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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)
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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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1.1. Test Facility

Study Director (b) (6)

Test Facility Management (b) (6)

1.2. Individual Scientists (IS) at Test Facility

Analytical Chemistry (b) (6)
Charles River Laboratories Montreal ULC
Senneville Site (CR MTL)
Senneville, QC

Bioanalysis
(mRNA Quantitation) (b) (6)
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
Sherbrooke, QC

Pathology
(Necropsy Only) (b) (6)
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
Sherbrooke, QC

1.3. IS at Sponsor Test Site

Toxicokinetic
Interpretation (b) (6)
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:	Gibco
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 Charles River Canada Inc., St. Constant, QC, Canada. 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, $AUC_{(0-t)}$ 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 $AUC_{(0-t)}$ 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 $AUC_{(0-t)}$ 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 **Dose (µg/dose)** **No. Animals Examined**	**1 (day 1)** **100** **15**	**1 (day 2)** **100** **5**	**1 (day 3)** **100** **5**	**1 (day 4)** **100** **5**	**1 (day 6)** **100** **5**	**1 (total)** **100** **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

(b) (6)

Date: 13 Dec 2017

Study Director

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

Appendix 1

charles river

FINAL STUDY PLAN

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
Sherbrooke, QC J1E 0B5
Canada

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1. OBJECTIVES

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

1.1. Study Classification

Study Category:	PK
Study Type:	Distribution; Single Dose PK
Study Design:	Parallel
Primary Treatment CAS Registry Number:	Not Available
Primary Treatment Unique Ingredient ID:	Not Available
Class of Compound:	mRNA

2. PROPOSED STUDY SCHEDULE

Proposed study dates are listed below. Actual applicable dates will be included in the Final Report.

Animal Arrival:	28 Jun 2017
Initiation of Dosing:	10 Jul 2017
Completion of In-life:	15 Jul 2017 (Last date of necropsy)
Draft Report:	25 Oct 2017 (69 working days following completion of in-life)
Final Report:	25 Apr 2018 (Expected date of Study Director signature, default 6 months from Draft Report)

3. GUIDELINES FOR STUDY DESIGN

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

4. REGULATORY COMPLIANCE

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

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5. SPONSOR

Sponsor Representative

(b) (6)

Address as cited for Sponsor
Tel: (b) (6)
E-mail: (b) (6)

6. RESPONSIBLE PERSONNEL

Study Director

(b) (6)

Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
Address as cited for Test Facility
Tel: (b) (6)
Fax: (b) (6)
E-mail: (b) (6)

Management Contact

(b) (6)

Address as cited for Test Facility
Tel: (b) (6)
Fax: (b) (6)
E-mail: (b) (6)

Individual Scientists (IS) at the Test Facility

Pathology	Will be added by amendment
Analytical Chemistry	(b) (6) Senior Research Scientist II Charles River Laboratories Montreal ULC Senneville Site (CR MTL) 22022 Transcanadienne Senneville, QC H9X 3R3 Canada Tel: (b) (6) E-mail: (b) (6)
Bioanalysis (mRNA quantitation)	(b) (6) Senior Research Scientist I Charles River Laboratories Montreal ULC Sherbrooke Site (CR SHB)

Appendix 1

Address as cited for Test Facility
Tel: (b) (6)
E-mail: (b) (6)

Each IS is required to report any deviations or other circumstances that could affect the quality or integrity of the study to the Study Director in a timely manner. Each IS will provide a report addressing their assigned phase of the study, which will be included as an appendix to the Final Report. The phase report will include the following:

- A listing of critical computerized systems used in the conduct and/or interpretation of the assigned study phase

IS at Sponsor Test Site

Toxicokinetic
Analysis/Interpretation (b) (6)
Moderna Therapeutics
200 Technology Sq, 3rd Floor
Cambridge MA 02138, USA
Email : (b) (6)

- Each PI is required to report any deviations or other circumstances that could affect the quality or integrity of the study to the Study Director in a timely manner. Each PI will provide a report addressing their assigned phase of the study, which will be included as an appendix to the Final Report. The phase report will include the following:
- The archive site for all records, samples, specimens and reports generated from the phase or segment (alternatively, details regarding the retention of the materials may be provided to the Study Director for inclusion in the Final Report)
- A listing of critical computerized systems used in the conduct and/or interpretation of the assigned study phase

7. TEST ITEM AND VEHICLE

7.1. Test Item

Identification:	mRNA-1647
Supplier:	Moderna Therapeutics, Inc
Batch (Lot) Number:	Will be added by amendment
Concentration:	Will be added by amendment
Retest Date:	Will be added by amendment
Physical Description:	White to off-white lipid nanoparticle dispersion
Storage Conditions:	Kept in a freezer set to maintain -20°C

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7.2. Vehicle

Identification:	Phosphate-buffered Saline (PBS) pH 7.2
Supplier:	Will be included in the Final Report
Batch (Lot) Number:	Will be included in the Final Report
Expiration Date:	Will be included in the Final Report
Physical Description:	Liquid
Storage Conditions:	Kept in a controlled temperature area set to maintain 21°C

7.3. Test Item Characterization

The Sponsor will provide to the Test Facility documentation of the identity, strength, purity and composition for the Test Item. A Certificate of Analysis or equivalent documentation will be provided for inclusion in the Final Report. The Sponsor will also provide information concerning the regulatory standard that was followed for these evaluations.

The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the Test Item, and this information is available to the appropriate regulatory agencies should it be requested.

7.4. Analysis of Test Item

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

7.5. Reserve Samples

Reserve samples will not be collected during this study.

7.6. Test Item and Vehicle Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of Test Item and Vehicle will be maintained. All unused Sponsor-supplied bulk Test Item will be returned to the Sponsor on dry ice (after completion of dosing).

Shipping Contact

(b) (6)
Moderna Therapeutics
500 Technology Sq, 8th Floor
Cambridge MA 02138, USA
E-mail: (b) (6)

8. SAFETY

The safety precautions for the Test Item and dose formulations will be documented in a Test Material Safety Data Sheet (TMSDS) based on the information provided by the Sponsor either by an MSDS or similar document.

Appendix 1

9. DOSE FORMULATION AND ANALYSIS

9.1. Preparation of Vehicle

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

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

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

9.2. Preparation of Test Item

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

Test Item dosing formulations will be diluted with Phosphate Buffered Saline, pH 7.2, as necessary for administration. The dosing formulations will be prepared on the day of dosing and will be stored in a refrigerator set to maintain 4°C. The dose formulations will be allowed to warm to room temperature for at least 30 minutes prior to dosing. Alternatively, the aliquots can be transferred directly to room temperature.

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

9.3. Sample Collection and Analysis

Dose formulation samples will be collected for analysis as indicated in the following table. Additional samples may be collected and analyzed at the discretion of the Study Director.

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 will be averaged and utilized as the concentration results.

Samples to be analyzed will be submitted as soon as possible following collection.

All samples to be analyzed will be transferred (on ice pack) to the analytical laboratory.

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

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9.3.1. Analytical Method

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

9.3.1.1. Concentration and Homogeneity Analysis

Samples for Analysis:	Duplicate top, middle, and bottom samples; sent for analysis as noted in Section 9.3.
Backup Samples:	Triplicate top, middle, and bottom samples; maintained at the Test Facility. Backup samples may be analyzed at the discretion of the Study Director.
Sampling Containers:	Appropriate sized glass containers.
Sample Volume:	0.5 mL for analysis and backup samples.
Storage Conditions:	Kept in a refrigerator set to maintain 4°C.
Acceptance Criteria:	For concentration, the criteria for acceptability will be mean sample concentration results within or equal to ± 15% of theoretical concentration. Each individual sample concentration result within or equal to ± 20%. For homogeneity, the criteria for acceptability will be a relative standard deviation (RSD) of concentrations of ≤ 15%.

9.3.1.2. Stability Analysis

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

10. TEST SYSTEM

Species:	Rat
Strain:	Crl:CD(SD) Sprague-Dawley rat
Source:	Charles River Canada Inc., St. Constant, QC, Canada
Number of Males Ordered:	38
Target Age at Arrival:	4 to 8 weeks
Target Weight at Arrival:	126 to 150 g

The actual age, weight, and number of animals received will be listed in the Final Report.

10.1. Justification of 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.

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The total number of animals to be used in this study is 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.

10.2. Animal Identification

Each animal will be identified using a subcutaneously implanted electronic identification chip.

10.3. Environmental Acclimation

A minimum acclimation period of 10 days will be allowed between animal receipt and the start of dosing in order to accustom the animals to the laboratory environment.

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

At arrival, animals will have their number randomly assigned. Animals in poor health will not be assigned to groups.

Before the initiation of dosing, any assigned animals considered unsuitable for use in the study will be replaced by alternate animals obtained from the same shipment and maintained under the same environmental conditions.

After initiation of dosing, study animals may be replaced during the replacement period with alternate animals in the event of accidental injury, non-Test Item-related health issues, or similar circumstances.

The alternate animals may be used as replacements on the study within 1 day.

The disposition of all animals will be documented in the study records.

11. HUSBANDRY

11.1. Housing

Animals will be group housed (up to 3 animals) in polycarbonate cages containing appropriate bedding equipped with an automatic watering valve. These housing conditions will be maintained unless deemed inappropriate by the Study Director and/or Clinical Veterinarian. The room in which the animals will be kept will be documented in the study records.

Animals will be separated during designated procedures/activities. Each cage will be clearly labeled with a color-coded cage card indicating study, group, animal number(s), and sex.

11.2. Environmental Conditions

The targeted conditions for animal room environment will be as follows:

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Temperature:	19°C to 25°C
Humidity:	30% to 70%
Light Cycle:	12 hours light and 12 hours dark (except during designated procedures)

11.3. Food

PMI Nutrition International Certified Rodent Chow No. 5CR4 will be provided ad libitum throughout the study, except during designated procedures. The same diet in meal form may be provided to individual animals as warranted by clinical signs (e.g., broken/damaged incisors or other health changes).

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

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

11.4. Water

Municipal tap water after treatment by reverse osmosis and ultraviolet irradiation will be freely available to each animal via an automatic watering system (except during designated procedures). Water bottles can be provided, if required.

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

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

11.5. Animal Enrichment

Animals will be socially housed for psychological/environmental enrichment and will be provided with items such as a hiding tube and a chewing object, except during study procedures/activities.

11.6. Veterinary Care

Veterinary care will be available throughout the course of the study and animals will be examined by the veterinary staff as warranted by clinical signs or other changes. All veterinary examinations and recommended therapeutic treatments, if any, will be documented in the study records.

In the event that animals show signs of illness or distress, the responsible veterinarian may make initial recommendations about treatment of the animal(s) and/or alteration of study procedures, which must be approved by the Study Director or Scientific designate. All such actions will be properly documented in the study records and, when appropriate, by study plan amendment.

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Treatment of the animal(s) for minor injuries or ailments may be approved without prior consultation with the Sponsor representative when such treatment does not impact fulfillment of the study objectives. If the condition of the animal(s) warrants significant therapeutic intervention or alterations in study procedures, the Sponsor representative will be contacted, when possible, to discuss appropriate action. If the condition of the animal(s) is such that emergency measures must be taken, the Study Director and/or clinical veterinarian will attempt to consult with the Sponsor representative prior to responding to the medical crisis, but the Study Director and/or veterinarian has authority to act immediately at his/her discretion to alleviate suffering. The Sponsor representative will be fully informed of any such events.

12. EXPERIMENTAL DESIGN

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

12.1. Administration of Test Item

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

The injection area will be marked as frequently as required to allow appropriate visualization of administration sites. Hair may be clipped or shaved if required to improve visualization of the injection sites. The injection site will be documented in the raw data.

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

13. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS

The in-life procedures, observations, and measurements listed below will be performed for all main study animals. During the study, additional evaluations to those described below and/or scheduled, and considered necessary by the Study Director and/or Veterinarian to assess health status will be conducted and duly documented. More frequent observations may be undertaken if considered appropriate.

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13.1. Mortality/Moribundity Checks

Frequency:	Twice daily, once in the morning and once in the afternoon, throughout the study.
Procedure:	Animals will be observed for general health/mortality and moribundity. Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

13.2. Clinical Observations

13.2.1. Cage Side Observations

Frequency:	Once on Day -1 and once daily throughout the study; target time of 4 to 6 hours postdose on day of dosing and approximately the same time each day thereafter.
Procedure:	Animals will not be removed from the cage during observation, unless necessary for identification or confirmation of possible findings.

13.2.2. Detailed Clinical Observations

Frequency:	Weekly
Procedure:	Animals removed from the cage for examination.

13.3. Body Weights

Frequency:	Weekly
Procedure:	Animals will be individually weighed. A fasted weight will be recorded on the day of necropsy. Terminal body weights will not be collected from animals found dead or euthanized moribund.

Appendix 1

14. LABORATORY EVALUATIONS

14.1. Bioanalysis and Toxicokinetic Evaluation

Blood and tissue samples will be collected according to the following table (± 15 minutes).

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 to be collected; - = Not applicable.
[a] Sample will be collected before dosing.
[b] TK time point starts at the perfusion.

Any residual/retained bioanalytical samples will be maintained for a minimum of 6 months following issuance of the Draft Report after which samples will be discarded. Alternatively, residual/retained samples will be discarded prior to the 6 month period should the issuance of the Final Report occur prior to the end of the 6 month retention period. An earlier discard of these residual/retained samples may also be requested and authorized by the Study Director.

14.1.1. Bioanalytical Blood Sample Collection

Blood will be collected from jugular venipuncture at termination and, if possible, from animals that are preterminally euthanized.

Target Blood Volume:	1.0 mL
Anticoagulant:	K_2EDTA
Processing:	To plasma; blood samples will be kept on wet ice prior to processing. The samples will be 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 will be aliquoted into 2 x 100 µL aliquot and a leftover (if available). Aliquots will be snap frozen in liquid nitrogen and put on dry ice.
Storage conditions:	Samples will be stored in a freezer set to maintain -80°C until analysis.
Disposition:	Plasma samples will be used for mRNA quantitation by the Immunology department using a bDNA method. The procedure to

Appendix 1

be followed during the course of this study along with the assay for acceptance criteria will be detailed in the appropriate analytical procedure. Samples will be analyzed in duplicate.

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

14.1.2. Bioanalytical Tissue Sample Collection

Lung, liver, heart, right kidney, axillary distal lymph nodes (pooled to a target mass of 1.5 mg per animal), proximal popliteal and inguinal lymph nodes (pooled to a target mass of 1.5 mg per animal), spleen, brain, stomach, testes (right testicle), eye (left), bone marrow (bilateral pooled in the same aliquot), jejunum, and injection site muscle (homogenized and split in 3 aliquots) will be collected following isoflurane anesthesia for terminal collection. Samples collected from all study animals at the scheduled necropsy will be analyzed. No samples will be collected from animals that are found dead or preterminally euthanized.

Target weight:	2 x 50 mg
Processing:	Animal will be 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 will be then collected, rinsed with 1X PBS, dried on paper towel, weighed, and immediately snap frozen on liquid nitrogen (target of 1 minute after collection), and kept on dry ice. Feces from bowel tissues will be removed before processing.
Storage conditions:	Samples will be stored in a freezer set to maintain -80°C until analysis.
Disposition:	Samples collected from all study animals at the scheduled necropsy will be analyzed. Samples (2 x 50 mg) will be used for mRNA quantitation by the Immunology department using a bDNA method. The procedures to be followed during the course of this study along with the assay for acceptance criteria will be detailed in the appropriate analytical procedures. Samples will be analyzed in duplicate.

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

14.1.3. Toxicokinetic Evaluation

Toxicokinetic (TK) parameters will be estimated using Phoenix pharmacokinetic software. A non-compartmental approach consistent with the intramuscular route of administration will be used for parameter estimation. All parameters will be generated from mRNA-1647 concentrations in plasma and tissues from all TK occasions, whenever practical.

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Parameters to be 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.

When data permits, the slope of the terminal elimination phase of each arithmetic mean concentration versus time curve will be determined by log-linear regression, and the following additional parameters will also be estimated.

Additional Parameters to be Estimated

Parameter	Description of Parameter
T1/2	The apparent terminal elimination half life.

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

15. TERMINAL PROCEDURES

Terminal procedures are summarized in the following table:

Terminal Procedures for Main Study Animals

Group No.	No. of Animals	Scheduled Euthanasia Day	Necropsy Procedures		
	Males		Necropsy	Tissue Collection	Sample Tissue Weights
1	15	1	X	X[a]	X
	5	2			
	5	3			
	5	4			
	5	6			
Unscheduled Deaths			X	Standard Diagnostic List	-
Replaced animals (prestudy)			X	Standard Diagnostic List	-
Replaced animals (after dosing start)			X	Standard Diagnostic List	-

X = Procedure to be conducted; - = Not applicable.

[a] Consisting of blood, lung, liver, heart, right kidney, axillary distal lymph nodes (pooled to a target mass of 1.5 mg per animal), proximal popliteal and inguinal lymph nodes (pooled to a target mass of 1.5 mg per animal), spleen, brain, stomach, testes (right testicle), eye (left), bone marrow (bilateral pooled in the same aliquot), jejunum, and injection site muscle (homogenized and split in 3 aliquots).

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15.1. Unscheduled Deaths

If a main study animal dies on study, a complete necropsy examination will be conducted and limited tissue (standard diagnostic tissue list) will be retained. If necessary, the animal will be refrigerated to minimize autolysis.

Main study animals may be euthanized for humane reasons as per Test Facility SOPs. The samples for laboratory evaluations will be obtained if possible as specified in Section 14. These animals will undergo exsanguination by incision from the abdominal aorta following isoflurane anesthesia unless deemed inappropriate by the Study Director and/or the clinical veterinarian. These animals will undergo necropsy, and limited tissues (standard diagnostic tissue list) will be retained. If necessary, the animal will be refrigerated (set to maintain 4°C) to minimize autolysis.

Animals found dead or euthanized before the initiation of dosing will be subject to complete necropsy examination and limited tissue retention (standard diagnostic tissue list). Any animal replaced after the start of dosing will be subject to complete necropsy examination and limited tissue retention (standard diagnostic tissue list), and any data generated will not be included in the report unless deemed appropriate by the Study Director.

15.2. Scheduled Euthanasia

Main study animals surviving until scheduled euthanasia will have a terminal body weight recorded, blood samples for laboratory evaluations will be collected (as appropriate), and will undergo isoflurane anesthesia followed by whole-body perfusion with NaCl 0.9 %, Heparin (1000 IU/L), 1 % sodium nitrite and then PBS 1X. Animals will be fasted overnight before their scheduled necropsy.

15.3. Necropsy

Main study animals will be subjected to a complete necropsy examination, which will include 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 will be performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology. A veterinary pathologist, or other suitably qualified person, will be available.

At the discretion of the necropsy supervising pathologist, images may be generated for illustration of or consultation on gross observations. Generation of such images will be documented and communicated to the Study Director. Images and associated documentation will be retained and archived.

Appendix 1

15.4. Sample Tissue Weights

Lung, liver, heart, right kidney, axillary distal lymph nodes (pooled to a target mass of 1.5 mg per animal), proximal popliteal and inguinal lymph nodes (pooled to a target mass of 1.5 mg per animal), spleen, brain, stomach, testes (right testicle), eye (left), bone marrow (bilateral pooled in the same aliquot), jejunum, and injection site muscle (homogenized and split in 3 aliquots) will be weighed at necropsy for all scheduled euthanasia animals. Sample tissue weights will not be recorded for animals found dead or euthanized in poor condition or in extremis.

16. STATISTICAL ANALYSIS

Means and standard deviations will be calculated for all numerical data.

17. COMPUTERIZED SYSTEMS

The following critical computerized systems may be used in the study. The actual critical computerized systems used will be specified in the Final Report.

Data for parameters not required by study plan, which are automatically generated by analytical devices used will be retained on file but not reported. Statistical analysis results that are generated by the program but are not required by study plan and/or are not scientifically relevant will be retained on file but will not be included in the tabulations.

Critical Computerized Systems

System Name	Description of Data Collected and/or Analyzed
Provantis	In-life; postmortem
Dispense	Test Material receipt, accountability
Mesa Laboratories AmegaView CMS	Continuous Monitoring System. Monitoring of standalone fridges, freezers, incubators, and selected laboratories to measure temperature, relative humidity, and CO2, as appropriate
Johnson Controls Metasys	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)	Data acquisition for dose formulation analysis, including regression analysis and measurement of concentration and recovery of dose formulations using HPLC
Phoenix	Computation of non-compartmental analysis, descriptive statistics and ratios, as well as graphical and tabular output
Analyst (AB Sciex)	Bioanalytical data collection
Watson Laboratory Information Management system (Thermo Scientific)	Regression analysis and descriptive statistics of bioanalytical data
Bio-Plex Manager	Data acquisition and regression for Luminex data
SOFTmax® PRO (Molecular Devices Corporation)	Bioanalytical data collection and/or regression analysis

Appendix 1

18. AMENDMENTS AND DEVIATIONS

Changes to the approved study plan shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary study plan changes in advance with the Sponsor.

All study plan and SOP deviations will be documented in the study records. Deviations from the study plan and/or SOP related to the phase(s) of the study conducted at a Test Site shall be documented, acknowledged by the PI/IS, and reported to the Study Director for authorization/acknowledgement. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

19. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, electronic data, documentation, study plan, retained samples and specimens, and interim (if applicable) and final reports will be archived by no later than the date of final report issue. All materials generated by Charles River from this study will be transferred to CR-MTL archive. One year after issue of the draft report, the Sponsor will be contacted to determine the disposition of materials associated with the study.

Records to be maintained will include, but will not be limited to, documentation and data for the following:

- Study Plan, study plan amendments, and deviations
- Study schedule
- Study-related correspondence
- Test system receipt, health, and husbandry
- Test Item and Vehicle receipt, identification, preparation, and analysis
- In-life measurements and observations
- Clinical pathology sample collection and evaluation
- Laboratory evaluations sample collection and evaluation
- Gross observations and related data
- Statistical analysis results

20. REPORTING

A comprehensive Draft Report will be prepared following completion of the study and will be finalized following consultation with the Sponsor. The report will include all information necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

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The Sponsor will receive an electronic version of the Draft and Final Report provided in Adobe Acrobat PDF format (hyperlinked and searchable at final) along with a Microsoft Word version of the text. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Test Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. An original copy of the report with the Test Facility's handwritten signatures will be retained.

Reports should be finalized within 6 months of issue of the Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the report will be finalized by the Test Facility unless other arrangements are made by the Sponsor.

21. ANIMAL WELFARE

21.1. Institutional Animal Care and Use Committee Approval

The study plan and any amendment(s) or procedures involving the care and use of animals in this study will be reviewed and approved by CR SHB Institutional Animal Care and Use Committee (IACUC). During the study, the care and use of animals will be conducted with guidance from the USA National Research Council and the Canadian Council on Animal Care (CCAC).

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TEST FACILITY APPROVAL

The signature below indicates that Test Facility Management approves the Study Director identified in this study plan.

(b) (6)

Date: 28 Jun 2017

Test Facility Management

The signature below indicates that the Study Director approves the study plan.

(b) (6)

Date: 28 Jun 2017

Study Director

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SPONSOR APPROVAL

The Study Plan was approved by the Sponsor by email on 28 Jun 2017. The signature below confirms the approval of the Study Plan by the Sponsor Representative

(b) (6) _____ Date: 16Oct17

Sponsor Representative

Appendix 1

STUDY PLAN AMENDMENT 1

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
Sherbrooke, QC J1E 0B5
Canada

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SUMMARY OF CHANGES AND JUSTIFICATIONS

Study Plan effective date: 28-Jun-2017

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
Amendment 1	
6. RESPONSIBLE PERSONNEL	To include the pathologist's contact information.
7.1. TEST ITEM AND VEHICLE	To complete the Test Item information (Botch/lot number, concentration and retest date).
14.1.2. Bioanalytical Tissue Sample Collection	To clarify the samples of tissues that should be collected, the target weight and the processing.
15. TERMINAL PROCEDURES	To clarify the samples of tissues that should be collected.
15.4. Sample Tissue Weights	To clarify the samples of tissues that should be weight.

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1. OBJECTIVES

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

1.1. Study Classification

Study Category:	PK
Study Type:	Distribution; Single Dose PK
Study Design:	Parallel
Primary Treatment CAS Registry Number:	Not Available
Primary Treatment Unique Ingredient ID:	Not Available
Class of Compound:	mRNA

2. PROPOSED STUDY SCHEDULE

Proposed study dates are listed below. Actual applicable dates will be included in the Final Report.

Animal Arrival:	28 Jun 2017
Initiation of Dosing:	10 Jul 2017
Completion of In-life:	15 Jul 2017 (Last date of necropsy)
Draft Report:	25 Oct 2017 (69 working days following completion of in-life)
Final Report:	25 Apr 2018 (Expected date of Study Director signature, default 6 months from Draft Report)

3. GUIDELINES FOR STUDY DESIGN

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

4. REGULATORY COMPLIANCE

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

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5. SPONSOR

Sponsor Representative

(b) (6)
Address as cited for Sponsor
Tel: (b) (6)
E-mail: (b) (6)

6. RESPONSIBLE PERSONNEL

Study Director

(b) (6)
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
Address as cited for Test Facility
Tel: (b) (6)
Fax: (b) (6)
E-mail: (b) (6)

Management Contact

(b) (6)
Address as cited for Test Facility
Tel: (b) (6)
Fax: (b) (6)
E-mail: (b) (6)

Individual Scientists (IS) at the Test Facility

Pathology — (b) (6)
Senior Scientific Director
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
1580 Ida-Metivier
Sherbrooke, QC J1E 0B5
Tel: (b) (6)
E-mail: (b) (6)
~~**Will be added by amendment**~~

Analytical Chemistry — (b) (6)
Senior Research Scientist II
Charles River Laboratories Montreal ULC
Senneville Site (CR MTL)
22022 Transcanadienne
Senneville, QC H9X 3R3
Canada

Appendix 1

Tel: (b) (6)
E-mail: (b) (6)

Bioanalysis
(mRNA quantitation)

(b) (6)
Senior Research Scientist I
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
Address as cited for Test Facility
Tel: (b) (6)
E-mail: (b) (6)

Each IS is required to report any deviations or other circumstances that could affect the quality or integrity of the study to the Study Director in a timely manner. Each IS will provide a report addressing their assigned phase of the study, which will be included as an appendix to the Final Report. The phase report will include the following:

- A listing of critical computerized systems used in the conduct and/or interpretation of the assigned study phase

IS at Sponsor Test Site

Toxicokinetic
Analysis/Interpretation

(b) (6)
Moderna Therapeutics
200 Technology Sq, 3rd Floor
Cambridge MA 02138, USA
Email : (b) (6)

- Each PI is required to report any deviations or other circumstances that could affect the quality or integrity of the study to the Study Director in a timely manner. Each PI will provide a report addressing their assigned phase of the study, which will be included as an appendix to the Final Report. The phase report will include the following:
- The archive site for all records, samples, specimens and reports generated from the phase or segment (alternatively, details regarding the retention of the materials may be provided to the Study Director for inclusion in the Final Report)
- A listing of critical computerized systems used in the conduct and/or interpretation of the assigned study phase

7. TEST ITEM AND VEHICLE

7.1. Test Item

Identification: mRNA-1647

Supplier: Moderna Therapeutics, Inc

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Batch (Lot) Number:	**MTDP17048**~~**Will be added by amendment**~~
Concentration:	**1.9 mg/mL**~~**Will be added by amendment**~~
Retest Date:	**20 Apr 2018**~~**Will be added by amendment**~~
Physical Description:	White to off-white lipid nanoparticle dispersion
Storage Conditions:	Kept in a freezer set to maintain -20°C

7.2. Vehicle

Identification:	Phosphate-buffered Saline (PBS) pH 7.2
Supplier:	Will be included in the Final Report
Batch (Lot) Number:	Will be included in the Final Report
Expiration Date:	Will be included in the Final Report
Physical Description:	Liquid
Storage Conditions:	Kept in a controlled temperature area set to maintain 21°C

7.3. Test Item Characterization

The Sponsor will provide to the Test Facility documentation of the identity, strength, purity and composition for the Test Item. A Certificate of Analysis or equivalent documentation will be provided for inclusion in the Final Report. The Sponsor will also provide information concerning the regulatory standard that was followed for these evaluations.

The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the Test Item, and this information is available to the appropriate regulatory agencies should it be requested.

7.4. Analysis of Test Item

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

7.5. Reserve Samples

Reserve samples will not be collected during this study.

7.6. Test Item and Vehicle Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of Test Item and Vehicle will be maintained. All unused Sponsor-supplied bulk Test Item will be returned to the Sponsor on dry ice (after completion of dosing).

Shipping Contact
(b) (6)
Moderna Therapeutics

Appendix 1

500 Technology Sq, 8th Floor
Cambridge MA 02138, USA
E-mail: (b) (6)

8. SAFETY

The safety precautions for the Test Item and dose formulations will be documented in a Test Material Safety Data Sheet (TMSDS) based on the information provided by the Sponsor either by an MSDS or similar document.

9. DOSE FORMULATION AND ANALYSIS

9.1. Preparation of Vehicle

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

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

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

9.2. Preparation of Test Item

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

Test Item dosing formulations will be diluted with Phosphate Buffered Saline, pH 7.2, as necessary for administration. The dosing formulations will be prepared on the day of dosing and will be stored in a refrigerator set to maintain 4°C. The dose formulations will be allowed to warm to room temperature for at least 30 minutes prior to dosing. Alternatively, the aliquots can be transferred directly to room temperature.

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

9.3. Sample Collection and Analysis

Dose formulation samples will be collected for analysis as indicated in the following table. Additional samples may be collected and analyzed at the discretion of the Study Director.

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 will be averaged and utilized as the concentration results.

Samples to be analyzed will be submitted as soon as possible following collection.

All samples to be analyzed will be transferred (on ice pack) to the analytical laboratory.

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

9.3.1. Analytical Method

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

9.3.1.1. Concentration and Homogeneity Analysis

Samples for Analysis:	Duplicate top, middle, and bottom samples; sent for analysis as noted in Section 9.3.
Backup Samples:	Triplicate top, middle, and bottom samples; maintained at the Test Facility. Backup samples may be analyzed at the discretion of the Study Director.
Sampling Containers:	Appropriate sized glass containers.
Sample Volume:	0.5 mL for analysis and backup samples.
Storage Conditions:	Kept in a refrigerator set to maintain 4°C.
Acceptance Criteria:	For concentration, the criteria for acceptability will be mean sample concentration results within or equal to ± 15% of theoretical concentration. Each individual sample concentration result within or equal to ± 20%. For homogeneity, the criteria for acceptability will be a relative standard deviation (RSD) of concentrations of ≤ 15%.

9.3.1.2. Stability Analysis

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

10. TEST SYSTEM

Species:	Rat
Strain:	Crl:CD(SD) Sprague-Dawley rat
Source:	Charles River Canada Inc., St. Constant, QC, Canada
Number of Males Ordered:	38
Target Age at Arrival:	4 to 8 weeks
Target Weight at Arrival:	126 to 150 g

The actual age, weight, and number of animals received will be listed in the Final Report.

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10.1. Justification of 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 is 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.

