DEPARTMENT OF HEALTH & HUMAN SERVICES **Public Health Service**

Food and Drug Administration
Rockville, MD 20857

April 13, 2022

Kathryn Blankenberg
Paul J. Orfanedes
Judicial Watch, Inc.
425 Third Street SW
Suite 800
Washington, DC 20024

Sent via email: kblankenberg@judicialwatch.org
porfanedes@judicialwatch.org

Re: FDA FOIA Request 2021-4379; *Judicial Watch, Inc. v. U.S. Department of Health and Human Services,* 21-cv-2418

Dear Ms. Blankenberg and Mr. Orfanedes,

This is in response to the Freedom of Information Act (FOIA) request number **2021-4379** that is the subject of the Complaint filed in *Judicial Watch, Inc. v. U.S. Department of Health and Human Services,* 21-cv-2418, now pending in the U.S. District Court for the District of Columbia.

Enclosed are 699 pages of records, some of which contain redaction.

We have withheld portions of pages under Exemption (b)(4), 5 U.S.C. § 552(b)(4). That exemption permits the withholding of trade secrets and commercial or financial information that was obtained from a person outside the government and that is privileged or confidential.

In addition, we have withheld portions of pages under Exemption (b)(6), 5 U.S.C. § 552(b)(6). That exemption protects information from disclosure when its release would cause a clearly unwarranted invasion of personal privacy. FOIA Exemption 6 is available to protect information in personnel or medical files and similar files. This requires a balancing of the public's right to disclosure against the individual's right to privacy.

Please direct any questions regarding this response to Jonathan Silberman, Associate Chief Counsel, Food and Drug Administration, telephone number (240) 731-9982 or email Jonathan.Silberman@fda.hhs.gov.

Sincerely,

Beth Brockner Ryan
Chief, Access Litigation and Freedom of Information Branch
Division of Disclosure and Oversight Management
Office of Communication Outreach and Development
Center for Biologics Evaluation and Research

Enclosure(s)

charles river

NON-GLP FINAL REPORT AMENDMENT NO. 01

Test Facility Study No. 5002121

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

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

TEST FACILITY:
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
1580 Ida-Metivier
Sherbrooke, QC J1E 0B5
Canada

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.

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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

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.

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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

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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

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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

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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

Appendix 1

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

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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).

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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phone 617-714-6500 • fax 617-583-1998

Summary of Analysis

(b) (4)

Appendix 2

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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

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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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.

Appendix 3

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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Analytical Procedure (AP.5002121.SP.01)

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Analytical Procedure (AP.5002121.SP.01)

(b) (4)

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(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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500 Tech Square • Cambridge, MA 02139
Phone 617-714-6500 • fax 617-583-1998

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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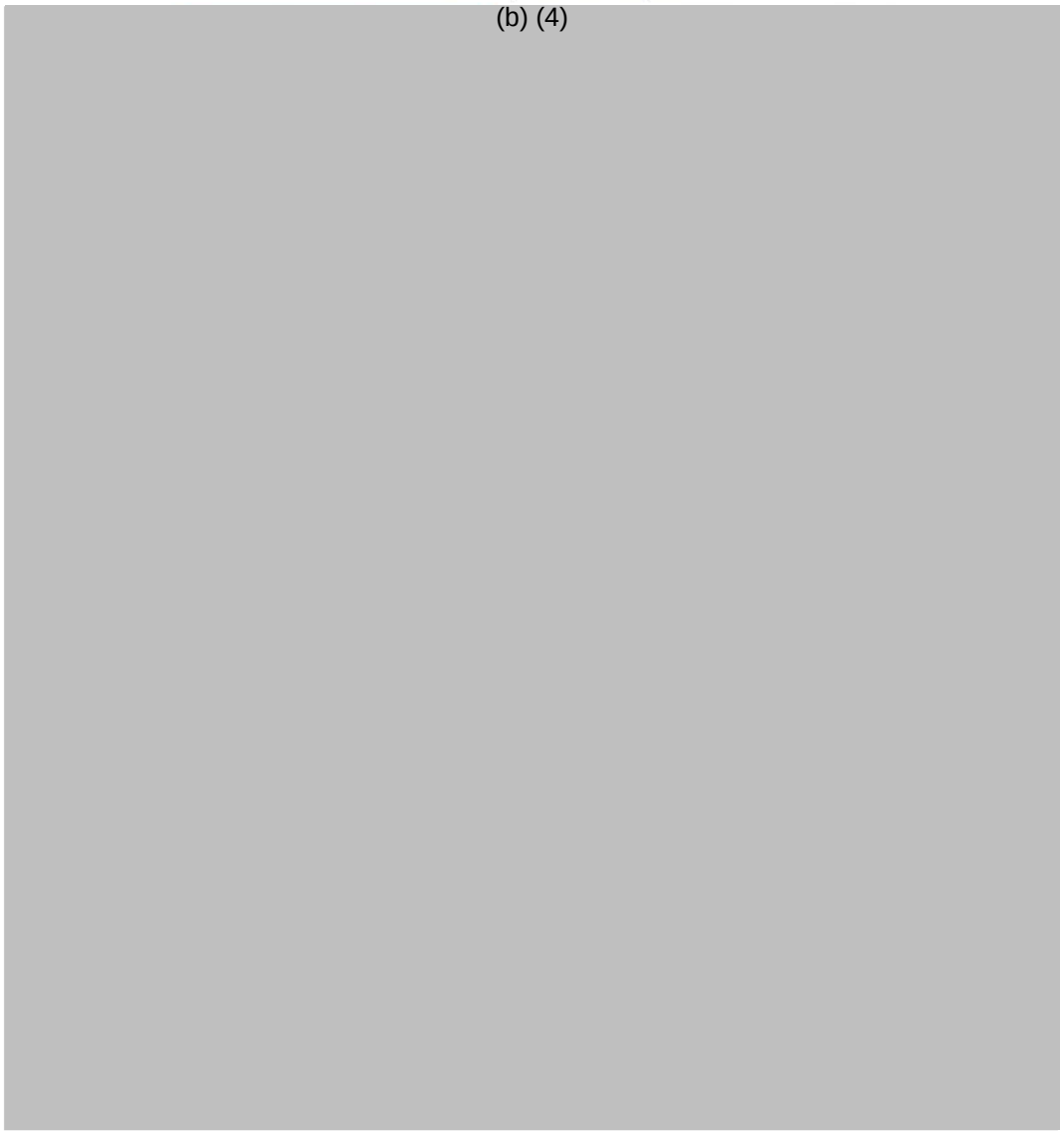
Summary of Analysis

(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

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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.

Appendix 7

Appendix 2
AP.5002121.bDNAp.03

Appendix 7

ANALYTICAL PROCEDURE

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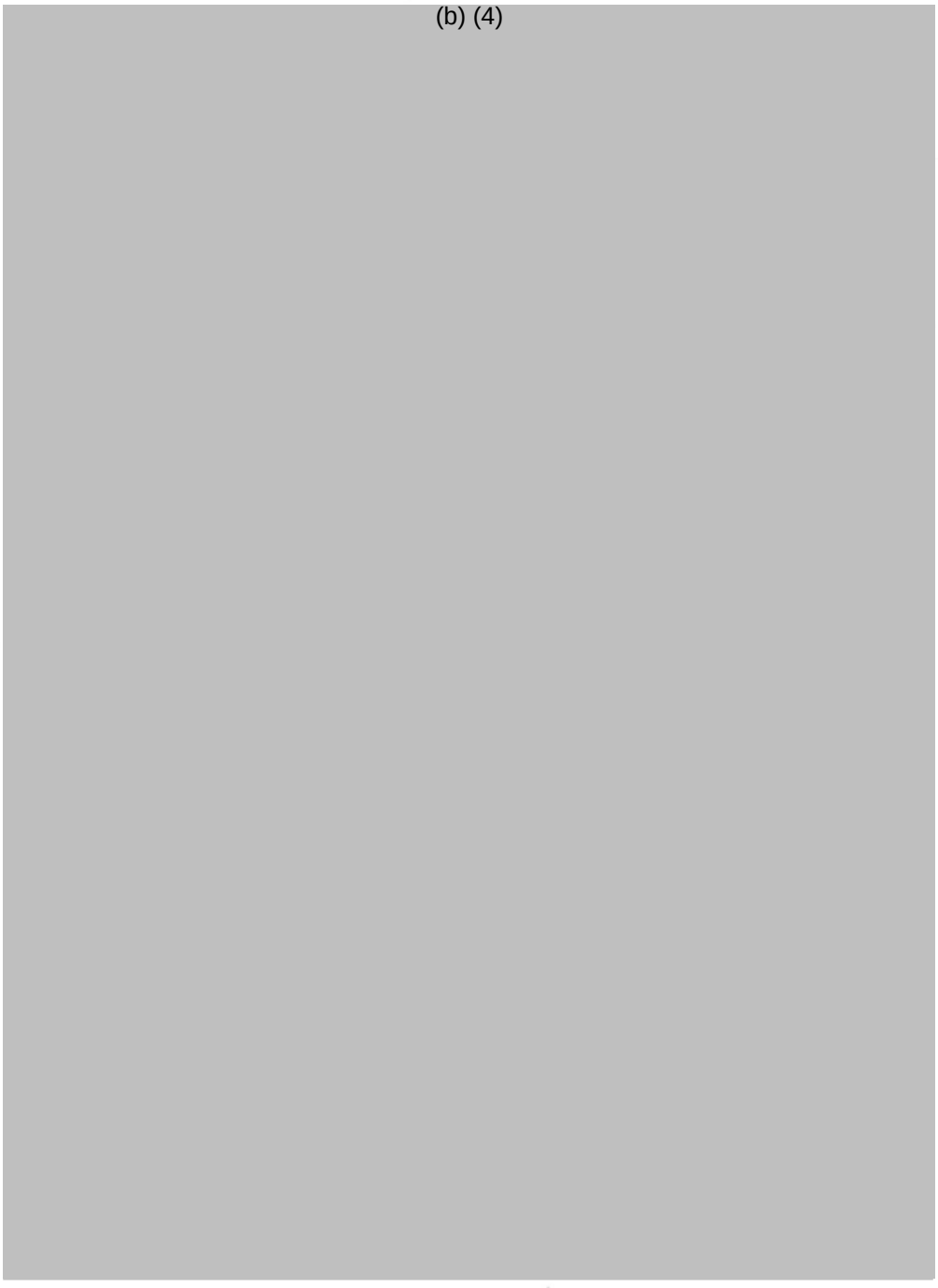
Appendix 7



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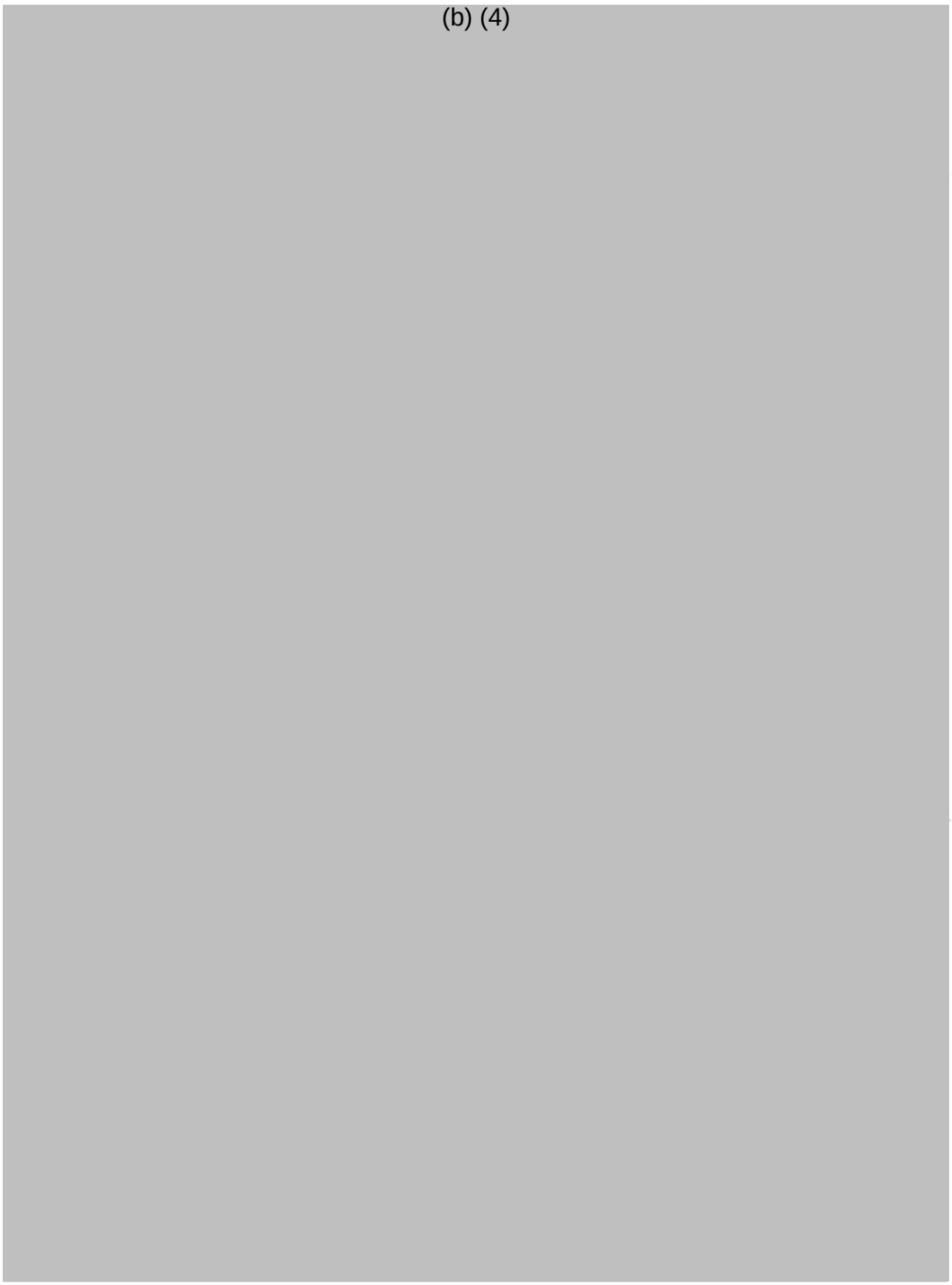

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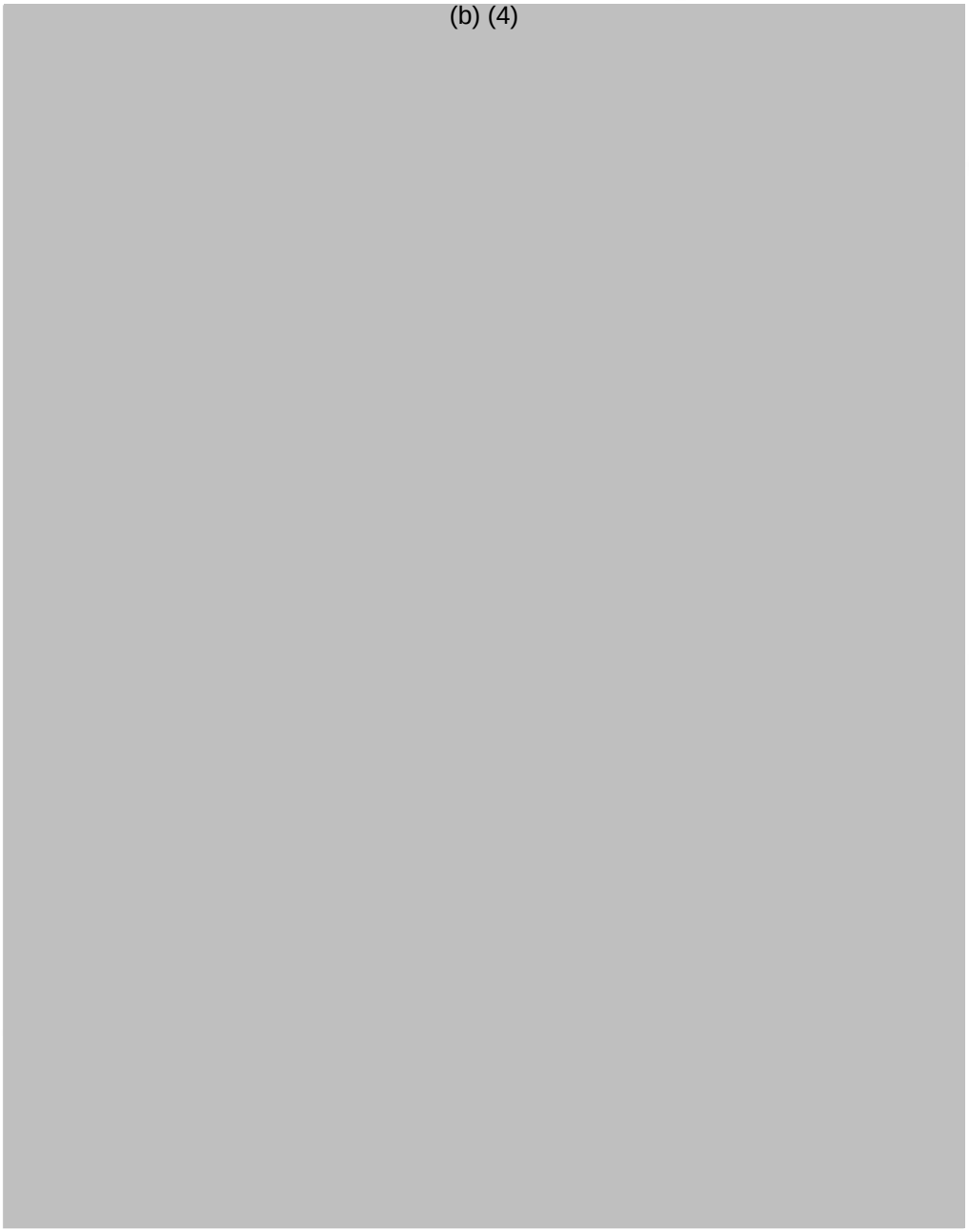

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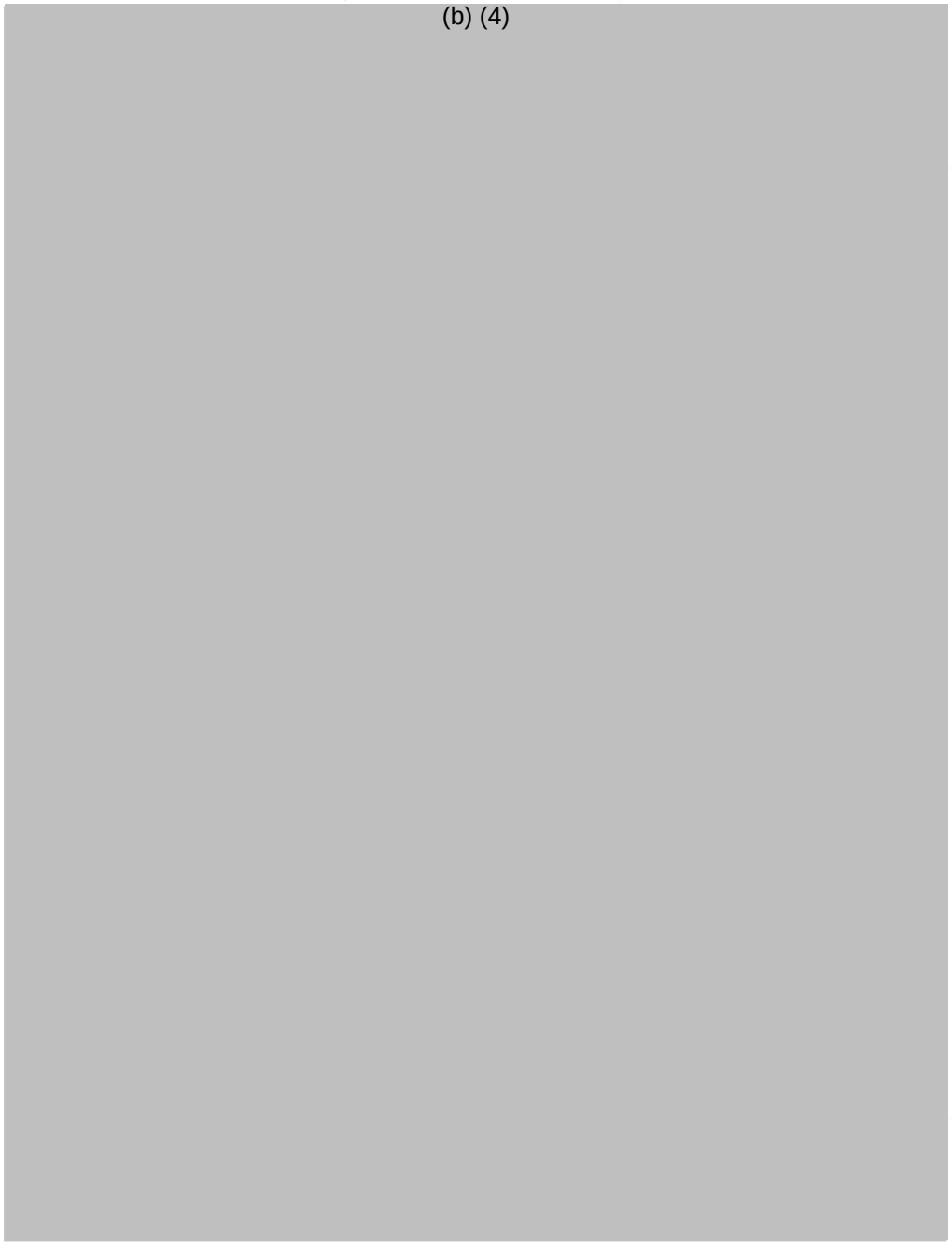

