Chemistry Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 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	64.3	37.6	148.3	2.0	257.0	0.083	13.4
	SD	11.8	4.4	19.5	0.0	184.3	0.011	1.7
	N	10	10	10	10	10	10	10
2M	Mean	75.5	37.6	132.6	2.0	385.6	0.096	14.8
	SD	18.6	6.2	19.9	0.0	244.1	0.031	1.8
	N	10	10	10	10	10	10	10
	%Diff G1	17.4	0.0	-10.6	0.0	50.0	15.663	10.4
3M	Mean	85.7	40.5	147.5	2.0	345.4	0.105	15.6a
	SD	39.4	9.9	17.7	0.0	346.8	0.026	2.0
	N	10	10	10	10	10	10	10
	%Diff G1	33.3	7.7	-0.5	0.0	34.4	26.506	16.4
4M	Mean	82.4	36.0	143.2	2.0	459.9	0.098	13.6
	SD	31.2	5.1	27.8	0.0	360.3	0.026	2.1
	N	10	10	10	10	10	10	10
	%Diff G1	28.1	-4.3	-3.4	0.0	78.9	18.072	1.5

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 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	0.36	209.1	75.3	70.3	5.79	3.83	1.96
	SD	0.05	31.0	5.3	48.0	0.22	0.16	0.18
	N	10	10	10	10	10	10	10
2M	Mean	0.34	162.6b	70.6	49.0	6.04	3.41c	2.63c
	SD	0.05	36.0	12.6	12.9	0.28	0.15	0.20
	N	10	10	10	10	10	10	10
	%Diff G1	-5.56	-22.2	-6.2	-30.3	4.32	-10.97	34.18
3M	Mean	0.37	168.7a	73.8	52.5	6.08a	3.36c	2.72c
	SD	0.05	26.6	15.2	12.1	0.18	0.11	0.14
	N	10	10	10	10	10	10	10
	%Diff G1	2.78	-19.3	-2.0	-25.3	5.01	-12.27	38.78
4M	Mean	0.39	167.7a	73.3	62.6	6.05	3.28c	2.77c
	SD	0.06	24.3	12.4	10.5	0.29	0.12	0.21
	N	10	10	10	10	10	10	10
	%Diff G1	8.33	-19.8	-2.7	-11.0	4.49	-14.36	41.33

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		A/G ratio	CA mg/dL	PHOS mg/dL	NA mmol/L	K mmol/L	CL mmol/L
1M	Mean	1.98	10.95	7.92	140.1	5.09	100.2
	SD	0.22	0.21	0.49	1.6	0.36	1.7
	N	10	10	10	10	10	10
2M	Mean	1.31a	11.17	8.43	140.1	5.14	100.4
	SD	0.10	0.26	0.31	1.6	0.38	2.5
	N	10	10	10	10	10	10
	%Diff G1	-33.84	2.01	6.44	0.0	0.98	0.2
3M	Mean	1.24c	11.12	8.63	140.2	5.47	100.2
	SD	0.07	0.14	0.77	1.7	0.30	2.0
	N	10	10	10	10	10	10
	%Diff G1	-37.37	1.55	8.96	0.1	7.47	0.0
4M	Mean	1.18c	11.15	9.20c	140.3	5.69e	100.0
	SD	0.09	0.22	0.85	1.6	0.37	1.6
	N	10	10	10	10	10	10
	%Diff G1	-40.40	1.83	16.16	0.1	11.79	-0.2

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 8

Summary of Clinical Chemistry Values: Day 30

Group 1 - Reference Item
Group 2 - mRNA-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 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	86.1	37.6	83.1	2.0	232.3	0.087	14.6
	SD	41.3	10.1	17.2	0.0	126.1	0.036	1.9
	N	10	10	10	10	10	10	10
2F	Mean	78.9	41.4	80.2	2.0	302.7	0.059	17.4
	SD	19.0	11.8	18.2	0.0	153.3	0.035	3.0
	N	10	10	10	10	10	10	10
	%Diff G1	-8.4	10.1	-3.5	0.0	30.3	-32.184	19.2
3F	Mean	103.8	36.6	89.8	2.0	610.4	0.077	16.5
	SD	24.4	9.1	20.0	0.0	394.5	0.026	4.0
	N	10	10	10	10	10	10	10
	%Diff G1	20.6	-2.7	8.1	0.0	162.8	-11.494	13.0
4F	Mean	92.3	51.4	107.1a	2.0	280.8	0.061	15.3
	SD	46.4	41.3	14.5	0.0	218.9	0.011	3.0
	N	10	10	10	10	10	10	10
	%Diff G1	7.2	36.7	28.9	0.0	20.9	-29.885	4.8

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 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	0.38	186.4	79.9	53.9	6.27	4.31	1.96
	SD	0.06	31.1	12.5	19.1	0.39	0.27	0.18
	N	10	10	10	10	10	10	10
2F	Mean	0.39	168.8	76.1	40.9	6.31	3.97a	2.34b
	SD	0.07	32.5	15.5	8.8	0.26	0.24	0.25
	N	10	10	10	10	10	10	10
	%Diff G1	2.63	-9.4	-4.8	-24.1	0.64	-7.89	19.39
3F	Mean	0.43	140.9b	71.7	42.3	6.38	3.94a	2.44c
	SD	0.05	26.2	16.3	5.7	0.39	0.27	0.28
	N	10	10	10	10	10	10	10
	%Diff G1	13.16	-24.4	-10.3	-21.5	1.75	-8.58	24.49
4F	Mean	0.41	147.3a	70.2	53.7	6.26	3.79c	2.47c
	SD	0.06	21.2	19.1	12.5	0.31	0.30	0.17
	N	10	10	10	10	10	10	10
	%Diff G1	7.89	-21.0	-12.1	-0.4	-0.16	-12.06	26.02

