charles river

NON-GLP FINAL REPORT AMENDMENT NO. 01

Test Facility Study No. 5002121

A Single Dose Intramuscular Injection Tissue Distribution Study of mRNA-1647 in Male Sprague-Dawley Rats

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

TEST FACILITY:
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
1580 Ida-Metivier
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SUMMARY OF CHANGES AND JUSTIFICATIONS

Note: When applicable, additions are indicated in bold underlined text and deletions are indicated in bold strikethrough text in the affected sections of the document.

Item or Section(s)	Justification
Final Amended Report 1	
2. SUMMARY	To correct the average value of terminal half-life for the muscle (i.e. injection site) based on the results of the toxicokinetic evaluation.
8.5. Toxicokinetic Evaluations	To correct the average value of terminal half-life for the muscle (i.e. injection site) based on the results of the toxicokinetic evaluation.
Toxicokinetic Report	To include a clarification page to correct the average value of terminal half-life for the muscle (i.e. injection site) based on the results of the toxicokinetic evaluation.

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1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Study Director

Test Facility Management

[redacted]

1.2. Individual Scientists (IS) at Test Facility

Analytical Chemistry

[redacted]

Charles River Laboratories Montreal ULC
Senneville Site (CR MTL)
Senneville, QC

Bioanalysis
(mRNA Quantitation)

[redacted]

Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
Sherbrooke, QC

Pathology
(Necropsy Only)

[redacted]

Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
Sherbrooke, QC

1.3. IS at Sponsor Test Site

Toxicokinetic
Interpretation

[redacted]

Moderna Therapeutics
Cambridge MA 02138, USA

2. SUMMARY

The objective of this study was to determine the tissue distribution of mRNA-1647, when given once by intramuscular injection to rats. In addition, the toxicokinetic characteristics of mRNA-1647 were determined.

This study was not within the scope of regulations governing the conduct of nonclinical laboratory studies and is not intended to comply with such regulations.

The study design was as follows:

Text Table 1
Experimental Design

Group No.	Test Item	Dose Level (µg)	Dose Volume (µL)	Dose Concentration (mg/mL)	No. of Animals Main Study Males
1	mRNA-1647	100	200	0.5	35

The following parameters and end points were evaluated in this study: clinical signs, body weights, toxicokinetic evaluation (mRNA-1647 quantitation in plasma and tissues) and gross necropsy findings.

Mean plasma concentrations of mRNA-1647 were quantifiable up to 24 hours following a single intramuscular injection at a dose level of 100 µg. All six mRNA-1647 constructs, gB, gH, gL, UL130, UL131A, and UL128 levels measured in plasma and tissues demonstrated nearly identical pharmacokinetic behavior. The highest mRNA-1647 exposure was observed in muscle (i.e. site of injection), followed by proximal (popliteal) lymph nodes, axillary distal lymph nodes and spleen, suggesting the mRNA-1647 distribution to the circulation by lymph flow. All other tissues tested, except for kidney and eye, have demonstrated exposures comparable or below that measured in plasma. Exposure observed for the eye was only slightly higher than that in plasma while no mRNA-1647 constructs were detected at any time point in the kidney. Concentrations of mRNA-1647 were quantifiable in the majority of tissues examined and in plasma at the first time point collected (i.e. 2 hours postdose) and peak concentrations were reached between 2 and 24 hours postdose in tissues with exposures above that of plasma. The $t_{1/2}$ of mRNA-1647 was reliably estimated in muscle (i.e. site of injection), proximal popliteal and axillary distal lymph nodes and spleen with average values for all construct $t_{1/2}$ of **14.9** ~~8.39~~, 34.8, 31.1, and 63.0 hours, respectively.

There were no mortalities during the course of the study and no mRNA-1647-related changes in body weight.

mRNA-1647-related clinical signs consisted of slight to severe swelling noted at the injection site (i.e. right hindlimb) from Day 2 to 4 with a decreasing severity on Day 4. This clinical sign was no longer observed on Days 5 and 6 which suggests that animals had fully recovered.

mRNA-1647-related macroscopic findings were limited to observations noted at the intramuscular injection site (i.e. right thigh) and draining lymph nodes. From Day 1 through Day 4, macroscopic findings of swelling, firmness and/or dark foci were observed at the injection site and enlargement and/or dark foci were noted at the lymph nodes draining the injection site (i.e. right popliteal and inguinal). These changes were consistent with a local reaction to the intramuscular injection of mRNA-1647 and/or were secondary to the changes

seen at the injection site. Apparent recovery of these findings was seen on Day 4 with only 1 male (No. 1034) with dark foci noted on the right inguinal lymph on Day 6.