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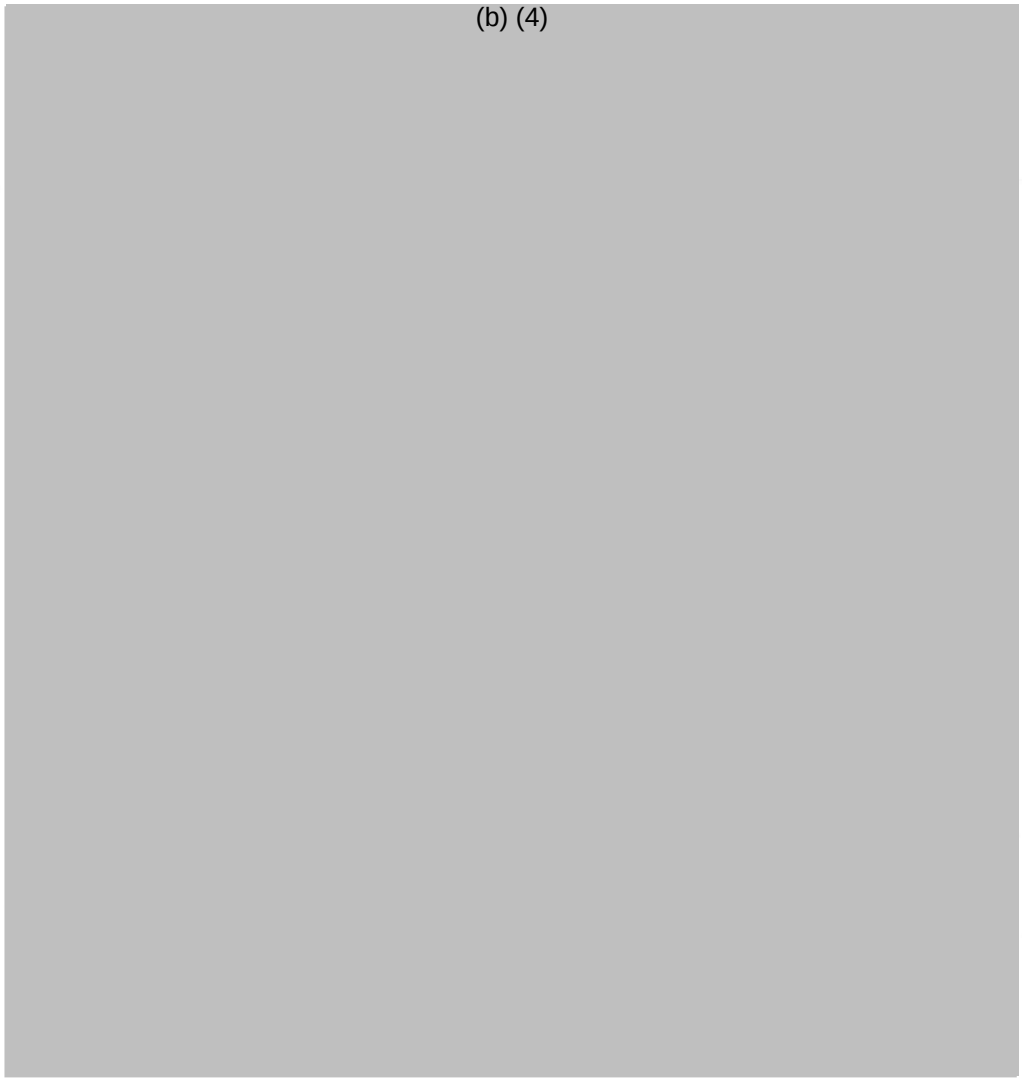

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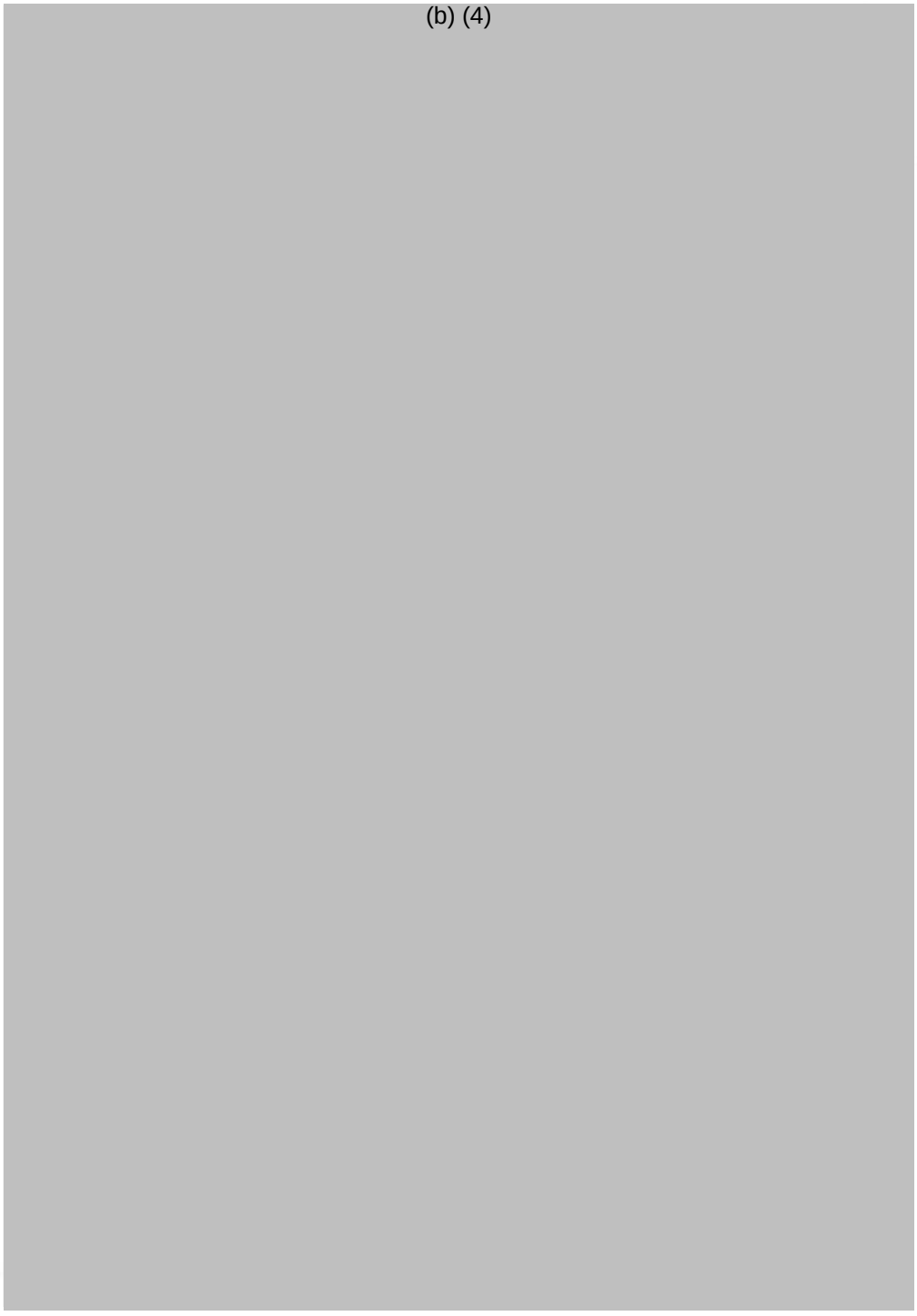

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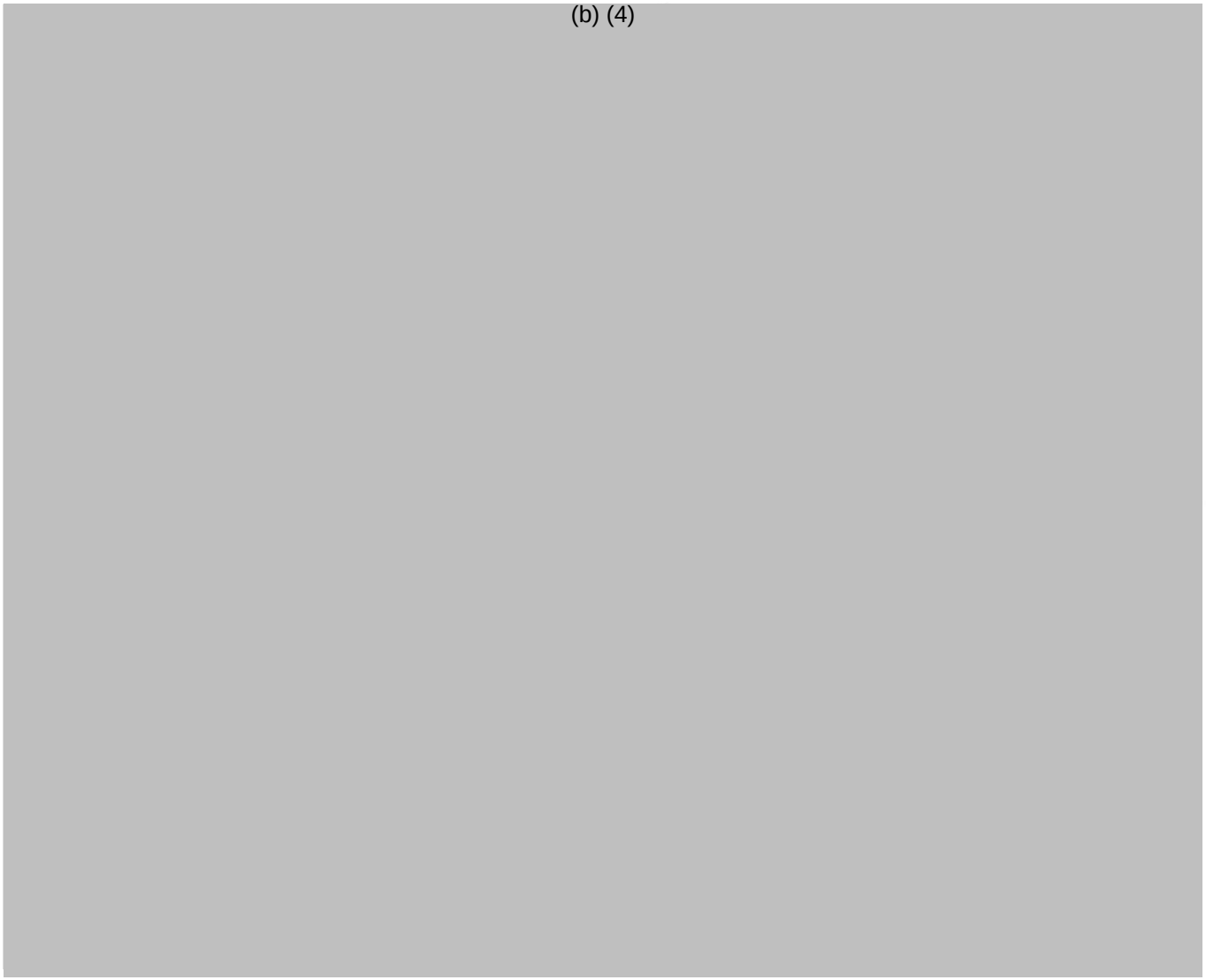

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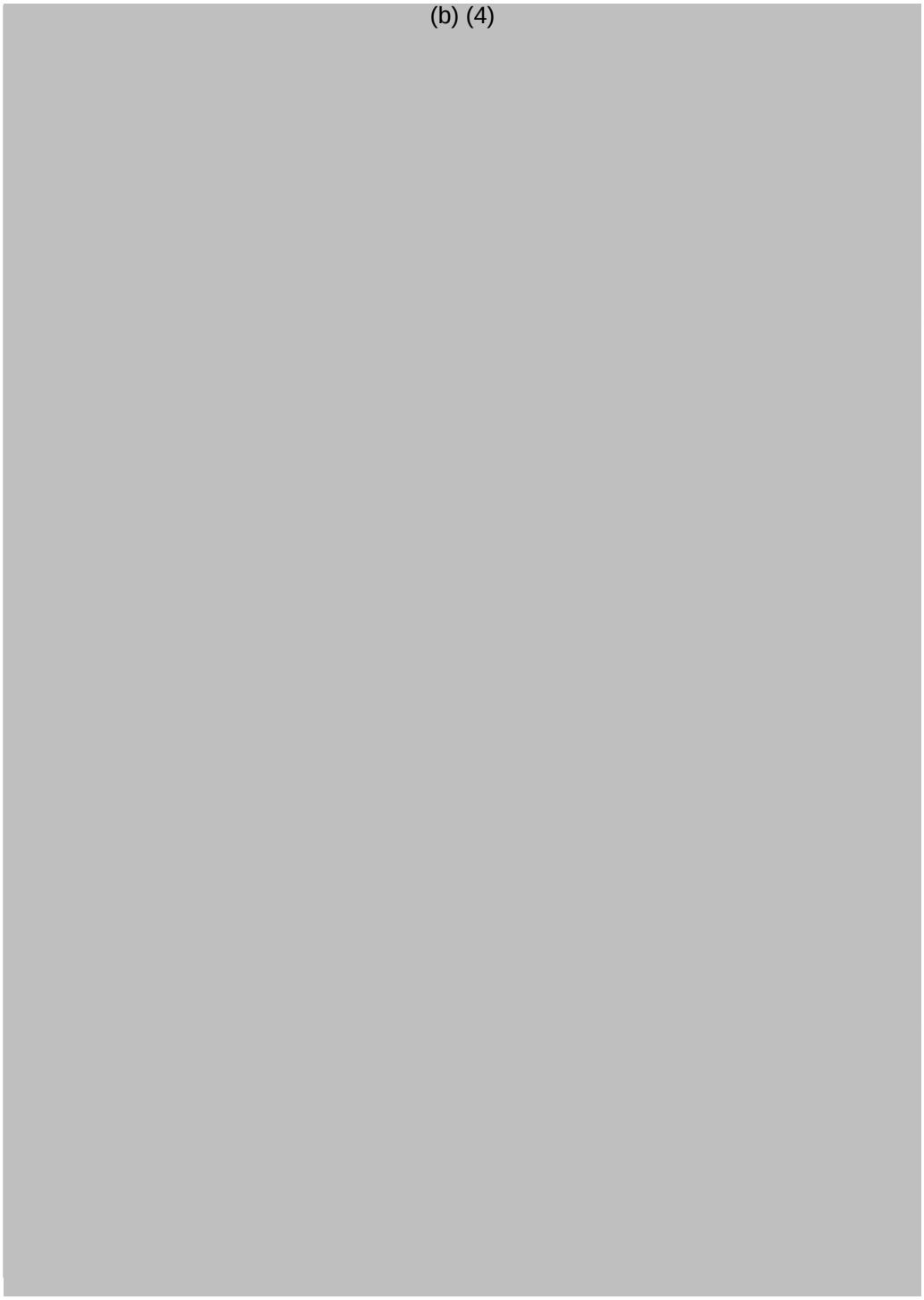

Appendix 7

Appendix 3
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Appendix 7

ANALYTICAL PROCEDURE

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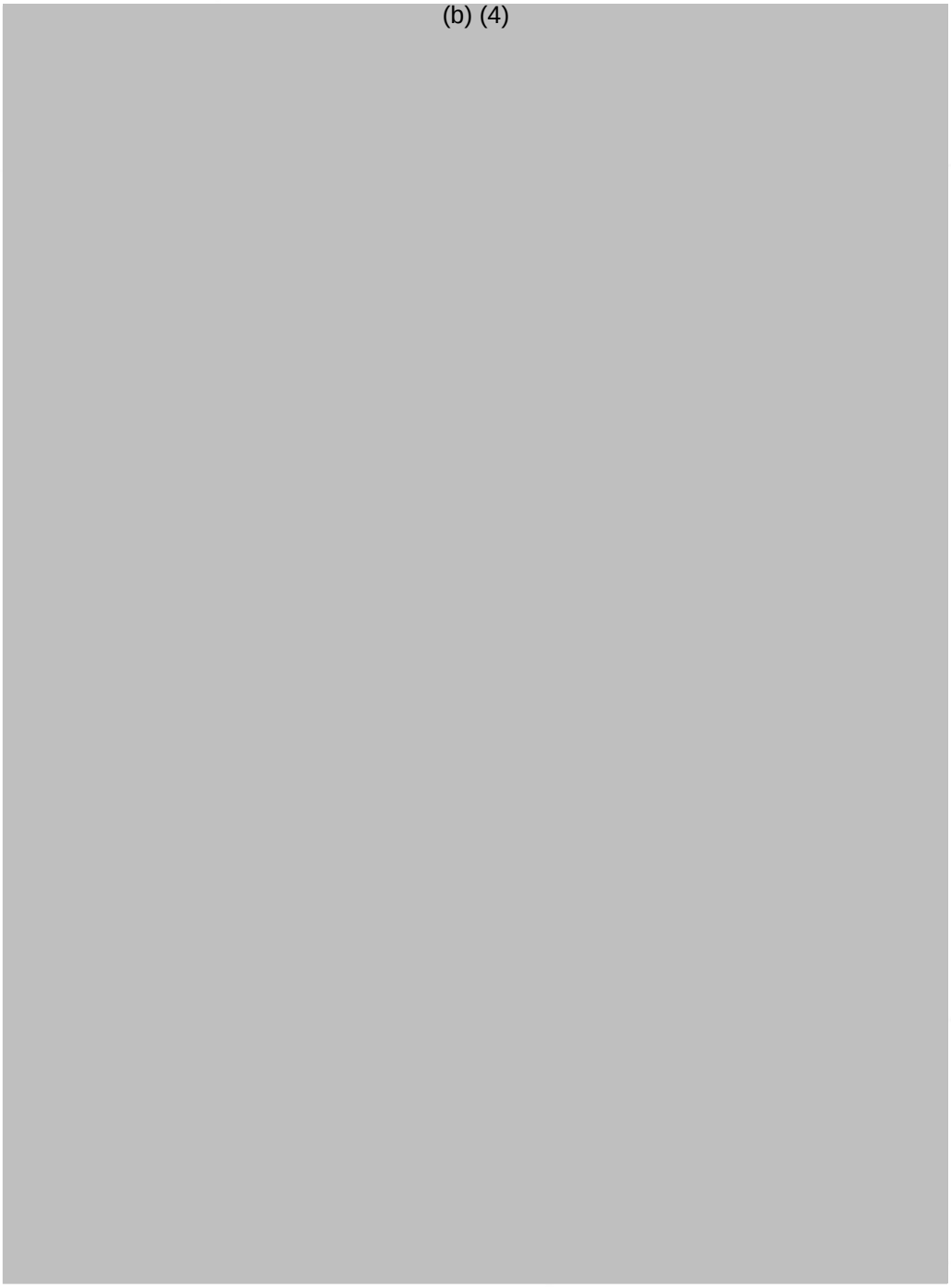