10.2. Animal Identification

Each animal will be identified using a subcutaneously implanted electronic identification chip.

10.3. Environmental Acclimation

A minimum acclimation period of 10 days will be allowed between animal receipt and the start of dosing in order to accustom the animals to the laboratory environment.

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

At arrival, animals will have their number randomly assigned. Animals in poor health will not be assigned to groups.

Before the initiation of dosing, any assigned animals considered unsuitable for use in the study will be replaced by alternate animals obtained from the same shipment and maintained under the same environmental conditions.

After initiation of dosing, study animals may be replaced during the replacement period with alternate animals in the event of accidental injury, non-Test Item-related health issues, or similar circumstances.

The alternate animals may be used as replacements on the study within 1 day.

The disposition of all animals will be documented in the study records.

11. HUSBANDRY

11.1. Housing

Animals will be group housed (up to 3 animals) in polycarbonate cages containing appropriate bedding equipped with an automatic watering valve. These housing conditions will be maintained unless deemed inappropriate by the Study Director and/or Clinical Veterinarian. The room in which the animals will be kept will be documented in the study records.

Animals will be separated during designated procedures/activities. Each cage will be clearly labeled with a color-coded cage card indicating study, group, animal number(s), and sex.

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11.2. Environmental Conditions

The targeted conditions for animal room environment will be as follows:

Temperature:	19°C to 25°C
Humidity:	30% to 70%
Light Cycle:	12 hours light and 12 hours dark (except during designated procedures)

11.3. Food

PMI Nutrition International Certified Rodent Chow No. 5CR4 will be provided ad libitum throughout the study, except during designated procedures. The same diet in meal form may be provided to individual animals as warranted by clinical signs (e.g., broken/damaged incisors or other health changes).

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

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

11.4. Water

Municipal tap water after treatment by reverse osmosis and ultraviolet irradiation will be freely available to each animal via an automatic watering system (except during designated procedures). Water bottles can be provided, if required.

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

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

11.5. Animal Enrichment

Animals will be socially housed for psychological/environmental enrichment and will be provided with items such as a hiding tube and a chewing object, except during study procedures/activities.

11.6. Veterinary Care

Veterinary care will be available throughout the course of the study and animals will be examined by the veterinary staff as warranted by clinical signs or other changes. All veterinary examinations and recommended therapeutic treatments, if any, will be documented in the study records.

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In the event that animals show signs of illness or distress, the responsible veterinarian may make initial recommendations about treatment of the animal(s) and/or alteration of study procedures, which must be approved by the Study Director or Scientific designate. All such actions will be properly documented in the study records and, when appropriate, by study plan amendment. Treatment of the animal(s) for minor injuries or ailments may be approved without prior consultation with the Sponsor representative when such treatment does not impact fulfillment of the study objectives. If the condition of the animal(s) warrants significant therapeutic intervention or alterations in study procedures, the Sponsor representative will be contacted, when possible, to discuss appropriate action. If the condition of the animal(s) is such that emergency measures must be taken, the Study Director and/or clinical veterinarian will attempt to consult with the Sponsor representative prior to responding to the medical crisis, but the Study Director and/or veterinarian has authority to act immediately at his/her discretion to alleviate suffering. The Sponsor representative will be fully informed of any such events.

12. EXPERIMENTAL DESIGN

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

12.1. Administration of Test Item

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

The injection area will be marked as frequently as required to allow appropriate visualization of administration sites. Hair may be clipped or shaved if required to improve visualization of the injection sites. The injection site will be documented in the raw data.

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

13. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS

The in-life procedures, observations, and measurements listed below will be performed for all main study animals. During the study, additional evaluations to those described below and/or scheduled, and considered necessary by the Study Director and/or Veterinarian to assess health

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status will be conducted and duly documented. More frequent observations may be undertaken if considered appropriate.

13.1. Mortality/Moribundity Checks

Frequency:	Twice daily, once in the morning and once in the afternoon, throughout the study.
Procedure:	Animals will be observed for general health/mortality and moribundity. Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

13.2. Clinical Observations

13.2.1. Cage Side Observations

Frequency:	Once on Day -1 and once daily throughout the study; target time of 4 to 6 hours postdose on day of dosing and approximately the same time each day thereafter.
Procedure:	Animals will not be removed from the cage during observation, unless necessary for identification or confirmation of possible findings.

13.2.2. Detailed Clinical Observations

Frequency:	Weekly
Procedure:	Animals removed from the cage for examination.

13.3. Body Weights

Frequency:	Weekly
Procedure:	Animals will be individually weighed. A fasted weight will be recorded on the day of necropsy. Terminal body weights will not be collected from animals found dead or euthanized moribund.

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14. LABORATORY EVALUATIONS

14.1. Bioanalysis and Toxicokinetic Evaluation

Blood and tissue samples will be collected according to the following table (± 15 minutes).

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 to be collected; - = Not applicable.
[a] Sample will be collected before dosing.
[b] TK time point starts at the perfusion.

Any residual/retained bioanalytical samples will be maintained for a minimum of 6 months following issuance of the Draft Report after which samples will be discarded. Alternatively, residual/retained samples will be discarded prior to the 6 month period should the issuance of the Final Report occur prior to the end of the 6 month retention period. An earlier discard of these residual/retained samples may also be requested and authorized by the Study Director.

14.1.1. Bioanalytical Blood Sample Collection

Blood will be collected from jugular venipuncture at termination and, if possible, from animals that are preterminally euthanized.

Target Blood Volume:	1.0 mL
Anticoagulant:	K_2EDTA
Processing:	To plasma; blood samples will be kept on wet ice prior to processing. The samples will be 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 will be aliquoted into 2 x 100 µL aliquot and a leftover (if available). Aliquots will be snap frozen in liquid nitrogen and put on dry ice.
Storage conditions:	Samples will be stored in a freezer set to maintain -80°C until analysis.
Disposition:	Plasma samples will be used for mRNA quantitation by the Immunology department using a bDNA method. The procedure to

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be followed during the course of this study along with the assay for acceptance criteria will be detailed in the appropriate analytical procedure. Samples will be analyzed in duplicate.

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

14.1.2. 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, if possible**), proximal popliteal and inguinal lymph nodes (**bilateral** pooled to a target mass of 1.5 mg per animal**; 1 aliquot or 2, if 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) will be collected following isoflurane anesthesia for terminal collection. Samples collected from all study animals at the scheduled necropsy will be analyzed. No samples will be collected from animals that are found dead or preterminally euthanized.

Target weight:	2 x 50 mg **or maximum obtainable if less than 2 x 50 mg; except for the bone marrow (1 aliquot) and the injection site (3 aliquots).**
Processing:	Animal will be 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 will be 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 will be removed before processing.
Storage conditions:	Samples will be stored in a freezer set to maintain -80°C until analysis.
Disposition:	Samples collected from all study animals at the scheduled necropsy will be analyzed. Samples (2 x 50 mg) will be used for mRNA quantitation by the Immunology department using a bDNA method. The procedures to be followed during the course of this study along with the assay for acceptance criteria will be detailed in the appropriate analytical procedures. Samples will be analyzed in duplicate.

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

14.1.3. Toxicokinetic Evaluation

Toxicokinetic (TK) parameters will be estimated using Phoenix pharmacokinetic software. A non-compartmental approach consistent with the intramuscular route of administration will be used for parameter estimation. All parameters will be generated from mRNA-1647 concentrations in plasma and tissues from all TK occasions, whenever practical.

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Parameters to be 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.

When data permits, the slope of the terminal elimination phase of each arithmetic mean concentration versus time curve will be determined by log-linear regression, and the following additional parameters will also be estimated.

Additional Parameters to be Estimated

Parameter	Description of Parameter
T1/2	The apparent terminal elimination half life.

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

15. TERMINAL PROCEDURES

Terminal procedures are summarized in the following table:

Terminal Procedures for Main Study Animals

Group No.	No. of Animals	Scheduled Euthanasia Day	Necropsy Procedures		
	Males		Necropsy	Tissue Collection	Sample Tissue Weights
1	15	1	X	X[a]	X
	5	2			
	5	3			
	5	4			
	5	6			
Unscheduled Deaths			X	Standard Diagnostic List	-
Replaced animals (prestudy)			X	Standard Diagnostic List	-
Replaced animals (after dosing start)			X	Standard Diagnostic List	-

X = Procedure to be 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, if possible**), proximal popliteal and inguinal lymph nodes (**bilateral** pooled to a target mass of 1.5 mg per animal**; 1 aliquot or 2, if possible**), spleen, brain **(left hemisphere)**, stomach **(glandular region)**, testes (right testicle), eye (left), bone

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Group No.	No. of Animals	Scheduled Euthanasia Day	Necropsy Procedures		
	Males		Necropsy	Tissue Collection	Sample Tissue Weights

marrow **femur** (bilateral pooled in the same aliquot), jejunum **(middle region)**, and injection site muscle (homogenized and split in 3 aliquots).

15.1. Unscheduled Deaths

If a main study animal dies on study, a complete necropsy examination will be conducted and limited tissue (standard diagnostic tissue list) will be retained. If necessary, the animal will be refrigerated to minimize autolysis.

Main study animals may be euthanized for humane reasons as per Test Facility SOPs. The samples for laboratory evaluations will be obtained if possible as specified in Section 14. These animals will undergo exsanguination by incision from the abdominal aorta following isoflurane anesthesia unless deemed inappropriate by the Study Director and/or the clinical veterinarian. These animals will undergo necropsy, and limited tissues (standard diagnostic tissue list) will be retained. If necessary, the animal will be refrigerated (set to maintain 4°C) to minimize autolysis.

Animals found dead or euthanized before the initiation of dosing will be subject to complete necropsy examination and limited tissue retention (standard diagnostic tissue list). Any animal replaced after the start of dosing will be subject to complete necropsy examination and limited tissue retention (standard diagnostic tissue list), and any data generated will not be included in the report unless deemed appropriate by the Study Director.

15.2. Scheduled Euthanasia

Main study animals surviving until scheduled euthanasia will have a terminal body weight recorded, blood samples for laboratory evaluations will be collected (as appropriate), and will undergo isoflurane anesthesia followed by whole-body perfusion with NaCl 0.9 %, Heparin (1000 IU/L), 1 % sodium nitrite and then PBS 1X. Animals will be fasted overnight before their scheduled necropsy.

15.3. Necropsy

Main study animals will be subjected to a complete necropsy examination, which will include 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 will be performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology. A veterinary pathologist, or other suitably qualified person, will be available.

At the discretion of the necropsy supervising pathologist, images may be generated for illustration of or consultation on gross observations. Generation of such images will be

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documented and communicated to the Study Director. Images and associated documentation will be retained and archived.

15.4. Sample Tissue Weights

Samples of ~~L~~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, if possible**), proximal popliteal and inguinal lymph nodes (**bilateral** pooled to a target mass of 1.5 mg per animal**; 1 aliquot or 2, if 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) will be weighed at necropsy for all scheduled euthanasia animals. Sample tissue weights will not be recorded for animals found dead or euthanized in poor condition or in extremis.

16. STATISTICAL ANALYSIS

Means and standard deviations will be calculated for all numerical data.

17. COMPUTERIZED SYSTEMS

The following critical computerized systems may be used in the study. The actual critical computerized systems used will be specified in the Final Report.

Data for parameters not required by study plan, which are automatically generated by analytical devices used will be retained on file but not reported. Statistical analysis results that are generated by the program but are not required by study plan and/or are not scientifically relevant will be retained on file but will not be included in the tabulations.

Critical Computerized Systems

System Name	Description of Data Collected and/or Analyzed
Provantis	In-life; postmortem
Dispense	Test Material receipt, accountability
Mesa Laboratories AmegaView CMS	Continuous Monitoring System. Monitoring of standalone fridges, freezers, incubators, and selected laboratories to measure temperature, relative humidity, and CO2, as appropriate
Johnson Controls Metasys	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)	Data acquisition for dose formulation analysis, including regression analysis and measurement of concentration and recovery of dose formulations using HPLC
Phoenix	Computation of non-compartmental analysis, descriptive statistics and ratios, as well as graphical and tabular output
Analyst (AB Sciex)	Bioanalytical data collection
Watson Laboratory Information Management system (Thermo Scientific)	Regression analysis and descriptive statistics of bioanalytical data

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Bio-Plex Manager	Data acquisition and regression for Luminex data
SOFTmax® PRO (Molecular Devices Corporation)	Bioanalytical data collection and/or regression analysis

18. AMENDMENTS AND DEVIATIONS

Changes to the approved study plan shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary study plan changes in advance with the Sponsor.

All study plan and SOP deviations will be documented in the study records. Deviations from the study plan and/or SOP related to the phase(s) of the study conducted at a Test Site shall be documented, acknowledged by the PI/IS, and reported to the Study Director for authorization/acknowledgement. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

19. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, electronic data, documentation, study plan, retained samples and specimens, and interim (if applicable) and final reports will be archived by no later than the date of final report issue. All materials generated by Charles River from this study will be transferred to CR-MTL archive. One year after issue of the draft report, the Sponsor will be contacted to determine the disposition of materials associated with the study.

Records to be maintained will include, but will not be limited to, documentation and data for the following:

- Study Plan, study plan amendments, and deviations
- Study schedule
- Study-related correspondence
- Test system receipt, health, and husbandry
- Test Item and Vehicle receipt, identification, preparation, and analysis
- In-life measurements and observations
- Clinical pathology sample collection and evaluation
- Laboratory evaluations sample collection and evaluation
- Gross observations and related data
- Statistical analysis results

20. REPORTING

A comprehensive Draft Report will be prepared following completion of the study and will be finalized following consultation with the Sponsor. The report will include all information

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necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

The Sponsor will receive an electronic version of the Draft and Final Report provided in Adobe Acrobat PDF format (hyperlinked and searchable at final) along with a Microsoft Word version of the text. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Test Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. An original copy of the report with the Test Facility's handwritten signatures will be retained.

Reports should be finalized within 6 months of issue of the Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the report will be finalized by the Test Facility unless other arrangements are made by the Sponsor.

21. ANIMAL WELFARE

21.1. Institutional Animal Care and Use Committee Approval

The study plan and any amendment(s) or procedures involving the care and use of animals in this study will be reviewed and approved by CR SHB Institutional Animal Care and Use Committee (IACUC). During the study, the care and use of animals will be conducted with guidance from the USA National Research Council and the Canadian Council on Animal Care (CCAC).

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AMENDMENT APPROVAL

(b) (6)

Date: 07 Jul 2017

Study Director

Appendix 1

SPONSOR APPROVAL

The Study Plan Amendment was approved by the Sponsor by email on 06 Jul 2017.

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STUDY PLAN AMENDMENT 2

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
Sherbrooke, QC J1E 0B5
Canada

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SUMMARY OF CHANGES AND JUSTIFICATIONS

Study Plan effective date: 28-Jun-2017

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
Amendment 1	**Date: 07-Jul-2017**
6. RESPONSIBLE PERSONNEL	To include the pathologist's contact information.
7.1. TEST ITEM AND VEHICLE	To complete the Test Item information (Botch/lot number, concentration and retest date).
14.1.2. Bioanalytical Tissue Sample Collection	To clarify the samples of tissues that should be collected, the target weight and the processing.
15. TERMINAL PROCEDURES	To clarify the samples of tissues that should be collected.
15.4. Sample Tissue Weights	To clarify the samples of tissues that should be weight.
Amendment 2	
6. RESPONSIBLE PERSONNEL	To clarify that no pathology report is required.

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1. OBJECTIVES

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

1.1. Study Classification

Study Category:	PK
Study Type:	Distribution; Single Dose PK
Study Design:	Parallel
Primary Treatment CAS Registry Number:	Not Available
Primary Treatment Unique Ingredient ID:	Not Available
Class of Compound:	mRNA

2. PROPOSED STUDY SCHEDULE

Proposed study dates are listed below. Actual applicable dates will be included in the Final Report.

Animal Arrival:	28 Jun 2017
Initiation of Dosing:	10 Jul 2017
Completion of In-life:	15 Jul 2017 (Last date of necropsy)
Draft Report:	25 Oct 2017 (69 working days following completion of in-life)
Final Report:	25 Apr 2018 (Expected date of Study Director signature, default 6 months from Draft Report)

3. GUIDELINES FOR STUDY DESIGN

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

4. REGULATORY COMPLIANCE

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

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5. SPONSOR

Sponsor Representative

(b) (6)
Address as cited for Sponsor
Tel: (b) (6)
E-mail: (b) (6)

6. RESPONSIBLE PERSONNEL

Study Director

(b) (6)
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
Address as cited for Test Facility
Tel: (b) (6)
Fax: (b) (6)
E-mail: (b) (6)

Management Contact

(b) (6)
Address as cited for Test Facility
Tel: (b) (6)
Fax: (b) (6)
E-mail: (b) (6)

Individual Scientists (IS) at the Test Facility

Pathology
(Necropsy only)

(b) (6)
Senior Scientific Director
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
1580 Ida-Metivier
Sherbrooke, QC J1E 0B5
Tel: (b) (6)
E-mail: (b) (6)

Analytical Chemistry

(b) (6)
Senior Research Scientist II
Charles River Laboratories Montreal ULC
Senneville Site (CR MTL)
22022 Transcanadienne
Senneville, QC H9X 3R3
Canada

Appendix 1

Tel: (b) (6)
E-mail: (b) (6)

Bioanalysis
(mRNA quantitation)

(b) (6)
Senior Research Scientist I
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
Address as cited for Test Facility
Tel: (b) (6)
E-mail: (b) (6)

Each IS is required to report any deviations or other circumstances that could affect the quality or integrity of the study to the Study Director in a timely manner. Each IS will provide a report addressing their assigned phase of the study, **with the exception of the pathologist,** which will be included as an appendix to the Final Report. The phase report will include the following:

- A listing of critical computerized systems used in the conduct and/or interpretation of the assigned study phase

IS at Sponsor Test Site

Toxicokinetic
Analysis/Interpretation

(b) (6)
Moderna Therapeutics
200 Technology Sq, 3rd Floor
Cambridge MA 02138, USA
Email : (b) (6)

- Each PI is required to report any deviations or other circumstances that could affect the quality or integrity of the study to the Study Director in a timely manner. Each PI will provide a report addressing their assigned phase of the study, which will be included as an appendix to the Final Report. The phase report will include the following:
- The archive site for all records, samples, specimens and reports generated from the phase or segment (alternatively, details regarding the retention of the materials may be provided to the Study Director for inclusion in the Final Report)
- A listing of critical computerized systems used in the conduct and/or interpretation of the assigned study phase

7. TEST ITEM AND VEHICLE

7.1. Test Item

Identification: mRNA-1647

Supplier: Moderna Therapeutics, Inc

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Batch (Lot) Number:	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

7.2. Vehicle

Identification:	Phosphate-buffered Saline (PBS) pH 7.2
Supplier:	Will be included in the Final Report
Batch (Lot) Number:	Will be included in the Final Report
Expiration Date:	Will be included in the Final Report
Physical Description:	Liquid
Storage Conditions:	Kept in a controlled temperature area set to maintain 21°C

7.3. Test Item Characterization

The Sponsor will provide to the Test Facility documentation of the identity, strength, purity and composition for the Test Item. A Certificate of Analysis or equivalent documentation will be provided for inclusion in the Final Report. The Sponsor will also provide information concerning the regulatory standard that was followed for these evaluations.

The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the Test Item, and this information is available to the appropriate regulatory agencies should it be requested.

7.4. Analysis of Test Item

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

7.5. Reserve Samples

Reserve samples will not be collected during this study.

7.6. Test Item and Vehicle Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of Test Item and Vehicle will be maintained. All unused Sponsor-supplied bulk Test Item will be returned to the Sponsor on dry ice (after completion of dosing).

Shipping Contact
(b) (6)
Moderna Therapeutics

Appendix 1

500 Technology Sq, 8th Floor
Cambridge MA 02138, USA
E-mail: (b) (6)

8. SAFETY

The safety precautions for the Test Item and dose formulations will be documented in a Test Material Safety Data Sheet (TMSDS) based on the information provided by the Sponsor either by an MSDS or similar document.

9. DOSE FORMULATION AND ANALYSIS

9.1. Preparation of Vehicle

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

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

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

9.2. Preparation of Test Item

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

Test Item dosing formulations will be diluted with Phosphate Buffered Saline, pH 7.2, as necessary for administration. The dosing formulations will be prepared on the day of dosing and will be stored in a refrigerator set to maintain 4°C. The dose formulations will be allowed to warm to room temperature for at least 30 minutes prior to dosing. Alternatively, the aliquots can be transferred directly to room temperature.

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

9.3. Sample Collection and Analysis

Dose formulation samples will be collected for analysis as indicated in the following table. Additional samples may be collected and analyzed at the discretion of the Study Director.

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 will be averaged and utilized as the concentration results.

Samples to be analyzed will be submitted as soon as possible following collection.

All samples to be analyzed will be transferred (on ice pack) to the analytical laboratory.

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

9.3.1. Analytical Method

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

9.3.1.1. Concentration and Homogeneity Analysis

Samples for Analysis:	Duplicate top, middle, and bottom samples; sent for analysis as noted in Section 9.3.
Backup Samples:	Triplicate top, middle, and bottom samples; maintained at the Test Facility. Backup samples may be analyzed at the discretion of the Study Director.
Sampling Containers:	Appropriate sized glass containers.
Sample Volume:	0.5 mL for analysis and backup samples.
Storage Conditions:	Kept in a refrigerator set to maintain 4°C.
Acceptance Criteria:	For concentration, the criteria for acceptability will be mean sample concentration results within or equal to ± 15% of theoretical concentration. Each individual sample concentration result within or equal to ± 20%. For homogeneity, the criteria for acceptability will be a relative standard deviation (RSD) of concentrations of ≤ 15%.

9.3.1.2. Stability Analysis

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

10. TEST SYSTEM

Species:	Rat
Strain:	Crl:CD(SD) Sprague-Dawley rat
Source:	Charles River Canada Inc., St. Constant, QC, Canada
Number of Males Ordered:	38
Target Age at Arrival:	4 to 8 weeks
Target Weight at Arrival:	126 to 150 g

The actual age, weight, and number of animals received will be listed in the Final Report.

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10.1. Justification of 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 is 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.

10.2. Animal Identification

Each animal will be identified using a subcutaneously implanted electronic identification chip.

10.3. Environmental Acclimation

A minimum acclimation period of 10 days will be allowed between animal receipt and the start of dosing in order to accustom the animals to the laboratory environment.

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

At arrival, animals will have their number randomly assigned. Animals in poor health will not be assigned to groups.

Before the initiation of dosing, any assigned animals considered unsuitable for use in the study will be replaced by alternate animals obtained from the same shipment and maintained under the same environmental conditions.

After initiation of dosing, study animals may be replaced during the replacement period with alternate animals in the event of accidental injury, non-Test Item-related health issues, or similar circumstances.

The alternate animals may be used as replacements on the study within 1 day.

The disposition of all animals will be documented in the study records.

11. HUSBANDRY

11.1. Housing

Animals will be group housed (up to 3 animals) in polycarbonate cages containing appropriate bedding equipped with an automatic watering valve. These housing conditions will be maintained unless deemed inappropriate by the Study Director and/or Clinical Veterinarian. The room in which the animals will be kept will be documented in the study records.

Animals will be separated during designated procedures/activities. Each cage will be clearly labeled with a color-coded cage card indicating study, group, animal number(s), and sex.

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11.2. Environmental Conditions

The targeted conditions for animal room environment will be as follows:

Temperature:	19°C to 25°C
Humidity:	30% to 70%
Light Cycle:	12 hours light and 12 hours dark (except during designated procedures)

11.3. Food

PMI Nutrition International Certified Rodent Chow No. 5CR4 will be provided ad libitum throughout the study, except during designated procedures. The same diet in meal form may be provided to individual animals as warranted by clinical signs (e.g., broken/damaged incisors or other health changes).

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

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

11.4. Water

Municipal tap water after treatment by reverse osmosis and ultraviolet irradiation will be freely available to each animal via an automatic watering system (except during designated procedures). Water bottles can be provided, if required.

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

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

11.5. Animal Enrichment

Animals will be socially housed for psychological/environmental enrichment and will be provided with items such as a hiding tube and a chewing object, except during study procedures/activities.

11.6. Veterinary Care

Veterinary care will be available throughout the course of the study and animals will be examined by the veterinary staff as warranted by clinical signs or other changes. All veterinary examinations and recommended therapeutic treatments, if any, will be documented in the study records.

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In the event that animals show signs of illness or distress, the responsible veterinarian may make initial recommendations about treatment of the animal(s) and/or alteration of study procedures, which must be approved by the Study Director or Scientific designate. All such actions will be properly documented in the study records and, when appropriate, by study plan amendment. Treatment of the animal(s) for minor injuries or ailments may be approved without prior consultation with the Sponsor representative when such treatment does not impact fulfillment of the study objectives. If the condition of the animal(s) warrants significant therapeutic intervention or alterations in study procedures, the Sponsor representative will be contacted, when possible, to discuss appropriate action. If the condition of the animal(s) is such that emergency measures must be taken, the Study Director and/or clinical veterinarian will attempt to consult with the Sponsor representative prior to responding to the medical crisis, but the Study Director and/or veterinarian has authority to act immediately at his/her discretion to alleviate suffering. The Sponsor representative will be fully informed of any such events.

12. EXPERIMENTAL DESIGN

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

12.1. Administration of Test Item

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

The injection area will be marked as frequently as required to allow appropriate visualization of administration sites. Hair may be clipped or shaved if required to improve visualization of the injection sites. The injection site will be documented in the raw data.

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

13. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS

The in-life procedures, observations, and measurements listed below will be performed for all main study animals. During the study, additional evaluations to those described below and/or scheduled, and considered necessary by the Study Director and/or Veterinarian to assess health

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status will be conducted and duly documented. More frequent observations may be undertaken if considered appropriate.

13.1. Mortality/Moribundity Checks

Frequency: Twice daily, once in the morning and once in the afternoon, throughout the study.

Procedure: Animals will be observed for general health/mortality and moribundity. Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

13.2. Clinical Observations

13.2.1. Cage Side Observations

Frequency: Once on Day -1 and once daily throughout the study; target time of 4 to 6 hours postdose on day of dosing and approximately the same time each day thereafter.

Procedure: Animals will not be removed from the cage during observation, unless necessary for identification or confirmation of possible findings.

13.2.2. Detailed Clinical Observations

Frequency: Weekly

Procedure: Animals removed from the cage for examination.

13.3. Body Weights

Frequency: Weekly

Procedure: Animals will be individually weighed. A fasted weight will be recorded on the day of necropsy. Terminal body weights will not be collected from animals found dead or euthanized moribund.

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14. LABORATORY EVALUATIONS

14.1. Bioanalysis and Toxicokinetic Evaluation

Blood and tissue samples will be collected according to the following table (± 15 minutes).

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 to be collected; - = Not applicable.
[a] Sample will be collected before dosing.
[b] TK time point starts at the perfusion.

Any residual/retained bioanalytical samples will be maintained for a minimum of 6 months following issuance of the Draft Report after which samples will be discarded. Alternatively, residual/retained samples will be discarded prior to the 6 month period should the issuance of the Final Report occur prior to the end of the 6 month retention period. An earlier discard of these residual/retained samples may also be requested and authorized by the Study Director.

14.1.1. Bioanalytical Blood Sample Collection

Blood will be collected from jugular venipuncture at termination and, if possible, from animals that are preterminally euthanized.

Target Blood Volume:	1.0 mL
Anticoagulant:	K_2EDTA
Processing:	To plasma; blood samples will be kept on wet ice prior to processing. The samples will be 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 will be aliquoted into 2 x 100 µL aliquot and a leftover (if available). Aliquots will be snap frozen in liquid nitrogen and put on dry ice.
Storage conditions:	Samples will be stored in a freezer set to maintain -80°C until analysis.
Disposition:	Plasma samples will be used for mRNA quantitation by the Immunology department using a bDNA method. The procedure to

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be followed during the course of this study along with the assay for acceptance criteria will be detailed in the appropriate analytical procedure. Samples will be analyzed in duplicate.

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

14.1.2. 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, if possible), proximal popliteal and inguinal lymph nodes (bilateral pooled to a target mass of 1.5 mg per animal; 1 aliquot or 2, if 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) will be collected following isoflurane anesthesia for terminal collection. Samples collected from all study animals at the scheduled necropsy will be analyzed. No samples will be collected from animals that are found dead or preterminally euthanized.

Target weight:	2 x 50 mg or maximum obtainable if less than 2 x 50 mg; except for the bone marrow (1 aliquot) and the injection site (3 aliquots).
Processing:	Animal will be 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 will be 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 will be removed before processing.
Storage conditions:	Samples will be stored in a freezer set to maintain -80°C until analysis.
Disposition:	Samples collected from all study animals at the scheduled necropsy will be analyzed. Samples (2 x 50 mg) will be used for mRNA quantitation by the Immunology department using a bDNA method. The procedures to be followed during the course of this study along with the assay for acceptance criteria will be detailed in the appropriate analytical procedures. Samples will be analyzed in duplicate.

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

14.1.3. Toxicokinetic Evaluation

Toxicokinetic (TK) parameters will be estimated using Phoenix pharmacokinetic software. A non-compartmental approach consistent with the intramuscular route of administration will be used for parameter estimation. All parameters will be generated from mRNA-1647 concentrations in plasma and tissues from all TK occasions, whenever practical.

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Parameters to be 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.

When data permits, the slope of the terminal elimination phase of each arithmetic mean concentration versus time curve will be determined by log-linear regression, and the following additional parameters will also be estimated.

Additional Parameters to be Estimated

Parameter	Description of Parameter
T1/2	The apparent terminal elimination half life.

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

15. TERMINAL PROCEDURES

Terminal procedures are summarized in the following table:

Terminal Procedures for Main Study Animals

Group No.	No. of Animals	Scheduled Euthanasia Day	Necropsy Procedures		
	Males		Necropsy	Tissue Collection	Sample Tissue Weights
1	15	1	X	X[a]	X
	5	2			
	5	3			
	5	4			
	5	6			
Unscheduled Deaths			X	Standard Diagnostic List	-
Replaced animals (prestudy)			X	Standard Diagnostic List	-
Replaced animals (after dosing start)			X	Standard Diagnostic List	-

X = Procedure to be 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, if possible), proximal popliteal and inguinal lymph nodes (bilateral pooled to a target mass of 1.5 mg per animal; 1 aliquot or 2, if possible), spleen, brain (left hemisphere), stomach (glandular region), testes (right testicle), eye (left), bone

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Group No.	No. of Animals	Scheduled Euthanasia Day	Necropsy Procedures		
	Males		Necropsy	Tissue Collection	Sample Tissue Weights

marrow femur (bilateral pooled in the same aliquot), jejunum (middle region), and injection site muscle (homogenized and split in 3 aliquots).

15.1. Unscheduled Deaths

If a main study animal dies on study, a complete necropsy examination will be conducted and limited tissue (standard diagnostic tissue list) will be retained. If necessary, the animal will be refrigerated to minimize autolysis.