In conclusion, the administration of 100 µg mRNA-1647 by a single intramuscular injection to male rats was clinically well-tolerated. Clinical signs were limited to firm swelling noted at the injection site and correlated with macroscopic anatomical changes observed at the injection site (swelling, firmness and/or dark foci) with secondary changes in the draining lymph nodes (enlargement and/or dark foci). These changes were consistent with a local reaction to the intramuscular injection of mRNA-1647 and were fully or partially resolved at the end of the study. Concentrations of mRNA-1647 were quantifiable in the majority of tissues examined and in plasma 2 hours postdose and peak concentrations were reached between 2 and 24 hours postdose in tissues with exposures above that of plasma. The highest mRNA-1647 exposure was observed in muscle (i.e. site of injection), followed by proximal (popliteal) lymph nodes, axillary distal lymph nodes and spleen, suggesting the mRNA-1647 distribution to the circulation by lymph flow. All other tissues tested, except for spleen (higher than plasma) and eye (slightly higher than plasma), have demonstrated exposures comparable or below that measured in plasma.

3. INTRODUCTION

The objective of this study was to determine the tissue distribution of mRNA-1647, when given once by intramuscular injection to rats. In addition, the toxicokinetic characteristics of mRNA-1647 were determined.

The design of this study was based on the study objective and the overall product development strategy for the Test Item.

The Study Director signed the study plan on 28 Jun 2017, and dosing was initiated on 10 Jul 2017. The study plan, the last amended study plan, and deviations are presented in Appendix 1.

4. MATERIALS AND METHODS

4.1. Test Item and Vehicle

4.1.1. Test Item

Identification:	mRNA-1647
Supplier:	Moderna Therapeutics, Inc.
Batch (Lot) No.:	MTDP17048
Concentration:	1.9 mg/mL
Retest Date:	20 Apr 2018
Physical Description:	White to off-white lipid nanoparticle dispersion
Storage Conditions:	Kept in a freezer set to maintain -20°C

4.2. Vehicle

Identification:	Phosphate-buffered Saline (PBS) pH 7.2
Supplier:	[redacted]
Batch (Lot) No.:	1854892
Expiration Date:	Dec 2018
Physical Description:	Liquid
Storage Conditions:	Kept in a controlled temperature area set to maintain 21°C

4.3. Test and Reference Item Characterization

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

4.4. Analysis of Test Item

The stability of the bulk Test Item was not determined during the course of this study.

4.5. Reserve Samples

Reserve samples were not collected during this study.

4.6. Test Item and Vehicle Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of Test Item and Vehicle were maintained. All unused Sponsor-supplied bulk Test Item was returned to Moderna Therapeutics, Cambridge MA 02138, USA, on dry ice (after completion of dosing).

4.7. Dose Formulation and Analysis

4.7.1. Preparation of Vehicle

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

The Vehicle, Phosphate Buffered Saline pH 7.2, was dispensed on day of dosing as required to dilute the bulk Test Item for administration to Group 1 animals.

Any residual volumes were discarded unless otherwise requested by the Study Director.

4.7.2. 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 the day of dosing 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.

Any residual volumes of formulated Test Item were stored in a refrigerator set at 4°C and were discarded prior to report finalization.

4.7.3. Sample Collection and Analysis

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

Text Table 2
Dose Formulation Sample Collection Schedule

Interval	Homogeneity	Concentration	Sampling From
Day 1	Group 1[a]	Group 1	Dosing container

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

Samples to be analyzed were submitted on 11 Jul 2017 (on ice pack) to the Test Facility analytical laboratory.

Any residual/retained analytical samples (and Test Item used in analysis) were discarded before issue of the Final Report.

4.7.3.1. Analytical Method

Analyses described below were performed by IEX-HPLC using a validated analytical procedure (CR MTL Study No. 1802050).

4.7.3.2. Concentration and HomogeneityAnalysis

Duplicate sets of samples (0.5 mL) were sent to the analytical laboratory; Triplicate sets of samples (0.5 mL) were retained at the Test Facility as backup samples. Concentration results were considered acceptable when mean sample concentration results were within or equal to ± 15% of theoretical concentration. The result of each individual sample concentration was considered acceptable within or equal to ± 20%. Homogeneity results were considered acceptable when the relative standard deviation of the mean value at each sampling location was ≤ 15%. After acceptance of the analytical results, backup samples were discarded.