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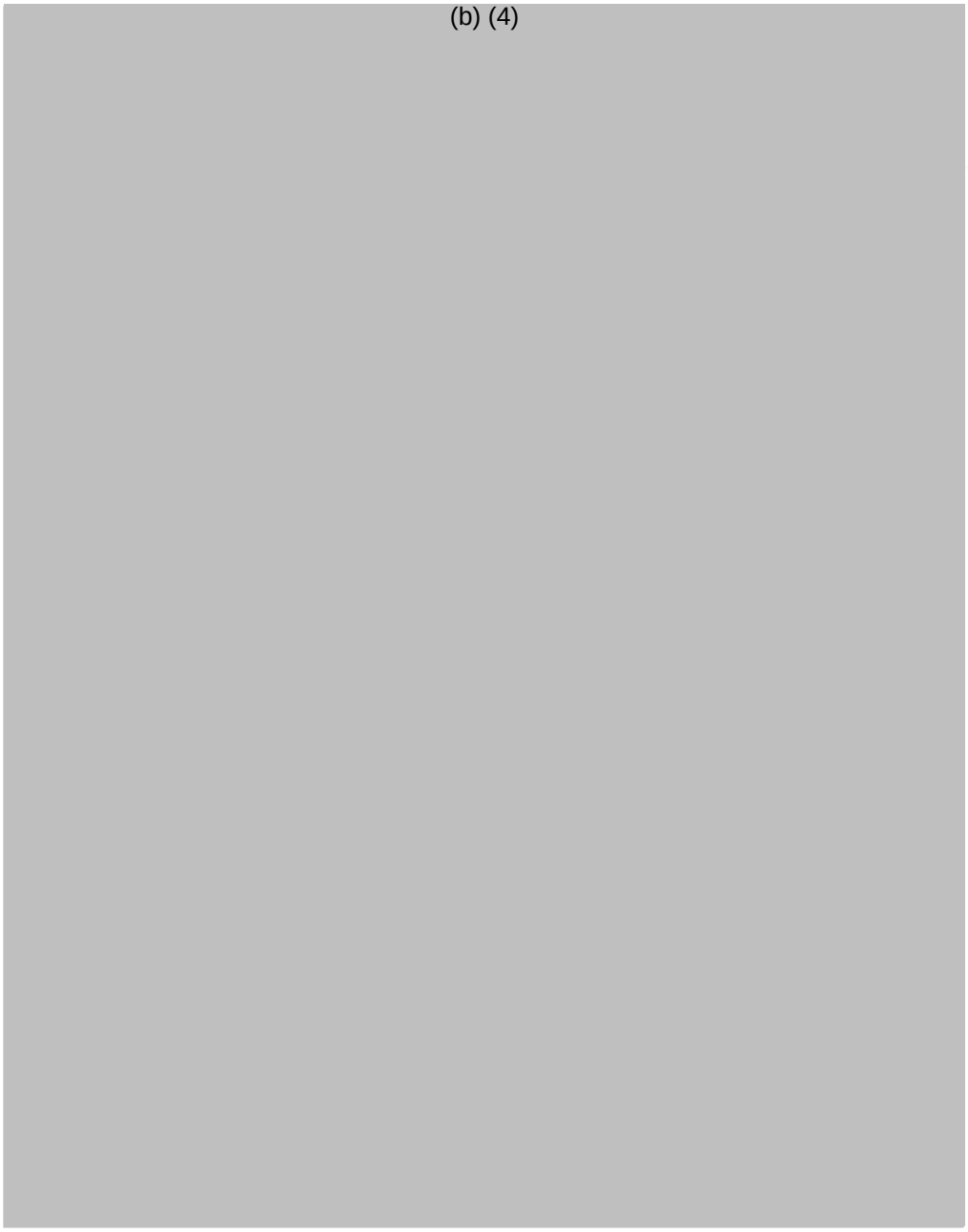

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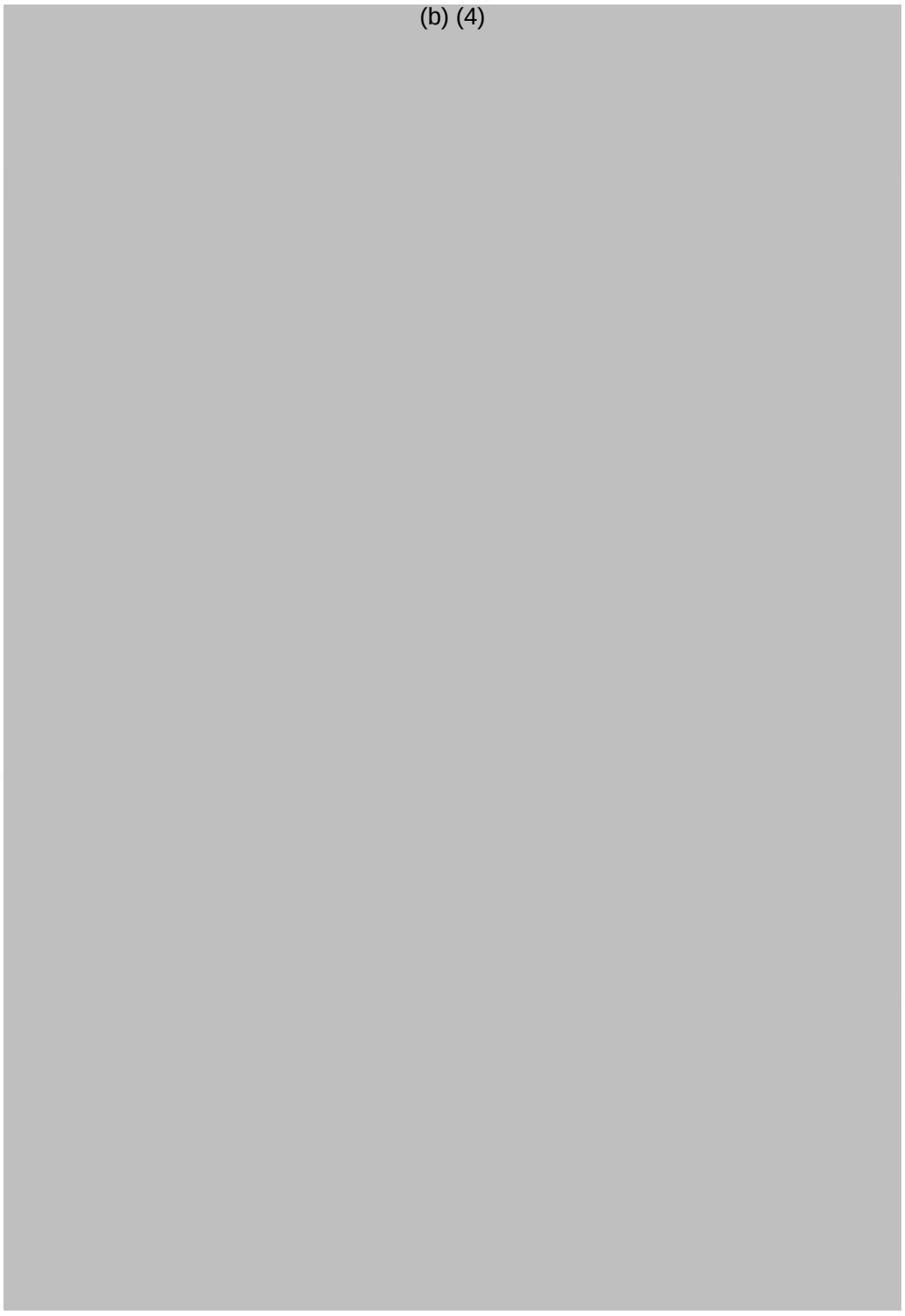

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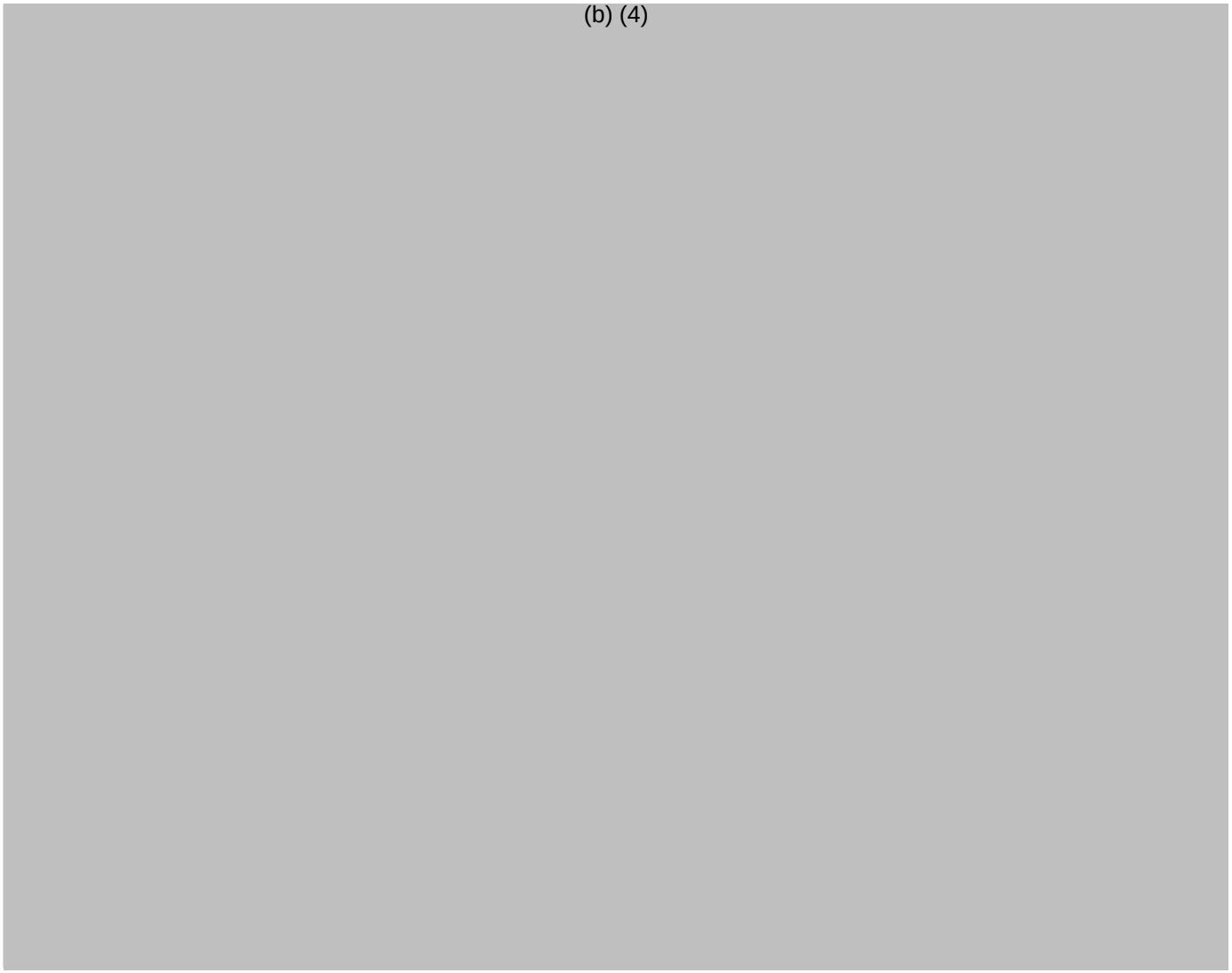

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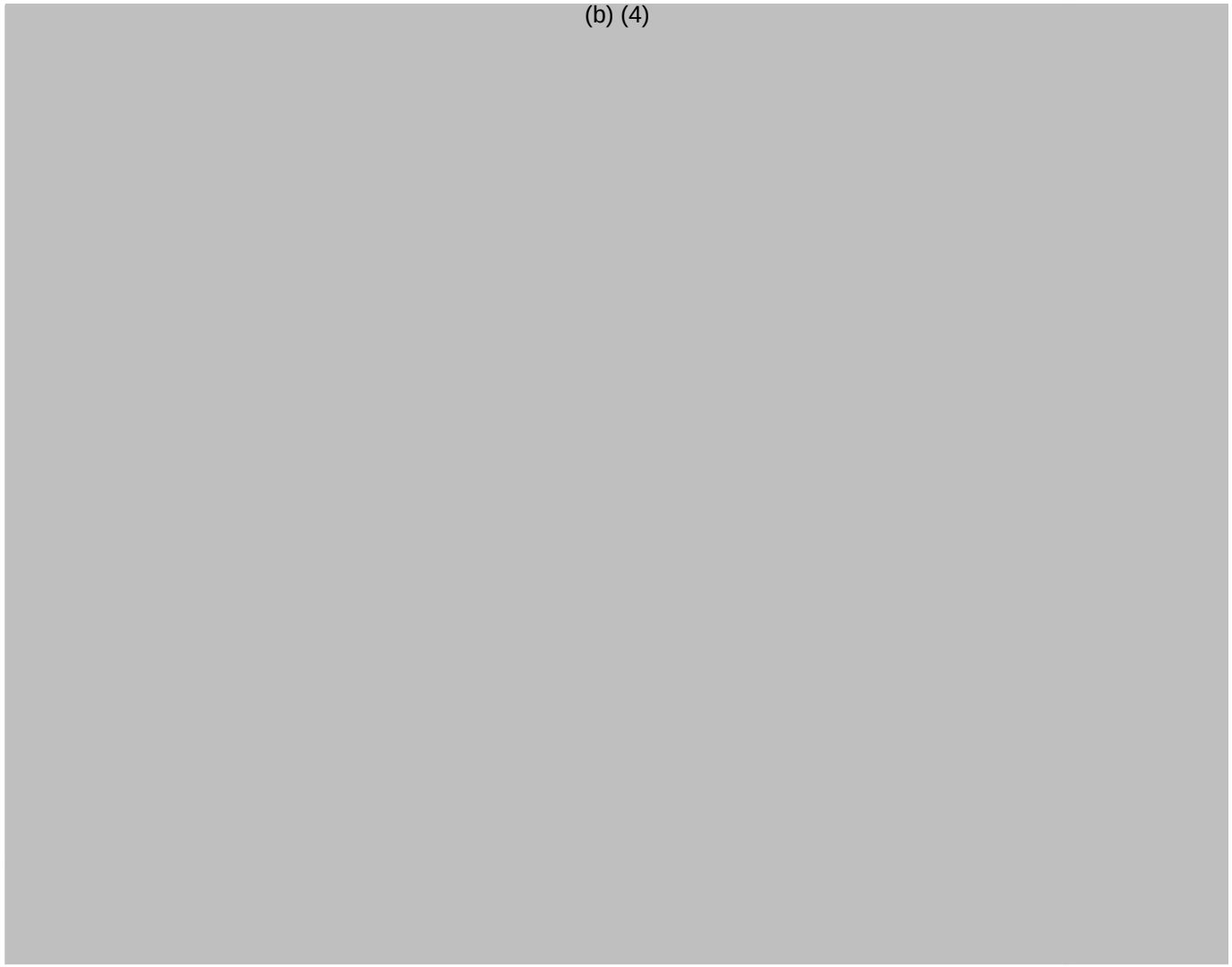

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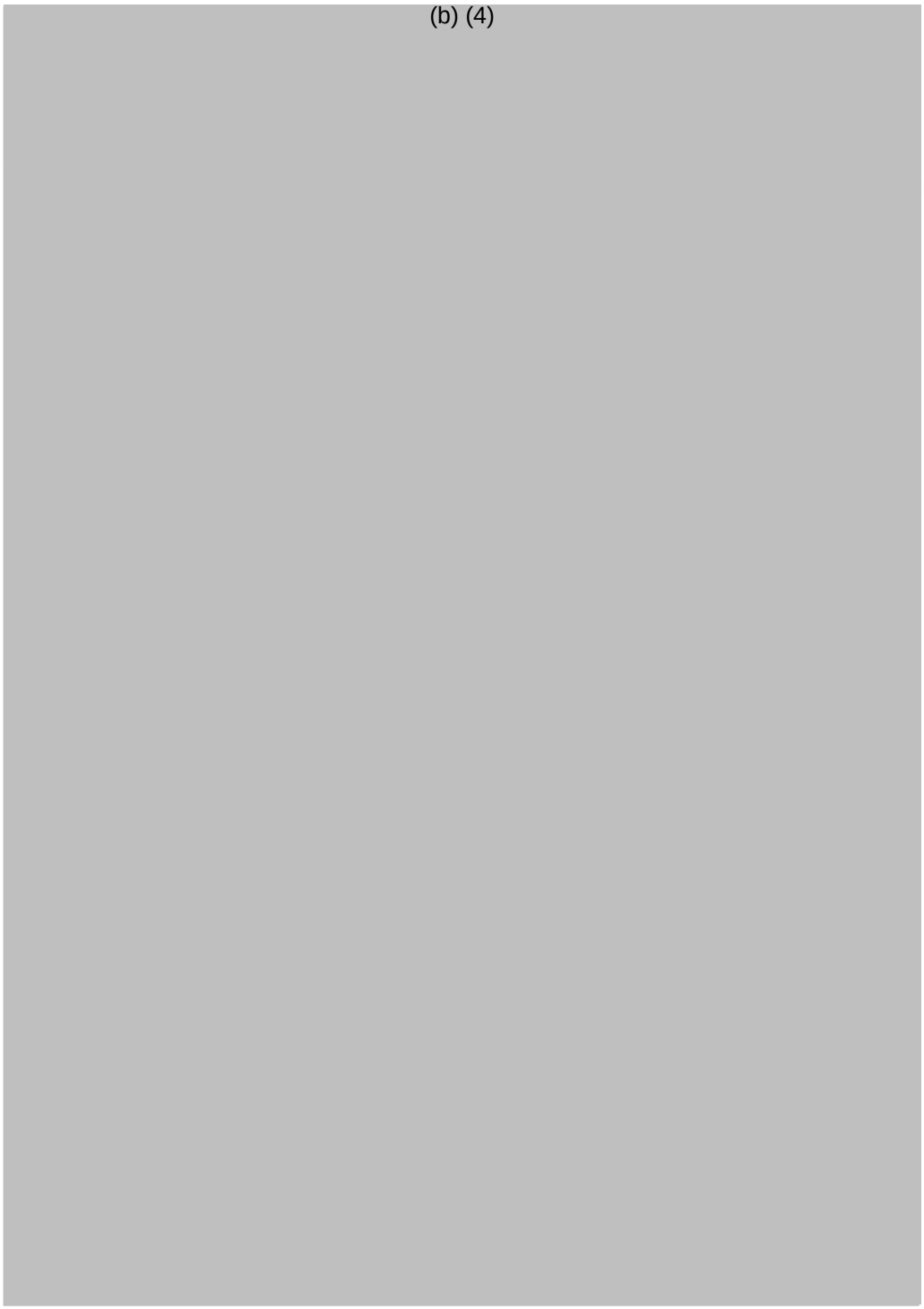

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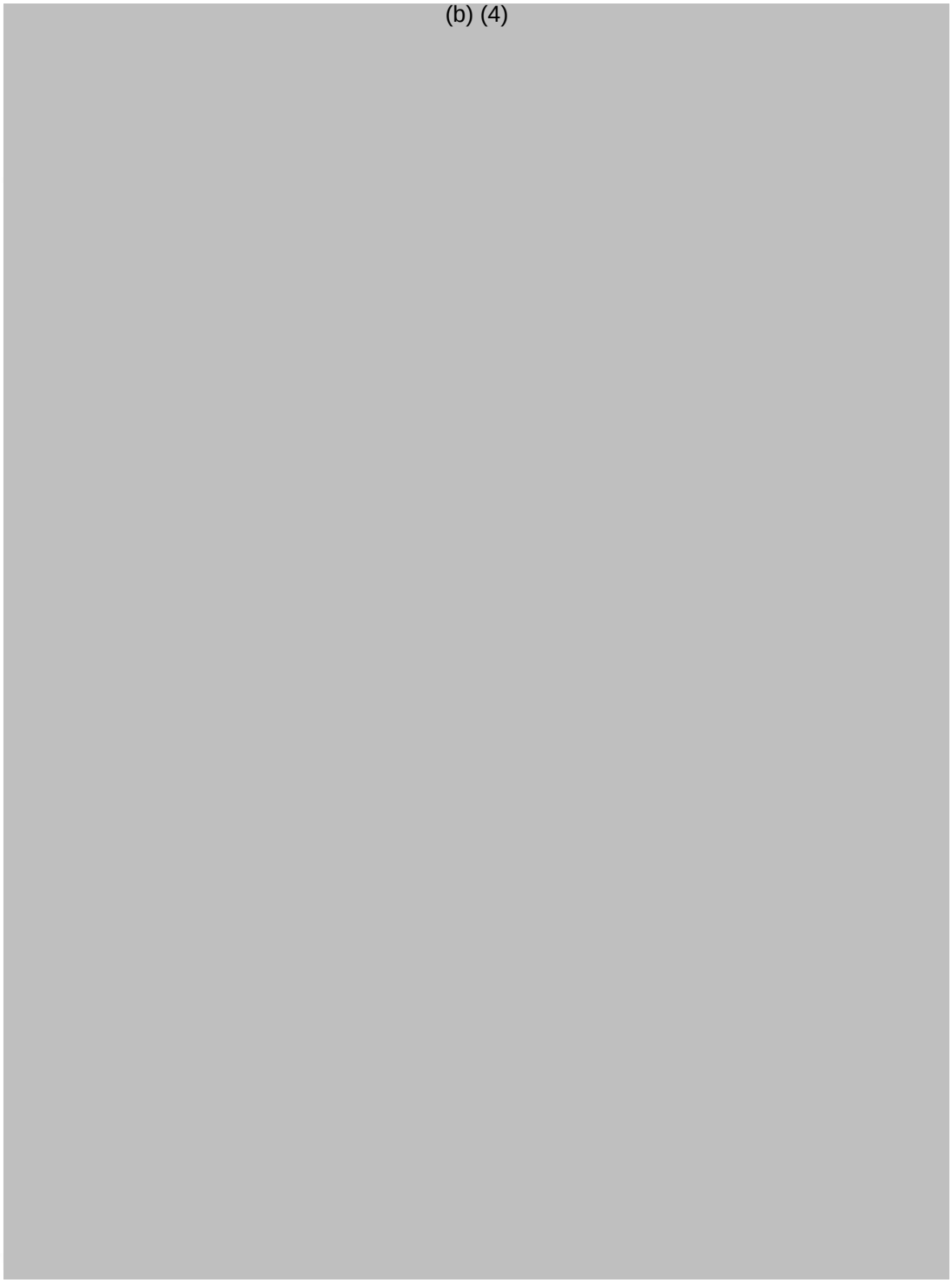