Main study animals may be euthanized for humane reasons as per Test Facility SOPs. The samples for laboratory evaluations will be obtained if possible as specified in Section 14. These animals will undergo exsanguination by incision from the abdominal aorta following isoflurane anesthesia unless deemed inappropriate by the Study Director and/or the clinical veterinarian. These animals will undergo necropsy, and limited tissues (standard diagnostic tissue list) will be retained. If necessary, the animal will be refrigerated (set to maintain 4°C) to minimize autolysis.

Animals found dead or euthanized before the initiation of dosing will be subject to complete necropsy examination and limited tissue retention (standard diagnostic tissue list). Any animal replaced after the start of dosing will be subject to complete necropsy examination and limited tissue retention (standard diagnostic tissue list), and any data generated will not be included in the report unless deemed appropriate by the Study Director.

15.2. Scheduled Euthanasia

Main study animals surviving until scheduled euthanasia will have a terminal body weight recorded, blood samples for laboratory evaluations will be collected (as appropriate), and will undergo isoflurane anesthesia followed by whole-body perfusion with NaCl 0.9 %, Heparin (1000 IU/L), 1 % sodium nitrite and then PBS 1X. Animals will be fasted overnight before their scheduled necropsy.

15.3. Necropsy

Main study animals will be subjected to a complete necropsy examination, which will include 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 will be performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology. A veterinary pathologist, or other suitably qualified person, will be available.

At the discretion of the necropsy supervising pathologist, images may be generated for illustration of or consultation on gross observations. Generation of such images will be

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documented and communicated to the Study Director. Images and associated documentation will be retained and archived.

15.4. Sample Tissue Weights

Samples of 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, if possible), proximal popliteal and inguinal lymph nodes (bilateral pooled to a target mass of 1.5 mg per animal; 1 aliquot or 2, if 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) will be weighed at necropsy for all scheduled euthanasia animals. Sample tissue weights will not be recorded for animals found dead or euthanized in poor condition or in extremis.

16. STATISTICAL ANALYSIS

Means and standard deviations will be calculated for all numerical data.

17. COMPUTERIZED SYSTEMS

The following critical computerized systems may be used in the study. The actual critical computerized systems used will be specified in the Final Report.

Data for parameters not required by study plan, which are automatically generated by analytical devices used will be retained on file but not reported. Statistical analysis results that are generated by the program but are not required by study plan and/or are not scientifically relevant will be retained on file but will not be included in the tabulations.

Critical Computerized Systems

System Name	Description of Data Collected and/or Analyzed
Provantis	In-life; postmortem
Dispense	Test Material receipt, accountability
Mesa Laboratories AmegaView CMS	Continuous Monitoring System. Monitoring of standalone fridges, freezers, incubators, and selected laboratories to measure temperature, relative humidity, and CO2, as appropriate
Johnson Controls Metasys	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)	Data acquisition for dose formulation analysis, including regression analysis and measurement of concentration and recovery of dose formulations using HPLC
Phoenix	Computation of non-compartmental analysis, descriptive statistics and ratios, as well as graphical and tabular output
Analyst (AB Sciex)	Bioanalytical data collection
Watson Laboratory Information Management system (Thermo Scientific)	Regression analysis and descriptive statistics of bioanalytical data
Bio-Plex Manager	Data acquisition and regression for Luminex data

Appendix 1

SOFTmax® PRO (Molecular Devices Corporation)	Bioanalytical data collection and/or regression analysis

18. AMENDMENTS AND DEVIATIONS

Changes to the approved study plan shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary study plan changes in advance with the Sponsor.

All study plan and SOP deviations will be documented in the study records. Deviations from the study plan and/or SOP related to the phase(s) of the study conducted at a Test Site shall be documented, acknowledged by the PI/IS, and reported to the Study Director for authorization/acknowledgement. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

19. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, electronic data, documentation, study plan, retained samples and specimens, and interim (if applicable) and final reports will be archived by no later than the date of final report issue. All materials generated by Charles River from this study will be transferred to CR-MTL archive. One year after issue of the draft report, the Sponsor will be contacted to determine the disposition of materials associated with the study.

Records to be maintained will include, but will not be limited to, documentation and data for the following:

- Study Plan, study plan amendments, and deviations
- Study schedule
- Study-related correspondence
- Test system receipt, health, and husbandry
- Test Item and Vehicle receipt, identification, preparation, and analysis
- In-life measurements and observations
- Clinical pathology sample collection and evaluation
- Laboratory evaluations sample collection and evaluation
- Gross observations and related data
- Statistical analysis results

20. REPORTING

A comprehensive Draft Report will be prepared following completion of the study and will be finalized following consultation with the Sponsor. The report will include all information

Appendix 1

necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

The Sponsor will receive an electronic version of the Draft and Final Report provided in Adobe Acrobat PDF format (hyperlinked and searchable at final) along with a Microsoft Word version of the text. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Test Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. An original copy of the report with the Test Facility's handwritten signatures will be retained.

Reports should be finalized within 6 months of issue of the Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the report will be finalized by the Test Facility unless other arrangements are made by the Sponsor.

21. ANIMAL WELFARE

21.1. Institutional Animal Care and Use Committee Approval

The study plan and any amendment(s) or procedures involving the care and use of animals in this study will be reviewed and approved by CR SHB Institutional Animal Care and Use Committee (IACUC). During the study, the care and use of animals will be conducted with guidance from the USA National Research Council and the Canadian Council on Animal Care (CCAC).

Appendix 1

AMENDMENT APPROVAL

(b) (6)

Date: 26 Jul 2017

Study Director

Appendix 1

SPONSOR APPROVAL

The Study Plan Amendment was approved by the Sponsor by email on 25 Jul 2017.

Appendix 1

DEVIATIONS

All deviations that occurred during the study have been authorized/acknowledged by the Study Director, assessed for impact, and documented in the study records. Only minor SOP deviations that did not impact the quality or integrity of the study occurred during the course of the study.

Appendix 2

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Summary of Analysis

(b) (4)

Appendix 2

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(b) (4)

Data Approved: (b) (6) Senior Scientist, DP Analytical Development (b) (6) Date: 06Jul2017

Appendix 3

charles river

NON-GLP FINAL REPORT

Study Phase: Analytical Chemistry

Test Facility Study No. 5002121

TEST FACILITY:
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)

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Appendix 3

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Appendix 3

LIST OF TABLES

LIST OF APPENDICES

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1. SUMMARY

Dose formulation samples have been analyzed by Ion Exchange High Performance Liquid Chromatography (IEX-HPLC) for the determination of mRNA-1647.

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

2. INTRODUCTION

This report describes the analytical evaluation of mRNA-1647 in dose formulations (phosphate-buffered saline (PBS) pH 7.2) in the bulk test item from Study 5002121.

For the work detailed in this report, the analytical phase experimental start date was 10 Jul 2017, and the analytical phase experimental completion date was 11 Jul 2017.

3. EXPERIMENTAL DESIGN

3.1. Dose Formulation Analysis

Analysis of dose formulations was carried out with regard to concentration and homogeneity.

On Day 1 of the study, duplicate samples were collected from the top, middle and bottom strata of Group 1 dose formulation. The samples were shipped on ice packs and analyzed on the same day.

4. MATERIALS AND METHODS

4.1. Materials

4.1.1. Reference Standard

Identification:	CX-0005128 mRNA
Physical Description:	Clear, colorless solution
Batch/Lot No.:	MTDS16027
Concentration:	1.95 mg/mL (used for calculations)
Retest Date:	Oct 2017
Storage Conditions:	Kept in a freezer set to maintain -20°C
Supplier:	Moderna Therapeutics, Inc.

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4.1.2. Reference Material

Identification:	mRNA-1647
Physical Description:	0.5 mL per vial, white to off-white lipid nanoparticle dispersion
Batch/Lot No.:	MTDP17015
Concentration:	2.4 mg/mL (used for calculations)
Date of Manufacture:	24 Feb 2017
Retest Date:	24 Feb 2018
Storage Conditions:	Kept in a freezer set to maintain -20°C
Supplier:	Moderna Therapeutics, Inc.

4.1.3. Characterization of Reference Standard and Reference Material

The Sponsor provided the documentation for the identity, strength, purity, composition, and stability for the reference standard and reference material. Copies of the supplied Summary of Analysis (SoA) or equivalent documentation are presented in Appendix 2.

4.1.4. Inventory and Disposition of Reference Standard and Reference Material

Records of the receipt, distribution, and storage of the reference standard and reference material were maintained. All unused Sponsor-supplied reference standard and reference material were retained for use on subsequent studies for the Sponsor.

4.2. Methods

4.2.1. Analytical Procedures

The method for concentration analysis is documented in Analytical Procedure AP.5002121.SP.01 (Appendix 1) and was previously validated under Study Nos. 1802050. Concentration stability data were generated by the department of Analytical Chemistry, Charles River, CR MTL for 1 day, 6 days, and 8 days, for formulation samples stored at ambient temperature, in a refrigerator set to maintain 4°C and in a freezer set to maintain a temperature of -20°C, respectively, over the concentration range of 0.00888 - 2.40 mg/mL, under Study No. 1802050.

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4.3. Computerized Systems

Critical computerized systems used in this study phase are listed below (see Text Table 1).

Text Table 1
Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
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 CO_2, 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

5. RESULTS AND DISCUSSIONS

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.

5.1. Dose Formulation Analysis

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. Results are presented in Table 1.

For homogeneity, the RSD of concentrations for all samples in each group tested was within the acceptance criteria of ≤ 5%. Results are presented in Table 1.

6. CONCLUSION

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

Appendix 3

7. REPORT APPROVAL

(b) (6)

Date: 31 Oct 2017

Individual Scientist, Analytical Chemistry

Appendix 3

Table 1 Study Samples - Concentration and Homogeneity

Occasion (Sampling Date)	Group	Theoretical Concentration (mg/mL)	Sampling Location	Measured Concentration (mg/mL)	Percent of Theoretical	RSD (%)
Day 1 (10 Jul 2017)	1	0.5	Top	0.560	112	4.9
				0.504	101	
			Middle	0.494	98.7	
				0.500	100	
			Bottom	0.505	101	
				0.497	99.4	
			Mean	0.510	102	

Appendix 3

Appendix 1
Analytical Procedure

Appendix 3

Analytical Procedure (AP.5002121.SP.01)

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Appendix 3

Analytical Procedure (AP.5002121.SP.01)

(b) (4)

Appendix 3

Analytical Procedure (AP.5002121.SP.01)

(b) (4)

Appendix 3

(b) (4)

Appendix 3

Analytical Procedure (AP.5002121.SP.01)

(b) (4)

Appendix 3

(b) (4)

Appendix 3

Analytical Procedure (AP.5002121.SP.01)

(b) (4)

AP Version Control

Initial version.

Verified by (b) (6) Date 06 Jul 2017

Approved by (b) (6) Date 06 Jul 2017

Authorized by (b) (6) Date 06 Jul 2017

Scientific Director ☑

Appendix 3

Appendix 2
Certificates of Analysis

Appendix 3

Document Number: DSAD-SOA-0025 Version: 3.0 Final Date: 13 Apr 2017
CX-005128 MTDS16027 SoA

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SUMMARY OF ANALYSIS

(b) (4), (b) (6)

Appendix 3

Document Number: DSAD-SOA-0025 Version: 3.0 Final Date: 13 Apr 2017
CX-005128 MTDS16027 SoA

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(b) (4), (b) (6)

Signatures:

(b) (6)

Generated by: (b) (6) Date: 12 Apr 17

(b) (6)

Reviewed by: (b) (6) Date: 12 APR 2017

Appendix 3

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Summary of Analysis

(b) (4)

Author:	(b) (6)	Principal Scientist, DP Analytical Development	(b) (6)	Date: 30 May 2017
Data reviewed:	(b) (6)	Senior Director, DP Analytical Development	(b) (6)	Date: 31-MAY-17

Data generated in accordance with standard Moderna Therapeutics laboratory Practices and have been verified for accuracy

Doc: DPAD-SOA-0002 *Page 1 of 2*

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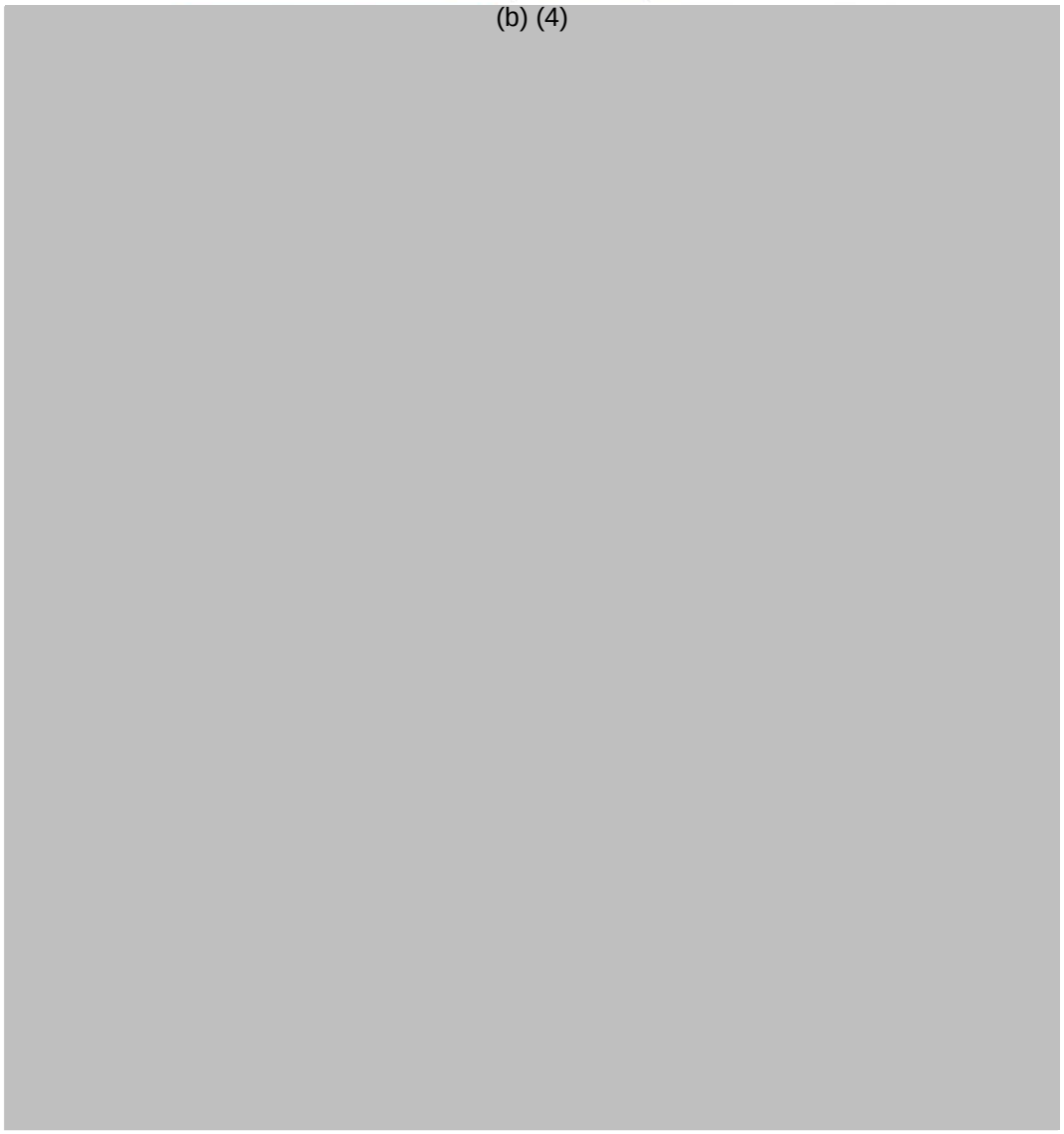
Summary of Analysis

(b) (4)



Doc: DPAD-SOA-0001.4 *Page 1 of 2*

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(b) (4)

Data Approved: (b) (6) Senior Scientist, DP Analytical Development (b) (6) Date: 23 Jun 2017

Doc: DPAD-SOA-0001.4 *Page 2 of 2*

Appendix 4

Individual Animal Mortality Explanation Page

Abbreviation	Description	Abbreviation	Description
AD or ACCD	Accidental death	REC	Recovery euthanasia
FD	Found dead	REL	Released
INTM	Interim	TE or TERM	Terminal euthanasia
NR	Not recorded	UE or UNSC	Unscheduled euthanasia

Note: This is a comprehensive list of abbreviations. All of the abbreviations listed may not be applicable to this report.

Note: Removal Time represents the time the removal was entered into the Provantis system and may not be representative of the time of death.

Dosing Information

Dosing information is abbreviated on various data outputs; the following represents the dosing information for this study.

Group No.	Test Item	Dose Level (µg)
1	mRNA-1647	100

Appendix 4

Individual Animal Mortality

5002121

Group	Dose Level	Sex	Animal	Cage	Removal Day	Removal Week	Removal Date	Removal Time	Time Slot	Removal Symptom	Pathology Reason
1	100 ug	Male	1001	1001	1	1	10JUL2017	9:15	.	.	TERM
			1002	1001	1	1	10JUL2017	10:01	.	.	TERM
			1003	1001	1	1	10JUL2017	10:38	.	.	TERM
			1004	1004	1	1	10JUL2017	11:10	.	.	TERM
			1005	1004	1	1	10JUL2017	11:40	.	.	TERM
			1006	1006	1	1	10JUL2017	13:03	.	.	TERM
			1007	1006	1	1	10JUL2017	13:47	.	.	TERM
			1008	1006	1	1	10JUL2017	14:23	.	.	TERM
			1009	1009	1	1	10JUL2017	14:56	.	.	TERM
			1010	1009	1	1	10JUL2017	15:29	.	.	TERM
			1011	1011	1	1	10JUL2017	19:05	.	.	TERM
			1012	1011	1	1	10JUL2017	19:40	.	.	TERM
			1013	1011	1	1	10JUL2017	20:09	.	.	TERM
			1014	1014	1	1	10JUL2017	20:50	.	.	TERM
			1015	1014	1	1	10JUL2017	21:27	.	.	TERM
			1016	1016	2	1	11JUL2017	11:07	.	.	TERM
			1017	1016	2	1	11JUL2017	11:44	.	.	TERM
			1018	1016	2	1	11JUL2017	12:16	.	.	TERM
			1019	1019	2	1	11JUL2017	12:51	.	.	TERM
			1020	1019	2	1	11JUL2017	13:26	.	.	TERM
			1021	1021	3	1	12JUL2017	11:13	.	.	TERM
			1022	1021	3	1	12JUL2017	11:42	.	.	TERM
			1023	1021	3	1	12JUL2017	12:17	.	.	TERM
			1024	1024	3	1	12JUL2017	12:56	.	.	TERM
			1025	1024	3	1	12JUL2017	13:27	.	.	TERM
			1026	1026	4	1	13JUL2017	10:57	.	.	TERM
			1027	1026	4	1	13JUL2017	11:41	.	.	TERM
			1028	1026	4	1	13JUL2017	12:20	.	.	TERM
			1029	1029	4	1	13JUL2017	12:54	.	.	TERM
			1030	1029	4	1	13JUL2017	13:29	.	.	TERM
			1031	1031	6	1	15JUL2017	11:12	.	.	TERM
			1032	1031	6	1	15JUL2017	11:38	.	.	TERM
			1033	1031	6	1	15JUL2017	12:12	.	.	TERM
			1034	1034	6	1	15JUL2017	12:46	.	.	TERM
			1035	1034	6	1	15JUL2017	13:24	.	.	TERM

Appendix 5

Individual Clinical Observations Explanation Page

Abbreviation	Description	Abbreviation	Description
AM SIRT	Signs of ill health or reaction to treatment check in the morning	PM SIRT	Signs of ill health or reaction to treatment check in the afternoon
CSO	Cage side observation	PostRx #	Observation post dosing
DE	Detailed examination	PreRx #	Observation predosing
During Rx/R #	Observation during dosing	Unsc #	Unscheduled examination
Vet Aid	Anything observed by Vet Aid	#	Number to avoid using the same timeslot/animal/day

Note: This is a comprehensive list of abbreviations. All of the abbreviations listed may not be applicable to this report.

Note: Only animals with findings are presented in this appendix.

Dosing Information

Dosing information is abbreviated on various data outputs; the following represents the dosing information for this study.

Group No.	Test Item	Dose Level (µg)
1	mRNA-1647	100

Appendix 5

Individual Clinical Observations

5002121

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	-1 DE	1 DE	2 DE	3 DE	4 DE
1	m	1012	Skin, Scab	Hindpaw, Left	X	X	.	.	.
		1016	Swollen Firm	Hindlimb, Right	.	.	3	.	.
		1017	Swollen Firm	Hindlimb, Right	.	.	2	.	.
		1018	Swollen Firm	Hindlimb, Right	.	.	2	.	.
		1019	Swollen Firm	Hindlimb, Right	.	.	2	.	.
		1020	Swollen Firm	Hindlimb, Right	.	.	3	.	.
		1021	Swollen Firm	Hindlimb, Right	.	.	.	2	.
		1022	Swollen Firm	Hindlimb, Right	.	.	.	2	.
		1023	Swollen Firm	Hindlimb, Right	.	.	.	2	.
		1024	Swollen Firm	Hindlimb, Right	.	.	.	2	.
			Skin, Scab	Treatment Site No.01	.	.	.	X	.
		1025	Swollen Firm	Hindlimb, Right	.	.	.	2	.
			Skin, Scab	Treatment Site No.01	.	.	.	X	.
		1026	Swollen Firm	Hindlimb, Right	.	.	.	.	1
		1027	Swollen Firm	Hindlimb, Right	.	.	.	.	1
		1028	Swollen Firm	Hindlimb, Right	.	.	.	.	1
		1029	Swollen Firm	Hindlimb, Right	.	.	.	.	1
		1030	Swollen Firm	Hindlimb, Right	.	.	.	.	1

Severity Codes: X = Present; 1 = Slight; 2 = Moderate; 3 = Severe

Group 1 - 100 ug

Appendix 6

Individual Body Weights Explanation Page

Abbreviation	Description	Abbreviation	Description
--	Not scheduled to be performed / dead	TERR	Technical error
AVS	Suspected aberrant value	UPTD	Unable to perform due to technical difficulty
OA	Omitted activity	X	Excluded from mean

Note: This is a comprehensive list of abbreviations. All of the abbreviations listed may not be applicable to this report.

Dosing Information

Dosing information is abbreviated on various data outputs; the following represents the dosing information for this study.

Group No.	Test Item	Dose Level (µg)
1	mRNA-1647	100

Appendix 6

Individual Body Weights

5002121

Sex: Male Bodyweight (g)

100 ug Group 1	Day(s) Relative to Start Date						
	-6	-1	1	2	3	4	6
1001	267	306	309	-	-	-	-
1002	291	335	343	-	-	-	-
1003	268	308	315	-	-	-	-
1004	288	335	339	-	-	-	-
1005	292	344	353	-	-	-	-
1006	286	337	341	-	-	-	-
1007	294	340	346	-	-	-	-
1008	287	323	333	-	-	-	-
1009	281	325	329	-	-	-	-
1010	282	320	324	-	-	-	-
1011	279	318	326	-	-	-	-
1012	279	314	321	-	-	-	-
1013	271	311	317	-	-	-	-
1014	286	340	347	-	-	-	-
1015	267	311	316	-	-	-	-
1016	281	322	-	317 !1	-	-	-
1017	285	324	-	326	-	-	-
1018	280	332	-	341	-	-	-
1019	268	305	-	299 !1	-	-	-

1 [RC:VALUE CONFIRMED]

Appendix 6

Individual Body Weights

5002121

Sex: Male Bodyweight (g)

100 ug Group 1	Day(s) Relative to Start Date						
	-6	-1	1	2	3	4	6
1020	288	331	-	339	-	-	-
1021	272	313	-	-	320	-	-
1022	290	323	-	-	317	-	-
1023	287	326	-	-	330	-	-
1024	279	329	-	-	341	-	-
1025	281	327	-	-	328	-	-
1026	278	311	-	-	-	320	-
1027	293	339	-	-	-	352	-
1028	294	346	-	-	-	361	-
1029	283	317	-	-	-	324	-
1030	281	332	-	-	-	355	-
1031	272	302	-	-	-	-	329
1032	271	307	-	-	-	-	335
1033	267	308	-	-	-	-	332
1034	293	346	-	-	-	-	391
1035	276	313	-	-	-	-	338
Mean	281.1	323.4	330.6	324.4	327.2	342.4	345.0
SD	8.6	12.9	13.7	17.3	9.4	19.0	25.9
N	35	35	15	5	5	5	5

Appendix 7

charles river

NON-GLP FINAL REPORT

Study Phase: Bioanalytical Report (mRNA Quantitation)

Test Facility Study No. 5002121

TEST FACILITY:
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)

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Appendix 7

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Appendix 7

LIST OF APPENDICES

Appendix 7

1. INTRODUCTION

This report describes the evaluation of mRNA-1647 (uL131, uL128, uL130, gL, gH and gB) in rat plasma (K_2EDTA) and tissue samples from Study No. 5002121 titled *" A Single Dose Intramuscular Injection Tissue Distribution Study of mRNA-1647 in Male Sprague-Dawley Rats"*.

For the work detailed in this report, the bioanalysis (mRNA-1647 quantitation) phase experimental start and end dates were 01 Aug 2017, and 24 Aug 2017, respectively.

2. EXPERIMENTAL PROCEDURES

2.1. Materials and Methods

2.1.1. Reference Standard

Identification: mRNA-1647

Physical Description: Opaque milky suspension

Lot No.: MTDP17048

RNA Content: 1.9 mg/mL

Retested Date: 20 Apr 2018 (1 year from manufacturing date: 20 Apr 2017)

Storage Conditions: Kept in a freezer set to maintain -20°C

Supplier: Moderna Therapeutics, Inc.

2.1.2. Methods

The methodology and materials used for the mRNA-1647 quantitation (uL131, uL128, uL130, gL, gH, and gB) analyses were detailed in the analytical procedures listed in the table below, only the latest version is appended:

Analyte	Matrix	Analytical Procedure(s) No.
mRNA-1647 (uL131, uL128, uL130, gL, gH and gB)	Plasma quantitation	AP.5002121.bDNAp.01, AP.5002121.bDNAp-02 and AP.5002121.bDNAp.03
	Tissue mRNA quantitation	AP.5002121.bDNAt.01, AP. 5002121.bDNAt-02 and AP.5002121.bDNAt.03
	Tissue sample processing	AP.5002121.EXT.01 and AP. 5002121.EXT.02

Appendix 7

2.2. Computerized Systems

Critical computerized systems used in this study phase are listed below (see Text Table 1).

Text Table 1
Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
Bio-Plex Manager	4.1 and 6.1	Data acquisition for mRNA quantitation
Watson LIMS	7.4.2 SP1	mRNA quantitation data regression
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 4.0.4.	Building Automation System. Control of HVAC and other building systems, as well as temperature/humidity control and trending in selected laboratories and animal rooms

3. RESULTS AND DISCUSSIONS

3.1. Standards and Quality Control Samples for mRNA-1647 Quantitation

Standard, Quality control (QC) preparation and acceptance criteria are described in the analytical procedure (Appendix 2). Standard curve and quality control specifications are presented in Text Table 2.

Text Table 2
mRNA Standard Curve and Quality Controls Specifications

mRNA-1647	Range of the Curve (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)	LQC (pg/mL)	MQC (pg/mL)	HQC (pg/mL)
uL131, uL128, gL and gH	0.10 to 50.00	0.10	50.00	0.30	10.00	40.00
uL130 and gB	0.10* to 50.00	0.50	50.00	1.50	10.00	40.00

* Accessory standard to help define the lower end of the calibration curve.

A total of 3 mRNA-1647 quantitation assays for plasma samples were performed and all assays met the method acceptance criteria. All results were reported from the assays that met the acceptance criteria.

A total of 23 mRNA-1647 quantitation assays for tissue samples were performed and all assays met the method acceptance criteria with the exception of four assays where several mRNAs failed to meet acceptance criteria. Root causes of these failures where due to probable technical oversights while spiking or loading the QC samples. All results were reported from the assays that met the acceptance criteria.

3.2. Study Samples

All study samples received for mRNA-1647 quantitation were processed and analyzed. One sample did not meet the acceptance criterion between replicate values (%CV > 25%), sample 1011 injection site for mRNA gB only. The mRNA gB results obtained were considered

Appendix 7

to be appropriate for reporting since the concentrations observed were similar to the other animals from the same timepoint and therefore this did not impact the mRNA quantitation reported results.

Appendix 7

4. CONCLUSION

All samples collected for the mRNA-1647 quantitation analyses were analyzed using a qualified bDNA method. Based on the acceptable performance of the standards and QCs during sample analysis, it is concluded that the concentration values reported for the study samples are valid. The study sample results are presented in the toxicology report.

Appendix 7

5. REPORT APPROVAL

(b) (6)

Date: 31 Oct 2017

Individual Scientist, Immunology

Appendix 7

Appendix 1
Deviations

Appendix 7

DEVIATIONS

All deviations that occurred during this study phase have been acknowledged by the Study Director, assessed for impact, and documented in the study records. No Study Plan deviations related occurred during this study phase, however there were deviations to the analytical procedures. None of the deviations were considered to have impacted the overall integrity of this study phase results.