4.7.3.3. Stability Analysis

There was no stability analysis performed for concentration used on this study.

4.8. Test System

4.8.1. Receipt

On 28 Jun 2017, 38 Crl:CD(SD) Spargue-Dawley male rats were received from [redacted] At dosing inititation, the animals were 8 weeks old and weighed between 302 and 346 grams.

4.8.2. Justification for Test System and Number of Animals

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

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

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

4.8.3. Animal Identification

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

4.8.4. Environmental Acclimation

A minimum acclimation period of 12 days was allowed between animal receipt and the start of dosing in order to accustom the animals to the laboratory environment.

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

At arrival, animals had their number randomly assigned.

The disposition of all animals was documented in the study records.

4.8.6. Husbandry

4.8.6.1. Housing

Animals were group housed (up to 3 animals) in polycarbonate cages containing appropriate bedding equipped with an automatic watering valve. These housing conditions were maintained throughout the study. 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.

4.8.6.2. 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, except when interrupted for designated procedures. Ten or greater air changes per hour with 100% fresh air (no air recirculation) were maintained in the animal rooms.

4.8.6.3. Food

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

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 would interfere with the objectives of the study.

4.8.6.4. Water

Municipal tap water after treatment by reverse osmosis and ultraviolet irradiation was freely available to each animal via an automatic watering system (except during designated procedures).

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 interfere with the outcome of the study.

4.8.6.5. Animal Enrichment

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

4.8.6.6. Veterinary Care

Veterinary care was available throughout the course of the study. No veterinary treatments were provided during the study.

4.9. Experimental Design

Text Table 3
Experimental Design

Group No.	Test Item	Dose Level (µg)	Dose Volume (µL)	Dose Concentration (mg/mL)	Animal Nos. Main Study Males
1	mRNA-1647	100	200	0.5	1001-1035

All rats remaining unassigned to groups after Day 1 were released from the study and their disposition was documented.

4.9.1. Administration of Test Materials

The Test Item was administered to the appropriate animals via intramuscular injection into the lateral compartment of the thigh once on Day 1. The volume for each dose was administered using a syringe/needle. The day of dosing was designated as Day 1.

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

On one occasion during the study, a spillage was noted for Animal No. 1034. Since this was single occurrence, this event was considered to have no impact on the study outcome.

4.9.2. Justification of Route and Dose Levels

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

The dose levels selected in this study were based upon pharmacologically active dose levels determined in rodent studies administered via this route. These dose levels were expected to produce sufficient tissue concentrations for quantitation in this tissue distribution study.

4.10. In-life Procedures, Observations, and Measurements

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

4.10.1. Mortality/Moribundity Checks

Throughout the study, animals were observed for general health/mortality and moribundity twice daily, once in the morning and once in the afternoon. Animals were not removed from cage during observation.

4.10.2. Clinical Observations

4.10.2.1. Cage Side Observations

Cage side observations were performed once daily throughout the study, beginning on Day -1. On the day of dosing, these observations were performed 4 to 6 hours postdose and approximately the same time each day thereafter. Animals were not removed from cage during observation.

4.10.2.2. Detailed Clinical Observations

The animals were removed from the cage, and a detailed clinical observation was performed weekly, beginning during Week -1.

4.10.3. Body Weights

Animals were weighed individually weekly, beginning during Week -1. A fasted weight was recorded on the day of necropsy.

4.11. Laboratory Evaluations

4.12. Bioanalysis and Toxicokinetic Evaluation

Blood and tissue samples were collected (± 15 minutes) according to Text Table 4.

Text Table 4
TK Sample Collection Schedule

Group No.	Subgroup	No. of Males	Sample Collection Time Points (Time Postdose[b]) on Day 1						
			0[a] hr	2 hrs	8 hrs	24 hrs	48 hrs	72 hrs	120 hrs
1	A	5	X	-	-	-	-	-	-
	B	5	-	X	-	-	-	-	-
	C	5	-	-	X	-	-	-	-
	D	5	-	-	-	X	-	-	-
	E	5	-	-	-	-	X	-	-
	F	5	-	-	-	-	-	X	-
	G	5	-	-	-	-	-	-	X

x = Sample collected; - = Not applicable.
[a] Sample collected before dosing.
[b] TK time point started at the perfusion.