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Appendix 4
AP.5002121.EXT.02

Appendix 7

ANALYTICAL PROCEDURE

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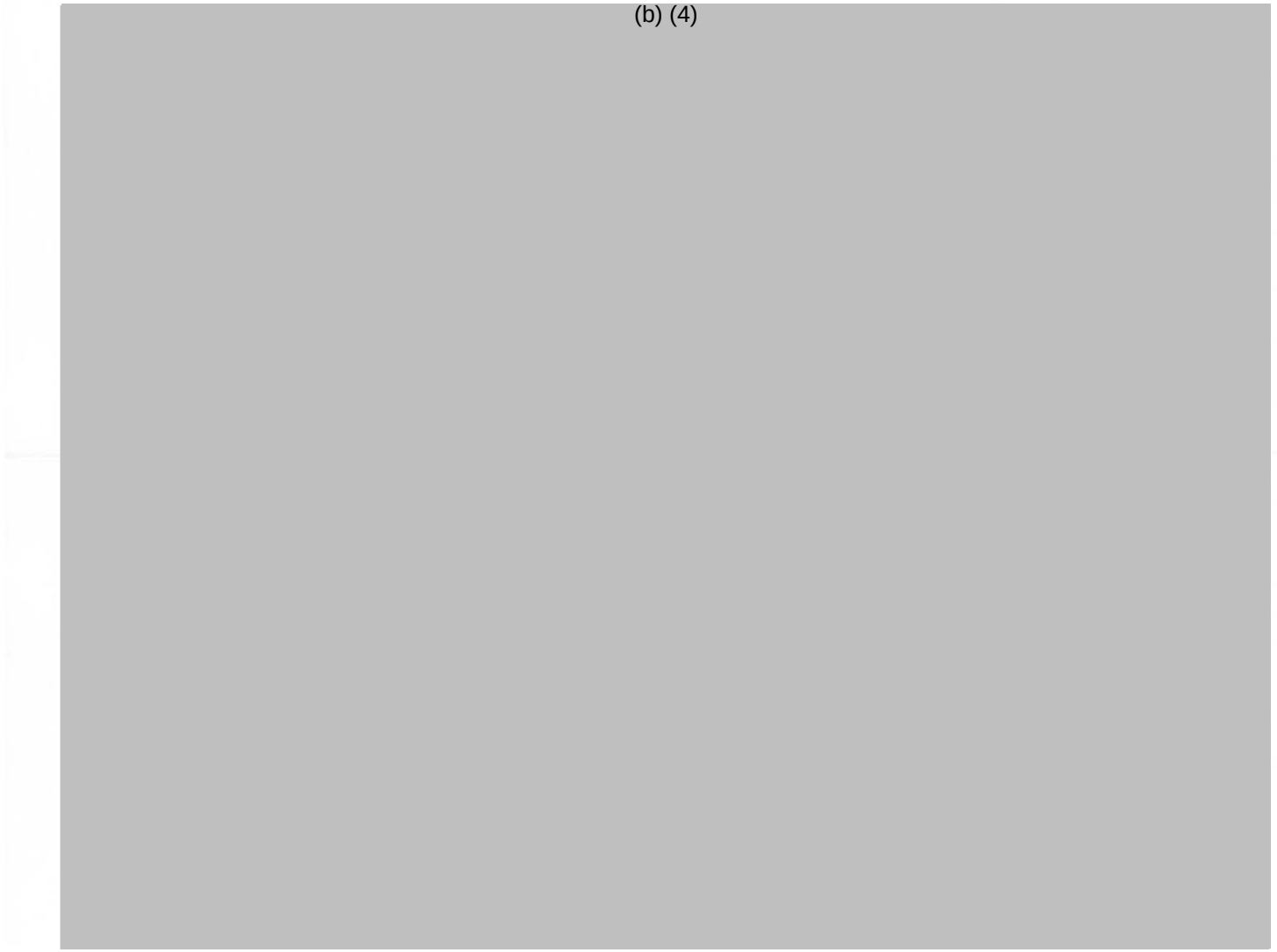

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Appendix 5
Certificate of Analysis

Appendix 7

moderna
messenger therapeutics

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

Summary of Analysis

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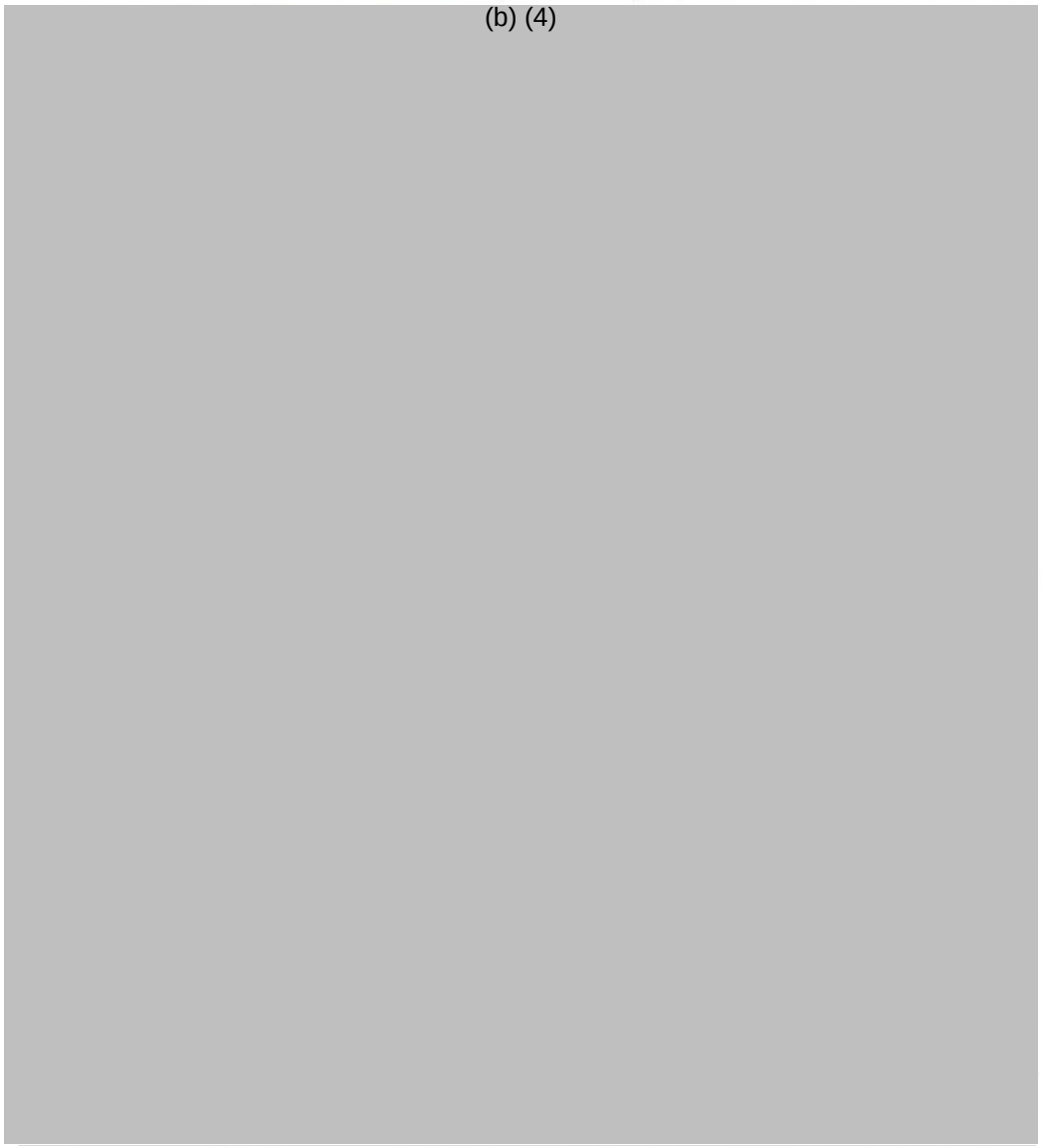

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moderna
messenger therapeutics

200 Tech Square • Cambridge, MA 02139
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*

Appendix 8

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

Appendix 8

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Appendix 8

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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

Appendix 8

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

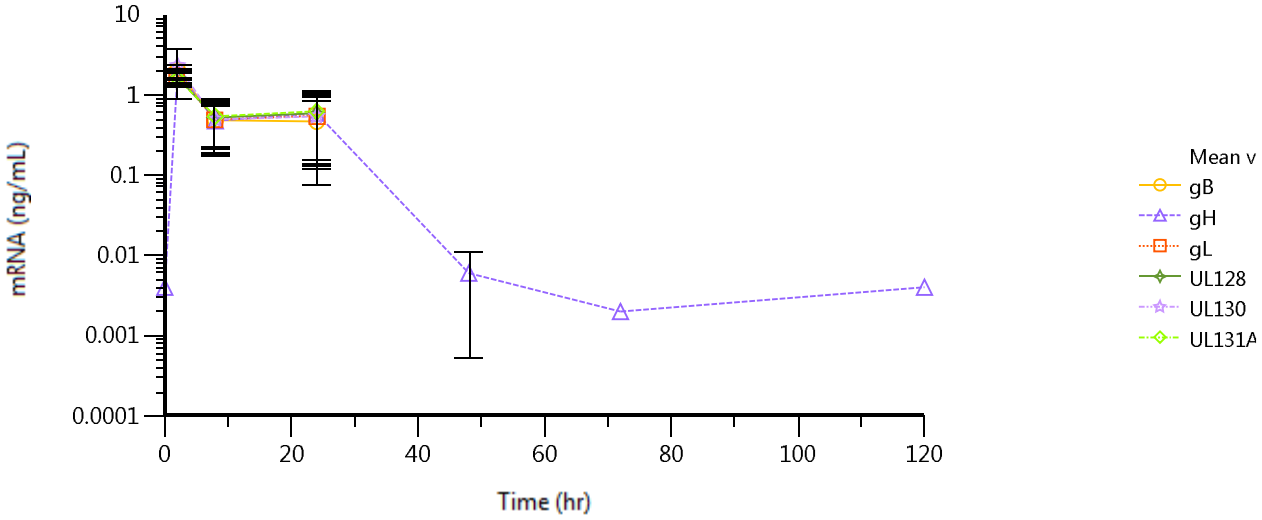

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Figure 1.2: Summary (± SD) Male Sprague-Dawley Rat Bone Marrow mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

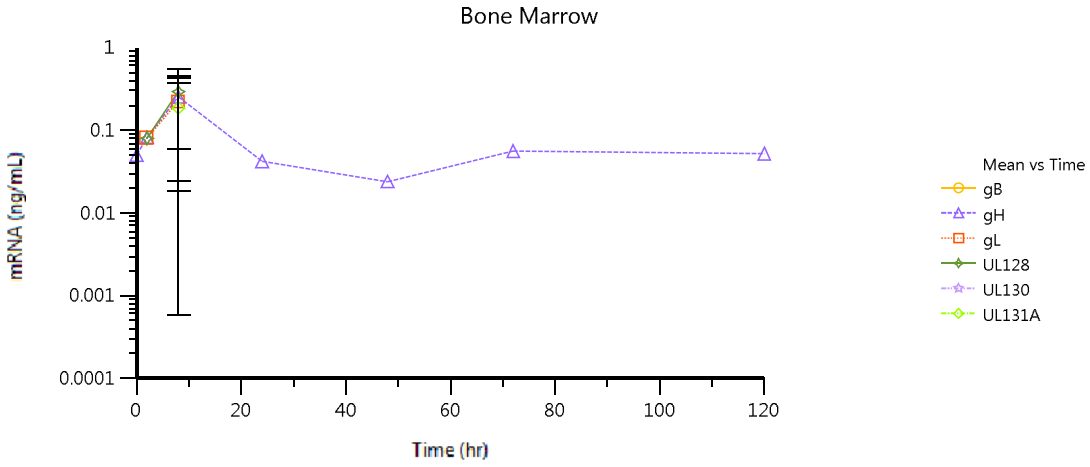

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Figure 1.3: Summary (± SD) Male Sprague-Dawley Rat Brain mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

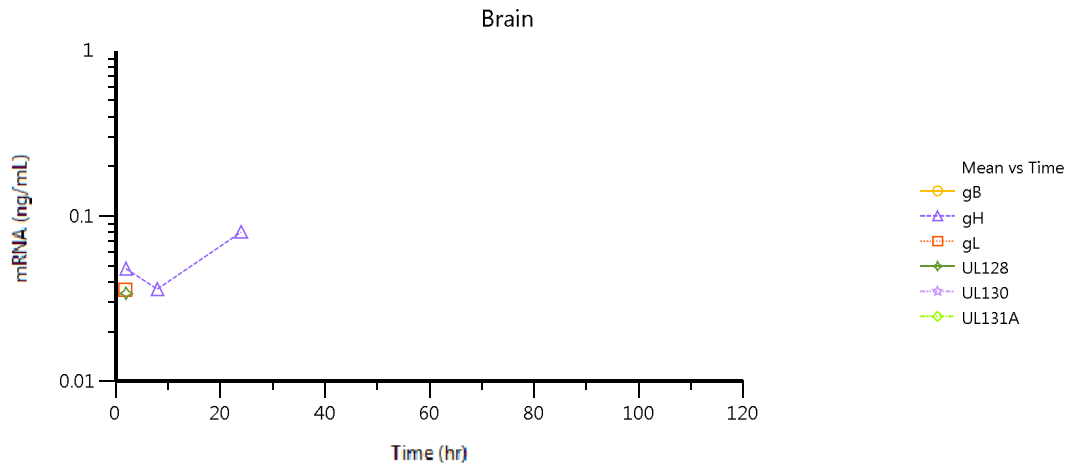

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Figure 1.4: Summary (± SD) Male Sprague-Dawley Rat Distal Lymph Nodes mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

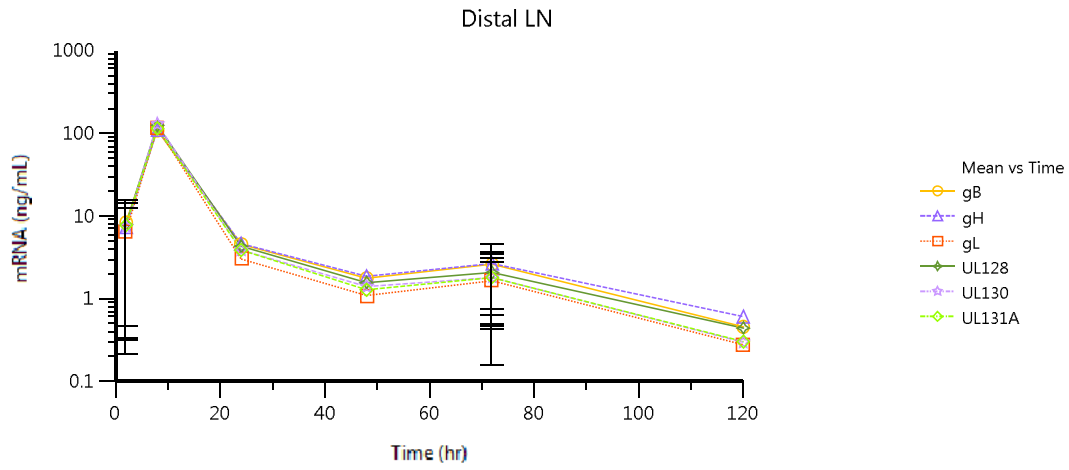

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Figure 1.5: Summary (± SD) Male Sprague-Dawley Rat Eye mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

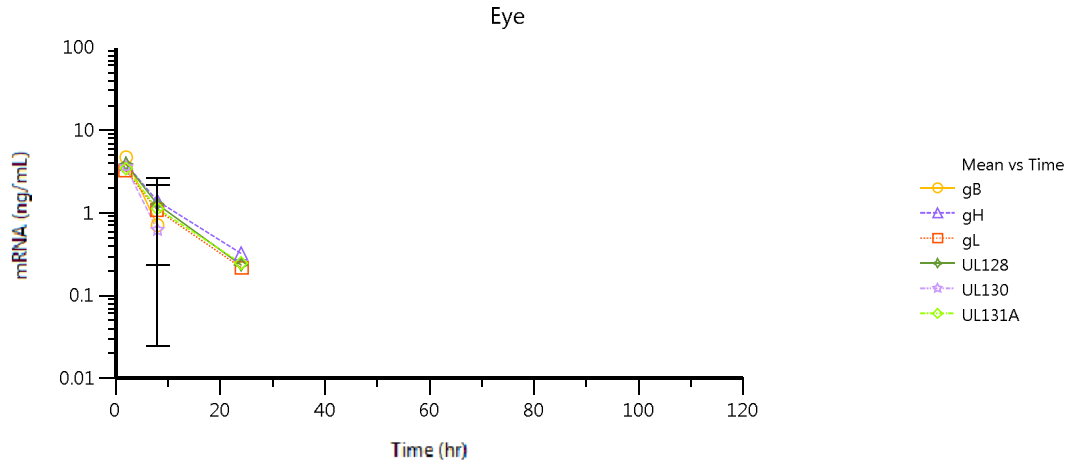

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Figure 1.6: Summary (± SD) Male Sprague-Dawley Rat Heart mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

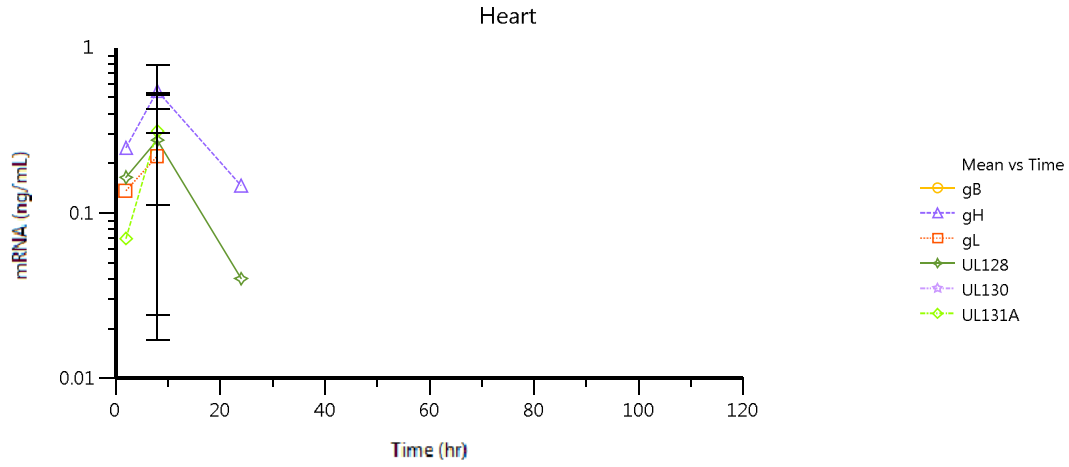

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Figure 1.7: Summary (± SD) Male Sprague-Dawley Rat Injection Site Muscle mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