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Appendix 7

ANALYTICAL PROCEDURE

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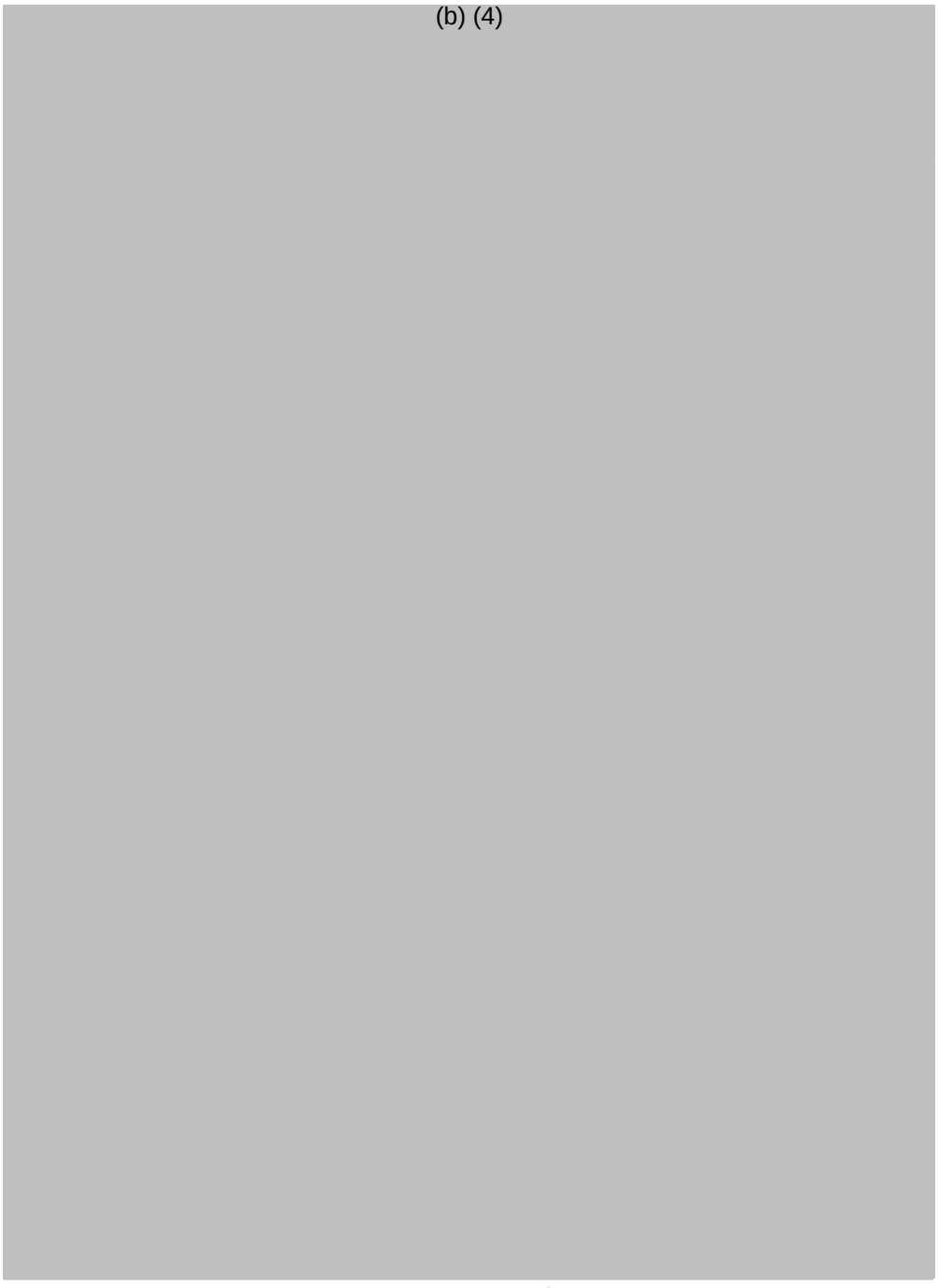

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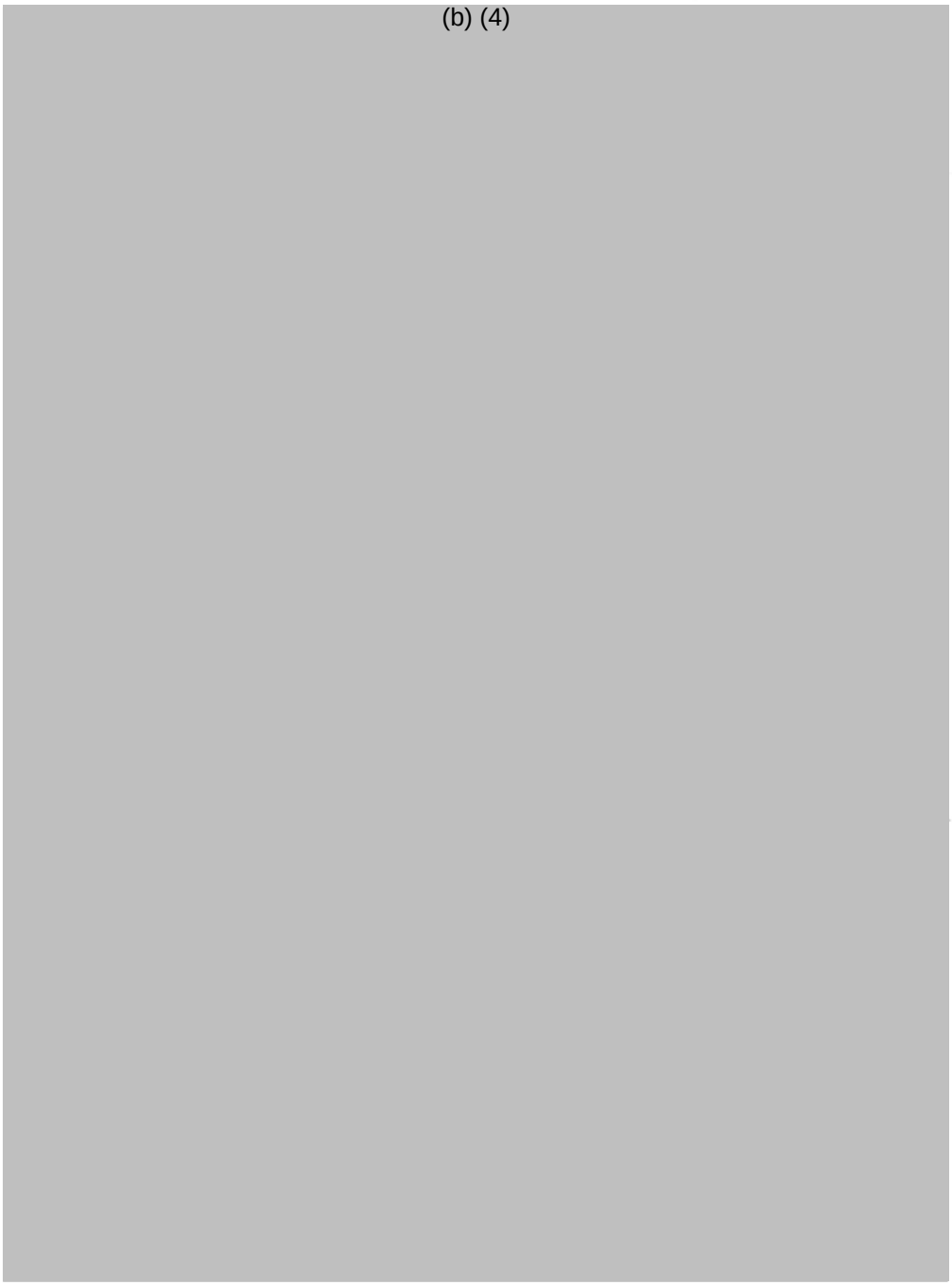

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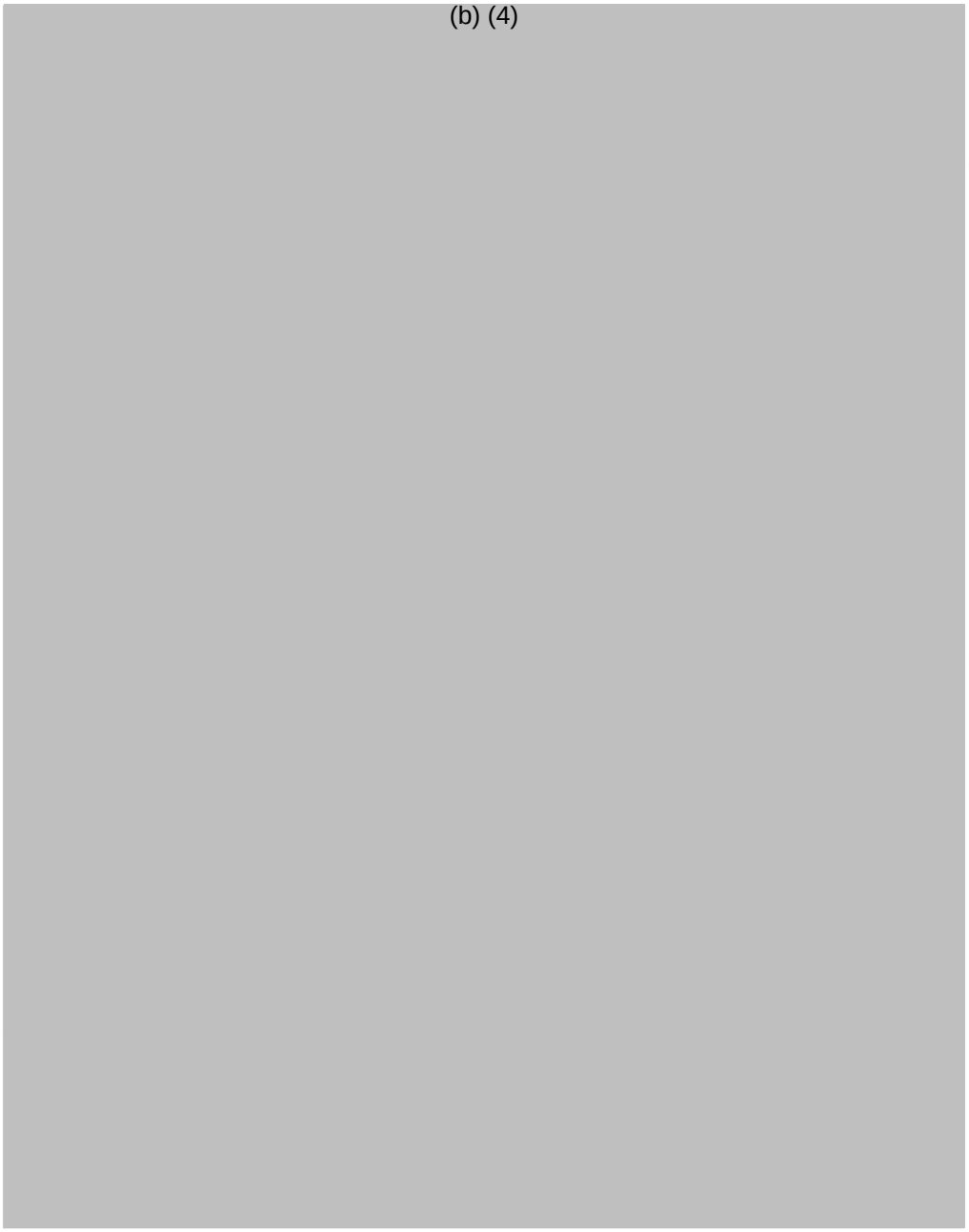

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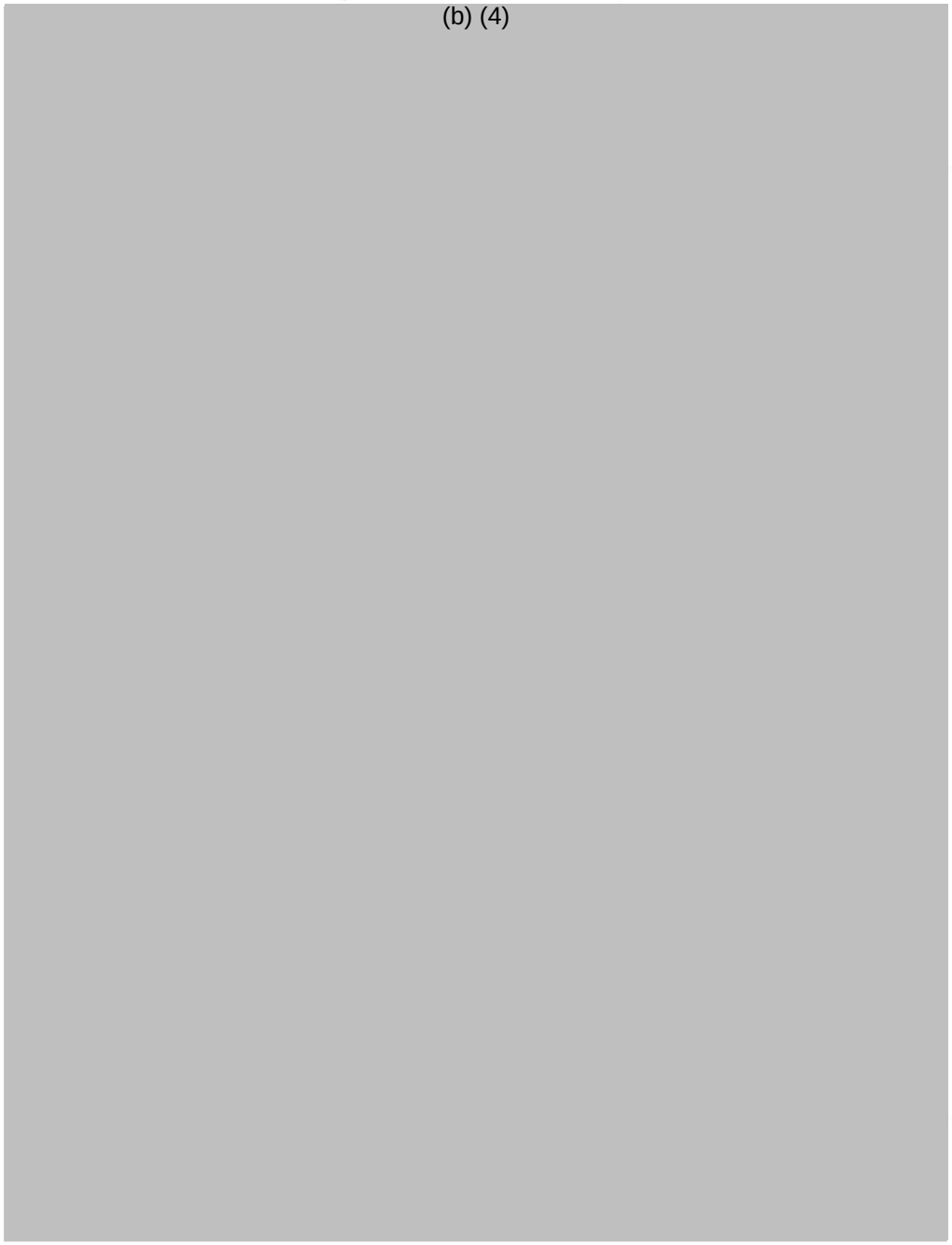

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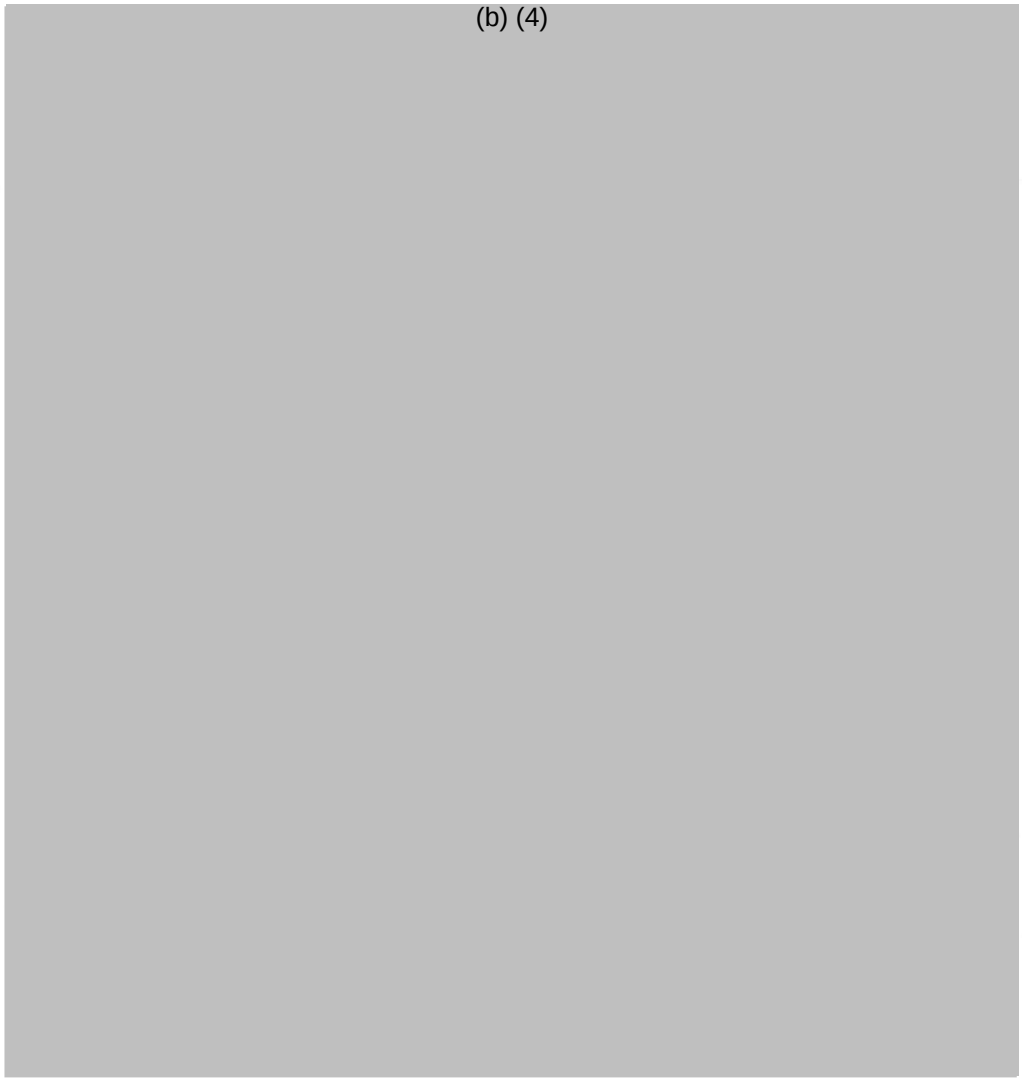

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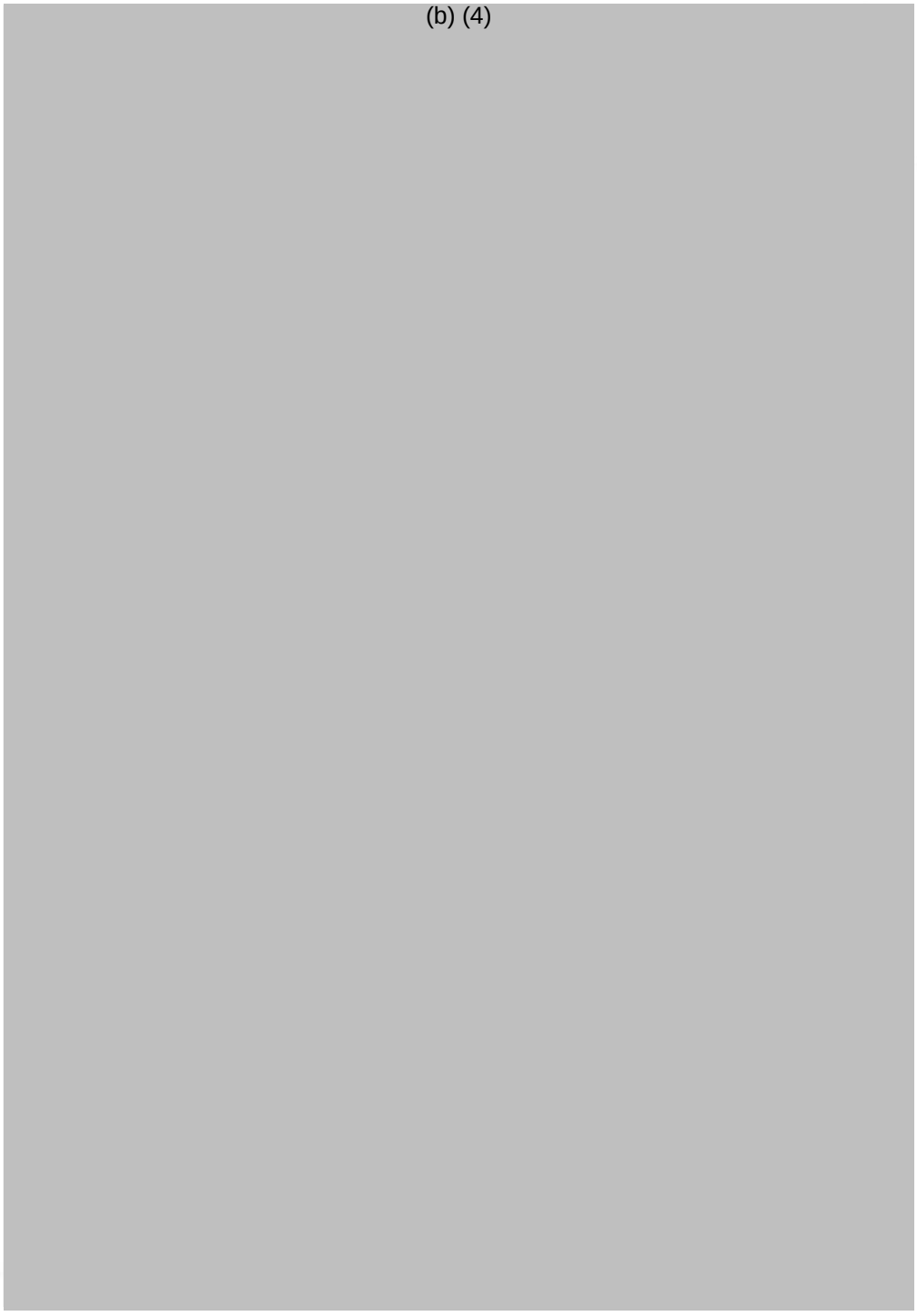

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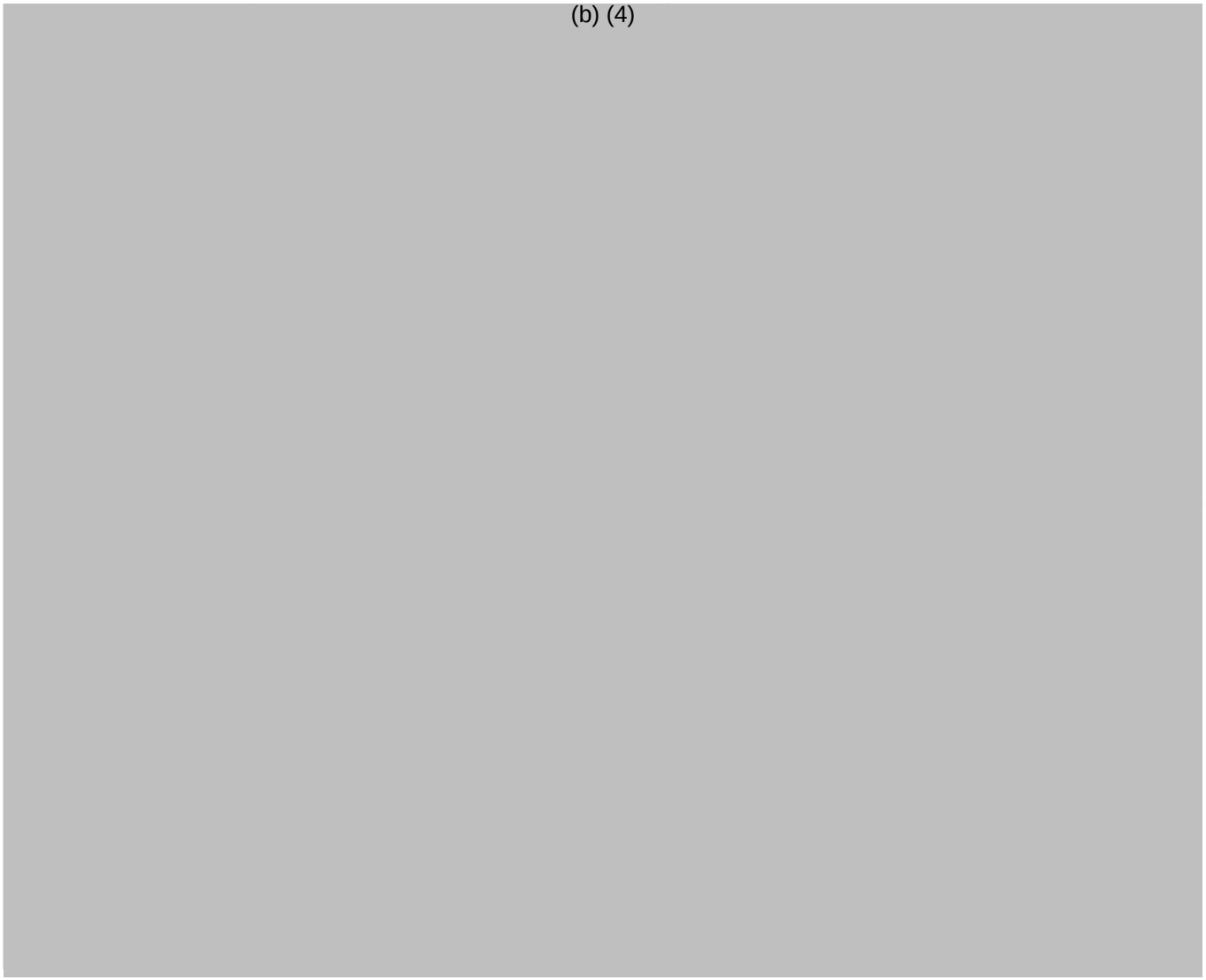

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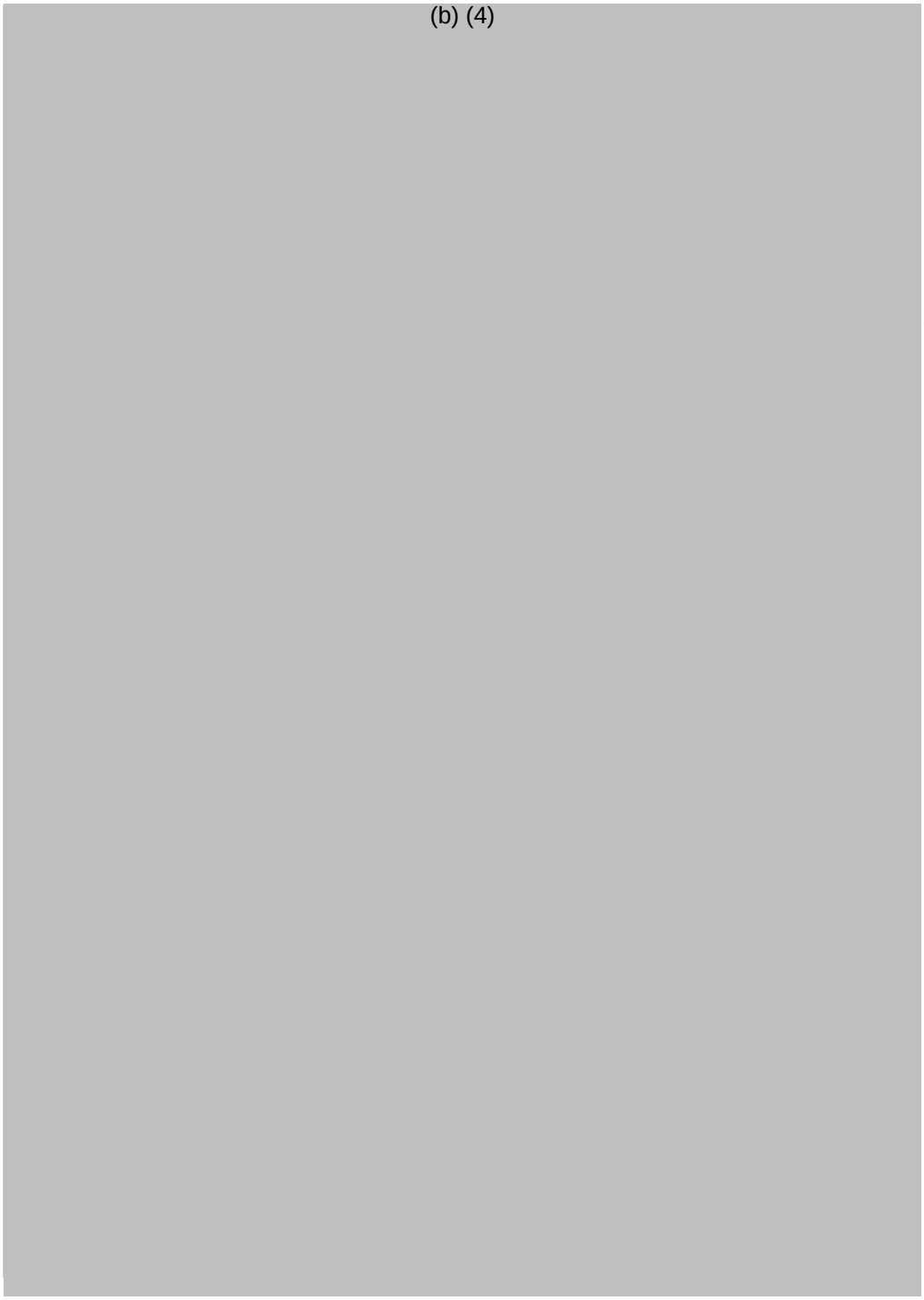

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ANALYTICAL PROCEDURE

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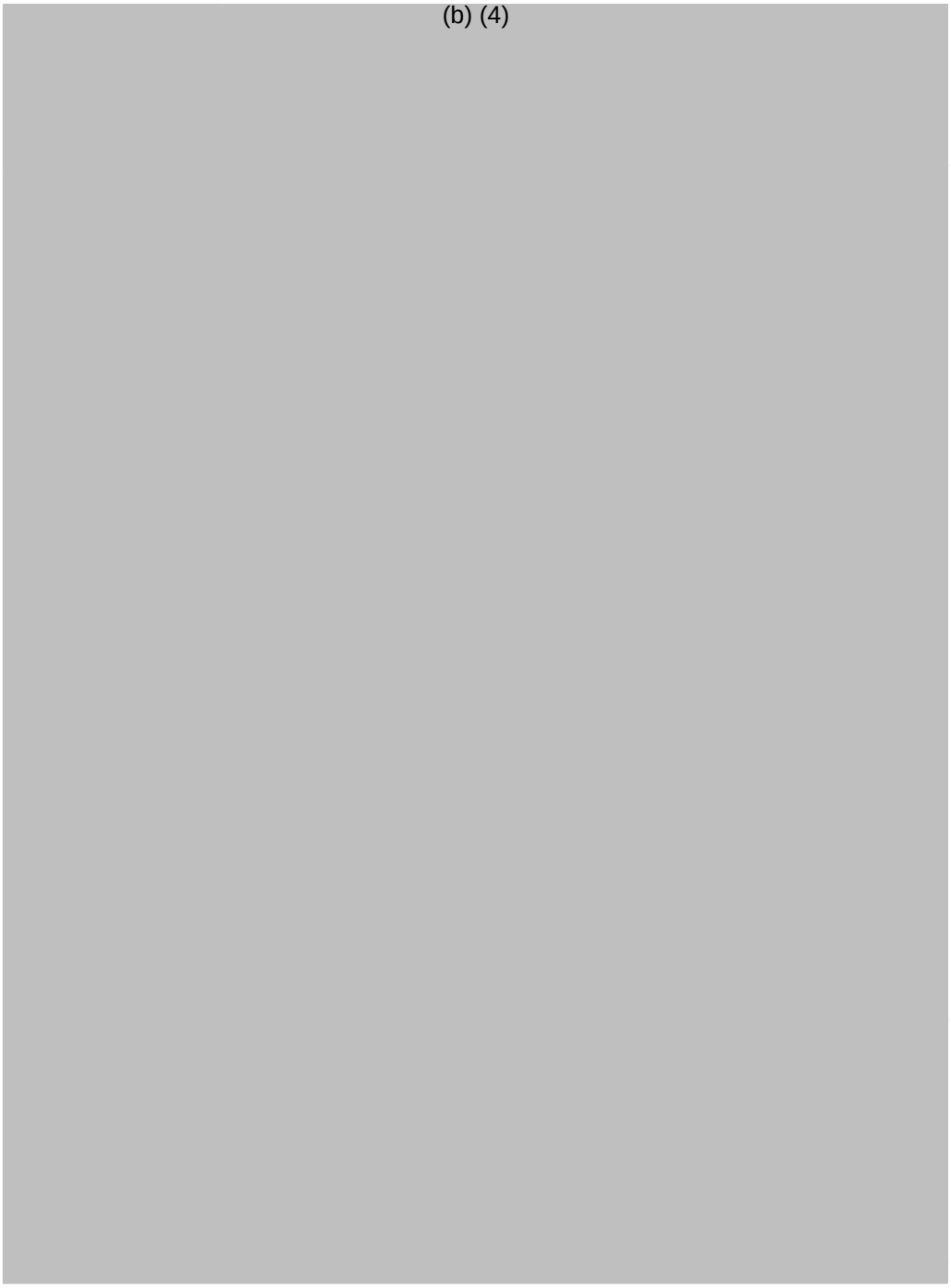