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-1653 10 µg/dose
Group 3 - mRNA-1653 50 µg/dose
Group 4 - mRNA-1653 150 µg/dose

Group / Sex		A/G ratio	CA mg/dL	PHOS mg/dL	NA mmol/L	K mmol/L	CL mmol/L
1F	Mean	2.22	11.03	7.28	140.9	4.66	101.5
	SD	0.18	0.31	0.63	1.7	0.26	1.4
	N	10	10	10	10	10	10
2F	Mean	1.72f	11.07	7.39	139.8	4.87	101.3
	SD	0.27	0.28	0.60	1.2	0.33	2.0
	N	10	10	10	10	10	10
	%Diff G1	-22.52	0.36	1.51	-0.8	4.51	-0.2
3F	Mean	1.63f	11.00	7.69	138.8b	4.87	99.2
	SD	0.23	0.42	0.40	0.6	0.38	2.3
	N	10	10	10	10	10	10
	%Diff G1	-26.58	-0.27	5.63	-1.5	4.51	-2.3
4F	Mean	1.55f	11.10	7.78	139.8	4.96	100.4
	SD	0.18	0.18	0.79	1.4	0.42	3.2
	N	10	10	10	10	10	10
	%Diff G1	-30.18	0.63	6.87	-0.8	6.44	-1.1

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-1653 150 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	112.8	44.8	124.6	2.0	806.0	0.070	15.8
	SD	36.8	4.7	17.9	0.0	488.9	0.023	1.5
	N	5	5	5	5	5	5	5
4M	Mean	93.6	47.6	152.4	2.0	458.0	0.050	14.0
	SD	12.4	5.5	26.6	0.0	214.3	0.016	1.7
	N	5	5	5	5	5	5	5
	%Diff G1	-17.0	6.3	22.3	0.0	-43.2	-28.571	-11.4

Table 8

Summary of Clinical Chemistry Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1653 150 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	176.4	77.6	84.2	6.00	3.82	2.18
	SD	0.04	30.3	16.1	30.7	0.28	0.19	0.15
	N	5	5	5	5	5	5	5
4M	Mean	0.36	200.8	64.8	64.2	6.12	3.82	2.30
	SD	0.05	40.2	6.1	22.4	0.13	0.08	0.07
	N	5	5	5	5	5	5	5
	%Diff G1	12.50	13.8	-16.5	-23.8	2.00	0.00	5.50

Table 8

Summary of Clinical Chemistry Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1653 150 µg/dose

Group / Sex		A/G ratio	CA mg/dL	PHOS mg/dL	NA mmol/L	K mmol/L	CL mmol/L
1M	Mean	1.76	10.18	8.04	141.6	5.38	102.2
	SD	0.15	0.13	0.83	1.1	0.26	2.2
	N	5	5	5	5	5	5
4M	Mean	1.66	10.18	7.80	141.2	5.28	102.4
	SD	0.05	0.29	0.77	0.8	0.28	1.3
	N	5	5	5	5	5	5
	%Diff G1	-5.68	0.00	-2.99	-0.3	-1.86	0.2

Table 8

Summary of Clinical Chemistry Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1653 150 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	86.0	35.0	65.8	2.0	412.6	0.066	16.6
	SD	12.8	5.1	10.4	0.0	197.0	0.026	2.8
	N	5	5	5	5	5	5	5
4F	Mean	104.0	44.2	78.0	2.0	433.0	0.054	17.0
	SD	37.9	14.0	13.2	0.0	303.0	0.018	4.7
	N	5	5	5	5	5	5	5
	%Diff G1	20.9	26.3	18.5	0.0	4.9	-18.182	2.4

Table 8

Summary of Clinical Chemistry Values: Day 43

Group 1 - Reference Item

Group 4 - mRNA-1653 150 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	0.40	190.2	70.8	67.6	6.24	4.40	1.84
	SD	0.00	40.9	7.4	17.8	0.15	0.07	0.13
	N	5	5	5	5	5	5	5
4F	Mean	0.40	211.2	75.2	71.2	6.66	4.52	2.14b
	SD	0.07	14.1	11.1	13.2	0.38	0.35	0.09
	N	5	5	5	5	5	5	5
	%Diff G1	0.00	11.0	6.2	5.3	6.73	2.73	16.30

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-1653 150 µg/dose

Group / Sex		A/G ratio	CA mg/dL	PHOS mg/dL	NA mmol/L	K mmol/L	CL mmol/L
1F	Mean	2.42	10.66	6.82	140.0	4.72	101.8
	SD	0.22	0.22	0.23	1.0	0.15	1.3
	N	5	5	5	5	5	5
4F	Mean	2.10a	10.62	6.74	138.6a	4.90	100.0
	SD	0.17	0.20	0.43	0.5	0.32	1.4
	N	5	5	5	5	5	5
	%Diff G1	-13.22	-0.38	-1.17	-1.0	3.81	-1.8

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