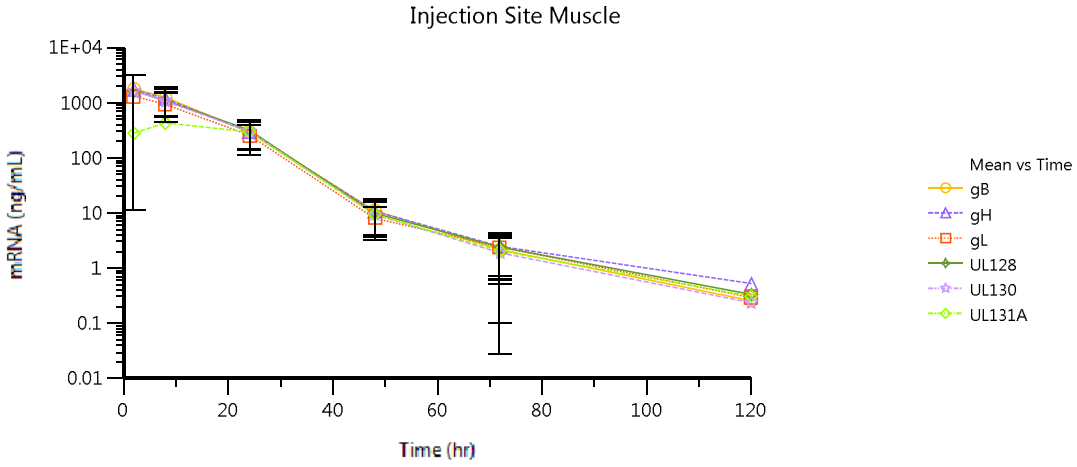

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Figure 1.8: Summary (± SD) Male Sprague-Dawley Rat Jejunum mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

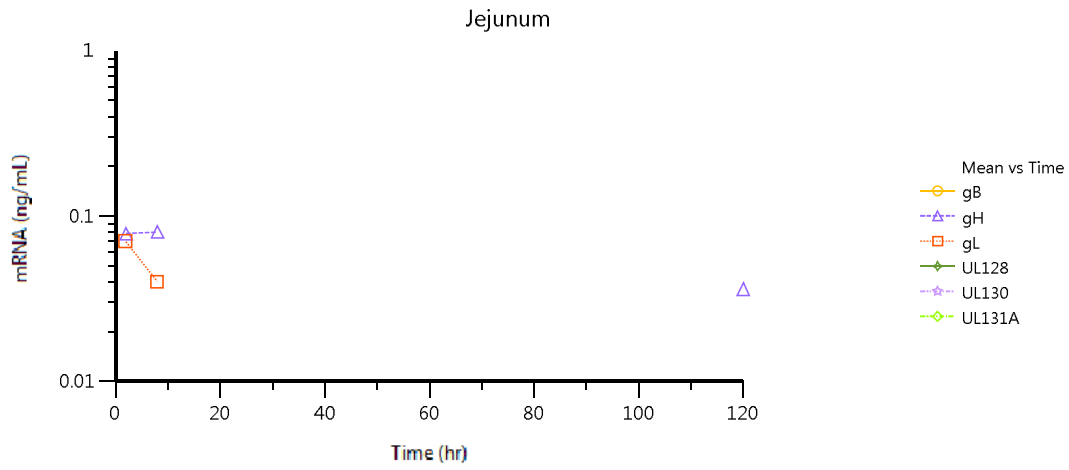

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Figure 1.9: Summary (± SD) Male Sprague-Dawley Rat Kidney mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

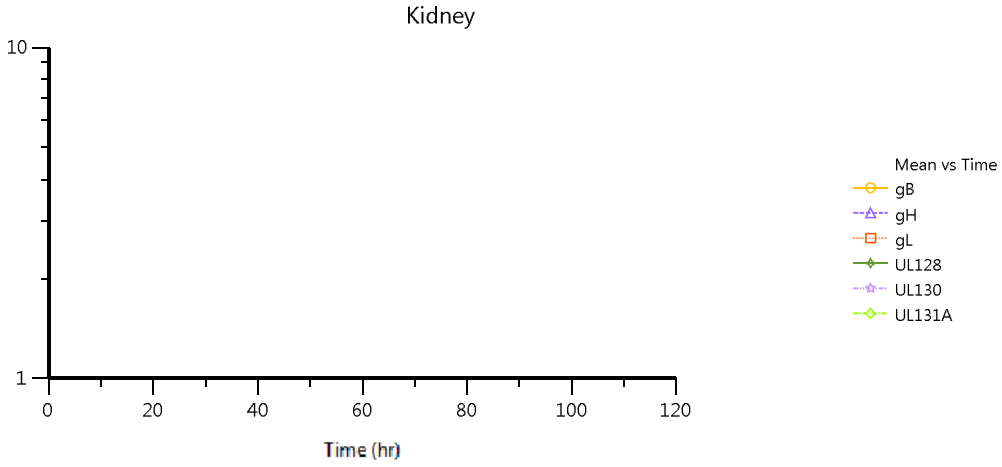

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Figure 1.10: Summary (± SD) Male Sprague-Dawley Rat Liver mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

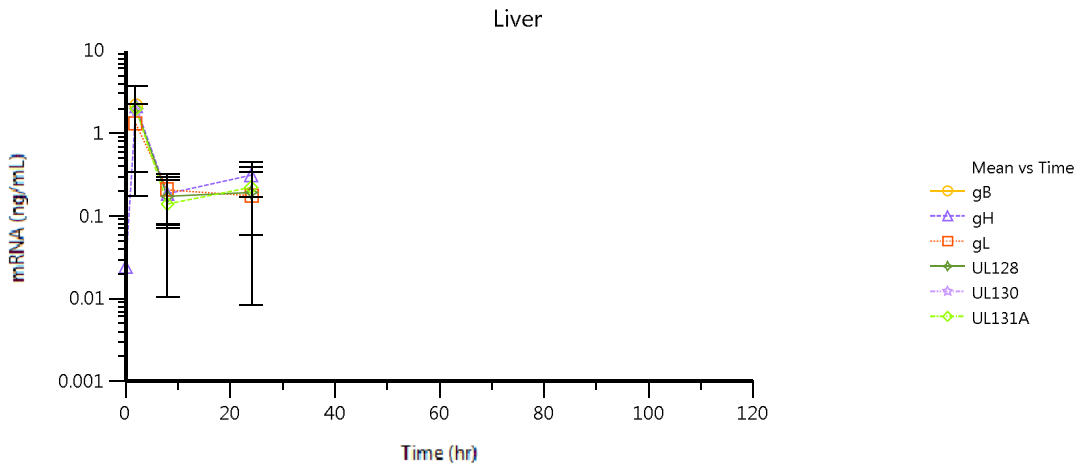

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Figure 1.11: Summary (± SD) Male Sprague-Dawley Rat Lung mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

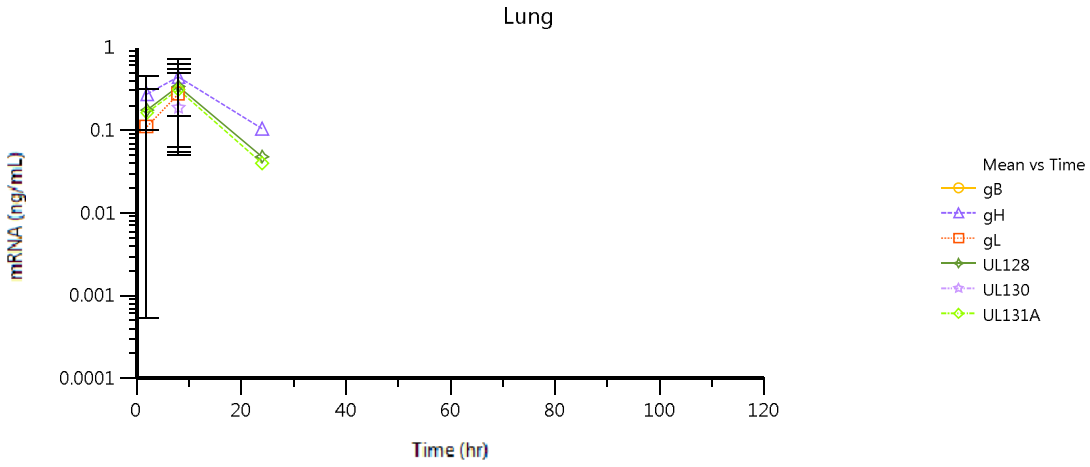

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Figure 1.12: Summary (± SD) Male Sprague-Dawley Rat Proximal Lymph node mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

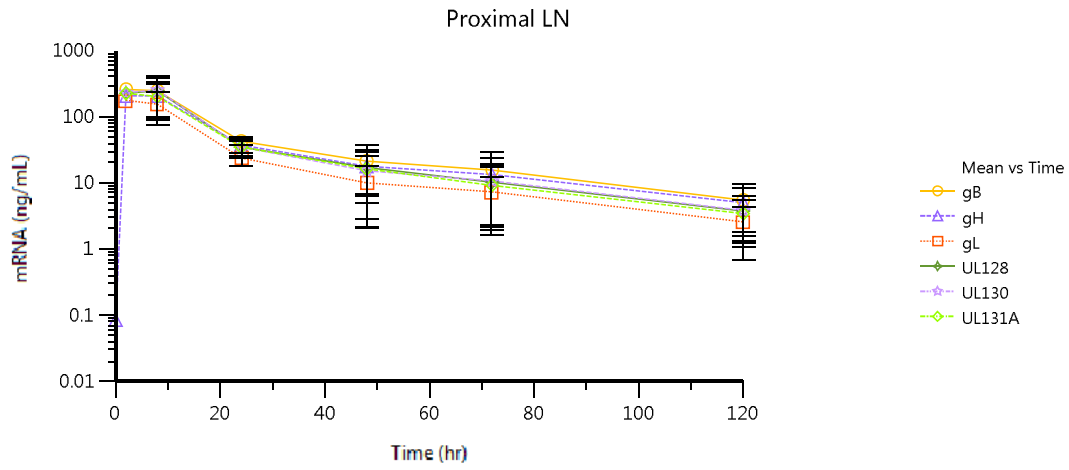

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Figure 1.13: Summary (± SD) Male Sprague-Dawley Rat Spleen mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

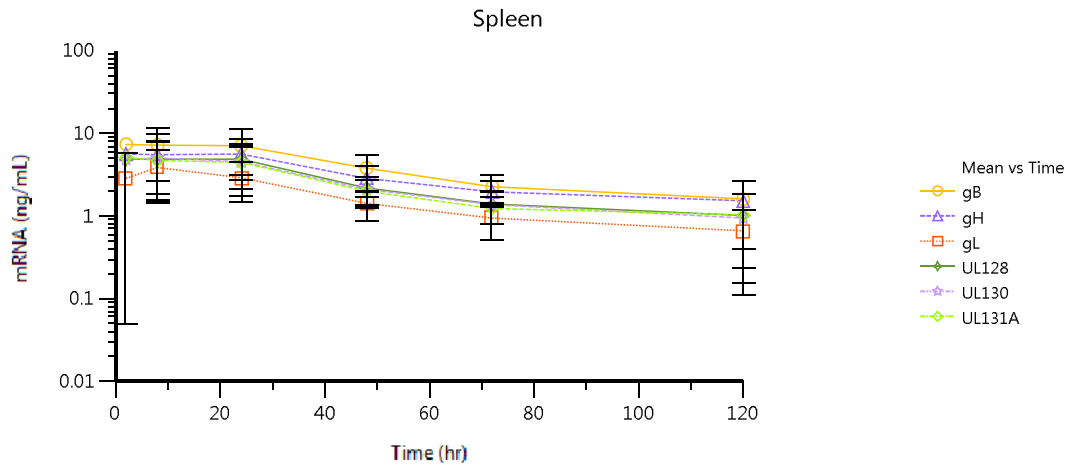

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Figure 1.14: Summary (± SD) Male Sprague-Dawley Rat Stomach mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

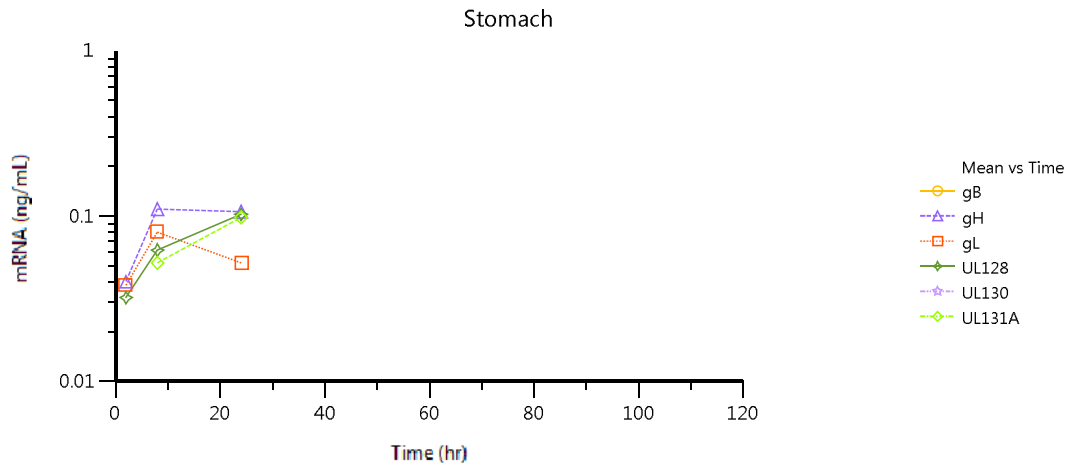

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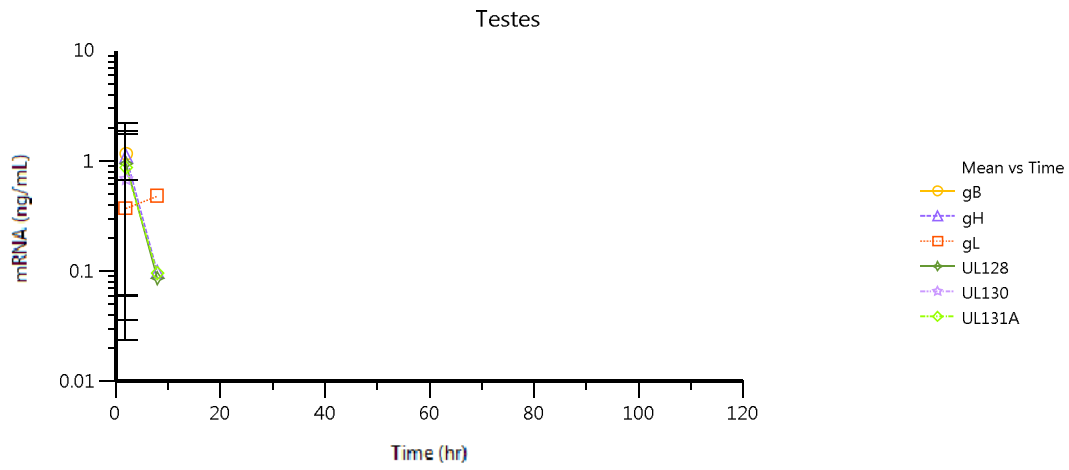
Figure 1.15: Summary (± SD) Male Sprague-Dawley Rat Testes mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1



Appendix 8

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

Appendix 8

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)

Appendix 8

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

Appendix 8

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

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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

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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

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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

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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

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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

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2.6.5.1 PHARMACOKINETICS: OVERVIEW

Type of Study	Test Article	Test System	Method of Administration	Testing Facility	Report Number	Location in eCTD
Distribution						
Single Dose IM tissue distribution study in male Sprague Dawley rats	mRNA-1647[a]	Rat, Sprague Dawley	Single IM Dose	Charles River Laboratories, Sherbrooke, QC, Canada	5002121 Amendment 1	4.2.2.3

Abbreviations: CMV = cytomegalovirus; gB = glycoprotein B; gH = glycoprotein; gL = glycoprotein L; eCTD = electronic common technical document; IM = intramuscular; mRNA = messenger RNA.

[a] mRNA-1647 contains 6 mRNAs that encode the full-length CMV gB and the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex. The 6 mRNAs are combined at a target mass ratio of 1:1:1:1:1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

2.6.5.2 ANALYTICAL METHODS AND VALIDATION REPORTS

Species/ Sample Matrix	Analyte	Calibration Range	Type of Assay	Method Utilized	Noteworthy Details (Qualification[a])	GLP Compliance	Report Number
Rat/ plasma and tissues	mRNA-1647	The LLOQs for plasma and tissues were set at 0.05 ng/mL for the gB and UL130 constructs and 0.01 ng/mL for the gH, gL, UL128, and UL131A constructs.	bDNA multiplex assay	QuantiGene 2.0 Plex assay	A multiplex bDNA assay is a hybridization-based method that combines multi-analyte profiling beads and bDNA signal amplification to enable the detection and quantitation of multiple RNA targets simultaneously. After preparation, a sample is combined with an array of fluorescent microspheres (capture beads) and probe sets specific for each RNA molecule of interest and allowed to incubate overnight. The capture beads are used as a support to capture RNA molecules, and the probe sets are used to quantify multiple target-specific RNA molecules within a single sample. Signal amplification is mediated by DNA amplification molecules that hybridize to one of the synthetic probes within each RNA-specific probe set. The capture beads are hybridized with pre-amplifier, amplifier, and label probe solutions. The label probes bind to streptavidin-conjugated R-phycoerythrin, and the resulting fluorescence signal associated with individual capture beads is read on a Luminex® flow cytometer. The signal is reported as the median fluorescence intensity and is proportional to the number of target RNA molecules present in the sample.	No	5002121 Amendment 1

Abbreviations: bDNA = branched DNA; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; LLOQ = lower limit of quantification.

[a] The method was not formally validated.

Source: Report 5002121 Amendment 1.