4.12.1. Bioanalytical Blood Sample Collection

Blood was collected from jugular venipuncture at termination.

Target Blood Volume: 1.0 mL

Anticoagulant: K_2EDTA

Processing: To plasma; blood samples were kept on wet ice prior to processing. The samples were centrifuged within 30 minutes in a refrigerated centrifuge (set to maintain 4°C) for 15 minutes at 3000 x g. Immediately after plasma collection, plasma was aliquoted into

2 x 100 µL aliquot and a leftover (when available). Aliquots were snap frozen in liquid nitrogen and put on dry ice.

Storage Conditions: Samples were stored in a freezer set to maintain -80°C until analysis.

Disposition: Plasma samples were used for mRNA quantitation by the Immunology department using a bDNA method. The procedure followed during the course of this study along with the assay for acceptance criteria were detailed in the appropriate analytical procedure. Samples were analyzed in duplicate.

Any residual/retained bioanalytical samples were discarded before issue of the Final Report.

4.12.2. Bioanalytical Tissue Sample Collection

Lung (left lobe), liver (left lateral), heart (ventricle bilateral), right kidney, axillary distal lymph nodes (bilateral pooled to a target mass of 1.5 mg per animal; 1 aliquot or 2, when possible), proximal popliteal and inguinal lymph nodes (bilateral pooled to a target mass of 1.5 mg per animal; 1 aliquot or 2, when possible), spleen, brain (left hemisphere), stomach (glandular region), testes (right testicle), eye (left), bone marrow femur (bilateral pooled in the same aliquot), jejunum (middle region), and injection site muscle (homogenized and split in 3 aliquots) were collected following isoflurane anesthesia for terminal collection. Samples collected from all study animals at the scheduled necropsy were analyzed.

Target Weight: 2 x 50 mg or maximum obtainable when less than 2 x 50 mg; except for the bone marrow (1 aliquot) and the injection site (3 aliquots).

Processing: Animal were flushed with Sodium chloride with Heparin and sodium nitrite solution to remove blood as much as possible in the tissues and then with PBS 1X. Tissues were then collected, rinsed with 1X PBS (except bone marrow), dried on paper towel (except bone marrow), weighed, and immediately snap frozen on liquid nitrogen (target of 1 minute after collection), and kept on dry ice. Feces from bowel tissues were removed before processing.

Storage Conditions: Samples were stored in a freezer set to maintain -80°C until analysis.

Disposition: Samples collected from all study animals at the scheduled necropsy were analyzed. Samples (2 x 50 mg) were used for mRNA quantitation by the Immunology department using a bDNA method. The procedures followed during the course of this study along with the assay for acceptance criteria were detailed in the appropriate analytical procedures. Samples were analyzed in duplicate.

Any residual/retained bioanalytical samples were discarded before issue of the Final Report.

4.12.3. Toxicokinetic Evaluation

Toxicokinetic (TK) parameters were estimated using Phoenix pharmacokinetic software. A non-compartmental approach consistent with the intramuscular route of administration was used

for parameter estimation. All parameters were generated from mRNA-1647 concentrations in plasma and tissues from all TK occasions, whenever practical.

Text Table 5
Parameters Estimated

Parameter	Description of Parameter
Tmax	The time after dosing at which the maximum observed concentration was observed.
Cmax	The maximum observed concentration measured after dosing.
AUC(0-t)	The area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed, using the linear or linear/log trapezoidal method.
T1/2	The apparent terminal elimination half life.

When data permits, the slope of the terminal elimination phase of each arithmetic mean concentration versus time curve was determined by log-linear regression.

Descriptive statistics (number, mean, median, standard deviation, standard error, etc.) were reported as deemed appropriate and when possible, as well as ratios for appropriate grouping and sorting variables were generated using Phoenix. TK table and graphs were also generated by Phoenix.

4.13. Terminal Procedures

Terminal procedures are summarized in Text Table 6.

Text Table 6
Terminal Procedures

Group No.	No. of Animals: Males	Scheduled Euthanasia Day	Necropsy Procedures: Necropsy	Necropsy Procedures: Tissue Collection	Necropsy Procedures: Sample Tissue Weights
1	15	1	X	X[a]	X
	5	2			
	5	3			
	5	4			
	5	6			

X = Procedure conducted; - = Not applicable.