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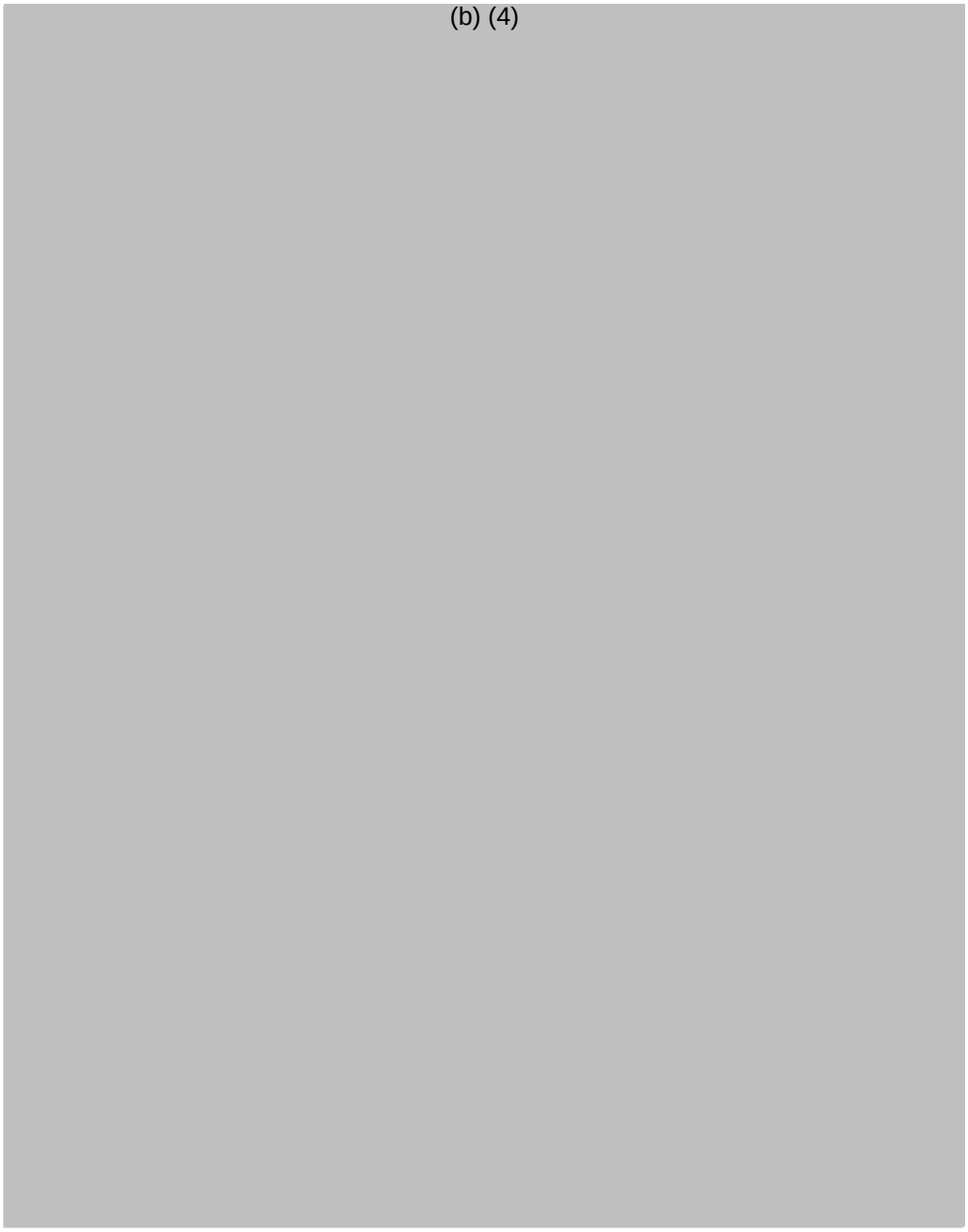

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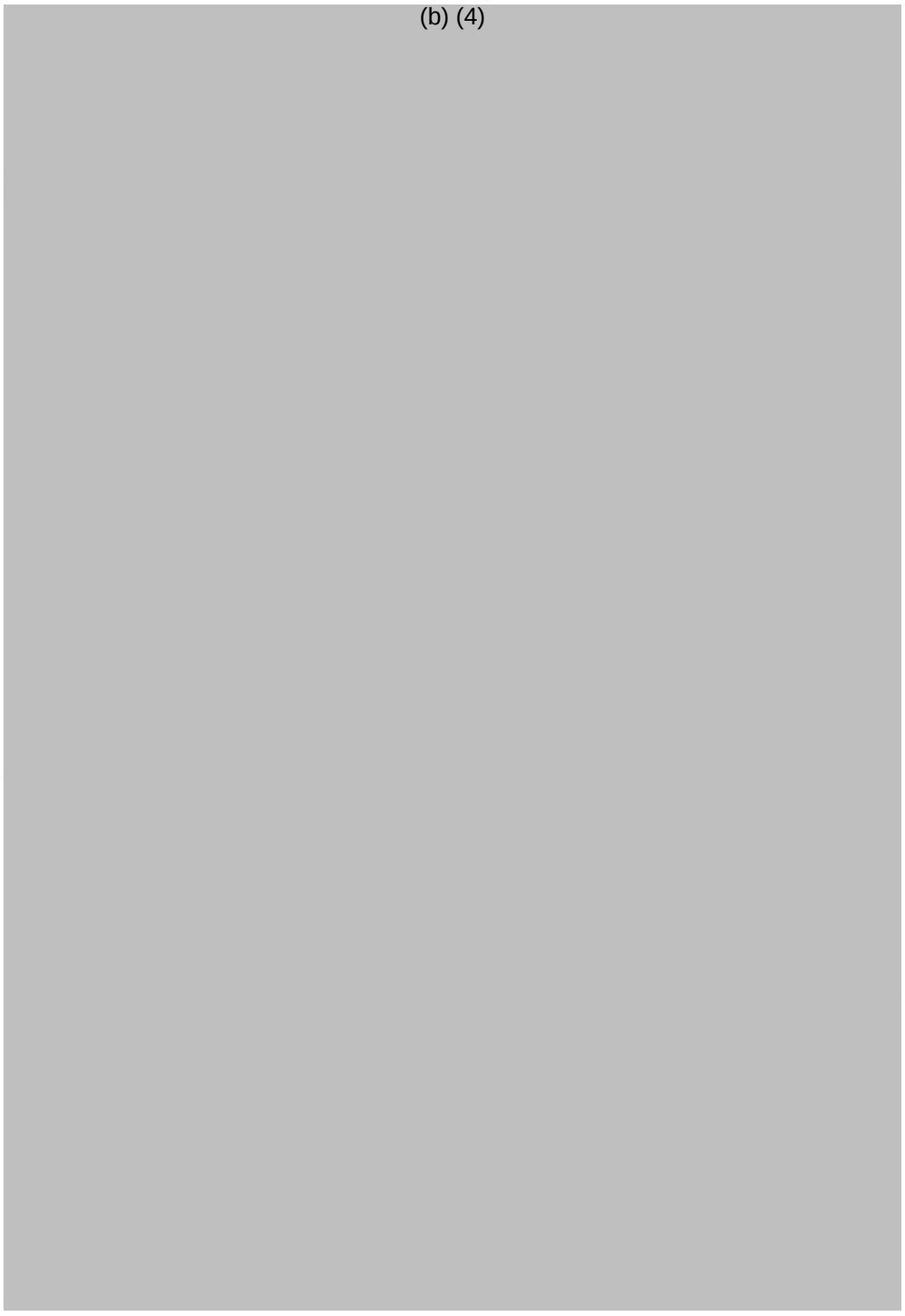

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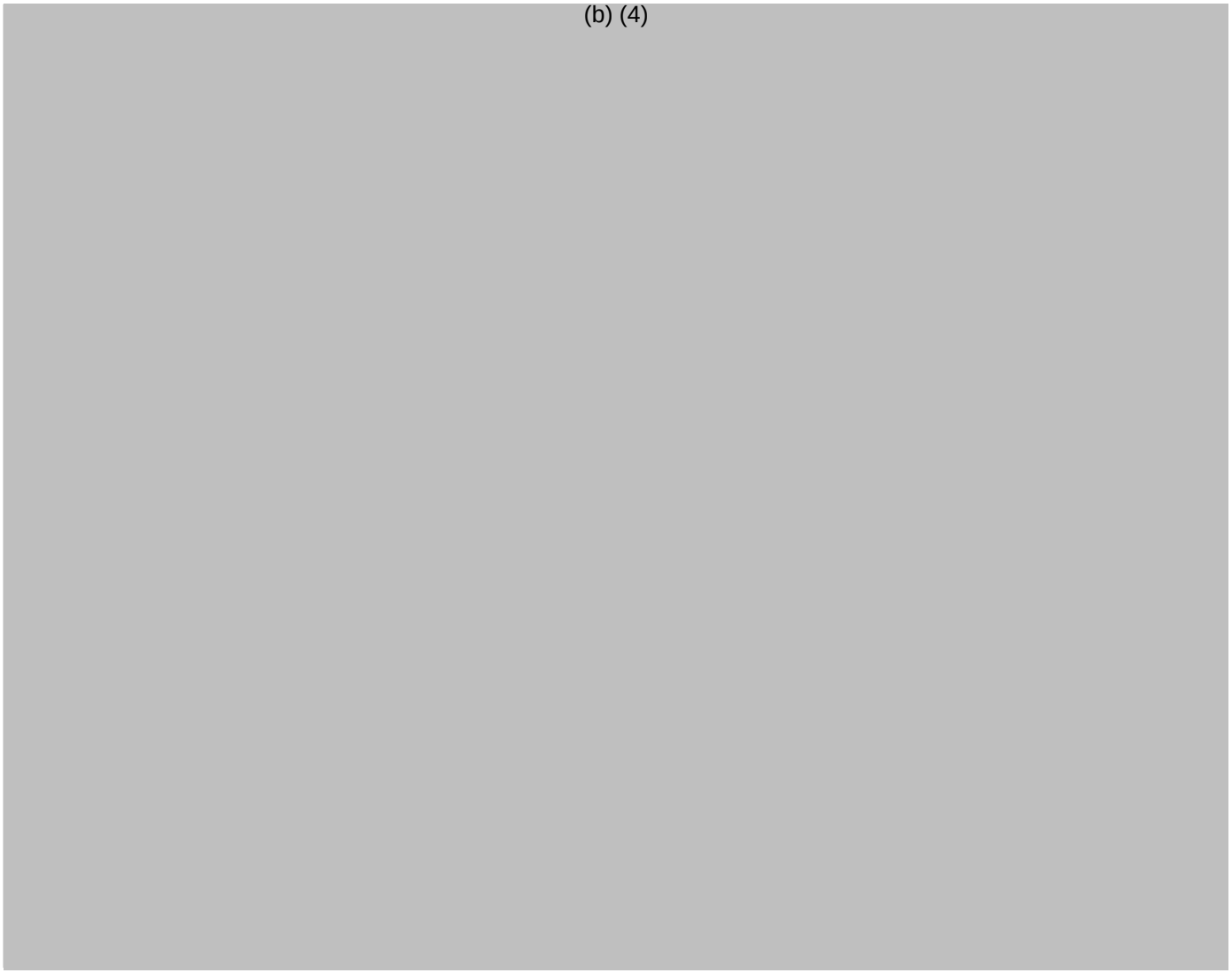

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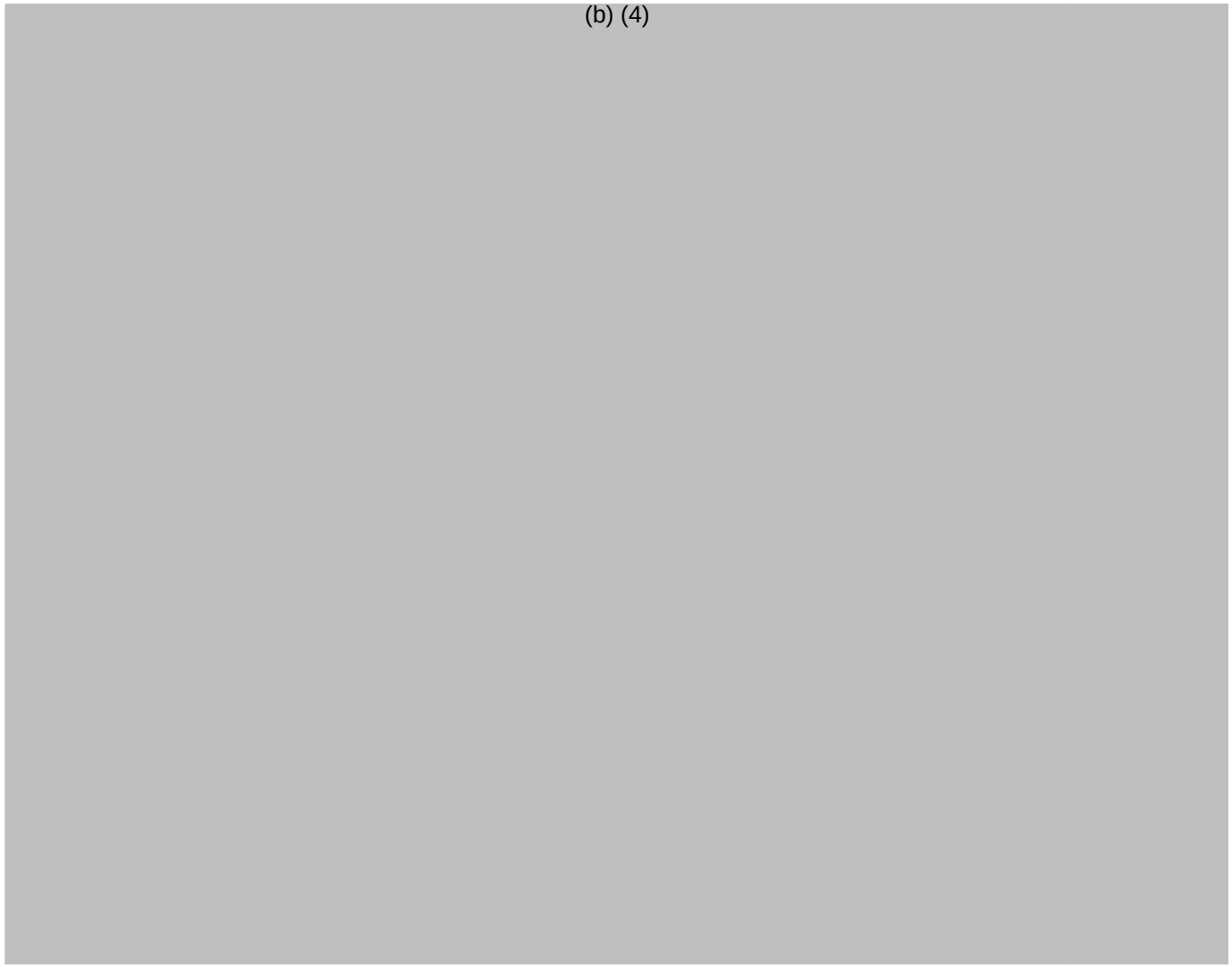

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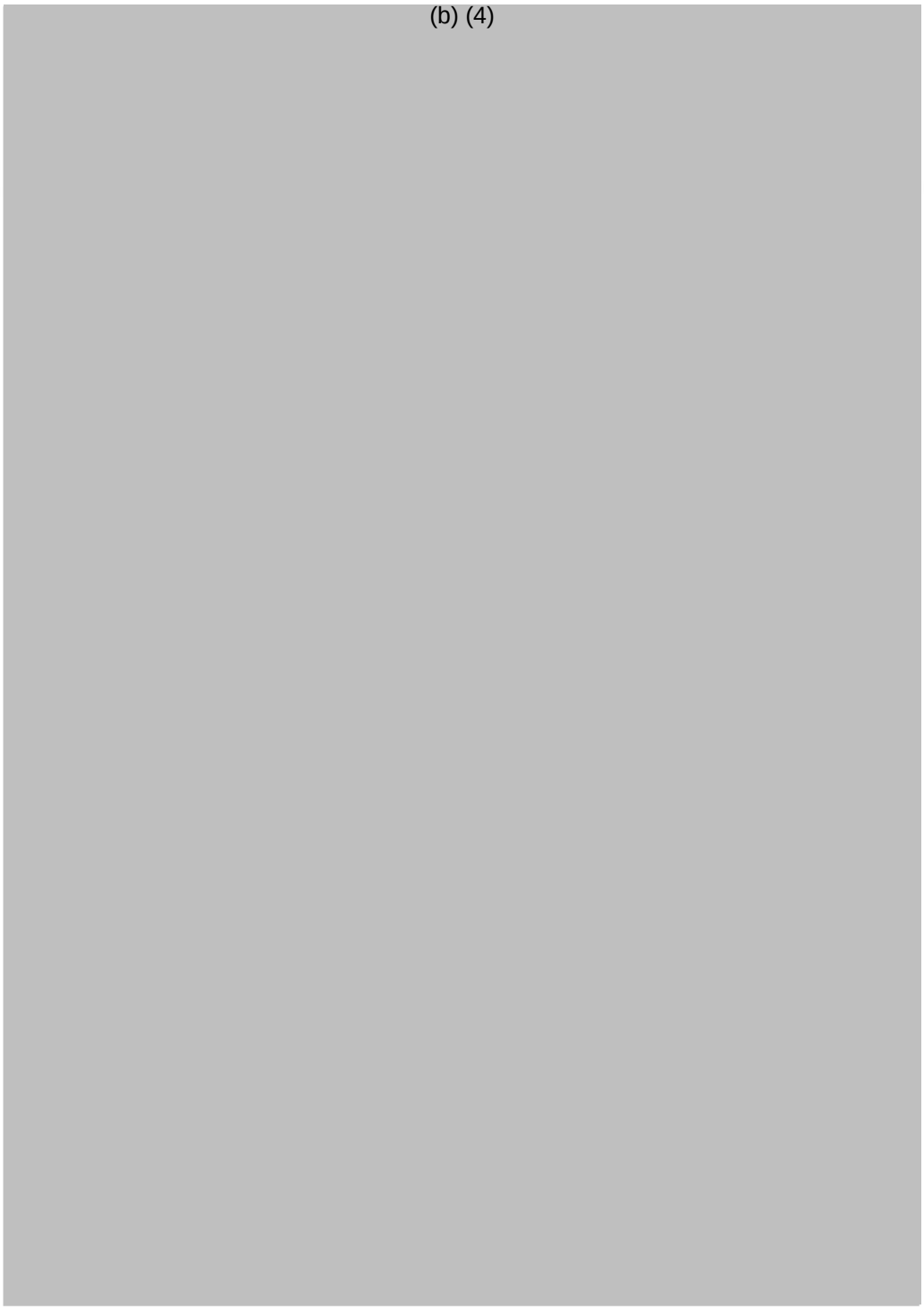

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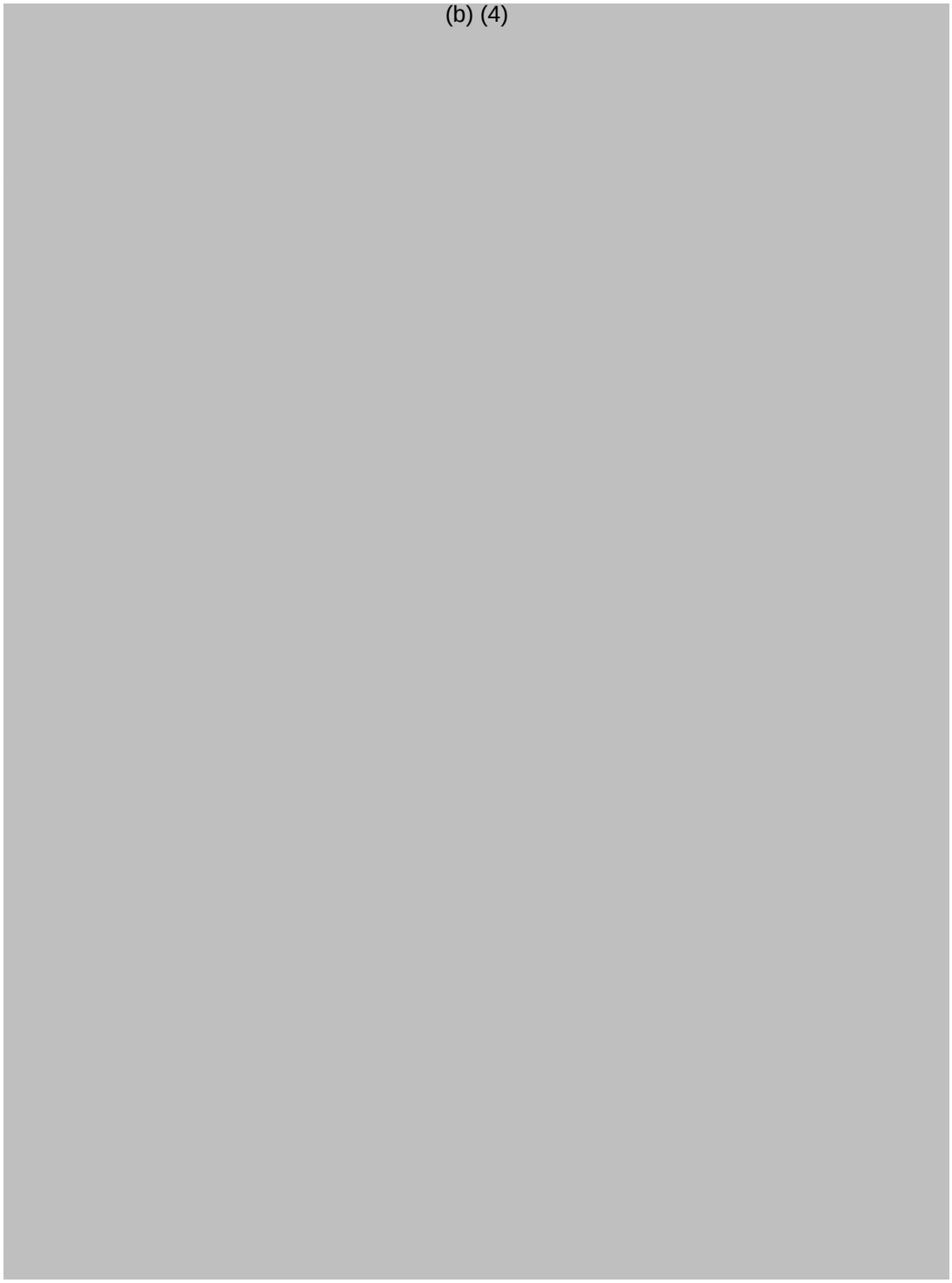

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ANALYTICAL PROCEDURE

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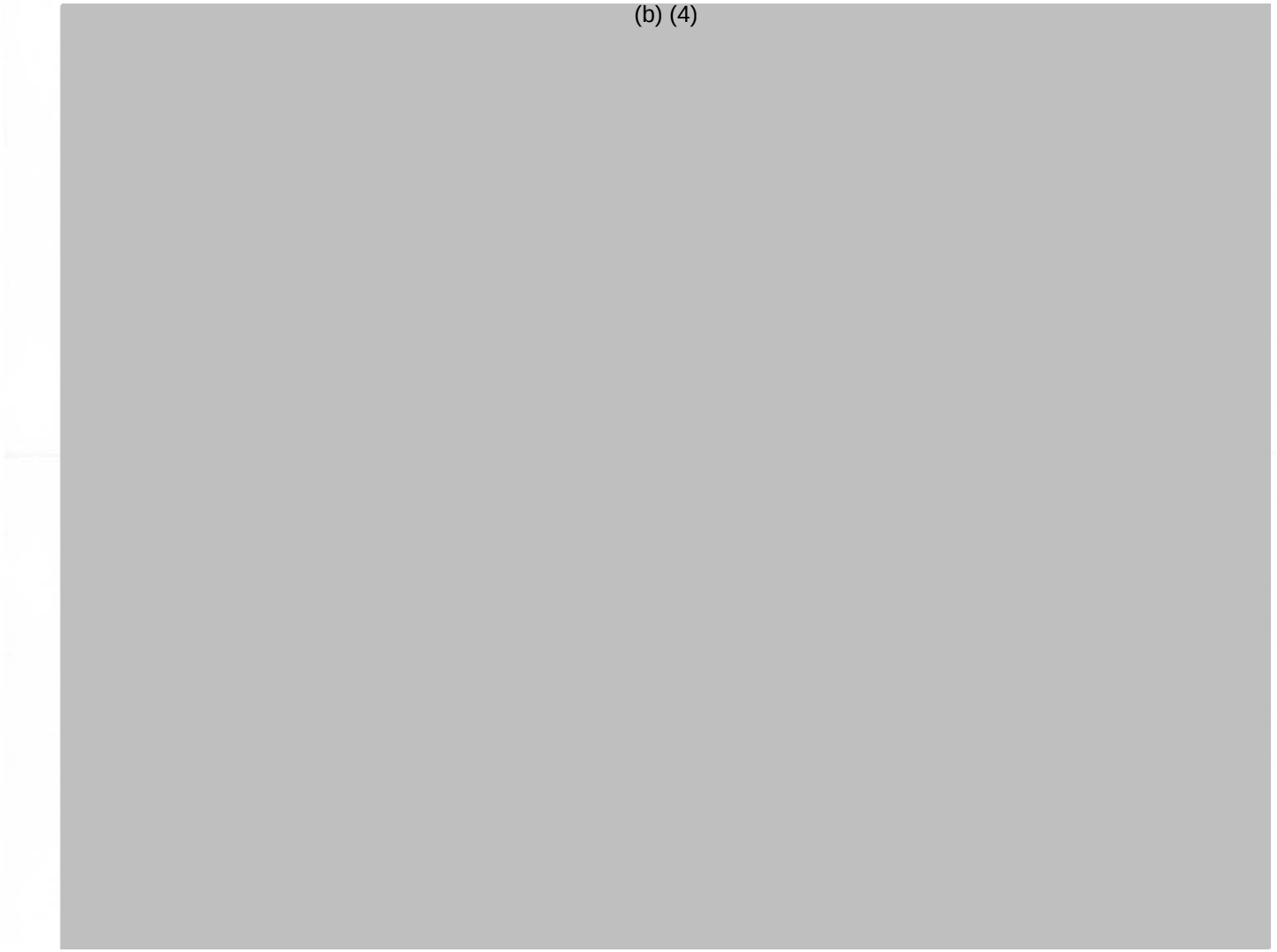

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Appendix 5
Certificate of Analysis

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moderna
messenger therapeutics

200 Tech Square • Cambridge, MA 02139
phone 617-714-6500 • fax 617-583-1998

Summary of Analysis

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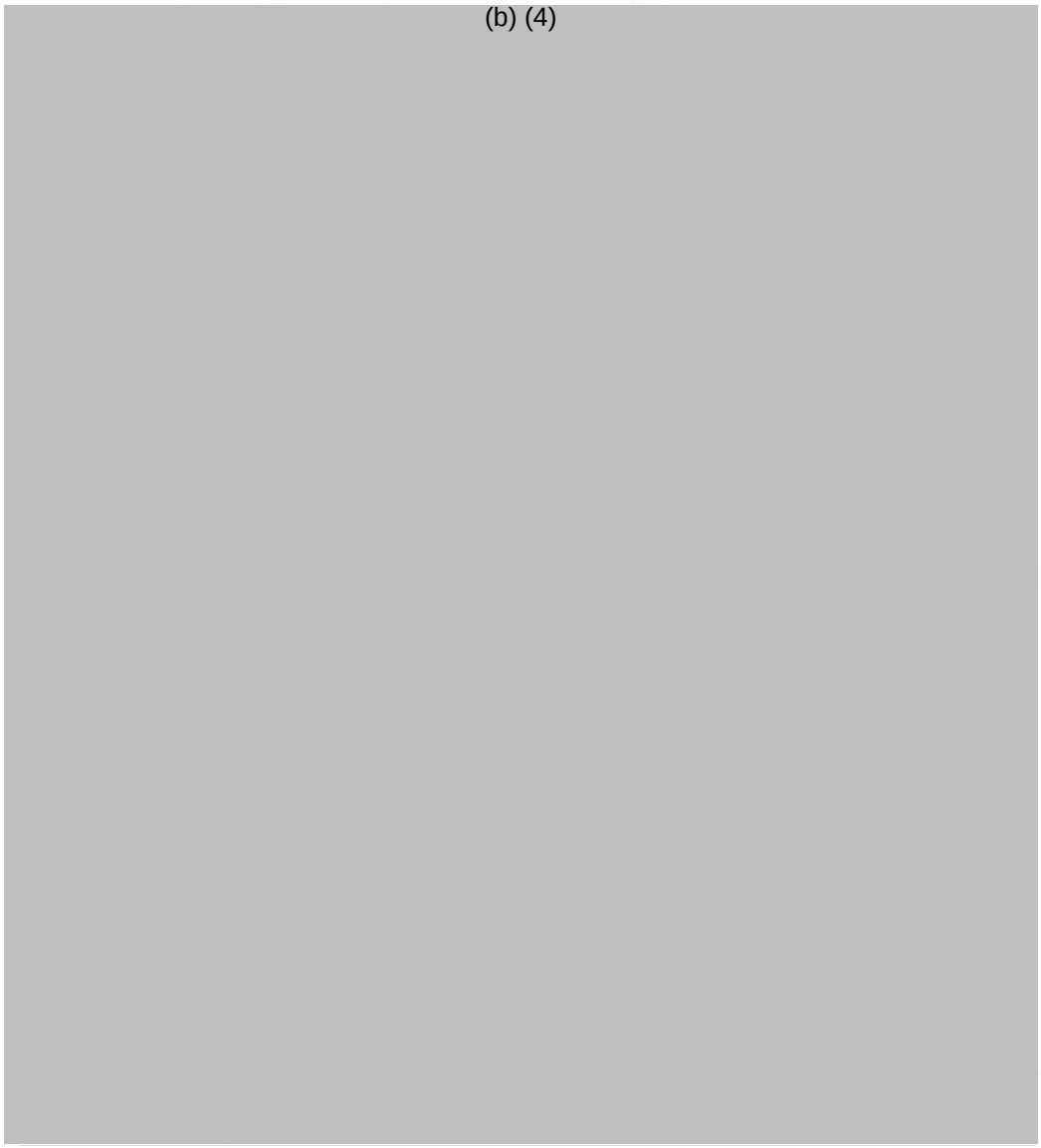

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phone 617-714-6500 • fax 617-583-1998

(b) (4)

Data Approved: (b) (6) Senior Scientist, DP Analytical Development (b) (6) Date: 06Jul2017

Doc: DPAD-SOA-0006.1 *Page 2 of 2*

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Toxicokinetic Report

Clarification

The average value of terminal half-life for the muscle (i.e. injection site) in Sections 4.2 and 5 of the toxicokinetic report should be read 14.9 instead of 8.39 based on the results of the toxicokinetic evaluation.