2.6.5.5 PHARMACOKINETICS: ORGAN DISTRIBUTION

Test Article: mRNA-1647

Study Title: A single dose intramuscular injection tissue distribution study of mRNA-1647 in male Sprague-Dawley rats

Species/Strain/Sex: Rat/Sprague Dawley/male **Location in eCTD:** 4.2.2.3

Number per Group: 5 animals terminated per time point (35 total) **Report Number:** 5002121 Amendment 1 **GLP Study:** No

Feeding Condition: Ad libitum **Dose Level:** 100 µg **Vehicle/Formulation:** PBS, pH 7.2 **Sample Matrix:** Plasma and tissues

Plasma and Tissue Collection Time Points: 0 (pre-dose), 2, 8, 24, 48, 72, and 120 h post-dose **Analyte:** mRNA-1647 (6 mRNA constructs)

Pharmacokinetic Parameters							
Sample Matrix	**mRNA Construct**	**T_{max} (h)[a]**	**C_{max} (ng/mL)[a]**	**$AUC_{(0-t)}$ (ng × h/mL)[a,b]**	**$T_{1/2}$ (h)[c]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[d]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average**
Plasma	gB	2.0	2.02 ± 0.181	22.7 ± 3.77	NC	NA	NA
	gH	2.0	1.91 ± 0.187	24.9 ± 4.49	NC	NA	
	gL	2.0	1.74 ± 0.177	23.4 ± 4.07	NC	NA	
	UL128	2.0	1.66 ± 0.151	24.1 ± 4.44	NC	NA	
	UL130	2.0	2.30 ± 0.621	25.5 ± 4.65	NC	NA	
	UL131A	2.0	1.60 ± 0.153	24.8 ± 4.59	NC	NA	
Tissue							
Bone marrow	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.254 ± 0.0871	7.85 ± 2.03	NC	0.316	
	gL	8.0	0.224 ± 0.0920	2.78 ± 1.03	NC	0.119	
	UL128	8.0	0.292 ± 0.120	3.53 ± 1.33	NC	0.147	
	UL130	NC	NC	NC	NC	NC	
	UL131A	8.0	0.186 ± 0.0829	2.05 ± 0.912	NC	0.0825	

Pharmacokinetic Parameters							
Sample Matrix	**mRNA Construct**	**T_{max} (h)[a]**	**C_{max} (ng/mL)[a]**	**$AUC_{(0-t)}$ (ng × h/mL)[a,b]**	**$T_{1/2}$ (h)[c]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[d]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average**
Brain	gB	NC	NC	NC	NC	NC	NR
	gH	24.0	0.0800 ± 0.0491	2.19 ± 1.08	NC	0.0880	
	gL	2.0	0.0360 ± 0.0360	0.144 ± 0.144	NC	0.00615	
	UL128	2.0	0.0340 ± 0.0340	0.136 ± 0.136	NC	0.00564	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Distal lymph node	gB	8.0	108 ± 101	1,460 ± 1,110	31.6	64.1	62.8
	gH	8.0	110 ± 102	1,490 ± 1,130	36.2	59.8	
	gL	8.0	117 ± 109	1,460 ± 1,200	30.6	62.6	
	UL128	8.0	125 ± 117	1,620 ± 1,290	32.1	67.1	
	UL130	8.0	129 ± 121	1,630 ± 1,330	27.9	64	
	UL131A	8.0	114 ± 108	1,470 ± 1,190	28.5	59.2	
Eye	gB	2.0	4.72 ± 2.77	26.7 ± 13.6	NC	1.18	1.24
	gH	2.0	3.92 ± 2.19	37.6 ± 11.0	NC	1.51	
	gL	2.0	3.23 ± 1.84	29.2 ± 9.75	NC	1.25	
	UL128	2.0	3.91 ± 2.19	34.5 ± 12.2	NC	1.43	
	UL130	2.0	3.61 ± 2.14	21.3 ± 11.0	NC	0.838	
	UL131A	2.0	3.43 ± 1.96	31.1 ± 10.2	NC	1.26	
Heart	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.548 ± 0.107	9.94 ± 1.85	NC	0.400	
	gL	8.0	0.220 ± 0.0907	2.96 ± 1.05	NC	0.127	
	UL128	8.0	0.276 ± 0.113	4.49 ± 1.51	NC	0.186	
	UL130	NC	NC	NC	NC	NC	
	UL131A	8.0	0.312 ± 0.0896	3.71 ± 1.02	NC	0.150	

Pharmacokinetic Parameters							
Sample Matrix	**mRNA Construct**	**T_{max} (h)[a]**	**C_{max} (ng/mL)[a]**	**$AUC_{(0-t)}$ (ng × h/mL)[a,b]**	**$T_{1/2}$ (h)[c]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[d]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average**
Injection site, muscle	gB	2.0	1,770 ± 803	27,100 ± 4,880	13.5	1190	939
	gH	2.0	1,720 ± 828	26,100 ± 4,700	17.1	1050	
	gL	2.0	1,310 ± 638	20,900 ± 3,720	15.2	893	
	UL128	2.0	1,620 ± 720	25,300 ± 4,090	14.9	1050	
	UL130	2.0	1,630 ± 777	24,500 ± 4,240	13.8	961	
	UL131A	8.0	427 ± 210	12,100 ± 2,830	15.0	487	
Jejunum	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.0800 ± 0.0490	2.06 ± 1.04	NC	0.0827	
	gL	2.0	0.0700 ± 0.0429	0.720 ± 0.472	NC	0.0308	
	UL128	NC	NC	NC	NC	NC	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Kidney	gB	NC	NC	NC	NC	NC	NR
	gH	NC	NC	NC	NC	NC	
	gL	NC	NC	NC	NC	NC	
	UL128	NC	NC	NC	NC	NC	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Liver	gB	2.0	2.16 ± 1.21	8.65 ± 4.83	NC	0.381	0.499
	gH	2.0	2.12 ± 0.982	16.8 ± 4.15	NC	0.674	
	gL	2.0	1.30 ± 0.432	11.0 ± 2.37	NC	0.470	
	UL128	2.0	2.00 ± 0.814	13.7 ± 3.72	NC	0.570	
	UL130	2.0	1.87 ± 1.01	7.46 ± 4.04	NC	0.293	
	UL131A	2.0	1.99 ± 0.928	13.9 ± 4.04	NC	0.562	

Pharmacokinetic Parameters							
Sample Matrix	**mRNA Construct**	**T_{max} (h)[a]**	**C_{max} (ng/mL)[a]**	**$AUC_{(0-t)}$ (ng × h/mL)[a,b]**	**$T_{1/2}$ (h)[c]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[d]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average**
Lung	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.442 ± 0.130	8.04 ± 1.96	NC	0.323	
	gL	8.0	0.274 ± 0.0984	3.45 ± 1.12	NC	0.148	
	UL128	8.0	0.340 ± 0.129	5.40 ± 1.74	NC	0.224	
	UL130	8.0	0.188 ± 0.188	2.07 ± 2.07	NC	0.0812	
	UL131A	8.0	0.310 ± 0.111	4.86 ± 1.49	NC	0.196	
Proximal lymph node	gB	2.0	260 ± 121	5,850 ± 949	33.5	257	201
	gH	8.0	206 ± 51.6	4,860 ± 722	38.2	195	
	gL	2.0	175 ± 81.9	3,460 ± 538	36.3	148	
	UL128	8.0	246 ± 66.6	5,190 ± 875	32.8	215	
	UL130	8.0	252 ± 67.2	5,240 ± 881	35.7	206	
	UL131A	2.0	225 ± 106	4,600 ± 719	32.2	185	
Spleen	gB	2.0	7.36 ± 3.81	460 ± 52.9	46.9	20.2	13.4
	gH	24.0	5.63 ± 1.28	371 ± 39.5	83.0	14.9	
	gL	8.0	3.83 ± 1.04	196 ± 21.0	68.2	8.36	
	UL128	24.0	4.87 ± 1.22	297 ± 34.8	68.8	12.3	
	UL130	8.0	5.03 ± 1.41	288 ± 33.0	64.9	11.3	
	UL131A	2.0	5.10 ± 2.64	277 ± 33.1	46.2	11.2	
Stomach	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.110 ± 0.0696	3.49 ± 1.59	NC	0.140	
	gL	8.0	0.0800 ± 0.0499	2.07 ± 1.19	NC	0.0886	
	UL128	24.0	0.102 ± 0.0648	2.85 ± 1.47	NC	0.118	
	UL130	NC	NC	NC	NC	NC	
	UL131A	24.0	0.0980 ± 0.0634	2.53 ± 1.39	NC	0.102	

Pharmacokinetic Parameters							
Sample Matrix	**mRNA Construct**	**T_{max} (h)[a]**	**C_{max} (ng/mL)[a]**	**$AUC_{(0-t)}$ (ng × h/mL)[a,b]**	**$T_{1/2}$ (h)[c]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[d]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average**
Testes	gB	2.0	1.16 ± 0.719	4.64 ± 2.88	NC	0.204	0.209
	gH	2.0	1.11 ± 0.480	5.52 ± 2.20	NC	0.222	
	gL	8.0	0.420 ± 0.335	6.08 ± 3.73	NC	0.260	
	UL128	2.0	0.946 ± 0.397	4.73 ± 1.85	NC	0.196	
	UL130	2.0	0.682 ± 0.442	2.73 ± 1.77	NC	0.107	
	UL131A	2.0	0.872 ± 0.380	4.54 ± 1.85	NC	0.183	

Abbreviations: eCTD = electronic common technical document; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; mRNA = messenger RNA; NA = not applicable; NC = not calculable (insufficient data points above lower limit of quantitation); NR = not reported (some constructs measured all samples as below limit of quantitation); PBS = phosphate-buffered saline.

[a] T_{max} and $T_{1/2}$ data reported as the mean; C_{max} and $AUC_{(0-t)}$ data reported as the mean ± standard error.

[b] 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.

[c] Due to the lack of a distinct elimination phase in plasma, the $T_{1/2}$ of the mRNA constructs could not be calculated; however, the $T_{1/2}$ was estimated to range from 2.7 to 3.8 hours.

[d] For $AUC_{(0-t)}$ Ratio, samples listed as NC were not calculable because all samples were below limit of quantitation.

Source: Report 5002121 Amendment 1 (Appendix 8, Table 2 and Table 3).

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List of Tables

List of Abbreviations

Abbreviation	Definition
AUC	area under the concentration versus time curve
$AUC_{(0-t)}$	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
bDNA	branched DNA
C_{max}	maximum plasma concentration
CoV	coronavirus
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
gB	glycoprotein B
gH	glycoprotein H
gL	glycoprotein L
GLP	Good Laboratory Practice
IM	intramuscular(ly)
LLOQ	lower limit of quantitation
LNP	lipid nanoparticle
mRNA	messenger RNA
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PG	propylene glycol
PK	pharmacokinetic
S	spike
S-2P	spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
SARS-CoV-2	2019 novel coronavirus
SM-102	heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate
$T_{1/2}$	half-life
Tris	tris(hydroxymethyl)aminomethane
T_{max}	time to peak (maximum) plasma concentration

2.6.4.1 BRIEF SUMMARY

ModernaTX, Inc. (Sponsor) has developed mRNA-1273, a novel lipid nanoparticle (LNP)-encapsulated messenger RNA (mRNA)-based vaccine against the 2019 novel coronavirus (CoV; SARS-CoV-2). mRNA-1273 contains a single mRNA that encodes the full-length spike (S) protein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilize the S protein into the prefusion conformation. The mRNA is combined in a mixture of 4 lipids common to the Sponsor's mRNA vaccine platform: SM-102, cholesterol, DSPC, and PEG2000-DMG.

The results of a biodistribution study of mRNA-1647 support the development of mRNA-1273. mRNA-1647 is a novel mRNA-based cytomegalovirus vaccine that contains 6 distinct mRNA sequences (1 that encodes the full-length cytomegalovirus glycoprotein B [gB], and 5 that encode the pentameric glycoprotein H [gH]/glycoprotein L [gL]/UL128/UL130/UL131A glycoprotein complex) combined at a target mass ratio of 1:1:1:1:1:1 in the Sponsor's standard proprietary SM-102–containing LNPs.

The biodistribution of mRNA-1647 was evaluated in a non-Good Laboratory Practice (GLP), single-dose, intramuscular (IM) injection study in Sprague Dawley rats. The objectives of this study were to determine the tissue distribution and pharmacokinetic (PK) characteristics of mRNA-1647 following IM administration. The biodistribution of mRNA-based vaccines in LNPs is predicted to be driven by the LNP characteristics. Therefore, mRNAs that are within an LNP of the same composition (eg, mRNA-1273 and mRNA-1647) are expected to distribute similarly.

Concentrations for all 6 mRNA-1647 constructs, gB, gH, gL, UL128, UL130, and UL131A, were detectable in plasma and tissues in a 1:1:1:1:1:1 ratio. After a single IM dose in male rats, the time after dosing at which the maximum concentration was observed in plasma (T_{max}) was 2 hours for all constructs and was followed by a rapid elimination phase, with a half-life ($T_{1/2}$) estimated to range from 2.7 to 3.8 hours. The maximum plasma concentration (C_{max}) ranged from 1.60 to 2.30 ng/mL, and the area under the concentration versus time curve (AUC) from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed ($AUC_{[0\text{-}t]}$) ranged from 22.7 to 25.5 ng × h/mL.

Concentrations for all 6 mRNA-1647 constructs were detected at levels above the lower limit of quantitation (LLOQ) in most tissues analyzed, except for the kidney, where all levels were below the LLOQ. For highly exposed tissues (injection site [muscle], lymph nodes [proximal and

distal], and spleen), the C_{max} was observed between 2 and 24 hours post-dose. The $T_{1/2}$ was calculated using the average tissue $T_{1/2}$ values for all 6 constructs. The results were 14.9 hours for injection site (muscle), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

As observed with other IM delivered vaccines, the highest mRNA concentrations were observed at the injection site followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0.

Overall, only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for all constructs ranged from 14.9 to 63.0 hours.. The completed nonclinical PK and biodistribution study is presented in Table 1.

Table 1: Nonclinical Biodistribution Study Supporting Development of mRNA-1273

Study Type	Test Article	Species, Strain	Method of Administration, Dose	GLP	Report Number
Single-dose tissue distribution study	mRNA-1647	Rat, Sprague Dawley	IM injection, dose of 100 µg on Day 1	No	5002121 Amendment 1

Abbreviations: CMV = cytomegalovirus; gB = glycoprotein B; gH = glycoprotein; gL = glycoprotein L; GLP = Good Laboratory Practice; IM = intramuscular; mRNA = messenger RNA; .

[a] mRNA-1647 contains 6 mRNAs that encode the full-length CMV gB and the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex. The 6 mRNAs are combined at a target mass ratio of 1:1:1:1:1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

2.6.4.2 METHODS OF ANALYSIS

The procedure followed during the course of this study, along with the assay acceptance criteria, was detailed in a bioanalytical protocol. The LLOQs for plasma and tissues was set at 0.05 ng/mL for the gB and UL130 constructs and 0.01 ng/mL for the gH, gL, UL128, and UL131A constructs. Samples were analyzed in duplicate. Details on how biological samples were collected and processed are provided in the study report (Report 5002121 Amendment 1 Section 4.12).

Samples were analyzed for all 6 mRNA constructs (gB, gH, gL, UL128, UL130, and UL131A) present in mRNA-1647. To quantify these multiple constructs in mRNA-1647, a multiplex branched DNA (bDNA) assay was used. This assay is a hybridization-based method that combines multi-analyte profiling beads and bDNA signal amplification to enable the detection and quantitation of multiple RNA targets simultaneously. After preparation, a sample is combined with an array of fluorescent microspheres (capture beads) and probe sets specific for each RNA molecule of interest and allowed to incubate overnight. The capture beads are used as a support to capture RNA molecules, and the probe sets are used to quantify multiple target-specific RNA molecules within a single sample. Signal amplification is mediated by DNA amplification molecules that hybridize to one of the synthetic probes within each RNA-specific probe set. The capture beads are hybridized with pre-amplifier, amplifier, and label probe solutions. The label probes bind to streptavidin-conjugated R-phycoerythrin, and the resulting fluorescence signal associated with individual capture beads is read on a Luminex® flow cytometer. The signal is reported as the median fluorescence intensity and is proportional to the number of target RNA molecules present in the sample.

2.6.4.3 ABSORPTION

No absorption studies with mRNA-1273 have been performed.

2.6.4.4 DISTRIBUTION

2.6.4.4.1 Tissue Distribution Studies

The objective of this non-GLP study was to determine the tissue distribution of mRNA-1647 when given once by IM injection to rats. The PK characteristics of mRNA-1647 were determined in plasma and tissue. A group of 35 male Sprague Dawley rats was given a single IM injection of 100 µg of mRNA-1647 in a dose volume of 200 µL (dose concentration of 0.5 mg/mL) on Day 1. Subgroups of 5 rats each were sacrificed pre-dose and 2, 8, 24, 48, 72, and 120 hours after IM dosing. Blood and tissues were collected and processed for quantitation of the 6 mRNA constructs (gB, gH, gL, UL128, UL130, and UL131A) present in mRNA-1647 using a qualified bDNA multiplex method (Section 2.6.4.2). The overall design of this study is presented in Table 2.

Table 2: A Single-Dose IM Pharmacokinetic and Biodistribution Study of mRNA-1647 in Sprague Dawley Rats

Group Number	Test Article (Method of Administration)	Species/ Strain	Number of Animals/Sex	Dose Level (µg)	Dose Volume (µL)	Dose Concentration (mg/mL)	Sample Collection Time Points (h)
1	mRNA-1647 (single IM injection)	Rats/ Sprague Dawley	35/male	100	200	0.5	0 (pre-dose), 2, 8, 24, 48, 72, and 120

Abbreviations: IM = intramuscular.
Source: Report 5002121 Amendment 1 (Text Table 3 and Text Table 4).

No quantifiable concentrations for any of the mRNA constructs were observed in plasma or tissue in pre-dose samples, with the exception of 2 plasma samples for which the gH construct concentration was slightly above the LLOQ. For all 6 mRNA constructs present in mRNA-1647, post-dose levels were detectable in plasma and tissues in a 1:1:1:1:1:1 ratio. Mean plasma concentrations were quantifiable up to 24 hours with an interanimal coefficient of variation from 21.8% and 79.8%. The only quantifiable plasma samples beyond 24 hours were 6 gH constructs that were slightly above the LLOQ.

After a single IM dose in male rats, the T_{max} for all 6 mRNA constructs was 2 hours, followed by a rapid elimination phase. Mean concentrations became undetectable for all constructs after 24 hours with the exception of gH, which was detectable up to the last time point of 120 hours. Due to the lack of a distinct elimination phase, the $T_{1/2}$ of the mRNA constructs could not be calculated; however, the $T_{1/2}$ was estimated to range from 2.7 to 3.8 hours. The C_{max} and $AUC_{(0\text{-}t)}$ ranged from 1.60 to 2.30 ng/mL and from 22.7 to 25.5 ng × h/mL, respectively (Table 3).

Table 3: Plasma Pharmacokinetic Parameters for a Single IM Dose of 100 µg of mRNA-1647 in Male Sprague Dawley Rats

Matrix	Construct	T_{max} (h)[a]	C_{max} (ng/mL)[a]	$AUC_{(0-t)}$ (ng × h/mL)[a]	$T_{1/2}$ (h)[b]
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
	UL131A	2.0	1.60 ± 0.153	24.8 ± 4.59	NC

Abbreviations: gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; IM = intramuscular; NC = not calculable (insufficient data points above the lower limit of quantification).

[a] T_{max} data reported as the mean; C_{max} and $AUC_{(0-t)}$ data reported as the mean ± standard error.

[b] Due to the lack of a distinct elimination phase, the $T_{1/2}$ of the mRNA constructs could not be calculated; however, the $T_{1/2}$ was estimated to range from 2.7 to 3.8 hours.

Source: Report 5002121 Amendment 1 (Appendix 8, Table 2).

All constructs of mRNA-1647 were quantifiable in most tissues analyzed, except for the kidney, where all levels were below the LLOQ. For highly exposed tissues (injection site [muscle], lymph nodes [proximal and distal], and spleen), the C_{max} was observed between 2 and 24 hours post-dose. The $T_{1/2}$ was calculated using the average tissue $T_{1/2}$ values for all 6 constructs. The results were 14.9 hours injection site (muscle), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

As observed with other IM delivered vaccines, the highest mRNA concentrations were observed at the injection site (muscle) followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0.

Overall, only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for all constructs ranged from 14.9 to 63.0 hours. (Table 4).