[a] Consisting of blood, lung (left lobe), liver (left lateral), heart (ventricle bilateral), right kidney, axillary distal lymph nodes (bilateral pooled to a target mass of 1.5 mg per animal; 1 aliquot or 2, when possible), proximal popliteal and inguinal lymph nodes (bilateral pooled to a target mass of 1.5 mg per animal; 1 aliquot or 2, when possible), spleen, brain (left hemisphere), stomach (glandular region), testes (right testicle), eye (left), bone marrow femur (bilateral pooled in the same aliquot), jejunum (middle region), and injection site muscle (homogenized and split in 3 aliquots).

4.13.1. Unscheduled Deaths

No animals died during the course of the study.

4.13.2. Scheduled Euthanasia

Main study animals surviving until scheduled euthanasia had a terminal body weight recorded, blood samples for laboratory evaluations were collected, and underwent isoflurane anesthesia

followed by whole-body perfusion with NaCl 0.9%, Heparin (1000 IU/L), 1% sodium nitrite and then PBS 1X. Animals were fasted overnight before their scheduled necropsy.

4.13.3. 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.

4.13.4. Sample Tissue Weights

Lung (left lobe), liver (left lateral), heart (ventricle bilateral), right kidney, axillary distal lymph nodes (bilateral pooled to a target mass of 1.5 mg per animal), proximal popliteal and inguinal lymph nodes (bilateral pooled to a target mass of 1.5 mg per animal), spleen, brain (left hemisphere), stomach (glandular region), testes (right testicle), eye (left), bone marrow femur (bilateral pooled in the same aliquot), jejunum (middle region), and injection site muscle (homogenized and split in 3 aliquots) were weighed at necropsy for all scheduled euthanasia animals.

5. STATISTICAL ANALYSIS

Means and standard deviations were calculated for all numerical data.

6. 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 7
Critical Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
Provantis	8	In-life; postmortem
Dispense	8	Test Material receipt, accountability
SRS (CR MTL in-house application built with SAS and SAS system for Windows)	1.4	Statistical analyses of numerical in-life and terminal data
In-house reporting software Nevis 2012 (using SAS)	Nevis 2 (SAS 9.2)	Statistical analyses of numerical in-life and terminal data
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
Mesa Laboratories AmegaView CMS	v3.0 Build 1208.8	Continuous Monitoring System. Monitoring of standalone fridges, freezers, incubators, and selected laboratories to measure temperature, relative humidity, and CO2, as appropriate
Johnson Controls Metasys	MVE 7.0	Building Automation System. Control of HVAC and other building systems, as well as temperature/humidity control and trending in selected laboratories and animal rooms
Phoenix	7.0	Computation of non-compartmental analysis, descriptive statistics and ratios, as well as graphical and tabular output
Watson Laboratory Information Management system (Thermo Scientific)	7.4.2 SP1	mRNA quantitation data regression
Bio-Plex Manager	4.1 and 6.1	Data acquisition for mRNA quantitation

7. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, documentation, study plan, samples, specimens, and final reports from this study were archived a CR MTL archives 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 that the data collected using Provantis 8 and reporting files stored on SDMS, which were archived at the Charles River Laboratories facility location in Wilmington, MA.

All records, and reports generated from phases or segments performed by Sponsor-designated subcontractors were kept at the Test Site for archiving.

8. RESULTS

8.1. Dose Formulation Analyses

(Appendix 3)

All study samples analyzed had mean concentrations within or equal to the acceptance criteria of ± 15% (individual values within or equal to ± 20%) of their theoretical concentrations.

For homogeneity, the relative standard deviation (RSD) of concentrations for all samples in each group tested was within the acceptance criteria of ≤ 5%.

8.2. Mortality

(Appendix 4)

There were no mortalities during the course of the study.

8.3. Clinical Observations

(Table 1 and Appendix 5)

For some animals, on the day of scheduled necropsy, slight to severe firm swelling was noted at the injection site (i.e. right hindlimb). On Day 2, moderate to severe swelling was noted while, from Day 3 through Day 4, the severity of the swelling tended to decrease from moderate to slight. This clinical sign was no longer observed on Days 5 and 6 which suggests that animals had fully recovered. There were no other mRNA-1647-related clinical signs noted.

8.4. Body Weights

(Appendix 6)

There were no mRNA-1647-related body weight changes during the study.