Changes indicated below had no impact on the study conclusion.

Note: Additions are indicated in bold underlined text and deletions are indicated in bold strikethrough text in sections indicated below. Values were not updated directly in the toxicokinetic report.

Section 4.2. Pharmacokinetic Evaluation

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

Section 5. Conclusion

Concentrations of mRNA-1647 were quantifiable in the majority of tissues examined at the first time point collected (2 hours post dose) and peak concentrations were reached between 2 and 24 hours post dose in tissues with exposures above that of plasma. The $t_{1/2}$ of mRNA-1647 was reliably estimated in muscle (site of injection), proximal popliteal and axillary distal lymph nodes and spleen with average values for all construct $t_{1/2}$ of **<u>14.9</u>** ~~**8.39**~~, 34.8, 31.1 and 63.0 hours, respectively.

Appendix 8

NON-GLP FINAL REPORT

Study Phase: Pharmacokinetics

Test Facility Study No. 5002121

TEST SITE:
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
Sherbrooke, QC J1E 0B5
Canada

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1. INTRODUCTION

This report describes the pharmacokinetics (PK) of mRNA-1647 in male Crl:CD(SD) Sprague-Dawley rat plasma and tissues following a single intramuscular injection of 100 µg mRNA-1647.

For the work detailed in this report, the Pharmacokinetic phase experimental start date was 23 August 2017, and the Pharmacokinetic phase experimental completion date was 07 Sept 2017.

2. EXPERIMENTAL DESIGN

Experimental procedures applicable to PK analysis are summarized in Text Table 1.

Text Table 1
Experimental Design

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

The vehicle used for this study was phosphate buffered saline (PBS) (1X), pH 7.2.

The Test Article was administered to the appropriate animals via intramuscular injection into the lateral compartment of the thigh once on Day 1.

Blood samples and tissues were collected on Day 1 according to the schedule illustrated in Text Table 2.

Text Table 2
PK 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.

Animals 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 (lung [left lobe], liver [left lateral], heart [ventricle bilateral], right kidney, axillary distal lymph nodes [bilateral pooled], proximal popliteal and inguinal lymph nodes [bilateral pooled], spleen, brain [left

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hemisphere], stomach [glandular region], testes [right testicle], eye [left], bone marrow femur [bilateral pooled], jejunum [middle region], and injection site muscle) were collected, rinsed with 1X PBS, dried on paper towel, weighed, and immediately snap frozen on liquid nitrogen, and kept on dry ice. Feces from bowel tissues were removed before processing.

PK blood samples were processed to plasma and analyzed, along with tissues collected, using a qualified bDNA multiplex method. Samples were analyzed for all six mRNA constructs (gB, gH, gL, UL130, UL131A, and UL128) present in mRNA-1647. The lower limit of quantification was set at 0.05, 0.01, 0.01, 0.05, 0.01, and 0.01 ng/mL for gB, gH, gL, UL130, UL131A, and UL128 constructs, respectively for plasma and tissues.

3. MATERIALS AND METHODS

3.1. Data Analysis

PK parameters were estimated using Phoenix pharmacokinetic software (Certara, USA) using a non-compartmental approach consistent with the intramuscular (plasma and tissues) routes of administration. All parameters were generated from mRNA-1647 construct concentrations for individual constructs in plasma and tissues from Day 1. Parameters were estimated using nominal sampling times relative to the start of each dose administration. Concentration values reported as below the limit of quantitation (BQL) were assigned a value of zero. All derived PK parameters were reported to 3 significant digits, except for T_{max} and $t_{1/2}$ which were reported to one decimal place.

The area under the concentration vs. time curve (AUC) was calculated using the linear trapezoidal method with linear interpolation and sparse sampling. The AUC was not calculated for PK profiles with less than 3 quantifiable concentrations of Test Article at separate time points. When practical, the terminal elimination phase of each concentration versus time curve was estimated using at least three observed concentration values. The slope of the elimination phase was determined using log linear regression on the unweighted concentration data. The parameters described in Text Table 3 were reported.

Descriptive statistics (numbers, means, standard error and standard deviations, as appropriate) for appropriate grouping and sorting variables were generated

AUC tissue/AUC plasma ratios were calculated using Microsoft Excel 2016. For the calculation of tissue to plasma ratios, where tissue is in ng/g and plasma is in ng/mL units, 1 g is assumed to be equal to 1 mL.

Text Table 3
PK Parameters Estimated

Parameter	Description of Parameter
T_{max}	The time after dosing at which the maximum observed concentration was observed.
C_{max}	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 trapezoidal method.

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Parameter	Description of Parameter
$t_{1/2}$	The apparent terminal elimination half life.

3.2. Computerized Systems

Critical computerized systems used in the study by the Test Facility are listed in Text Table 4.

Text Table 4
Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
Phoenix	7.0	Computation of non-compartmental analysis, descriptive statistics and ratios, as well as graphical and tabular output
Microsoft Excel	2016	AUC tissue/AUC plasma ratios calculation

4. RESULTS AND DISCUSSIONS

4.1. Concentration Observations

(Table 1)

No quantifiable mRNA-1647 concentrations for any of the constructs were observed in plasma and tissues predose samples (BQL), with exception for 2 plasma samples in gH construct assay which were just above the LLOQ.

Mean plasma concentrations of mRNA-1647 were quantifiable up to 24 hours post dose 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 Article distributes from the site of administration proceeding through the lymphatic system. Test Article 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 (2 hours post dose) 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 (site of injection), lymph nodes and spleen, mRNA-1647 concentrations were quantifiable up to the last sampling collection time, 120 hours post dose. In general, high concentration variability was observed for all tissues examined.

4.2. Pharmacokinetic Evaluation

(Figure 1, Table 2 and Table 3)

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

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quantifiable mean concentrations and therefore, is an estimate. For highly exposed tissues, peak concentration (C_{max}) was observed between 2 hours and 8 hours post dose in muscle and lymph nodes and between 2 and 24 hours post dose 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 (site of injection), proximal popliteal and axillary distal lymph nodes and spleen with average values for all construct $t_{1/2}$ of 8.39, 34.8, 31.1 and 63.0 hours, respectively.

Peak mRNA-1647 plasma concentration was reached at the first sampling time point (2 hours post dose). 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 $AUC_{(0-t)}$ 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 (site of injection), followed by proximal (popliteal) and axillary distal lymph nodes, suggesting the Test Article 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 $AUC_{(0-t)}$ ratios for highly exposed tissues were 939, 201, 62.8, and 13.4 for muscle (injection site), the lymph nodes (proximal popliteal and axillary distal) and spleen, respectively.

5. CONCLUSION

The PK of mRNA-1647 in male Crl:CD(SD) Sprague-Dawley rat plasma and tissues were evaluated following a single intramuscular injection of mRNA-1647 at a dose level of 100 µg.

Overall, mRNA-1647 constructs demonstrated nearly identical PK behavior. For all six mRNA-1647 constructs, measured levels for gB, gH, gL, UL130, UL131A, and UL128 in plasma and tissues were measured in a 1:1:1:1:1:1 ratio.

The highest mRNA-1647 exposure was observed in muscle (site of injection), followed by proximal (popliteal) and axillary distal lymph nodes, suggesting the mRNA-1647 distribution to the circulation by lymph flow.

All other peripheral tissues have demonstrated exposures comparable or below that measured in plasma.

Concentrations of mRNA-1647 were quantifiable in the majority of tissues examined at the first time point collected (2 hours post dose) and peak concentrations were reached between 2 and 24 hours post dose in tissues with exposures above that of plasma. The $t_{1/2}$ of mRNA-1647 was reliably estimated in muscle (site of injection), proximal popliteal and axillary distal lymph

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nodes and spleen with average values for all construct $t_{1/2}$ of 8.39, 34.8, 31.1 and 63.0 hours, respectively.

Appendix 8

6. REPORT APPROVAL

(b) (6)

Date: 24 Oct 2017

Principal Scientist, Pharmacokinetics

Appendix 8

Figure 1
Concentration vs. Time Curves of mRNA-1647 in Male Crl:CD(SD) Sprague-Dawley Rat Plasma and Tissues

Appendix 8

Figure 1.1: Summary (± SD) Male Sprague-Dawley Rat Plasma mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

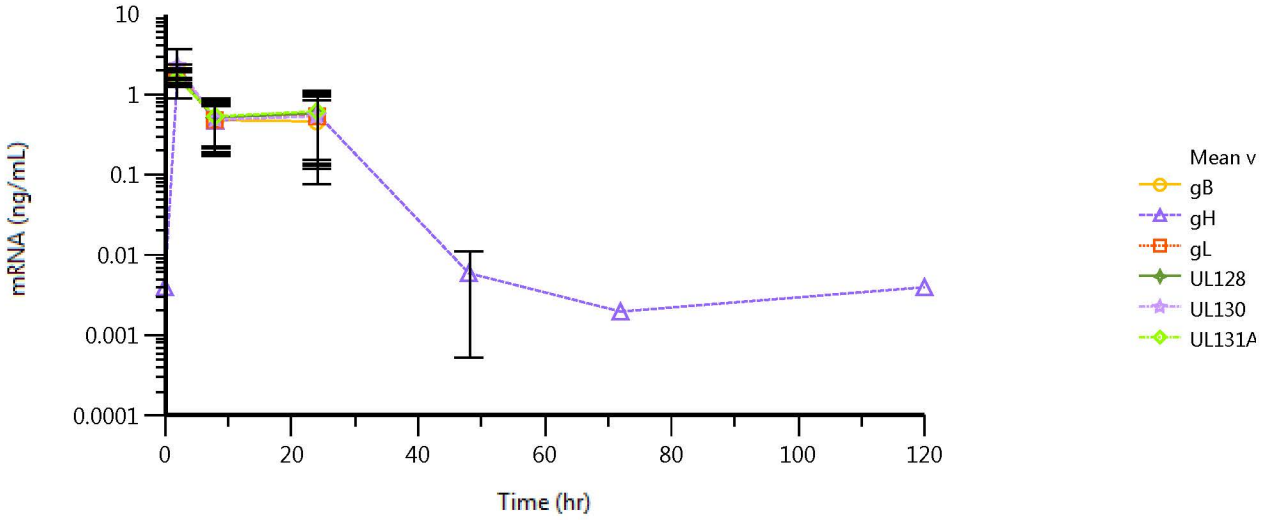

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Figure 1.2: Summary (± SD) Male Sprague-Dawley Rat Bone Marrow mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

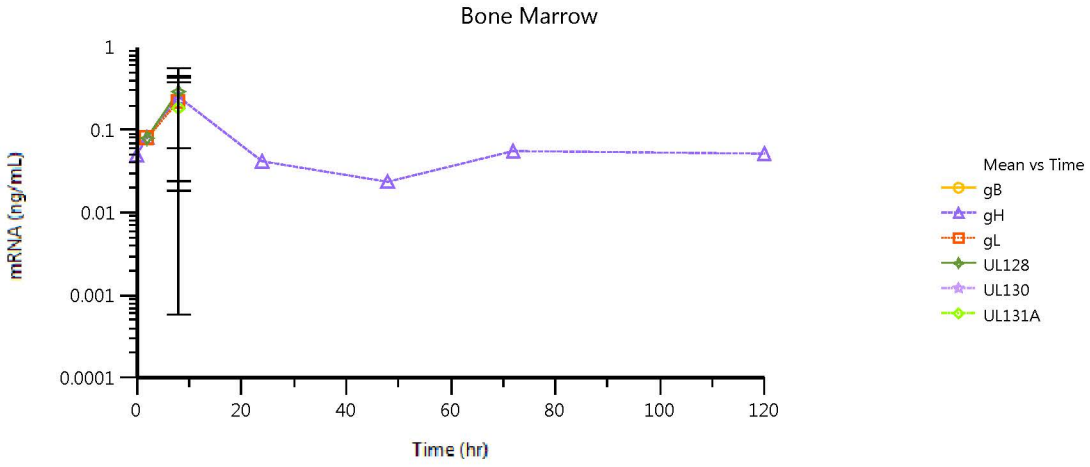

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Figure 1.3: Summary (± SD) Male Sprague-Dawley Rat Brain mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

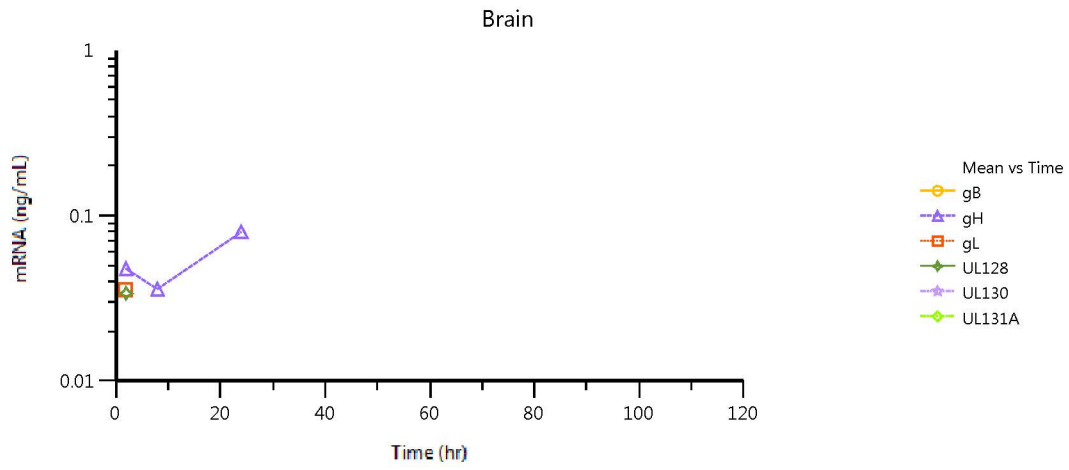

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Figure 1.4: Summary (± SD) Male Sprague-Dawley Rat Distal Lymph Nodes mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

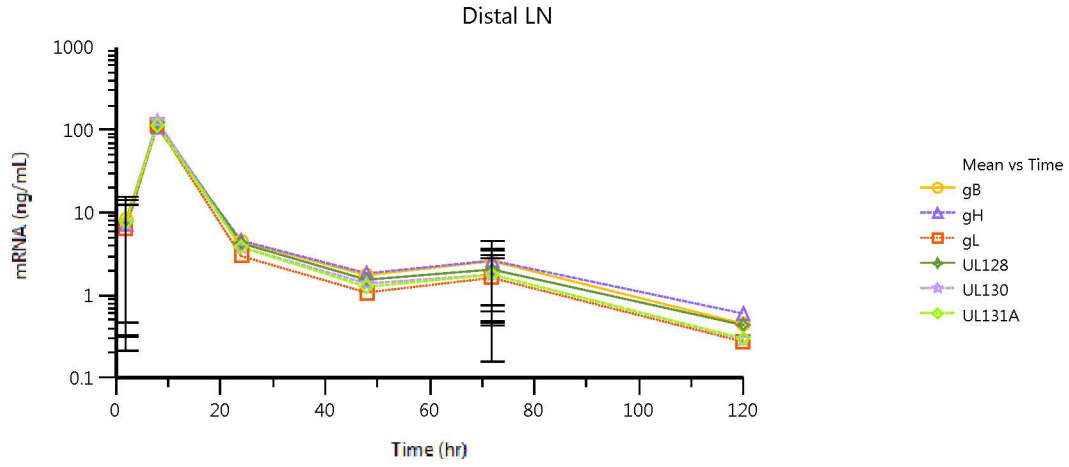

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Figure 1.5: Summary (± SD) Male Sprague-Dawley Rat Eye mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

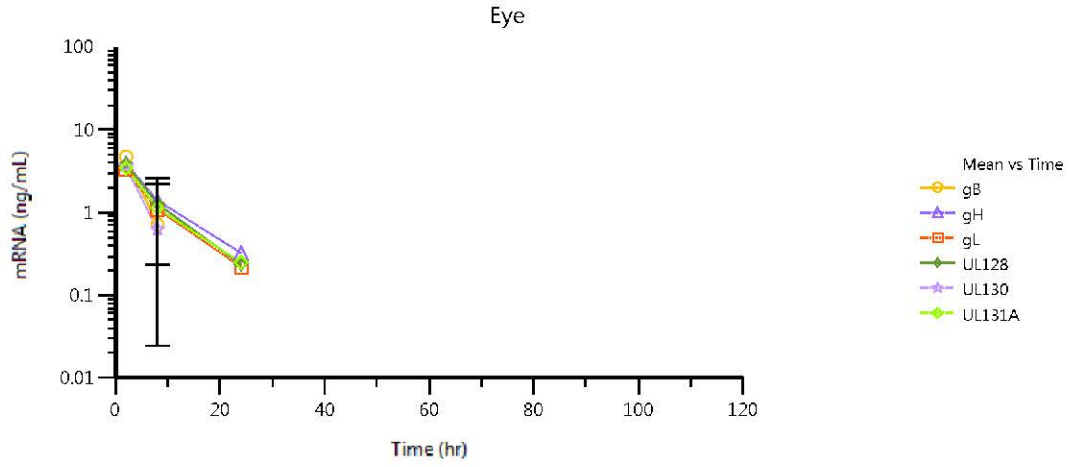

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Figure 1.6: Summary (± SD) Male Sprague-Dawley Rat Heart mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

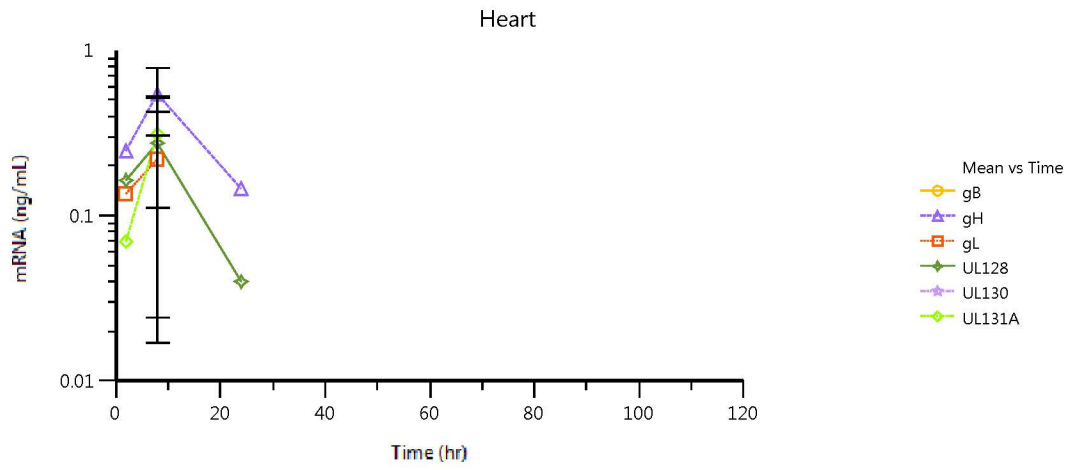

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Figure 1.7: Summary (± SD) Male Sprague-Dawley Rat Injection Site Muscle mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

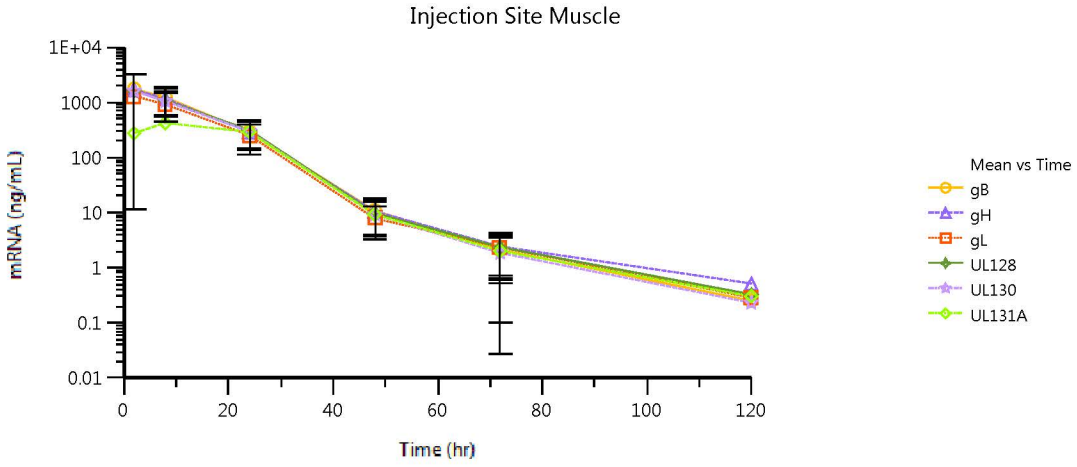

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Figure 1.8: Summary (± SD) Male Sprague-Dawley Rat Jejunum mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

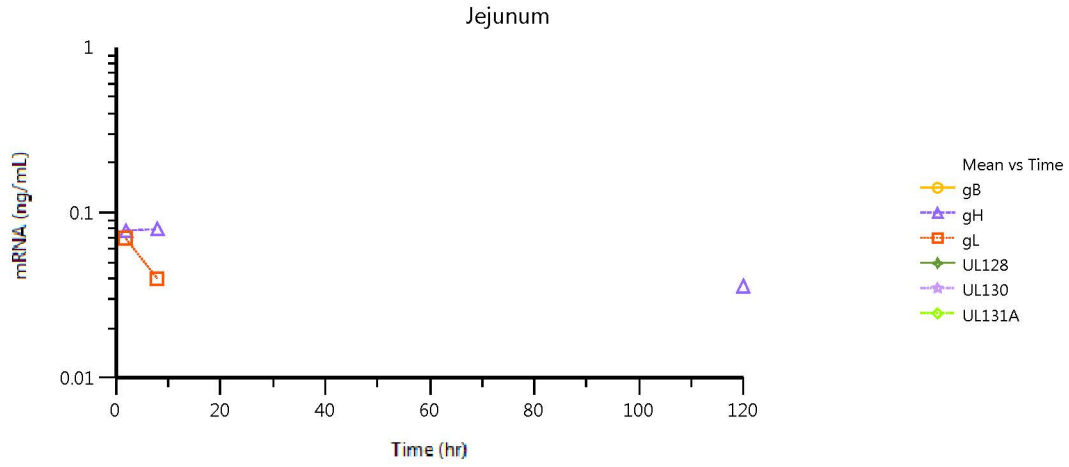

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Figure 1.9: Summary (± SD) Male Sprague-Dawley Rat Kidney mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

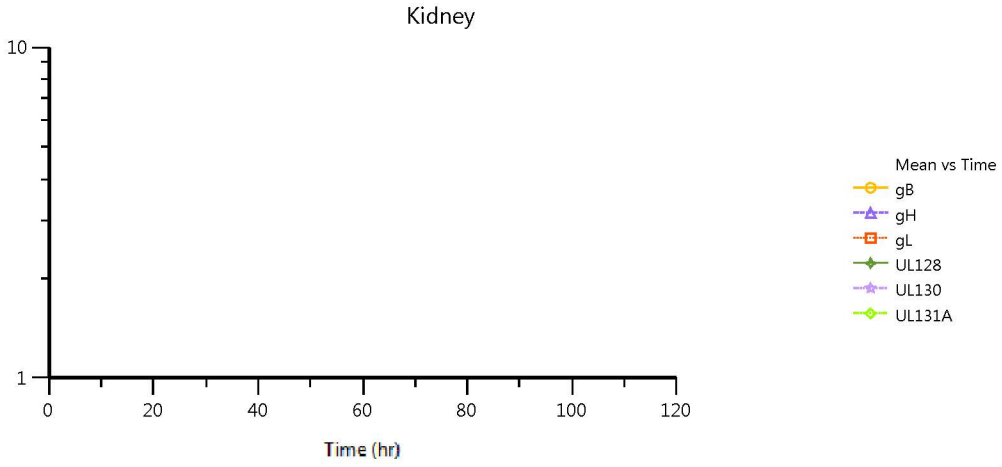

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Figure 1.10: Summary (± SD) Male Sprague-Dawley Rat Liver mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

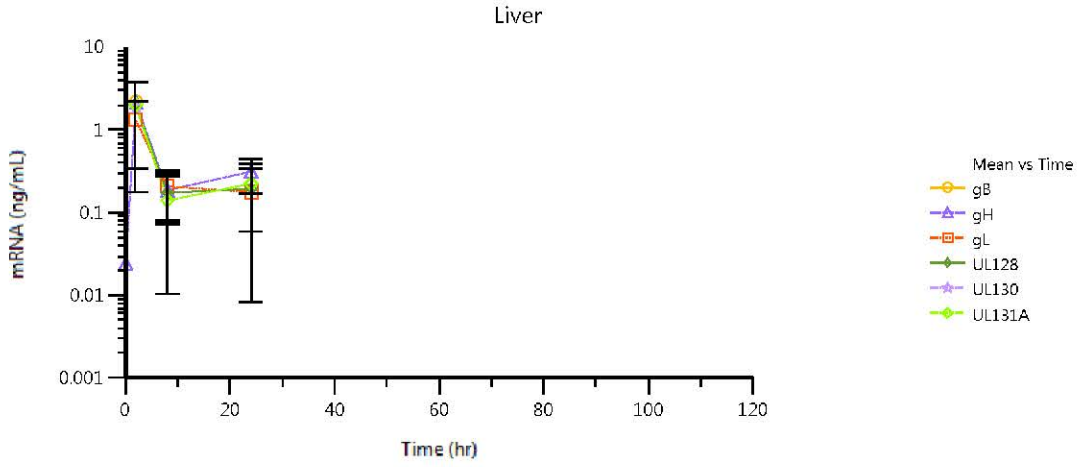

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Figure 1.11: Summary (± SD) Male Sprague-Dawley Rat Lung mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

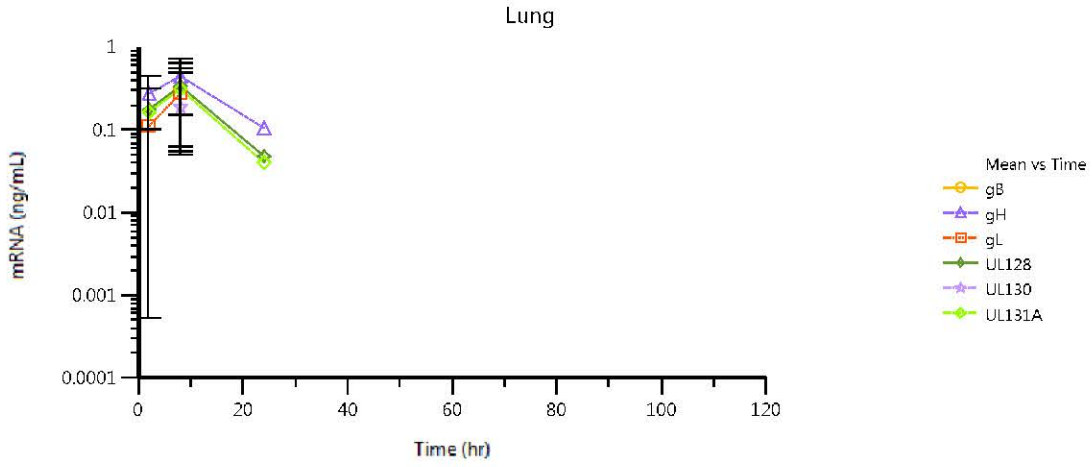

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Figure 1.12: Summary (± SD) Male Sprague-Dawley Rat Proximal Lymph node mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

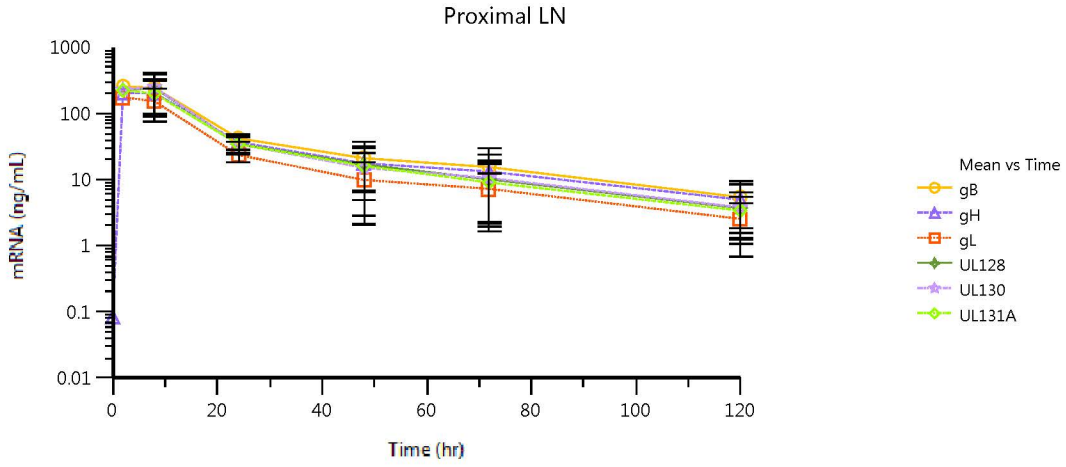

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Figure 1.13: Summary (± SD) Male Sprague-Dawley Rat Spleen mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

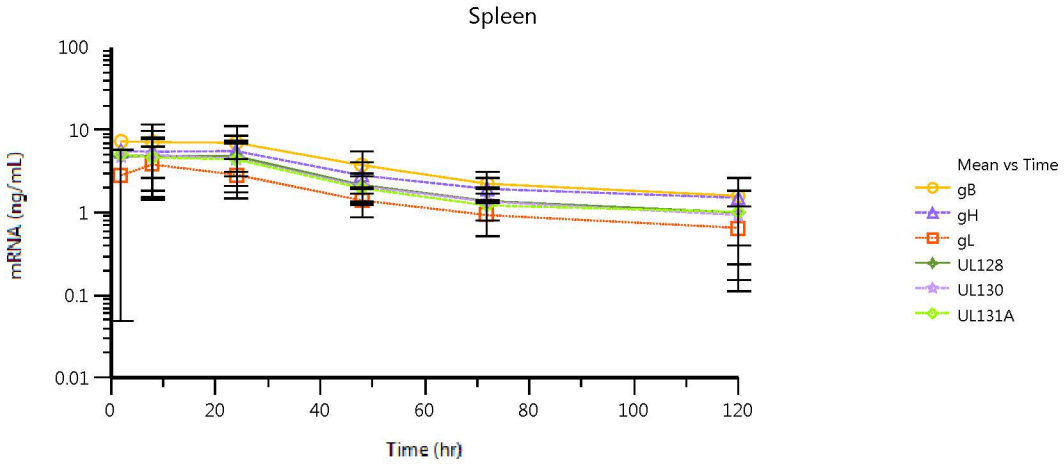

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Figure 1.14: Summary (± SD) Male Sprague-Dawley Rat Stomach mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

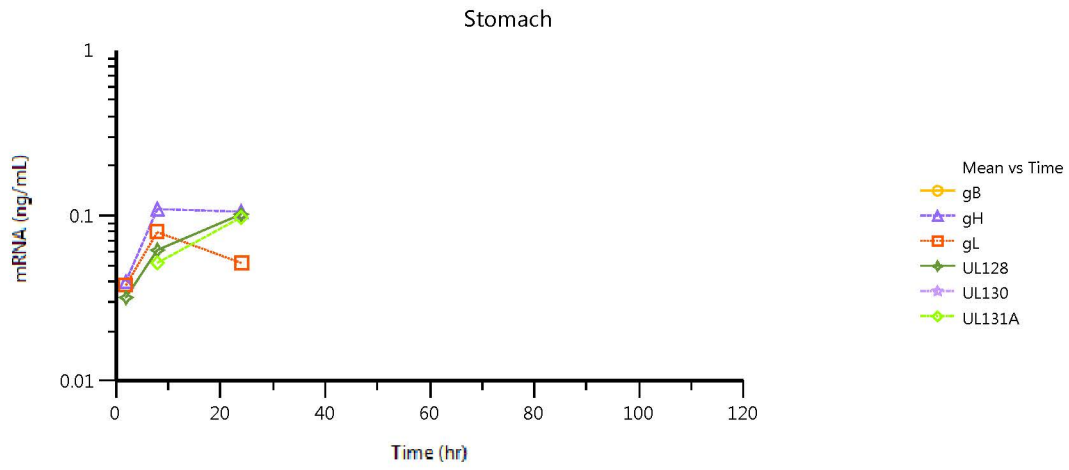

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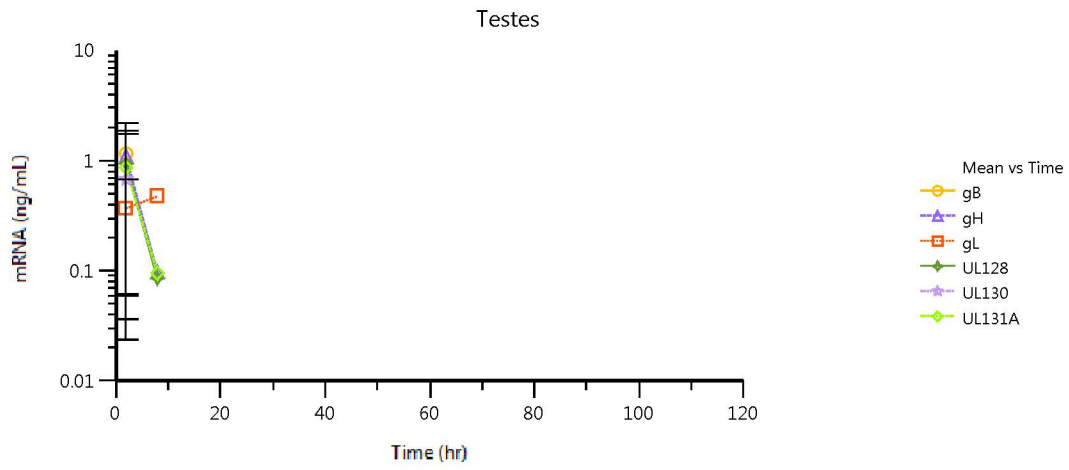
Figure 1.15: Summary (± SD) Male Sprague-Dawley Rat Testes mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1



Appendix 8

Table 1
Concentrations of mRNA-1647 in Male Crl:CD(SD) Sprague-Dawley Rat Plasma and Tissues

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Table 1.1: Mean Male Sprague-Dawley Rat Plasma mRNA-1647 Concentrations Following Intramuscular Administration of 100 µg mRNA-1647 on Day 1

Time (hr)	mRNA (ng/mL)											
	gB		gH		gL		UL128		UL130		UL131A	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.0	BQL	NA	0.00400[a]	0.00548	BQL	NA	BQL	NA	BQL	NA	BQL	NA
2.0	2.02	0.406	1.91	0.417	1.74	0.395	1.66	0.338	2.30	1.39	1.60	0.341
8.0	0.480	0.249	0.470	0.297	0.492	0.323	0.520	0.342	0.494	0.281	0.538	0.351
24.0	0.468	0.391	0.586	0.468	0.552	0.412	0.588	0.455	0.542	0.411	0.624	0.471
48.0	BQL	NA	0.00600[a]	0.00548	BQL	NA	BQL	NA	BQL	NA	BQL	NA
72.0	BQL	NA	0.00200[a]	0.00447	BQL	NA	BQL	NA	BQL	NA	BQL	NA
120.0	BQL	NA	0.00400[a]	0.00548	BQL	NA	BQL	NA	BQL	NA	BQL	NA

BQL = Below Quantifiable Limit (at 0.05, 0.01, 0.01, 0.05, 0.01, and 0.01 ng/mL for gB, gH, gL, UL130, UL131A, and UL128)
NA = not applicable; all values are BQL
a mean value was calculated with several BQL data points, hence the resulting value appears to be below the LLOQ.