Table 4: Tissue Pharmacokinetic Parameters for a Single IM Dose of 100 µg of mRNA-1647 in Male Sprague Dawley Rats

Matrix	Construct	T_{max} (h)[a]	C_{max} (ng/mL)[a]	$AUC_{(0-t)}$ (ng × h/mL)[a,b]	$T_{1/2}$ (h)[a]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[c]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average
Bone marrow	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.254 ± 0.0871	7.85 ± 2.03	NC	0.316	
	gL	8.0	0.224 ± 0.0920	2.78 ± 1.03	NC	0.119	
	UL128	8.0	0.292 ± 0.120	3.53 ± 1.33	NC	0.147	
	UL130	NC	NC	NC	NC	NC	
	UL131A	8.0	0.186 ± 0.0829	2.05 ± 0.912	NC	0.0825	
Brain	gB	NC	NC	NC	NC	NC	NR
	gH	24.0	0.0800 ± 0.0491	2.19 ± 1.08	NC	0.0880	
	gL	2.0	0.0360 ± 0.0360	0.144 ± 0.144	NC	0.00615	
	UL128	2.0	0.0340 ± 0.0340	0.136 ± 0.136	NC	0.00564	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Distal lymph node	gB	8.0	108 ± 101	1,460 ± 1,110	31.6	64.1	62.8
	gH	8.0	110 ± 102	1,490 ± 1,130	36.2	59.8	
	gL	8.0	117 ± 109	1,460 ± 1,200	30.6	62.6	
	UL128	8.0	125 ± 117	1,620 ± 1,290	32.1	67.1	
	UL130	8.0	129 ± 121	1,630 ± 1,330	27.9	64	
	UL131A	8.0	114 ± 108	1,470 ± 1,190	28.5	59.2	

Matrix	Construct	T_{max} (h)[a]	C_{max} (ng/mL)[a]	$AUC_{(0-t)}$ (ng × h/mL)[a,b]	$T_{1/2}$ (h)[a]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[c]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average
Eye	gB	2.0	4.72 ± 2.77	26.7 ± 13.6	NC	1.18	1.24
	gH	2.0	3.92 ± 2.19	37.6 ± 11.0	NC	1.51	
	gL	2.0	3.23 ± 1.84	29.2 ± 9.75	NC	1.25	
	UL128	2.0	3.91 ± 2.19	34.5 ± 12.2	NC	1.43	
	UL130	2.0	3.61 ± 2.14	21.3 ± 11.0	NC	0.838	
	UL131A	2.0	3.43 ± 1.96	31.1 ± 10.2	NC	1.26	
Heart	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.548 ± 0.107	9.94 ± 1.85	NC	0.400	
	gL	8.0	0.220 ± 0.0907	2.96 ± 1.05	NC	0.127	
	UL128	8.0	0.276 ± 0.113	4.49 ± 1.51	NC	0.186	
	UL130	NC	NC	NC	NC	NC	
	UL131A	8.0	0.312 ± 0.0896	3.71 ± 1.02	NC	0.150	
Injection site muscle	gB	2.0	1,770 ± 803	27,100 ± 4,880	13.5	1190	939
	gH	2.0	1,720 ± 828	26,100 ± 4,700	17.1	1050	
	gL	2.0	1,310 ± 638	20,900 ± 3,720	15.2	893	
	UL128	2.0	1,620 ± 720	25,300 ± 4,090	14.9	1050	
	UL130	2.0	1,630 ± 777	24,500 ± 4,240	13.8	961	
	UL131A	8.0	427 ± 210	12,100 ± 2,830	15.0	487	
Jejunum	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.0800 ± 0.0490	2.06 ± 1.04	NC	0.0827	
	gL	2.0	0.0700 ± 0.0429	0.720 ± 0.472	NC	0.0308	
	UL128	NC	NC	NC	NC	NC	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	

Matrix	Construct	T_{max} (h)[a]	C_{max} (ng/mL)[a]	$AUC_{(0-t)}$ (ng × h/mL)[a,b]	$T_{1/2}$ (h)[a]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[c]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average
Kidney	gB	NC	NC	NC	NC	NC	NR
	gH	NC	NC	NC	NC	NC	
	gL	NC	NC	NC	NC	NC	
	UL128	NC	NC	NC	NC	NC	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Liver	gB	2.0	2.16 ± 1.21	8.65 ± 4.83	NC	0.381	0.499
	gH	2.0	2.12 ± 0.982	16.8 ± 4.15	NC	0.674	
	gL	2.0	1.30 ± 0.432	11.0 ± 2.37	NC	0.470	
	UL128	2.0	2.00 ± 0.814	13.7 ± 3.72	NC	0.570	
	UL130	2.0	1.87 ± 1.01	7.46 ± 4.04	NC	0.293	
	UL131A	2.0	1.99 ± 0.928	13.9 ± 4.04	NC	0.562	
Lung	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.442 ± 0.130	8.04 ± 1.96	NC	0.323	
	gL	8.0	0.274 ± 0.0984	3.45 ± 1.12	NC	0.148	
	UL128	8.0	0.340 ± 0.129	5.40 ± 1.74	NC	0.224	
	UL130	8.0	0.188 ± 0.188	2.07 ± 2.07	NC	0.0812	
	UL131A	8.0	0.310 ± 0.111	4.86 ± 1.49	NC	0.196	
Proximal lymph nodes	gB	2.0	260 ± 121	5,850 ± 949	33.5	257	201
	gH	8.0	206 ± 51.6	4,860 ± 722	38.2	195	
	gL	2.0	175 ± 81.9	3,460 ± 538	36.3	148	
	UL128	8.0	246 ± 66.6	5,190 ± 875	32.8	215	
	UL130	8.0	252 ± 67.2	5,240 ± 881	35.7	206	
	UL131A	2.0	225 ± 106	4,600 ± 719	32.2	185	

Matrix	Construct	T_{max} (h)[a]	C_{max} (ng/mL)[a]	$AUC_{(0-t)}$ (ng × h/mL)[a,b]	$T_{1/2}$ (h)[a]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[c]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average
Spleen	gB	2.0	7.36 ± 3.81	460 ± 52.9	46.9	20.2	13.4
	gH	24.0	5.63 ± 1.28	371 ± 39.5	83.0	14.9	
	gL	8.0	3.83 ± 1.04	196 ± 21.0	68.2	8.36	
	UL128	24.0	4.87 ± 1.22	297 ± 34.8	68.8	12.3	
	UL130	8.0	5.03 ± 1.41	288 ± 33.0	64.9	11.3	
	UL131A	2.0	5.10 ± 2.64	277 ± 33.1	46.2	11.2	
Stomach	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.110 ± 0.0696	3.49 ± 1.59	NC	0.140	
	gL	8.0	0.0800 ± 0.0499	2.07 ± 1.19	NC	0.0886	
	UL128	24.0	0.102 ± 0.0648	2.85 ± 1.47	NC	0.118	
	UL130	NC	NC	NC	NC	NC	
	UL131A	24.0	0.0980 ± 0.0634	2.53 ± 1.39	NC	0.102	
Testes	gB	2.0	1.16 ± 0.719	4.64 ± 2.88	NC	0.204	0.209
	gH	2.0	1.11 ± 0.480	5.52 ± 2.20	NC	0.222	
	gL	8.0	0.420 ± 0.335	6.08 ± 3.73	NC	0.260	
	UL128	2.0	0.946 ± 0.397	4.73 ± 1.85	NC	0.196	
	UL130	2.0	0.682 ± 0.442	2.73 ± 1.77	NC	0.107	
	UL131A	2.0	0.872 ± 0.380	4.54 ± 1.85	NC	0.183	

Abbreviations: gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; IM = intramuscular; NC = not calculable (insufficient data points above the lower limit of quantitation); NR = not reported (some constructs measured all samples as below limit of quantitation).

[a] T_{max} and $T_{1/2}$ data reported as the mean; C_{max} and $AUC_{(0-t)}$ data reported as the mean ± standard error.

[b] 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.

[c] For $AUC_{(0-t)}$ Ratio, samples listed as NC were not calculable because all samples were below limit of quantitation.

Source: Report 5002121 Amendment 1 (Appendix 8, Table 2 and Table 3)

2.6.4.5 METABOLISM

No metabolism studies with mRNA-1273 have been performed.

2.6.4.6 EXCRETION

No excretion studies with mRNA-1273 have been performed.

2.6.4.7 PHARMACOKINETIC DRUG INTERACTIONS

No PK drug interaction studies with mRNA-1273 have been performed.

2.6.4.8 OTHER PHARMACOKINETIC STUDIES

No other PK studies with mRNA-1273 have been performed.

2.6.4.9 DISCUSSION AND CONCLUSION

A non-GLP biodistribution study was completed with mRNA-1647, an mRNA-based vaccine combined in SM-102–containing LNPs, in male Sprague Dawley rats and is provided to support the development of mRNA-1273 using the Sponsor's mRNA technology platform. The biodistribution of mRNA-based vaccines in LNPs is predicted to be driven by the LNP characteristics. Therefore, mRNAs that are within an LNP of the same composition (eg, mRNA-1273 and mRNA-1647) are expected to distribute similarly.

- Concentrations for mRNA constructs were detected at levels above the LLOQ in most tissues analyzed, except for the kidney, where all levels were below the LLOQ.

- As observed with other IM-delivered vaccines, the highest mRNA concentrations were observed at the injection site followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0.

- The T_{max} in plasma was achieved at 2 hours post-dose, with an estimated $T_{1/2}$ in plasma ranging from 2.7 to 3.8 hours. For highly exposed tissues, C_{max} was observed between 2 and 24 hours post-dose. The $T_{1/2}$ values, calculated using the average tissue $T_{1/2}$ values for all 6 constructs, were 14.9 hours for site of injection (muscle), 34.8 hours for

proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

Overall, only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for all constructs ranged from 14.9 to 63.0 hours.. The biodistribution of mRNA-based vaccines in LNPs is predicted to be driven by the LNP characteristics. Therefore, mRNAs that are within an LNP of the same composition (eg, mRNA-1273 and mRNA-1647) are expected to distribute similarly.

2.6.4.10 TABLES AND FIGURES

The tables and figures are included in the body of the document.

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List of Abbreviations

Abbreviation	Definition
CoV	coronavirus
COVID-19	coronavirus disease caused by the 2019 novel coronavirus
CMV	cytomegalovirus
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
ERD	enhanced respiratory disease
GLP	Good Laboratory Practice
ICH	International Council for Harmonisation
Ig	immunoglobulin
IM	intramuscular
LNP	lipid nanoparticle
mRNA	messenger RNA
NHP	nonhuman primate
OECD	Organisation for Economic Co-operation and Development
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
S	spike
S-2P	SARS-CoV-2 spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
SARS	severe acute respiratory syndrome
SARS-CoV-2	2019 novel coronavirus
SM-102	heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate
Th	T helper
Tris	tris(hydroxymethyl)aminomethane
WHO	World Health Organization

2.6.1 INTRODUCTION

Coronaviruses (CoVs) are part of a large family of viruses that cause illnesses ranging from the common cold to more severe diseases, such as Middle East respiratory syndrome and severe acute respiratory syndrome (SARS).

An outbreak of the CoV disease (COVID-19) caused by the 2019 novel CoV (2019-nCoV, later designated SARS-CoV-2) began in Wuhan, Hubei Province, China, in Dec 2019 and the disease has since spread globally (WHO 2020). Currently, there is no FDA-approved vaccine against SARS-CoV-2. Without further advances in the use of nonpharmaceutical interventions, over 2.5 million COVID-19 deaths are projected globally by 01 Mar 2021, with daily deaths peaking at about 15,000/day during this time (IHME 2020). Global efforts to evaluate novel antivirals and therapeutic strategies to treat severe SARS-CoV-2 infections have intensified, and there is an urgent public health need for rapid development of novel prophylactic therapies, including vaccines to prevent the spread of this disease.

ModernaTX, Inc. (Sponsor) has used its messenger RNA (mRNA)-based, rapid-response proprietary vaccine platform to develop mRNA-1273, a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine against SARS-CoV-2. mRNA-1273 contains a single mRNA that encodes the full-length SARS-CoV-2 spike (S) protein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilize the S protein into the prefusion conformation. The mRNA is combined in a mixture of 4 lipids common to the Sponsor's mRNA vaccine platform: SM-102, cholesterol, DSPC, and PEG2000-DMG. The mRNA-1273 Drug Product is provided as a sterile liquid for injection at a concentration of 0.20 mg/mL in 20 mM Tris buffer containing 87 g/L sucrose and 4.3 mM acetate, at pH 7.5.

The clinical development of mRNA-1273 to support its use in the adult population consists of 3 ongoing clinical trials being conducted in the US: a Phase 1, open-label, dose-ranging study (NCT04283461) sponsored by the National Institute of Allergy and Infectious Diseases and a Phase 2a, randomized, observer-blind, placebo-controlled, dose-confirmation study (NCT04405076) and a Phase 3 randomized, stratified, observer-blind, placebo-controlled study (NCT04470427) conducted by the Sponsor to evaluate the efficacy, safety, and immunogenicity of the vaccine. The development of mRNA-1273 has been accelerated to address the current COVID-19 outbreak, benefiting from the uniquely rapid and scalable manufacturing processes that have been developed for this vaccine.

2.6.1.1 Nonclinical Development Program for mRNA-1273

The nonclinical pharmacology, pharmacokinetics and tissue distribution, and toxicology studies conducted with mRNA-1273 or other mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs support the intended clinical use of mRNA-1273. The program was designed in accordance with guidelines applicable at the time the studies were conducted, including relevant International Council for Harmonisation (ICH) and other global regulatory guidelines, and Good Laboratory Practice (GLP) regulations. The pivotal nonclinical safety studies were conducted according to the Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (ENV/MC/CHEM[98]17) or GLP regulations in other countries that are signatories to the OECD Mutual Acceptance of Data agreement (eg, US Food and Drug Administration Code of Federal Regulations Title 21, Part 58: Good Laboratory Practice for Nonclinical Laboratory Studies).

The nonclinical studies were conducted in mice, rats, hamsters, and rhesus macaques (nonhuman primates [NHPs]), species determined to be relevant for the assessment of the immunogenicity, efficacy, and safety of mRNA-1273.

2.6.1.1.1 Nonclinical Pharmacology Program

Nonclinical primary pharmacology evaluations were conducted in young and aged mice (BALB/c, BALB/cJ, C57/BL6/J, and B6C3F1/J strains), golden Syrian hamsters, and rhesus macaques animal models to characterize the immunogenicity of mRNA-1273, as well as its effects on viral replication and disease progression after SARS-CoV-2 challenge, and to evaluate its safety profile and its potential to promote vaccine-associated enhanced respiratory disease (ERD) after viral challenge, which has previously been observed with vaccines against respiratory syncytial virus (Kim et al 1969), measles (Polack 2007), and in animal models of SARS-CoV vaccination (Czub et al 2005; Deming et al 2007; Bolles et al 2011; Corbett et al 2020). Additionally, the immunogenicity of mRNA-1273 was assessed as part of a non-GLP repeat-dose pharmacology study in Sprague Dawley rats.

Immunogenicity was characterized in young and aged mice, rats, hamsters, and NHPs through the evaluation of the humoral (immunoglobulin [Ig] G binding antibodies), cellular (T-cell cytokines and T helper [Th] 1-directed CD4+ and CD8+ responses), and/or neutralizing antibody responses elicited by prime-only or prime/boost immunization schedule with a range of mRNA-1273 dose levels.

Protection by mRNA-1273 immunization was assessed in young and aged mice, hamsters, and NHPs immunized with a prime-only or prime/boost schedule, followed by viral challenge with a high dose of SARS-CoV-2 (mice: mouse-adapted SARS-CoV-2 strain; hamsters and NHPs: WT SARS-CoV-2 strain, Washington state isolate). mRNA-1273 dose levels and immunization schedules predicted to drive optimal and suboptimal protection were included in these studies to identify immune signatures for each regimen and to assess the level of protection mediated by different dose levels. Suboptimal dose levels that confer only partial protection were also included to evaluate the theoretical risk of disease enhancement. Viral load and replication in the upper (nasal turbinates) and lower (lungs) airways, as well as lung pathology and inflammation, were evaluated after viral challenge.

The potential of mRNA-1273 to promote vaccine-associated ERD was assessed in young and aged mice, hamsters, and NHPs through the evaluation of immunogenicity endpoints (IgG1:IgG2a ratio, Th1/Th2 cytokine profiles, and the ratio of binding to neutralizing antibodies) indicative of a protective versus a disease enhancement phenotype and through monitoring of viral load, viral replication, and histopathological evaluation of lung tissues after viral challenge.

2.6.1.1.2 Nonclinical Pharmacokinetic Program

mRNA is degraded within minutes in biological fluids and is unlikely to persist in tissues; therefore, the biodistribution of mRNA-based vaccines formulated in LNPs is predicted to be driven by the LNP characteristics and mRNAs that are within LNPs of the same composition (ie, SM-102-containing LNPs) are expected to distribute similarly to the LNPs. Thus, the distribution of mRNA-1647, an mRNA-based cytomegalovirus (CMV) vaccine that contains 6 mRNA sequences combined in SM-102-containing LNPs, assessed in a non-GLP, single intramuscular (IM) dose biodistribution study supports the development of mRNA-1273.

2.6.1.1.3 Nonclinical Toxicology Program

The toxicological profile associated with mRNA-based vaccines formulated in SM-102-containing LNPs, including mRNA-1273, is driven primarily by the LNP composition and, to a lesser extent, by the biologic activity of the antigen(s) encoded by the mRNA. The safety and tolerability of 5 mRNA-based vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs (2 Zika virus vaccines: mRNA-1706 and mRNA-1893; 1 human metapneumovirus and parainfluenza virus type 3 vaccine: mRNA-1653; and 2 CMV vaccines: mRNA-1647 and mRNA-1443) have been evaluated in 6 GLP-compliant repeat-dose toxicity studies in Sprague Dawley rats. Additionally,

the Sponsor completed a non-GLP repeat-dose study in Sprague Dawley rats to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses.

SM-102, the novel lipid used in mRNA-1273, was evaluated in genotoxicity studies as an individual agent using a standard ICH S2 (R1) approach (ICH 2011), including a GLP-compliant bacterial reverse mutation (Ames) test in *Salmonella typhimurium* and *Escherichia coli* and a GLP-compliant in vitro micronucleus test in human peripheral blood lymphocytes. In addition, SM-102 was evaluated for in vivo genotoxicity risk in a GLP-compliant in vivo rat micronucleus test using an mRNA-based vaccine formulated in SM-102 LNPs (mRNA-1706) and a non-GLP-compliant in vivo rat micronucleus test using a reporter mRNA (nascent peptide imaging luciferase mRNA) formulated in SM-102 LNPs.

Overall, data from the nonclinical testing program presented in this submission demonstrate that mRNA-1273 is safe and well tolerated, is immunogenic, fully protects animals from viral challenge, and does not promote ERD at either optimal or suboptimal dose levels. These data support the clinical evaluation of the efficacy and safety of 100 µg of mRNA-1273 administered as 2 IM injections 28 days apart.

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List of Abbreviations

Abbreviation	Definition
ACE-2	angiotensin converting enzyme 2
BLA	biologics license application
CDC	Centers for Disease Control and Prevention
CMV	cytomegalovirus
CoV	coronavirus
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
DTPA	diethylenetriamine pentaacetic acid
ERD	enhanced respiratory disease
gB	glycoprotein B
gH	glycoprotein H
gL	glycoprotein L
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
hMPV	human metapneumovirus
ICH	International Council for Harmonisation
Ig	immunoglobulin
IM	intramuscular(ly)
LLOQ	lower limit of quantitation
LNP	lipid nanoparticle
mRNA	messenger RNA
NHP	nonhuman primate
NPI	nascent peptide imaging
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PIV3	parainfluenza virus type 3
S	spike
S-2P	spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
SARS	severe acute respiratory syndrome
SARS-CoV-1 DIV	double-inactivated severe acute respiratory syndrome coronavirus-1
SARS-CoV-2	2019 novel coronavirus
SM-102	heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate
Th	T helper

Abbreviation	Definition
Tris	tris(hydroxymethyl)aminomethane
WHO	World Health Organization
WT	wild type

2.4.1. OVERVIEW OF NONCLINICAL TESTING STRATEGY

2.4.1.1 Background

Coronaviruses (CoVs) are part of a large family of viruses that cause illnesses ranging from the common cold to more severe diseases, such as Middle East respiratory syndrome and severe acute respiratory syndrome (SARS).

An outbreak of the CoV disease (COVID-19) caused by the 2019 novel CoV (2019-nCoV, later designated SARS-CoV-2) began in Wuhan, Hubei Province, China, in Dec 2019 and the disease has since spread globally (WHO 2020a). The WHO declared COVID-19 a pandemic on 11 Mar 2020; however, widespread community transmission was already occurring in many locations. As of Nov 2020, more than 53 million cases and over 1.3 million deaths worldwide have been attributed to the COVID-19 pandemic (WHO 2020a; WHO 2020b). Widespread community transmission of SARS-CoV-2 has been reported in the Americas, Europe, Africa, and Southeast Asia, and clusters of cases continue to be reported throughout Asia and Australia (WHO 2020a). During the winter, the combination of re-opening of schools and increase in indoor activity, because of lower temperatures, is expected to further increases in COVID-19 cases and deaths in some parts of the world.

Current evidence suggests that SARS-CoV-2 is primarily transmitted via direct contact or person-to-person via respiratory droplets by coughing or sneezing from an infected individual (regardless of whether they are symptomatic) (Chen et al 2020; Licciardi et al 2020; Rothan and Byrareddy 2020; Shen et al 2020). Airborne transmission may be possible during certain medical procedures and in indoor, crowded, and poorly ventilated environments (WHO 2020c). Common symptoms of COVID-19 include fever and cough, and other symptoms include shortness of breath or difficulty breathing, muscle aches, chills, sore throat, headache, and loss of taste or smell. Individuals at highest risk of COVID-19 and severe COVID-19 are older adults (≥ 65 years old) and people of any age who have certain underlying medical conditions such as cancer, chronic kidney disease, chronic obstructive pulmonary disease, serious heart conditions, immunocompromised state, obesity, pregnancy, sickle cell disease, and type 2 diabetes mellitus; smokers are also at increased risk for severe COVID-19 disease (CDC 2020).

Currently, there is no FDA-approved vaccine against SARS-CoV-2. Without further advances in the use of nonpharmaceutical interventions, over 2.5 million COVID-19 deaths are projected globally by 01 Mar 2021, with daily deaths peaking at about 15,000/day during this time (IHME 2020). Global efforts to evaluate novel antivirals and therapeutic strategies to treat severe

SARS-CoV-2 infections have intensified, and there is an urgent public health need for rapid development of novel prophylactic therapies, including vaccines, to prevent the spread of this disease.