8.5. Toxicokinetic Evaluations

(Appendix 7 and Appendix 8)

No quantifiable mRNA-1647 concentrations were observed in the predose plasma and tissue samples (i.e. all results were below the limit of quantitation [BLQ]) for all constructs except gH, where 2 plasma samples were slightly above the lower limit of quantitation (LLOQ).

Mean plasma concentrations of mRNA-1647 were quantifiable up to 24 hours with inter-animal variability between 21.8 and 79.8 CV%. The only quantifiable plasma samples beyond 24 hours were 6 gH samples which were just above the LLOQ.

The gradient of mRNA-1647 constructs concentrations in evaluated tissues suggests that Test Item distributes from the site of administration proceeding through the lymphatic system. mRNA-1647 was retained at the site of administration and upon entry into circulation was primarily deposited in spleen. The amounts of mRNA-1647 detected in some peripheral tissues, although detectable, overall were negligible.

Concentrations of mRNA-1647 constructs were quantifiable by the first time point collected (i.e. 2 hours postdose) in highly exposed tissues (injection site muscle, lymph nodes, spleen). Other peripheral tissues have demonstrated varying concentrations of individual constructs

generally at low levels, except for kidneys where no mRNA-1647 constructs were detected at any time point. In muscle (i.e.site of injection), lymph nodes and spleen, mRNA-1647 concentrations were quantifiable up to the last sampling collection time, 120 hours postdose. In general, high concentration variability was observed for all tissues examined.

mRNA-1647 was detected in all of the analyzed tissues except for kidney. For the bone marrow, brain, jejunum, heart, liver, lung, stomach and testes, $AUC_{(0\text{-}t)}$ was calculated using less than 3 quantifiable mean concentrations and therefore, is an estimate. For highly exposed tissues, peak concentration (C_{max}) was observed between 2 hours and 8 hours postdose in muscle and lymph nodes and between 2 and 24 hours postdose in spleen. For all six mRNA-1647 constructs, measured levels for gB, gH, gL, UL130, UL131A, and UL128 in plasma and tissues were detectable in 1:1:1:1:1:1 ratio.

The half-life ($t_{1/2}$) of mRNA-1647 was reliably estimated in muscle (i.e. site of injection), proximal popliteal and axillary distal lymph nodes and spleen with average values for all construct $t_{1/2}$ of **14.9** ~~**8.39**~~, 34.8, 31.1, and 63.0 hours, respectively.

Peak mRNA-1647 plasma concentration was reached at the first sampling time point (i.e. 2 hours postdose). Peak concentration was followed by a rapid elimination phase. A rough estimation of $t_{1/2}$ for mRNA-1647 from initial data points of PK profile, including the C_{max} yielded values between 2.7 and 3.8 hours. The C_{max} and $AUC_{(0\text{-}t)}$ associated with a mRNA-1647 intramuscular administration of 100 µg in male Crl:CD(SD) Sprague-Dawley rats were between 1.60 and 2.30 ng/mL and between 22.7 and 25.5 hr*ng/mL, respectively.

The highest mRNA-1647 exposure was observed in muscle (i.e. site of injection), followed by proximal (popliteal) and axillary distal lymph nodes, suggesting the Test Item distribution to the circulation by lymph flow. All other tissues tested, except for spleen and eye, had exposures comparable to or below the measured plasma concentration (tissue to plasma AUC ratios below 1.0). Exposure observed for the eye was only slightly higher than that in plasma. Concentrations were no longer detectable after 24 hours.

The averaged for all constructs, mRNA-1647 tissue-to-plasma $AUC_{(0\text{-}t)}$ ratios for highly exposed tissues were 939, 201, 62.8, and 13.4 for muscle (i.e. injection site), the lymph nodes (proximal popliteal and axillary distal) and spleen, respectively.

8.6. Gross Pathology

(Table 2 and Appendix 9)

mRNA-1647-related gross pathology findings were noted at the intramuscular injection site (i.e. right thigh) and draining lymph nodes, and are summarized in Text Table 8.