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Table 1.2: Individual Male Sprague-Dawley Rat Plasma mRNA-1647 Concentrations Following Intramuscular Administration of 100 µg mRNA-1647 on Day 1

Time Postdose (hr)	Animal ID	mRNA (ng/mL)					
		UL130	UL131A	UL128	gB	gH	gL
Predose	1001	BLQ	BLQ	BLQ	BLQ	0.01	BLQ
	1002	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	1003	BLQ	BLQ	BLQ	BLQ	0.01	BLQ
	1004	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	1005	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
2	1006	1.26	1.22	1.34	1.61	1.45	1.27
	1007	2.10	1.96	2.01	2.36	2.32	2.22
	1008	2.06	1.96	2.03	2.54	2.39	2.06
	1009	4.69	1.44	1.51	1.83	1.74	1.63
	1010	1.39	1.41	1.39	1.76	1.67	1.50
8	1011	0.43	0.46	0.42	0.41	0.39	0.42
	1012	0.11	0.05	0.05	0.13	0.05	0.04
	1013	0.67	0.81	0.78	0.69	0.68	0.68
	1014	0.85	0.94	0.92	0.75	0.82	0.90
	1015	0.41	0.43	0.43	0.42	0.41	0.42
24	1016	0.15	0.17	0.16	0.18	0.19	0.15
	1017	0.27	0.32	0.31	0.25	0.27	0.29
	1018	1.21	1.39	1.34	1.15	1.37	1.22
	1019	0.56	0.64	0.59	0.38	0.57	0.58
	1020	0.52	0.60	0.54	0.38	0.53	0.52
48	1021	BLQ	BLQ	BLQ	BLQ	0.01	BLQ
	1022	BLQ	BLQ	BLQ	BLQ	0.01	BLQ
	1023	BLQ	BLQ	BLQ	BLQ	0.01	BLQ
	1024	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	1025	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
72	1026	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	1027	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	1028	BLQ	BLQ	BLQ	BLQ	0.01	BLQ
	1029	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	1030	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
120	1031	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	1032	BLQ	BLQ	BLQ	BLQ	0.01	BLQ
	1033	BLQ	BLQ	BLQ	BLQ	0.01	BLQ
	1034	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	1035	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

BQL = Below Quantifiable Limit (at 0.05, 0.01, 0.01, 0.05, 0.01, and 0.01 ng/mL for gB, gH, gL, UL130, UL131A, and UL128)

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Table 1.3: Mean Male Sprague-Dawley Rat Tissue mRNA-1647 Concentrations Following Intramuscular Administration of 100 µg mRNA-1647 on Day 1

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Tissue	Time	gB		gH		gL		UL128	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Bone Marrow	0 0	BQL	BQL	0 0500	0 112	BQL	BQL	BQL	BQL
	2 0	BQL	BQL	0 0820	0 112	0 0800	0 110	0 0800	0 110
	8 0	BQL	BQL	0 254	0 195	0 224	0 206	0 292	0 268
	24 0	BQL	BQL	0 0420	0 0939	BQL	BQL	BQL	BQL
	48 0	BQL	BQL	0 0240	0 0537	BQL	BQL	BQL	BQL
	72 0	BQL	BQL	0 0560	0 0767	BQL	BQL	BQL	BQL
	120 0	BQL	BQL	0 0520	0 0726	BQL	BQL	BQL	BQL
Brain	0 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	2 0	BQL	BQL	0 0480	0 107	0 0360	0 0805	0 0340	0 0760
	8 0	BQL	BQL	0 0360	0 0805	BQL	BQL	BQL	BQL
	24 0	BQL	BQL	0 0800	0 110	BQL	BQL	BQL	BQL
	48 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	72 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	120 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
Distal LN	0 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	2 0	8 36	8 37	7 29	6 98	6 40	6 19	7 84	7 36
	8 0	108	225	110	229	117	243	125	261
	24 0	4 54	9 59	4 63	9 03	3 01	5 83	4 29	8 25
	48 0	1 76	1 98	1 87	2 12	1 09	1 19	1 56	1 68
	72 0	2 61	1 96	2 67	1 89	1 64	1 21	2 06	1 60
	120 0	0 454	1 02	0 608	0 655	0 278	0 472	0 442	0 572
Eye	0 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	2 0	4 72	6 20	3 92	4 90	3 23	4 10	3 91	4 89
	8 0	0 710	1 59	1 40	1 17	1 08	1 14	1 28	1 45
	24 0	BQL	BQL	0 322	0 363	0 218	0 345	0 236	0 528
	48 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	72 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	120 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
Heart	0 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	2 0	BQL	BQL	0 248	0 273	0 136	0 191	0 164	0 179
	8 0	BQL	BQL	0 548	0 240	0 220	0 203	0 276	0 252
	24 0	BQL	BQL	0 146	0 149	BQL	BQL	0 0400	0 0894
	48 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	72 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	120 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
Injection Site Muscle	0 0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	2 0	1770	1800	1720	1850	1310	1430	1620	1610
	8 0	1240	689	1180	619	933	488	1100	506
	24 0	298	157	294	150	255	141	317	166
	48 0	11 1	7 23	10 7	6 68	8 04	4 75	9 91	6 25
	72 0	2 15	2 12	2 46	1 87	2 41	1 66	2 39	1 74

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	120.0	0.256	0.572	0.518	0.566	0.294	0.447	0.328	0.527
Jejunum	0.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	2.0	BQL	BQL	0.0780	0.107	0.0700	0.0959	BQL	BQL
	8.0	BQL	BQL	0.0800	0.110	0.0400	0.0894	BQL	BQL
	24.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	48.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	0.0360	0.0805	BQL	BQL	BQL	BQL
Kidney	0.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	2.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	8.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	24.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	48.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
Liver	0.0	BQL	BQL	0.0240	0.0537	BQL	BQL	BQL	BQL
	2.0	2.16	2.70	2.12	2.20	1.30	0.967	2.00	1.82
	8.0	BQL	BQL	0.186	0.109	0.206	0.124	0.172	0.100
	24.0	BQL	BQL	0.310	0.138	0.176	0.168	0.192	0.192
	48.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
Lung	0.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	2.0	BQL	BQL	0.274	0.172	0.110	0.151	0.176	0.177
	8.0	BQL	BQL	0.442	0.290	0.274	0.220	0.340	0.288
	24.0	BQL	BQL	0.104	0.147	BQL	BQL	0.0480	0.107
	48.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
Proximal LN	0.0	BQL	BQL	0.0840	0.188	BQL	BQL	BQL	BQL
	2.0	260	270	205	212	175	183	227	236
	8.0	249	156	206	115	156	82.6	246	149
	24.0	42.7	6.03	37.3	8.52	23.7	5.20	35.1	11.1
	48.0	21.5	14.8	17.9	11.7	9.97	7.87	17.2	14.3
	72.0	15.6	13.4	13.2	11.1	7.23	5.33	10.2	7.91
	120.0	5.50	3.72	4.98	3.40	2.59	1.91	3.74	2.68
Spleen	0.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL

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	2.0	7.36	8.52	5.59	6.04	2.87	2.82	4.86	5.27
	8.0	7.15	4.51	5.55	4.00	3.83	2.33	4.84	3.34
	24.0	7.06	3.91	5.63	2.86	2.92	1.45	4.87	2.73
	48.0	3.80	1.81	2.86	1.15	1.41	0.545	2.19	0.811
	72.0	2.26	0.830	1.95	0.645	0.940	0.428	1.40	0.613
	120.0	1.59	1.61	1.51	1.11	0.658	0.545	1.02	0.860
Stomach	0.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	2.0	BQL	BQL	0.0400	0.0894	0.0380	0.0850	0.0320	0.0716
	8.0	BQL	BQL	0.110	0.156	0.0800	0.112	0.0620	0.139
	24.0	BQL	BQL	0.106	0.155	0.0520	0.116	0.102	0.145
	48.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
Testes	0.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	2.0	1.16	1.61	1.11	1.07	0.366	0.324	0.946	0.887
	8.0	BQL	BQL	0.0980	0.219	0.420	0.750	0.0860	0.192
	24.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	48.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL

Tissue	Time	U130		U131A	
		Mean	SD	Mean	SD
Bone Marrow	0.0	BQL	BQL	BQL	BQL
	2.0	BQL	BQL	BQL	BQL
	8.0	BQL	BQL	0.186	0.185
	24.0	BQL	BQL	BQL	BQL
	48.0	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL
Brain	0.0	BQL	BQL	BQL	BQL
	2.0	BQL	BQL	BQL	BQL
	8.0	BQL	BQL	BQL	BQL
	24.0	BQL	BQL	BQL	BQL
	48.0	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL

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	120.0	BQL	BQL	BQL	BQL
Distal LN	0.0	BQL	BQL	BQL	BQL
	2.0	7.42	7.47	7.78	7.45
	8.0	129	271	114	241
	24.0	3.85	8.08	3.87	7.55
	48.0	1.40	1.66	1.28	1.35
	72.0	1.81	1.65	1.81	1.32
	120.0	0.294	0.657	0.302	0.515
Eye	0.0	BQL	BQL	BQL	BQL
	2.0	3.61	4.79	3.43	4.39
	8.0	0.626	1.40	1.13	1.11
	24.0	BQL	BQL	0.246	0.393
	48.0	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL
Heart	0.0	BQL	BQL	BQL	BQL
	2.0	BQL	BQL	0.0700	0.157
	8.0	BQL	BQL	0.312	0.200
	24.0	BQL	BQL	BQL	BQL
	48.0	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL
Injection Site Muscle	0.0	BQL	BQL	BQL	BQL
	2.0	1630	1740	277	379
	8.0	1050	507	427	470
	24.0	304	162	298	164
	48.0	9.37	6.00	9.39	6.12
	72.0	1.90	1.80	2.05	1.53
	120.0	0.232	0.519	0.310	0.520
Jejunum	0.0	BQL	BQL	BQL	BQL
	2.0	BQL	BQL	BQL	BQL
	8.0	BQL	BQL	BQL	BQL
	24.0	BQL	BQL	BQL	BQL
	48.0	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL
Kidney	0.0	BQL	BQL	BQL	BQL

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	2.0	BQL	BQL	BQL	BQL
	8.0	BQL	BQL	BQL	BQL
	24.0	BQL	BQL	BQL	BQL
	48.0	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL
Liver	0.0	BQL	BQL	BQL	BQL
	2.0	1.87	2.26	1.99	2.07
	8.0	BQL	BQL	0.140	0.130
	24.0	BQL	BQL	0.222	0.164
	48.0	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL
Lung	0.0	BQL	BQL	BQL	BQL
	2.0	BQL	BQL	0.162	0.161
	8.0	0.188	0.420	0.310	0.248
	24.0	BQL	BQL	0.0400	0.0894
	48.0	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL
Proximal LN	0.0	BQL	BQL	BQL	BQL
	2.0	233	243	225	236
	8.0	252	150	200	110
	24.0	34.8	10.7	34.4	9.44
	48.0	15.1	10.2	16.3	14.2
	72.0	10.8	8.57	9.21	7.60
	120.0	3.82	2.59	3.43	2.11
Spleen	0.0	BQL	BQL	BQL	BQL
	2.0	4.87	5.66	5.10	5.90
	8.0	5.03	3.15	4.69	3.24
	24.0	4.53	2.46	4.41	2.61
	48.0	2.10	0.819	2.01	0.749
	72.0	1.37	0.550	1.24	0.428
	120.0	0.942	0.953	1.02	0.784
Stomach	0.0	BQL	BQL	BQL	BQL
	2.0	BQL	BQL	BQL	BQL
	8.0	BQL	BQL	0.0520	0.116

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	24.0	BQL	BQL	0.0980	0.142
	48.0	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL
Testes	0.0	BQL	BQL	BQL	BQL
	2.0	0.682	0.988	0.872	0.849
	8.0	BQL	BQL	0.0960	0.215
	24.0	BQL	BQL	BQL	BQL
	48.0	BQL	BQL	BQL	BQL
	72.0	BQL	BQL	BQL	BQL
	120.0	BQL	BQL	BQL	BQL

BQL = Below Quantifiable Limit (at 0.05, 0.01, 0.01, 0.05, 0.01, and 0.01 ng/mL for gB, gH, gL, UL130, UL131A, and UL128)

Appendix 8

Table 1.4: Individual Male Sprague-Dawley Rat Tissue mRNA-1647 Concentrations Following Intramuscular Administration of 100 µg mRNA-1647 on Day 1

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Lung	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Lung	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Lung	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Lung	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
Lung	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Lung	2	1006	BQL	BQL	BQL	BQL	BQL	BQL
Lung	2	1007	BQL	BQL	BQL	BQL	0.27	BQL
Lung	2	1008	BQL	0.35	0.39	BQL	0.44	0.27
Lung	2	1009	BQL	0.29	0.31	BQL	0.4	0.28
Lung	2	1010	BQL	0.17	0.18	BQL	0.26	BQL
Lung	8	1011	BQL	BQL	BQL	BQL	0.22	BQL
Lung	8	1012	BQL	0.35	0.39	BQL	0.44	0.29
Lung	8	1013	BQL	0.35	0.37	BQL	0.43	0.27
Lung	8	1014	0.94	0.67	0.77	BQL	0.92	0.61
Lung	8	1015	BQL	0.18	0.17	BQL	0.2	0.2
Lung	24	1016	BQL	BQL	BQL	BQL	BQL	BQL
Lung	24	1017	BQL	BQL	BQL	BQL	0.21	BQL
Lung	24	1018	BQL	0.2	0.24	BQL	0.31	BQL
Lung	24	1019	BQL	BQL	BQL	BQL	BQL	BQL
Lung	24	1020	BQL	BQL	BQL	BQL	BQL	BQL
Lung	48	1021	BQL	BQL	BQL	BQL	BQL	BQL
Lung	48	1022	BQL	BQL	BQL	BQL	BQL	BQL
Lung	48	1023	BQL	BQL	BQL	BQL	BQL	BQL
Lung	48	1024	BQL	BQL	BQL	BQL	BQL	BQL
Lung	48	1025	BQL	BQL	BQL	BQL	BQL	BQL
Lung	72	1026	BQL	BQL	BQL	BQL	BQL	BQL
Lung	72	1027	BQL	BQL	BQL	BQL	BQL	BQL
Lung	72	1028	BQL	BQL	BQL	BQL	BQL	BQL
Lung	72	1029	BQL	BQL	BQL	BQL	BQL	BQL
Lung	72	1030	BQL	BQL	BQL	BQL	BQL	BQL
Lung	120	1031	BQL	BQL	BQL	BQL	BQL	BQL
Lung	120	1032	BQL	BQL	BQL	BQL	BQL	BQL
Lung	120	1033	BQL	BQL	BQL	BQL	BQL	BQL

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Lung	120	1034	BQL	BQL	BQL	BQL	BQL	BQL
Lung	120	1035	BQL	BQL	BQL	BQL	BQL	BQL
Liver	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Liver	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Liver	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Liver	0	1004	BQL	BQL	BQL	BQL	0.12	BQL
Liver	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Liver	2	1006	BQL	0.31	0.36	BQL	0.25	0.31
Liver	2	1007	BQL	0.67	0.86	BQL	0.65	0.65
Liver	2	1008	5.11	5.23	4.64	6.15	5.37	2.52
Liver	2	1009	3.29	2.89	3.14	3.71	3.4	2.13
Liver	2	1010	0.93	0.83	1	0.95	0.93	0.9
Liver	8	1011	BQL	0.21	0.21	BQL	0.24	0.24
Liver	8	1012	BQL	BQL	BQL	BQL	BQL	BQL
Liver	8	1013	BQL	0.27	0.26	BQL	0.28	0.3
Liver	8	1014	BQL	0.22	0.21	BQL	0.22	0.3
Liver	8	1015	BQL	BQL	0.18	BQL	0.19	0.19
Liver	24	1016	BQL	0.17	BQL	BQL	0.17	BQL
Liver	24	1017	BQL	BQL	BQL	BQL	0.21	BQL
Liver	24	1018	BQL	0.28	0.3	BQL	0.42	0.24
Liver	24	1019	BQL	0.21	0.22	BQL	0.26	0.27
Liver	24	1020	BQL	0.45	0.44	BQL	0.49	0.37
Liver	48	1021	BQL	BQL	BQL	BQL	BQL	BQL
Liver	48	1022	BQL	BQL	BQL	BQL	BQL	BQL
Liver	48	1023	BQL	BQL	BQL	BQL	BQL	BQL
Liver	48	1024	BQL	BQL	BQL	BQL	BQL	BQL
Liver	48	1025	BQL	BQL	BQL	BQL	BQL	BQL
Liver	72	1026	BQL	BQL	BQL	BQL	BQL	BQL
Liver	72	1027	BQL	BQL	BQL	BQL	BQL	BQL
Liver	72	1028	BQL	BQL	BQL	BQL	BQL	BQL
Liver	72	1029	BQL	BQL	BQL	BQL	BQL	BQL
Liver	72	1030	BQL	BQL	BQL	BQL	BQL	BQL
Liver	120	1031	BQL	BQL	BQL	BQL	BQL	BQL
Liver	120	1032	BQL	BQL	BQL	BQL	BQL	BQL
Liver	120	1033	BQL	BQL	BQL	BQL	BQL	BQL
Liver	120	1034	BQL	BQL	BQL	BQL	BQL	BQL
Liver	120	1035	BQL	BQL	BQL	BQL	BQL	BQL

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Heart	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Heart	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Heart	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Heart	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
Heart	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Heart	2	1006	BQL	BQL	BQL	BQL	BQL	BQL
Heart	2	1007	BQL	BQL	0.17	BQL	0.26	BQL
Heart	2	1008	BQL	0.35	0.43	BQL	0.66	0.4
Heart	2	1009	BQL	BQL	0.22	BQL	0.32	0.28
Heart	2	1010	BQL	BQL	BQL	BQL	BQL	BQL
Heart	8	1011	BQL	0.43	0.47	BQL	0.66	0.28
Heart	8	1012	BQL	0.22	BQL	BQL	0.26	0.01
Heart	8	1013	BQL	0.45	0.45	BQL	0.78	0.41
Heart	8	1014	BQL	0.46	0.46	BQL	0.72	0.4
Heart	8	1015	BQL	BQL	BQL	BQL	0.32	BQL
Heart	24	1016	BQL	BQL	BQL	BQL	BQL	BQL
Heart	24	1017	BQL	BQL	BQL	BQL	BQL	BQL
Heart	24	1018	BQL	BQL	BQL	BQL	0.21	BQL
Heart	24	1019	BQL	BQL	0.2	BQL	0.35	BQL
Heart	24	1020	BQL	BQL	BQL	BQL	0.17	BQL
Heart	48	1021	BQL	BQL	BQL	BQL	BQL	BQL
Heart	48	1022	BQL	BQL	BQL	BQL	BQL	BQL
Heart	48	1023	BQL	BQL	BQL	BQL	BQL	BQL
Heart	48	1024	BQL	BQL	BQL	BQL	BQL	BQL
Heart	48	1025	BQL	BQL	BQL	BQL	BQL	BQL
Heart	72	1026	BQL	BQL	BQL	BQL	BQL	BQL
Heart	72	1027	BQL	BQL	BQL	BQL	BQL	BQL
Heart	72	1028	BQL	BQL	BQL	BQL	BQL	BQL
Heart	72	1029	BQL	BQL	BQL	BQL	BQL	BQL
Heart	72	1030	BQL	BQL	BQL	BQL	BQL	BQL
Heart	120	1031	BQL	BQL	BQL	BQL	BQL	BQL
Heart	120	1032	BQL	BQL	BQL	BQL	BQL	BQL
Heart	120	1033	BQL	BQL	BQL	BQL	BQL	BQL
Heart	120	1034	BQL	BQL	BQL	BQL	BQL	BQL
Heart	120	1035	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	0	1002	BQL	BQL	BQL	BQL	BQL	BQL

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Kidney	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	2	1006	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	2	1007	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	2	1008	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	2	1009	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	2	1010	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	8	1011	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	8	1012	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	8	1013	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	8	1014	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	8	1015	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	24	1016	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	24	1017	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	24	1018	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	24	1019	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	24	1020	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	48	1021	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	48	1022	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	48	1023	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	48	1024	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	48	1025	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	72	1026	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	72	1027	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	72	1028	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	72	1029	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	72	1030	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	120	1031	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	120	1032	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	120	1033	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	120	1034	BQL	BQL	BQL	BQL	BQL	BQL
Kidney	120	1035	BQL	BQL	BQL	BQL	BQL	BQL
Distal LN	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Distal LN	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Distal LN	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Distal LN	0	1004	BQL	BQL	BQL	BQL	BQL	BQL

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Distal LN	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Distal LN	2	1006	8.05	7.28	8.2	9.42	7.38	6.4
Distal LN	2	1007	18.9	18.65	18.88	21.16	18.08	15.89
Distal LN	2	1008	8.62	11.03	9.87	9.5	8.72	7.9
Distal LN	2	1009	BQL	0.57	0.68	BQL	0.71	0.56
Distal LN	2	1010	1.55	1.36	1.55	1.71	1.55	1.24
Distal LN	8	1011	1.17	1	1.28	1.32	1.17	0.9
Distal LN	8	1012	1.74	1.66	2.13	1.75	1.66	2.5
Distal LN	8	1013	29.3	22.8	29.53	25.36	27.64	29.7
Distal LN	8	1014	BQL	0.57	0.66	BQL	0.71	0.52
Distal LN	8	1015	612.69	545.21	592.36	510.1	518.97	551.13
Distal LN	24	1016	0.95	0.81	1.02	1.04	0.96	0.72
Distal LN	24	1017	18.28	17.38	19.04	21.67	20.77	13.43
Distal LN	24	1018	BQL	0.37	0.44	BQL	0.38	0.37
Distal LN	24	1019	BQL	0.63	0.74	BQL	0.74	0.52
Distal LN	24	1020	BQL	0.18	0.23	BQL	0.28	BQL
Distal LN	48	1021	1.75	1.73	1.98	2.41	2.08	1.16
Distal LN	48	1022	BQL	BQL	BQL	BQL	BQL	BQL
Distal LN	48	1023	4.04	3.36	4.21	4.76	5.36	3.04
Distal LN	48	1024	1.2	1.09	1.3	1.61	1.51	0.96
Distal LN	48	1025	BQL	0.23	0.32	BQL	0.39	0.31
Distal LN	72	1026	2.95	2.68	2.97	3.74	3.94	2.36
Distal LN	72	1027	3.16	2.56	3.32	3.84	4.09	2.57
Distal LN	72	1028	BQL	BQL	BQL	BQL	BQL	BQL
Distal LN	72	1029	BQL	0.8	0.68	1.04	1.31	0.69
Distal LN	72	1030	2.93	2.99	3.34	4.43	3.99	2.56
Distal LN	120	1031	BQL	BQL	0.25	BQL	0.27	BQL
Distal LN	120	1032	1.47	1.19	1.44	2.27	1.76	1.09
Distal LN	120	1033	BQL	BQL	0.18	BQL	0.32	BQL
Distal LN	120	1034	BQL	0.32	0.34	BQL	0.51	0.3
Distal LN	120	1035	BQL	BQL	BQL	BQL	0.18	BQL
Proximal LN	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Proximal LN	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Proximal LN	0	1003	BQL	BQL	BQL	BQL	0.42	BQL
Proximal LN	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
Proximal LN	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Proximal LN	2	1006	449.93	443.15	460.98	478.26	396.66	346.36

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Proximal LN	2	1007	14.04	11.69	11.63	15.08	12.87	9.55
Proximal LN	2	1008	30.5	28.79	27.74	37.37	28.61	22.06
Proximal LN	2	1009	534.94	513.16	499.59	612.9	466.75	396.06
Proximal LN	2	1010	133.46	130.47	134.66	156.93	121.57	102.44
Proximal LN	8	1011	68.03	63.37	68.71	57.59	51.38	47.95
Proximal LN	8	1012	175.52	153.99	166.22	166.35	156.06	130.6
Proximal LN	8	1013	418.24	242.75	426.13	431.78	280.45	202.77
Proximal LN	8	1014	202.39	181.3	198.16	204.77	189.98	132.63
Proximal LN	8	1015	394.98	359.54	372.98	386.49	351.4	266.74
Proximal LN	24	1016	53.29	50.18	54.3	53.06	52.12	32.44
Proximal LN	24	1017	28.35	29.01	28.81	38.38	32.01	19.05
Proximal LN	24	1018	31.9	29.62	31.86	41.2	33.83	23.93
Proximal LN	24	1019	26.97	27.19	26.35	38.59	31.8	20.71
Proximal LN	24	1020	33.64	36.22	34.06	42.41	36.89	22.56
Proximal LN	48	1021	23.7	38.03	36.92	37.68	32.4	14.69
Proximal LN	48	1022	28.07	23.41	27.65	36.52	28.12	21.43
Proximal LN	48	1023	11.46	8.88	9.99	17.15	14.27	6.68
Proximal LN	48	1024	6.65	6.16	5.99	8.6	7.91	3.78
Proximal LN	48	1025	5.69	4.98	5.36	7.39	7.04	3.28
Proximal LN	72	1026	4.42	3.47	3.85	5.56	5.13	2.51
Proximal LN	72	1027	6.82	5.3	6.24	9.27	7.7	4.59
Proximal LN	72	1028	21.14	16.22	19.32	32.04	26.34	13.35
Proximal LN	72	1029	2.65	2.45	3.23	3.17	2.86	3.05
Proximal LN	72	1030	18.79	18.59	18.12	28.04	24	12.66
Proximal LN	120	1031	2.72	2.61	2.46	4.05	3.52	1.49
Proximal LN	120	1032	1.13	0.99	1.05	1.53	1.41	0.69
Proximal LN	120	1033	7.12	5.87	7.1	10.39	9.32	5.05
Proximal LN	120	1034	2.16	2.26	1.99	3.18	2.85	1.52
Proximal LN	120	1035	5.99	5.41	6.1	8.37	7.81	4.2
Spleen	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Spleen	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Spleen	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Spleen	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
Spleen	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Spleen	2	1006	0.84	0.8	0.85	1.31	1.16	0.61
Spleen	2	1007	BQL	0.33	0.49	BQL	0.31	0.69
Spleen	2	1008	5.28	5.51	5.29	9.19	6.38	2.83