Text Table 8
Summary of Gross Pathology Findings - Scheduled Euthanasia (Day 1, 2, 3, 4, and 6)

Males						
Group	**1 (day 1)**	**1 (day 2)**	**1 (day 3)**	**1 (day 4)**	**1 (day 6)**	**1 (total)**
Dose (µg/dose)	**100**	**100**	**100**	**100**	**100**	**100**
No. Animals Examined	**15**	**5**	**5**	**5**	**5**	**35**
Injection site (No. Examined)	(15)	(5)	(5)	(5)	(5)	(35)
Swelling	4	5	3	0	0	12
Firm	0	5	5	0	0	10
Focus; dark	0	0	4	1	0	5
Material accumulation; clot	0	0	1	0	0	1
Draining lymph nodes[a] (No. Examined)	(15)	(5)	(5)	(5)	(5)	(35)
Enlargement	1	2	2	0	0	5
Focus; dark	0	0	1	0	1	2

[a] Popliteal right and inguinal right only.

At the intramuscular injection site (i.e. right thigh), macroscopic findings of swelling, firmness and/or dark foci were observed in several animals euthanized from Day 1 through Day 4, with an apparent recovery of the findings starting on Day 4. In addition, material accumulation (i.e. clot) was observed at the injection site of one male (No. 1023) on Day 3. These changes were consistent with a local reaction to the intramuscular injection of mRNA-1647.

At the lymph nodes draining the injection site (i.e. right popliteal and inguinal), macroscopic changes of enlargement and/or dark foci were occasionally noted mainly in animals euthanized from Day 1 through Day 3, and were considered secondary to the changes seen at the injection site. Similarly, an apparent recovery of the findings was seen on Day 4 and 6 with only one male (No. 1034) with dark foci noted on the right inguinal lymph node on Day 6.

Other gross findings observed were considered incidental, and/or of the nature commonly observed in this strain and age of rats, and, therefore, were considered not mRNA-1647-related.

9. CONCLUSION

In conclusion, the administration of 100 µg mRNA-1647 by a single intramuscular injection to male rats was clinically well-tolerated. Clinical signs were limited to firm swelling noted at the injection site and correlated with macroscopic anatomical changes observed at the injection site (swelling, firmness and/or dark foci) with secondary changes in the draining lymph nodes (enlargement and/or dark foci). These changes were consistent with a local reaction to the intramuscular injection of mRNA-1647 and were fully or partially resolved at the end of the study. Concentrations of mRNA-1647 were quantifiable in the majority of tissues examined and in plasma 2 hours postdose and peak concentrations were reached between 2 and 24 hours postdose in tissues with exposures above that of plasma. The highest mRNA-1647 exposure was observed in muscle (i.e. site of injection), followed by proximal (popliteal) lymph nodes, axillary distal lymph nodes and spleen, suggesting the mRNA-1647 distribution to the circulation by lymph flow. All other tissues tested, except for spleen (higher than plasma) and eye (slightly higher than plasma) have demonstrated exposures comparable or below that measured in plasma.

10. REPORT APPROVAL

[redacted]

Date: [redacted]

Table 1

Summary of Clinical Observations

5002121

Day numbers relative to Start Date

Sex: Male

	100 ug
Swollen Firm	
Number of Observations	15
Number of Animals	15
Days from - to	2 4
Skin, Scab	
Number of Observations	4
Number of Animals	3
Days from - to	-1 3

Table 2

Incidence of Necropsy Findings by Organ/Group
5002121

Removal Reason: TERMINAL EUTHANASIA	Male 100 ug Group 1	
Number of Animals:	35	
KIDNEY		
Adhesion	1	
LYMPH NODE, AXILLARY		
Focus; dark	7	
LYMPH NODE, INGUINAL		
Enlargement	1	
Focus; dark	1	
LYMPH NODE, MANDIBULAR		
Focus; dark	5	
Enlargement	1	
LYMPH NODE, POPLITEAL		
Enlargement	5	
Focus; dark	1	
SITE, INJECTION		
Swelling	12	
Abnormal consistency; firm	10	
Focus; dark	5	
Material accumulation; clot	1	
STOMACH		
Focus; dark	2	
THYMUS		
Focus; dark	23	

Table 2

Incidence of Necropsy Findings by Organ/Group
5002121

Key Page

Measurement/Statistics

Measurement	Descriptive	Comparative	Arithmetic/Adjusted	Transformation
Pathology Observation	Count Positives			

Group Information

Short Name	Long Name	Report Headings		
1	1	100	ug	Group 1

Removal Reason Grouping

Grouping Name	Abbreviation	Removal Reasons
TERMINAL EUTHANASIA	TERM	TERMINAL EUTHANASIA