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Spleen	2	1009	14.21	14.93	13.53	21.18	15.44	7.55
Spleen	2	1010	4.02	3.94	4.13	5.1	4.68	2.66
Spleen	8	1011	2.28	2.24	3.02	3.69	2.38	2.92
Spleen	8	1012	2.72	1.37	1.33	2.37	1.87	1.79
Spleen	8	1013	6.93	7.1	6.5	10.67	7.43	4.45
Spleen	8	1014	3.58	3.77	3.54	6.11	4.53	2.38
Spleen	8	1015	9.62	8.97	9.79	12.89	11.53	7.6
Spleen	24	1016	1.01	0.94	1.05	1.82	1.6	0.77
Spleen	24	1017	4.78	4.72	4.94	6.77	5.4	3
Spleen	24	1018	4.39	3.94	4.68	7.53	5.97	2.93
Spleen	24	1019	7.95	8.27	8.76	12.81	9.66	4.87
Spleen	24	1020	4.5	4.16	4.92	6.38	5.52	3.02
Spleen	48	1021	1.19	1.16	1.24	2.42	1.99	0.84
Spleen	48	1022	2.86	2.76	3.05	6.62	3.93	1.81
Spleen	48	1023	1.97	2.18	1.99	3.29	2.45	1.36
Spleen	48	1024	1.47	1.29	1.65	2.2	1.71	0.95
Spleen	48	1025	3.03	2.65	3	4.45	4.23	2.11
Spleen	72	1026	1	0.89	0.91	1.53	1.52	0.53
Spleen	72	1027	2.04	1.75	2.19	3.14	2.53	1.5
Spleen	72	1028	0.64	0.71	0.67	1.24	1.09	0.5
Spleen	72	1029	1.54	1.4	1.7	2.6	2.03	1.17
Spleen	72	1030	1.62	1.45	1.53	2.8	2.58	1
Spleen	120	1031	BQL	0.17	BQL	BQL	0.26	BQL
Spleen	120	1032	0.98	1	0.96	1.81	1.26	0.6
Spleen	120	1033	2.14	2.17	2.15	3.72	2.99	1.35
Spleen	120	1034	BQL	0.45	0.42	BQL	0.78	0.3
Spleen	120	1035	1.59	1.32	1.55	2.44	2.27	1.04
Brain	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Brain	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Brain	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Brain	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
Brain	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Brain	2	1006	BQL	BQL	BQL	BQL	BQL	BQL
Brain	2	1007	BQL	BQL	BQL	BQL	BQL	BQL
Brain	2	1008	BQL	BQL	BQL	BQL	BQL	BQL
Brain	2	1009	BQL	BQL	0.17	BQL	0.24	0.18
Brain	2	1010	BQL	BQL	BQL	BQL	BQL	BQL

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Brain	8	1011	BQL	BQL	BQL	BQL	BQL	BQL
Brain	8	1012	BQL	BQL	BQL	BQL	0.18	BQL
Brain	8	1013	BQL	BQL	BQL	BQL	BQL	BQL
Brain	8	1014	BQL	BQL	BQL	BQL	BQL	BQL
Brain	8	1015	BQL	BQL	BQL	BQL	BQL	BQL
Brain	24	1016	BQL	BQL	BQL	BQL	BQL	BQL
Brain	24	1017	BQL	BQL	BQL	BQL	0.19	BQL
Brain	24	1018	BQL	BQL	BQL	BQL	0.21	BQL
Brain	24	1019	BQL	BQL	BQL	BQL	BQL	BQL
Brain	24	1020	BQL	BQL	BQL	BQL	BQL	BQL
Brain	48	1021	BQL	BQL	BQL	BQL	BQL	BQL
Brain	48	1022	BQL	BQL	BQL	BQL	BQL	BQL
Brain	48	1023	BQL	BQL	BQL	BQL	BQL	BQL
Brain	48	1024	BQL	BQL	BQL	BQL	BQL	BQL
Brain	48	1025	BQL	BQL	BQL	BQL	BQL	BQL
Brain	72	1026	BQL	BQL	BQL	BQL	BQL	BQL
Brain	72	1027	BQL	BQL	BQL	BQL	BQL	BQL
Brain	72	1028	BQL	BQL	BQL	BQL	BQL	BQL
Brain	72	1029	BQL	BQL	BQL	BQL	BQL	BQL
Brain	72	1030	BQL	BQL	BQL	BQL	BQL	BQL
Brain	120	1031	BQL	BQL	BQL	BQL	BQL	BQL
Brain	120	1032	BQL	BQL	BQL	BQL	BQL	BQL
Brain	120	1033	BQL	BQL	BQL	BQL	BQL	BQL
Brain	120	1034	BQL	BQL	BQL	BQL	BQL	BQL
Brain	120	1035	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	2	1006	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	2	1007	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	2	1008	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	2	1009	BQL	BQL	0.16	BQL	0.2	0.19
Stomach	2	1010	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	8	1011	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	8	1012	BQL	BQL	BQL	BQL	BQL	BQL

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Stomach	8	1013	BQL	BQL	BQL	BQL	0.22	0.17
Stomach	8	1014	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	8	1015	BQL	0.26	0.31	BQL	0.33	0.23
Stomach	24	1016	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	24	1017	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	24	1018	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	24	1019	BQL	0.31	0.31	BQL	0.34	0.26
Stomach	24	1020	BQL	0.18	0.2	BQL	0.19	BQL
Stomach	48	1021	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	48	1022	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	48	1023	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	48	1024	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	48	1025	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	72	1026	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	72	1027	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	72	1028	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	72	1029	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	72	1030	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	120	1031	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	120	1032	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	120	1033	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	120	1034	BQL	BQL	BQL	BQL	BQL	BQL
Stomach	120	1035	BQL	BQL	BQL	BQL	BQL	BQL
Testes	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Testes	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Testes	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Testes	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
Testes	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Testes	2	1006	BQL	0.48	0.47	BQL	0.57	BQL
Testes	2	1007	1.25	1.23	1.26	2.54	1.45	0.37
Testes	2	1008	BQL	0.48	0.46	BQL	0.49	0.87
Testes	2	1009	BQL	BQL	0.18	BQL	0.2	0.40
Testes	2	1010	2.16	2.17	2.36	3.26	2.84	0.19
Testes	8	1011	BQL	0.48	0.43	BQL	0.49	1.73
Testes	8	1012	BQL	BQL	BQL	BQL	BQL	0.37
Testes	8	1013	BQL	BQL	BQL	BQL	BQL	BQL
Testes	8	1014	BQL	BQL	BQL	BQL	BQL	BQL

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Testes	8	1015	BQL	BQL	BQL	BQL	BQL	BQL
Testes	24	1016	BQL	BQL	BQL	BQL	BQL	BQL
Testes	24	1017	BQL	BQL	BQL	BQL	BQL	BQL
Testes	24	1018	BQL	BQL	BQL	BQL	BQL	BQL
Testes	24	1019	BQL	BQL	BQL	BQL	BQL	BQL
Testes	24	1020	BQL	BQL	BQL	BQL	BQL	BQL
Testes	48	1021	BQL	BQL	BQL	BQL	BQL	BQL
Testes	48	1022	BQL	BQL	BQL	BQL	BQL	BQL
Testes	48	1023	BQL	BQL	BQL	BQL	BQL	BQL
Testes	48	1024	BQL	BQL	BQL	BQL	BQL	BQL
Testes	48	1025	BQL	BQL	BQL	BQL	BQL	BQL
Testes	72	1026	BQL	BQL	BQL	BQL	BQL	BQL
Testes	72	1027	BQL	BQL	BQL	BQL	BQL	BQL
Testes	72	1028	BQL	BQL	BQL	BQL	BQL	BQL
Testes	72	1029	BQL	BQL	BQL	BQL	BQL	BQL
Testes	72	1030	BQL	BQL	BQL	BQL	BQL	BQL
Testes	120	1031	BQL	BQL	BQL	BQL	BQL	BQL
Testes	120	1032	BQL	BQL	BQL	BQL	BQL	BQL
Testes	120	1033	BQL	BQL	BQL	BQL	BQL	BQL
Testes	120	1034	BQL	BQL	BQL	BQL	BQL	BQL
Testes	120	1035	BQL	BQL	BQL	BQL	BQL	BQL
Eye	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Eye	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Eye	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Eye	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
Eye	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Eye	2	1006	BQL	BQL	0.26	BQL	0.45	0.28
Eye	2	1007	BQL	BQL	BQL	BQL	BQL	BQL
Eye	2	1008	11.5	10.48	11.87	14.9	11.95	9.97
Eye	2	1009	4.57	4.72	5.06	6.02	5.01	4.11
Eye	2	1010	2	1.97	2.34	2.7	2.2	1.81
Eye	8	1011	BQL	1.62	1.59	BQL	2.09	1.7
Eye	8	1012	BQL	0.8	0.77	BQL	0.93	BQL
Eye	8	1013	BQL	BQL	BQL	BQL	0.49	0.44
Eye	8	1014	BQL	0.44	0.4	BQL	0.39	0.47
Eye	8	1015	3.13	2.81	3.66	3.55	3.11	2.78
Eye	24	1016	BQL	0.33	BQL	BQL	0.46	0.3

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Eye	24	1017	BQL	BQL	BQL	BQL	BQL	BQL
Eye	24	1018	BQL	BQL	BQL	BQL	BQL	BQL
Eye	24	1019	BQL	BQL	BQL	BQL	0.28	BQL
Eye	24	1020	BQL	0.9	1.18	BQL	0.87	0.79
Eye	48	1021	BQL	BQL	BQL	BQL	BQL	BQL
Eye	48	1022	BQL	BQL	BQL	BQL	BQL	BQL
Eye	48	1023	BQL	BQL	BQL	BQL	BQL	BQL
Eye	48	1024	BQL	BQL	BQL	BQL	BQL	BQL
Eye	48	1025	BQL	BQL	BQL	BQL	BQL	BQL
Eye	72	1026	BQL	BQL	BQL	BQL	BQL	BQL
Eye	72	1027	BQL	BQL	BQL	BQL	BQL	BQL
Eye	72	1028	BQL	BQL	BQL	BQL	BQL	BQL
Eye	72	1029	BQL	BQL	BQL	BQL	BQL	BQL
Eye	72	1030	BQL	BQL	BQL	BQL	BQL	BQL
Eye	120	1031	BQL	BQL	BQL	BQL	BQL	BQL
Eye	120	1032	BQL	BQL	BQL	BQL	BQL	BQL
Eye	120	1033	BQL	BQL	BQL	BQL	BQL	BQL
Eye	120	1034	BQL	BQL	BQL	BQL	BQL	BQL
Eye	120	1035	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	0	1005	BQL	BQL	BQL	BQL	0.25	BQL
Bone Marrow	2	1006	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	2	1007	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	2	1008	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	2	1009	BQL	BQL	0.19	BQL	0.21	0.2
Bone Marrow	2	1010	BQL	BQL	0.21	BQL	0.2	0.2
Bone Marrow	8	1011	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	8	1012	BQL	0.32	0.45	BQL	0.39	0.34
Bone Marrow	8	1013	BQL	0.41	0.52	BQL	0.5	0.38
Bone Marrow	8	1014	BQL	0.2	0.49	BQL	0.19	0.4
Bone Marrow	8	1015	BQL	BQL	BQL	BQL	0.19	BQL
Bone Marrow	24	1016	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	24	1017	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	24	1018	BQL	BQL	BQL	BQL	BQL	BQL

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Bone Marrow	24	1019	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	24	1020	BQL	BQL	BQL	BQL	0.21	BQL
Bone Marrow	48	1021	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	48	1022	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	48	1023	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	48	1024	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	48	1025	BQL	BQL	BQL	BQL	0.12	BQL
Bone Marrow	72	1026	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	72	1027	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	72	1028	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	72	1029	BQL	BQL	BQL	BQL	0.14	BQL
Bone Marrow	72	1030	BQL	BQL	BQL	BQL	0.14	BQL
Bone Marrow	120	1031	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	120	1032	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	120	1033	BQL	BQL	BQL	BQL	BQL	BQL
Bone Marrow	120	1034	BQL	BQL	BQL	BQL	0.11	BQL
Bone Marrow	120	1035	BQL	BQL	BQL	BQL	0.15	BQL
Jejunum	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	2	1006	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	2	1007	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	2	1008	BQL	BQL	BQL	BQL	0.21	BQL
Jejunum	2	1009	BQL	BQL	BQL	BQL	0.18	0.18
Jejunum	2	1010	BQL	BQL	BQL	BQL	BQL	0.17
Jejunum	8	1011	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	8	1012	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	8	1013	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	8	1014	BQL	BQL	BQL	BQL	0.20	0.20
Jejunum	8	1015	BQL	BQL	BQL	BQL	0.20	BQL
Jejunum	24	1016	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	24	1017	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	24	1018	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	24	1019	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	24	1020	BQL	BQL	BQL	BQL	BQL	BQL

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
Jejunum	48	1021	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	48	1022	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	48	1023	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	48	1024	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	48	1025	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	72	1026	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	72	1027	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	72	1028	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	72	1029	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	72	1030	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	120	1031	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	120	1032	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	120	1033	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	120	1034	BQL	BQL	BQL	BQL	BQL	BQL
Jejunum	120	1035	BQL	BQL	BQL	BQL	0.18	BQL
IS Muscle	0	1001	BQL	BQL	BQL	BQL	BQL	BQL
IS Muscle	0	1002	BQL	BQL	BQL	BQL	BQL	BQL
IS Muscle	0	1003	BQL	BQL	BQL	BQL	BQL	BQL
IS Muscle	0	1004	BQL	BQL	BQL	BQL	BQL	BQL
IS Muscle	0	1005	BQL	BQL	BQL	BQL	BQL	BQL
IS Muscle	2	1006	34.02	18.30	31.79	37.95	20.33	15.76
IS Muscle	2	1007	2173.31	1.96	2173.12	2384.16	2047.75	1661.86
IS Muscle	2	1008	945.36	802.87	1213.46	1250.57	1171.97	761.58
IS Muscle	2	1009	4400.23	4.12	4124.59	4582.69	4742.96	3624.67
IS Muscle	2	1010	584.95	559.73	566.82	610.75	596.43	485.86
IS Muscle	8	1011	926.3	907.98	978.76	1064.7[a]	963.80	789.70
IS Muscle	8	1012	1145.24	1.18	1215.63	1182.2	1237.03	1071.16
IS Muscle	8	1013	1235.85	945.69	1287.02	1479.56	1472.70	850.52
IS Muscle	8	1014	289.54	276.56	327.99	292.35	281.66	306.08
IS Muscle	8	1015	1675.84	1.56	1702.62	2193.22	1949.48	1648.81
IS Muscle	24	1016	315.88	303.68	338.92	314.57	295.81	277.33
IS Muscle	24	1017	50.36	50.53	52.41	49.29	51.84	42.34
IS Muscle	24	1018	306.33	291.79	328.5	291.54	312.67	247.38
IS Muscle	24	1019	501.67	511.07	513.88	479.66	461.61	437.29
IS Muscle	24	1020	344.41	334.44	350.66	354.36	346.35	271.29
IS Muscle	48	1021	18.01	18.29	19.15	21.44	19.76	14.68
IS Muscle	48	1022	12.29	11.96	12.55	13.98	14.36	10.53

Appendix 8

Tissue	Time	Subject	mRNA (ng/g tissue)					
			UL130	UL131A	UL128	gB	gH	gL
IS Muscle	48	1023	8.14	8.81	8.28	10.84	10.01	7.08
IS Muscle	48	1024	2.43	2.45	2.69	2.64	2.66	2.26
IS Muscle	48	1025	6.00	5.46	6.88	6.41	6.46	5.63
IS Muscle	72	1026	3.45	3.55	3.93	3.99	4.38	4.06
IS Muscle	72	1027	2.41	2.34	2.64	2.29	2.54	2.99
IS Muscle	72	1028	3.66	3.45	4.19	4.47	4.25	3.7
IS Muscle	72	1029	BQL	0.49	0.65	BQL	0.58	0.65
IS Muscle	72	1030	BQL	0.41	0.53	BQL	0.57	0.63
IS Muscle	120	1031	1.16	1.20	1.21	1.28	1.48	1.01
IS Muscle	120	1032	BQL	BQL	BQL	BQL	0.14	BQL
IS Muscle	120	1033	BQL	0.35	0.43	BQL	0.58	0.46
IS Muscle	120	1034	BQL	BQL	BQL	BQL	0.22	BQL
IS Muscle	120	1035	BQL	BQL	BQL	BQL	0.17	BQL

BQL = Below Quantifiable Limit (at 0.05, 0.01, 0.01, 0.05, 0.01, and 0.01 ng/mL for gB, gH, gL, UL130, UL131A, and UL128); IS = Injection Site.

a) Upon the QC, the value for the subject 1101 injection site muscle for gB was found to be approximately 30% CV for the replicates which is outside of the defined range for the passing criteria. The datapoint was used in all calculations as it appear to be within the range for the neighboring readouts and was not expected to affect the overall study conclusions.

Appendix 8

Table 2
Pharmacokinetic Parameters of mRNA-1647 in Male Crl:CD(SD) Sprague-Dawley Rat Following an Intramuscular Injection of mRNA-1647

Appendix 8

Table 2.1: Summary Mean (± SE) mRNA-1647 Pharmacokinetic Parameters in Sprague-Dawley Rat in Plasma and Tissues Following 100 µg Intramuscular Administration of mRNA-1647 on Day 1

Tissue	Construct	T_{max} (hr)	C_{max} (ng/mL)		$AUC_{(0-t)}$ (hr*ng/mL)		$t_{1/2}$ (hr)
			Mean	SE	Mean	SE	
Bone Marrow	gB	NC	NC	NC	NC	NC	NC
	gH	8.0	0.254	0.0871	7.85	2.03	NC
	gL	8.0	0.224	0.0920	2.78	1.03	NC
	UL128	8.0	0.292	0.120	3.53	1.33	NC
	UL130	NC	NC	NC	NC	NC	NC
	UL131A	8.0	0.186	0.0829	2.05	0.912	NC
Brain	gB	NC	NC	NC	NC	NC	NC
	gH	24.0	0.0800	0.0491	2.19	1.08	NC
	gL	2.0	0.0360	0.0360	0.144	0.144	NC
	UL128	2.0	0.0340	0.0340	0.136	0.136	NC
	UL130	NC	NC	NC	NC	NC	NC
	UL131A	NC	NC	NC	NC	NC	NC
Distal LN	gB	8.0	108	101	1460	1110	31.6
	gH	8.0	110	102	1490	1130	36.2
	gL	8.0	117	109	1460	1200	30.6
	UL128	8.0	125	117	1620	1290	32.1
	UL130	8.0	129	121	1630	1330	27.9
	UL131A	8.0	114	108	1470	1190	28.5
Eye	gB	2.0	4.72	2.77	26.7	13.6	NC
	gH	2.0	3.92	2.19	37.6	11.0	NC
	gL	2.0	3.23	1.84	29.2	9.75	NC
	UL128	2.0	3.91	2.19	34.5	12.2	NC
	UL130	2.0	3.61	2.14	21.3	11.0	NC
	UL131A	2.0	3.43	1.96	31.1	10.2	NC
Heart	gB	NC	NC	NC	NC	NC	NC
	gH	8.0	0.548	0.107	9.94	1.85	NC
	gL	8.0	0.220	0.0907	2.96	1.05	NC
	UL128	8.0	0.276	0.113	4.49	1.51	NC
	UL130	NC	NC	NC	NC	NC	NC
	UL131A	8.0	0.312	0.0896	3.71	1.02	NC
Injection Site Muscle	gB	2.0	1770	803	27100	4880	13.5

Appendix 8

Tissue	Construct	T_{max} (hr)	C_{max} (ng/mL)		$AUC_{(0-t)}$ (hr*ng/mL)		$t_{1/2}$ (hr)
			Mean	SE	Mean	SE	
	gH	2.0	1720	828	26100	4700	17.1
	gL	2.0	1310	638	20900	3720	15.2
	UL128	2.0	1620	720	25300	4090	14.9
	UL130	2.0	1630	777	24500	4240	13.8
	UL131A	8.0	427	210	12100	2830	15.0
Jejunum	gB	NC	NC	NC	NC	NC	NC
	gH	8.0	0.0800	0.0490	2.06	1.04	NC
	gL	2.0	0.0700	0.0429	0.720	0.472	NC
	UL128	NC	NC	NC	NC	NC	NC
	UL130	NC	NC	NC	NC	NC	NC
	UL131A	NC	NC	NC	NC	NC	NC
Kidney	gB	NC	NC	NC	NC	NC	NC
	gH	NC	NC	NC	NC	NC	NC
	gL	NC	NC	NC	NC	NC	NC
	UL128	NC	NC	NC	NC	NC	NC
	UL130	NC	NC	NC	NC	NC	NC
	UL131A	NC	NC	NC	NC	NC	NC
Liver	gB	2.0	2.16	1.21	8.65	4.83	NC
	gH	2.0	2.12	0.982	16.8	4.15	NC
	gL	2.0	1.30	0.432	11.0	2.37	NC
	UL128	2.0	2.00	0.814	13.7	3.72	NC
	UL130	2.0	1.87	1.01	7.46	4.04	NC
	UL131A	2.0	1.99	0.928	13.9	4.04	NC
Lung	gB	NC	NC	NC	NC	NC	NC
	gH	8.0	0.442	0.130	8.04	1.96	NC
	gL	8.0	0.274	0.0984	3.45	1.12	NC
	UL128	8.0	0.340	0.129	5.40	1.74	NC
	UL130	8.0	0.188	0.188	2.07	2.07	NC
	UL131A	8.0	0.310	0.111	4.86	1.49	NC
Plasma	gB	2.0	2.02	0.181	22.7	3.77	NC
	gH	2.0	1.91	0.187	24.9	4.49	NC
	gL	2.0	1.74	0.177	23.4	4.07	NC
	UL128	2.0	1.66	0.151	24.1	4.44	NC
	UL130	2.0	2.30	0.621	25.5	4.65	NC

Appendix 8

Tissue	Construct	T_{max} (hr)	C_{max} (ng/mL)		$AUC_{(0-t)}$ (hr*ng/mL)		$t_{1/2}$ (hr)
			Mean	SE	Mean	SE	
	UL131A	2.0	1.60	0.153	24.8	4.59	NC
Proximal LN	gB	2.0	260	121	5850	949	33.5
	gH	8.0	206	51.6	4860	722	38.2
	gL	2.0	175	81.9	3460	538	36.3
	UL128	8.0	246	66.6	5190	875	32.8
	UL130	8.0	252	67.2	5240	881	35.7
	UL131A	2.0	225	106	4600	719	32.2
Spleen	gB	2.0	7.36	3.81	460	52.9	46.9
	gH	24.0	5.63	1.28	371	39.5	83.0
	gL	8.0	3.83	1.04	196	21.0	68.2
	UL128	24.0	4.87	1.22	297	34.8	68.8
	UL130	8.0	5.03	1.41	288	33.0	64.9
	UL131A	2.0	5.10	2.64	277	33.1	46.2
Stomach	gB	NC	NC	NC	NC	NC	NC
	gH	8.0	0.110	0.0696	3.49	1.59	NC
	gL	8.0	0.0800	0.0499	2.07	1.19	NC
	UL128	24.0	0.102	0.0648	2.85	1.47	NC
	UL130	NC	NC	NC	NC	NC	NC
	UL131A	24.0	0.0980	0.0634	2.53	1.39	NC
Testes	gB	2.0	1.16	0.719	4.64	2.88	NC
	gH	2.0	1.11	0.480	5.52	2.20	NC
	gL	8.0	0.420	0.335	6.08	3.73	NC
	UL128	2.0	0.946	0.397	4.73	1.85	NC
	UL130	2.0	0.682	0.442	2.73	1.77	NC
	UL131A	2.0	0.872	0.380	4.54	1.85	NC

NC = Not Calculable, due to insufficient data points above LLOQ

Appendix 8

Table 3
Tissue-to-Plasma Ratios of mRNA-1647 in Male Crl:CD(SD) Sprague-Dawley Rat Following an Intramuscular Injection of mRNA-1647

Appendix 8

Table 3.1: Mean Tissue-to-Plasma Ratios in Sprague-Dawley Rat Following Intramuscular Administration of mRNA-1647

Matrix	**$AUC_{(0-t)}$ Ratio**						
	gB	gH	gL	UL128	UL130	UL131A	Average
Injection Site Muscle	1190	1050	893	1050	961	487	939
Proximal LN	257	195	148	215	206	185	201
Distal LN	64.1	59.8	62.6	67.1	64	59.2	62.8
Spleen	20.2	14.9	8.36	12.3	11.3	11.2	13.4
Eye	1.18	1.51	1.25	1.43	0.838	1.26	1.24
Liver	0.381	0.674	0.470	0.570	0.293	0.562	0.499
Testes	0.204	0.222	0.260	0.196	0.107	0.183	0.209
Bone Marrow	NC	0.316	0.119	0.147	NC	0.0825	NR
Brain	NC	0.0880	0.00615	0.00564	NC	NC	NR
Heart	NC	0.400	0.127	0.186	NC	0.150	NR
Jejunum	NC	0.0827	0.0308	NC	NC	NC	NR
Kidney	NC	NC	NC	NC	NC	NC	NR
Lung	NC	0.323	0.148	0.224	0.0812	0.196	NR
Stomach	NC	0.140	0.0886	0.118	NC	0.102	NR

NC = Not Calculable: all samples were BQL; NR = Not Reported: some constructs measured all samples as BLQ.

Appendix 9

Individual Gross Pathological Findings Explanation Page

Abbreviation	Description	Abbreviation	Description
AB	Abdominal region	LJ	Lower jaw
AX	Axillary region	LN	Lymph node
BC	Body cavity	LT	Left
BI	Bilateral	LU	Lumbar region
CGEP	Complete gross examination performed	MF	Multifocal
CR	Cranium	MU	Muzzle
DC	Dorsal cervical region	NBF	Neutral buffered formalin
DT	Dorsal thoracic region	Ø	In diameter
F	Focal	PO	Periorbital region
FL	Forelimb	RT	Right
FP	Forepaw	SA	Sacral region
G	Gross Pathology	SC	Scapular region
GALT	Gut associated lymphoid tissue	SI	Small intestine
GL	Gland	SR	Scrotum
HL	Hindlimb	TGL	Trackable Gross Lesion
HP	Hindpaw	UG	Urogenital region
IG	Inguinal region	VC	Ventral cervical region
IS	Interscapular region	VT	Ventral thoracic region
LI	Large Intestine		

Note: This is a comprehensive list of abbreviations. All of the abbreviations listed may not be applicable to this report.

Dosing Information

Dosing information is abbreviated on various data outputs; the following represents the dosing information for this study.

Group No.	Test Item	Dose Level (µg)
1	mRNA-1647	100

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1001	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 1 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

No observations found

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1002	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 1 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, MANDIBULAR : Focus; dark : >10, bilateral

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1003	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 1 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, MANDIBULAR : Focus; dark : >10, bilateral
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1004	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 1 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, MANDIBULAR : Focus; dark : >10, bilateral
LYMPH NODE, MANDIBULAR : Enlargement : Left

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1005	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 1 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, MANDIBULAR : Focus; dark : >10, bilateral
THYMUS : Focus; dark : 7

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1006	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 1 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

No observations found

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1007	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 1 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, AXILLARY : Focus; dark : 3, right.
LYMPH NODE, INGUINAL : Enlargement : right.
LYMPH NODE, POPLITEAL : Enlargement : right.

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1008	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 1 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, AXILLARY : Focus; dark : >10, bilateral.
THYMUS : Focus; dark : >10.

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1009	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 1 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

No observations found

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1010	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 1 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

KIDNEY : Adhesion : right to capsule.
LYMPH NODE, AXILLARY : Focus; dark : >10, left.

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1011	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 1 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1012	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 1 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, AXILLARY : Focus; dark : >10, left
SITE, INJECTION : Swelling : right

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1013	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 1 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

SITE, INJECTION : Swelling : right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1014	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 1 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, MANDIBULAR : Focus; dark : >10, bilateral
SITE, INJECTION : Swelling : right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1015	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 1 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

SITE, INJECTION : Swelling : right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1016	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 2 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, POPLITEAL : Enlargement : Right
SITE, INJECTION : Swelling : right
SITE, INJECTION : Abnormal consistency; firm : right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1017	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 2 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, POPLITEAL : Enlargement : Right
SITE, INJECTION : Swelling : right, extending into subcutis
SITE, INJECTION : Abnormal consistency; firm : right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1018	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 2 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, AXILLARY : Focus; dark : 1 to >10, bilateral
SITE, INJECTION : Swelling : Right
SITE, INJECTION : Abnormal consistency; firm : Right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1019	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 2 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

SITE, INJECTION : Swelling : right
SITE, INJECTION : Abnormal consistency; firm : right
STOMACH : Focus; dark : 2, mucosa, glandular
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1020	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 2 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

SITE, INJECTION : Swelling : right
SITE, INJECTION : Abnormal consistency; firm : right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1021	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 3 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

SITE, INJECTION : Abnormal consistency; firm : right
SITE, INJECTION : Focus; dark : 1, right
STOMACH : Focus; dark : 1, mucosa, glandular
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1022	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 3 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, AXILLARY : Focus; dark : 1, right
LYMPH NODE, POPLITEAL : Enlargement : right
SITE, INJECTION : Swelling : right
SITE, INJECTION : Abnormal consistency; firm : right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1023	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 3 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, POPLITEAL : Focus; dark : >10, right
SITE, INJECTION : Abnormal consistency; firm : right
SITE, INJECTION : Focus; dark : 2, right
SITE, INJECTION : Material accumulation; clot : right

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1024	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 3 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, POPLITEAL : Enlargement : right
SITE, INJECTION : Swelling : right
SITE, INJECTION : Abnormal consistency; firm : right
SITE, INJECTION : Focus; dark : >10, right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1025	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 3 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

SITE, INJECTION : Swelling : right
SITE, INJECTION : Abnormal consistency; firm : right
SITE, INJECTION : Focus; dark : >10, right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1026	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 4 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1027	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 4 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

No observations found

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1028	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 4 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

No observations found

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1029	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 4 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1030	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 4 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

SITE, INJECTION : Focus; dark : 1, right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal: 1031	Group: 1		Sex: Male
Species: Rat	Strain: Sprague-Dawley		
	Dose: 100 ug		
	Removal Reason: Terminal Euthanasia		
	Day (Week) of Death 6 (1)		

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1032	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 6 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1033	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 6 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

No observations found

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1034	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 6 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, INGUINAL : Focus; dark : >10, right
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Animal:	1035	Group: 1	Sex: Male
Species:	Rat	Strain: Sprague-Dawley	
		Dose: 100 ug	
		Removal Reason: Terminal Euthanasia	
		Day (Week) of Death 6 (1)	

Gross Pathology Animal Details:

Animal Complete gross examination was performed.
Animal Notes: EUTHANASIA VIA ANESTHESIA AND PERFUSION

Gross Pathology Observations:

LYMPH NODE, AXILLARY : Focus; dark : 8, left
THYMUS : Focus; dark : >10

Gross Pathology - The following Tissues were Not Examined:

None

Appendix 9

Individual Gross Pathological Findings
5002121

Key Page

Codes

(TGL) = Trackable Gross Lesion, (MPF) = Major Pathological Finding, (?) = Questionable, (E) = Excluded, (C) = Clinical Observation, (M) = Mass, (G) = Gross Pathology, (H) = Histo Pathology

Group Information

Short Name	Long Name
1	1