ModernaTX, Inc. (Sponsor) has developed a rapid-response proprietary vaccine platform based on a messenger RNA (mRNA) delivery system. The platform is based on the principle and observation that cells can take up mRNA, translate it, and then express viral antigen(s) on the cell surface. The delivered mRNA does not enter the nucleus or interact with the genome, is nonreplicating, and is expressed transiently. mRNA vaccines developed with the Sponsor's mRNA-based platform have been used to induce immune responses against infectious pathogens such as cytomegalovirus (CMV; NCT03382405), human metapneumovirus (hMPV) and parainfluenza virus type 3 (PIV3; NCT03392389), Zika virus (NCT04064905), and influenza virus (NCT03076385 and NCT03345043).

The Sponsor has used its mRNA-based platform to develop mRNA-1273, a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine against SARS-CoV-2. mRNA-1273 contains a single mRNA that encodes the full-length SARS-CoV-2 spike (S) protein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilize the S protein into the prefusion conformation. The CoV S protein mediates attachment and entry of the virus into host cells (by binding to the angiotensin converting enzyme 2 [ACE-2] receptor followed by membrane fusion), making it a primary target for neutralizing antibodies that prevent infection (Corti et al 2015; Wang et al 2015; Yu et al 2015; Johnson et al 2016; Chen et al 2017; Wang et al 2018; Kim et al 2019; Widjaja et al 2019). It has been confirmed that the stabilized SARS-CoV-2 S-2P mRNA expresses well in mammalian cells and is in the prefusion conformation (Wrapp et al 2020).

Nonclinical studies have demonstrated that CoV S proteins are immunogenic and that S protein-based vaccines, including those based on mRNA delivery platforms, are protective in animals (Corbett et al 2020a, Corbett et al 2020b, Graham et al 2020, Mercado et al 2020, Tian et al 2020, Tostanoski et al 2020, Vogel et al 2020). Prior clinical studies of vaccines targeting related CoVs and other viruses have assessed the immunogenicity and safety profiles of mRNA-based vaccines (Anderson et al 2020, Folegatti et al 2020, Jackson et al 2020, Keech et al 2020, Mulligan et al 2020, Sadoff et al 2020, Walsh et al 2020).

The clinical development of mRNA-1273 to support its use in the adult population consists of 3 ongoing clinical trials being conducted in the US: a Phase 1, open-label, dose-ranging study (NCT04283461) sponsored by the National Institute of Allergy and Infectious Diseases and a Phase 2a, randomized, observer-blind, placebo-controlled, dose-confirmation study

(NCT04405076) and a Phase 3 randomized, stratified, observer-blind, placebo-controlled study (NCT04470427) conducted by the Sponsor to evaluate the efficacy, safety, and immunogenicity of the vaccine. The development of mRNA-1273 has been accelerated to address the current COVID-19 outbreak, benefiting from the uniquely rapid and scalable manufacturing processes that have been developed for this vaccine.

2.4.1.2 Test Material

mRNA-1273 contains a single mRNA that encodes for SARS-CoV-2 S-2P combined in a mixture of 4 lipids common to the Sponsor's mRNA vaccine platform: SM-102, cholesterol, DSPC, and PEG2000-DMG, respectively. The mRNA-1273 Drug Product is provided as a sterile suspension for injection at a concentration of 0.20 mg/mL in 20 mM Tris buffer containing 87 g/L sucrose and 4.3 mM acetate, at pH 7.5.

The pivotal biologics license application (BLA)-enabling toxicology studies were conducted with mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs. A development mRNA-1273 lot (Lot AMPDP-200005), which was evaluated in the nonclinical pharmacology programs, was prepared with a manufacturing process representative of the GMP mRNA-1273 Drug Product evaluated in the Phase 3 clinical trial (as described in Module 3, Section 3.2.P.2.3 [Scale A process]) and was therefore representative of the clinical presentations. Lot AMPDP-200005 was manufactured at a concentration of 0.5 mg/mL in 20 mM Tris buffer containing 87 g/L sucrose and 10.7 mM acetate, at pH 7.5.

The distribution, toxicity, and genotoxicity associated with mRNA vaccines formulated in LNPs are driven primarily by the composition of the LNPs and, to a lesser extent, by the biologic activity of the antigen(s) encoded by the mRNA. Therefore, the distribution study, Good Laboratory Practice (GLP)-compliant toxicology studies, and in vivo GLP-compliant genotoxicity study conducted with mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs are considered supportive and BLA-enabling for mRNA-1273. SM-102, the novel lipid used in mRNA-1273, was evaluated as an individual agent in GLP-compliant in vitro genotoxicity studies. Additionally, the immunogenicity and toxicity profiles of mRNA-1273 were assessed in a non-GLP repeat-dose study.

2.4.1.3 Nonclinical Testing Program

The nonclinical testing program was designed to adhere to international regulatory guidelines, the intended clinical development program, and traditional pharmacology and toxicology principles and was consistent with International Council for Harmonisation (ICH) guidelines for biological drug development, including ICH S6(R1) (Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals) and appropriate GLP regulations that were applicable when studies were conducted. The pivotal nonclinical safety studies were conducted according to the OECD Principles of Good Laboratory Practice (ENV/MC/CHEM[98]17) or GLP regulations in other countries that are signatories to the OECD Mutual Acceptance of Data agreement (eg, US FDA Code of Federal Regulations Title 21, Part 58: Good Laboratory Practice for Nonclinical Laboratory Studies).

The route of administration of mRNA-1273 used in the nonclinical studies was intramuscular (IM), consistent with the clinical route.

Nonclinical primary pharmacology evaluations were conducted in young and aged mice (BALB/c, BALB/cJ, C57/BL6J, and B6C3F1/J strains), golden Syrian hamsters, and rhesus macaques (nonhuman primates [NHPs]) animal models to characterize the immunogenicity of mRNA-1273, as well as its effects on viral replication and disease progression after SARS-CoV-2 challenge, and to evaluate its safety profile and its potential to promote vaccine-associated enhanced respiratory disease (ERD) after viral challenge, which has previously been observed with vaccines against respiratory syncytial virus (Kim et al 1969), measles (Polack 2007), and in animal models of SARS-CoV vaccination (Czub et al 2005; Deming et al 2007; Bolles et al 2011; Corbett et al 2020a). Additionally, the immunogenicity of mRNA-1273 was assessed in a non-GLP repeat-dose pharmacology study in Sprague Dawley rats.

As SARS-CoV-2 is a newly emerged CoV, there were no established animal models for the evaluation of prophylactic vaccines and therapeutics. Therefore, nonclinical studies were initiated in multiple animal species in order to gain a comprehensive understanding of the effects of mRNA-1273 immunization. Wild-type (WT) mice are a convenient and easy-to-use model to assess vaccine immunogenicity; however, the ACE-2 receptor, the primary route for SARS-CoV-2 binding and entry, differs significantly between mice and humans and, as result, WT SARS-CoV-2 does not infect mice. Therefore, a mouse-adapted SARS-CoV-2 strain, which was developed by the laboratory of Dr. Ralph Baric at the University of North Carolina at Chapel Hill, was used to assess protection of immunized mice from SARS-CoV-2 challenge. Although this mouse-adapted strain infects young mice and induces mild disease symptoms, more severe

symptoms are evident in aged mice (> 12 months) (Dinnon et al 2020). Aged mice were therefore included in the nonclinical pharmacology program to further characterize the immune response and the level of protection from viral challenge. In addition, this model was used to characterize the quality of the immune response to determine if the mRNA-1273-induced immunity would be predicted to promote vaccine-associated ERD. The immunogenicity and protection study in aged mice was designed to directly address this concern through the evaluation of dose levels predicted to drive optimal or suboptimal protection from viral challenge. Golden Syrian hamsters were also selected as a relevant model for evaluation. Wild-type SARS-CoV-2 productively infects hamsters, causing weight loss and moderate to severe lung pathology. This model was selected because it is currently the only animal species in which severe respiratory disease is evident after virus challenge (Chan et al 2020). Nonhuman primates are the species most closely related to humans and have previously recapitulated several important aspects of SARS-CoV infection (Lu et al 2020). Although SARS-CoV-2 infection in NHPs result only in mild clinical symptoms, infection does cause illness with evidence of pneumonia (Johansen et al 2020).

mRNA is degraded within minutes in biological fluids and is unlikely to persist in tissues; therefore, the biodistribution of mRNA-based vaccines formulated in LNPs is predicted to be driven by the LNP characteristics and mRNAs that are within LNPs of the same composition (ie, SM-102-containing LNPs) are expected to distribute similarly to the LNPs. Thus, the distribution of mRNA-1647, an mRNA-based CMV vaccine that contains 6 mRNA sequences combined in SM-102-containing LNPs, assessed in a non-GLP, single IM dose biodistribution study supports the development of mRNA-1273.

The toxicological profile associated with mRNA-based vaccines formulated in SM-102-containing LNPs, including mRNA-1273, is driven primarily by the LNP composition and, to a lesser extent, by the biologic activity of the antigen(s) encoded by the mRNA. The safety and tolerability of 5 mRNA-based vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs (2 Zika virus vaccines: mRNA-1706 and mRNA-1893; 1 hMPV and PIV3 vaccine: mRNA-1653; and 2 CMV vaccines: mRNA-1647 and mRNA-1443) have been evaluated in 6 GLP-compliant repeat-dose toxicity studies in Sprague Dawley rats. Additionally, the Sponsor completed a non-GLP repeat-dose study in Sprague Dawley rats to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses.

The Sprague Dawley rat was selected as the animal model for the toxicity studies because it is an accepted rodent species for nonclinical toxicology testing by regulatory agencies and is a

relevant species to assess the toxicity and immunogenicity of mRNA vaccines, as evidenced by immunogenic responses.

Rats were administered doses up to the anticipated maximum tolerated dose of 150 µg/dose, where clinical observations included vocalization (at 100 µg/dose) and were accompanied by body weight loss and decrease in food consumption. The number of doses selected for the individual GLP studies was 1 more than the intended number of doses proposed for the individual clinical studies. The number of doses ranged from 3 to 4, and doses were administered every 2 weeks or as determined based on the frequency of the anticipated clinical dosing regimen.

SM-102, the novel lipid used in mRNA-1273, was evaluated in genotoxicity studies as an individual agent using a standard ICH S2 (R1) approach (ICH 2011), including a GLP-compliant bacterial reverse mutation (Ames) test in *Salmonella typhimurium* and *Escherichia coli* and a GLP-compliant in vitro micronucleus test in human peripheral blood lymphocytes. In addition, SM-102 was evaluated for in vivo genotoxicity risk in a GLP-compliant in vivo rat micronucleus test using an mRNA-based vaccine formulated in SM-102 LNPs (mRNA-1706) and a non-GLP-compliant in vivo rat micronucleus test using a reporter mRNA (nascent peptide imaging [NPI] luciferase mRNA) formulated in SM-102 LNPs.

2.4.2. PHARMACOLOGY

Table 1 summarizes the nonclinical pharmacology program for mRNA-1273. Pharmacology results are fully summarized in Module 2.6.2.

Table 1: Summary of Pharmacology Program for mRNA-1273

Study Type/Description	Test Article Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Evaluation of immunogenicity, protective capacity, and safety in young mice	mRNA-1273: 0.01, 0.1, 1 or 10 µg SARS-CoV-2 S-2P: 0.01, 0.1, or 1 µg (+ SAS-adjuvant)	Mouse (young), BALB/cJ, C57BL/6J, B6C3F1/J	IM; prime only prime/boost (3-week interval) prime/boost (4-week interval)	No	VRC01
Immunization and protein restimulation in young BALB/c mice with enhanced respiratory disease endpoint monitoring	mRNA-1273: 1 or 10 µg SARS-CoV-2 S-2P: 10 µg (+ alum)	Mouse (young), BALB/c	IM; prime/boost (2-week interval)	No	MOD-3937
Immunogenicity and determination of titer dynamic range in young BALB/c mice	mRNA-1273: 0.0025 through 20 µg	Mouse (young), BALB/c	IM; prime/boost (3-week interval)	No	MOD-3938/ MOD-3940
Immunogenicity and characterization of cellular response in young BALB/cJ mice	mRNA-1273: 0.1, 1, or 10 µg SARS-CoV-1 DIV: 0.2 or 1 µg (+ alum) CDS: 0.2 or 1 µg (+ alum)	Mouse (young), BALB/c	IM; prime/boost (3-week interval)	No	VRC05
Efficacy and enhanced respiratory disease in aged BALB/c mice	mRNA-1273: 0.1 or 1 µg SARS-CoV-1 DIV: 0.1 µg (+ alum)	Mouse (aged), BALB/c	IM; prime/boost (3-week interval)	No	VRC02
Five-week (2 doses: prime/boost) repeat-dose immunogenicity with safety endpoints	mRNA-1273: 0, 30, 60, or 100 µg	Rat, Sprague Dawley	IM; prime/boost (3-week interval)	No	2308-123
Protection from WT SARS-CoV-2 in hamsters using optimal and suboptimal doses	mRNA-1273: 1, 5, or 25 µg	Hamster, golden Syrian	IM; prime/boost (3-week interval)	No	UTMB01

Study Type/Description	Test Article Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Immunogenicity and protective efficacy in NHPs	mRNA-1273: 10 or 100 µg	NHP, rhesus macaque (Indian-origin)	IM; prime/boost (4-week interval)	No	VRC04
Evaluation of immunogenicity and efficacy from expanded dose range in NHPs	mRNA-1273: 2.5, 30, or 100 µg	NHP, rhesus macaque (Indian-origin)	IM; prime/boost (4-week interval)	No	VRC07

Abbreviations: alum = aluminum hydroxide; CDS = conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein; GLP = Good Laboratory Practice; IM = intramuscular; NHP = nonhuman primate; SARS-CoV-1 DIV = double-inactivated severe acute respiratory syndrome coronavirus-1; SARS-CoV-2 = 2019 novel coronavirus; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SAS = Sigma Adjuvant System®; WT = wild-type.

2.4.2.1 Primary Pharmacology

Nonclinical primary pharmacology studies were conducted in young and aged mice (BALB/c, BALB/cJ, C57BL/6J, and B6C3F1/J strains), golden Syrian hamsters, and rhesus macaques (NHPs) animal models to characterize the immunogenicity of mRNA-1273, as well as its effects on viral replication and disease progression after SARS-CoV-2 challenge, and to evaluate its safety profile and potential to promote vaccine-associated ERD after viral challenge (Module 2.6.2). Additionally, the immunogenicity of mRNA-1273 was evaluated in a non-GLP repeat-dose pharmacology study in Sprague Dawley rats (Module 2.6.6; Section 2.6.6.9).

Immunogenicity was characterized in young and aged mice, rats, hamsters, and NHPs through the evaluation of the humoral (immunoglobulin [Ig] G binding antibodies), cellular (T-cell cytokines and T helper [Th] 1-directed CD4+ and CD8+ responses), and/or neutralizing antibody responses elicited by prime-only or prime/boost immunization schedule with a range of mRNA-1273 dose levels.

Protection by mRNA-1273 immunization was assessed in young and aged mice, hamsters, and NHPs immunized with a prime-only or prime/boost schedule, followed by viral challenge with a high dose of SARS-CoV-2 (mouse-adapted SARS-CoV-2 strain; hamsters and NHPs: WT SARS-CoV-2 strain, Washington state isolate). mRNA-1273 dose levels and immunization schedules predicted to drive optimal and suboptimal protection were included in these studies to identify immune signatures for each regimen and to assess the level of protection mediated by different dose levels. Suboptimal dose levels that confer only partial protection were also

included to evaluate the theoretical risk of disease enhancement. Viral load and replication in the upper (nasal turbinates) and lower (lungs) airways, as well as lung pathology and inflammation, were evaluated after viral challenge.

The potential of mRNA-1273 to promote vaccine-associated ERD was assessed in young and aged mice, hamsters, and NHPs through the evaluation of immunogenicity endpoints (IgG1:IgG2a ratio, Th1/Th2 cytokine profiles, and the ratio of binding to neutralizing antibodies) indicative of a protective versus a disease enhancement phenotype, and through monitoring of viral load, viral replication, and histopathological evaluation of lung tissues after viral challenge. The immune signature of mice immunized with mRNA-1273 was compared to that of vaccines that have been associated with ERD (SARS-CoV-1 DIV and conformationally disrupted spike protein [CDS] in alum adjuvant) included as controls in 2 mouse studies.

These studies demonstrated that mRNA-1273 is immunogenic in all the species assessed, showing a dose-dependent response in IgG binding antibody titers and neutralizing antibody activities. Antigen-specific T-cell responses were observed in mice and NHPs. Direct measurement of Th1-directed responses in mice and NHPs, indirect measurement of Th1-directed responses (IgG2a/c:IgG1 antibody subclasses) in mice, and the high levels of neutralizing antibody in all species lessen the concerns regarding the risk of ERD associated with mRNA-1273 immunization. Additionally, a robust and dose-dependent CD8+ T-cell response in mice and a low CD8+ T-cell response in NHPs were observed after boosting with a second dose of mRNA-1273.

In addition to measurements of the immune response, mice, hamsters, and NHPs were challenged with a high dose of SARS-CoV-2 (mice: mouse-adapted SARS-CoV-2 strain; hamsters and NHPs: WT SARS-CoV-2 strain, Washington state isolate); mice and hamsters were challenged intranasally and NHPs were challenged intranasally and intratracheally. Dose levels predicted to be optimal (fully protective) and suboptimal (subprotective) were included in these studies. At higher doses, mice, hamsters, and NHPs were fully protected from viral replication in both lungs and nasal passages. At suboptimal dose levels, animals were either fully protected in the lungs or had reduced viral burden after challenge compared to control animals. There were no observations of increased viral load in animals immunized with suboptimal dose levels of mRNA-1273, which further supports that mRNA-1273 immunization does not promote ERD. Lung histopathology assessments were performed to verify reduction of inflammation, immune complex deposition, and immune cell invasion in response to viral challenge in animals immunized with mRNA-1273 compared to control (PBS) animals. In animals immunized with either optimal or suboptimal mRNA-1273 dose levels, histopathological evaluation of the lungs of mice and NHPs confirmed the lack of evidence of ERD, as demonstrated by minimal

inflammation and no noteworthy neutrophilic-associated alveolar disease or eosinophil-dominant inflammatory response, which have been historically associated with vaccine-associated ERD. In contrast, moderate to severe inflammation involving the small airways and the adjacent alveolar interstitia was elicited by SARS-CoV-2 infection in PBS-control animals.

Overall, nonclinical pharmacology studies demonstrated that mRNA-1273 is well tolerated, is immunogenic, and provides protection from SARS-CoV-2 challenge. In mice, hamsters, and NHPs, a prime-only immunization schedule induced robust SARS-CoV-2-specific binding and neutralizing antibody responses that significantly increased after boosting with a second dose of mRNA-1273. A prime/boost immunization schedule elicited a substantial dose-dependent binding antibody response in rats. In addition, Th1-directed antigen-specific CD4+ and CD8+ T-cell responses were observed in mice and a Th1-directed antigen-specific CD4+ T-cell response was observed in NHPs. mRNA-1273 was fully protective from viral challenge in immunized mice and hamsters when administered as a prime-only or prime/boost schedule at $\geq$ 1 µg/dose and in immunized NHPs when administered as a prime/boost schedule at $\geq$ 30 µg/dose. Furthermore, mRNA-1273 did not promote vaccine-associated ERD in mice, hamsters, and NHPs as demonstrated by balanced Th1/Th2-directed immune responses to immunization, the absence of increased lung pathology, and controlled viral replication after viral challenge when administered at doses predicted to be fully (optimal dose) or partially (suboptimal dose) protective.

2.4.3. PHARMACOKINETICS AND TISSUE DISTRIBUTION

Table 2 lists the nonclinical pharmacokinetics and tissue distribution study with mRNA-1647 in support of the development of mRNA-1273. Biodistribution results are fully summarized in Module 2.6.4.

Table 2: Summary of Pharmacokinetics Program for mRNA-1273

Study Type	Test Article	Species, Strain	Method of Administration, Dose	GLP	Report Number
Single-dose tissue distribution study	mRNA-1647[a]	Rat, Sprague Dawley	IM injection dose of 100 µg on Day 1	No	5002121 Amendment 1

Abbreviations: CMV = cytomegalovirus; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; IM = intramuscular; mRNA = messenger RNA.

[a] mRNA-1647 contains 6 mRNAs that encode the full-length CMV gB and the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex. The 6 mRNAs are combined at a target mass ratio of 1:1:1:1:1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

2.4.3.1 Pharmacokinetics and Tissue Distribution

The results of a biodistribution, non-GLP, single dose, IM injection study of mRNA-1647 in male Sprague Dawley rats (Table 2.6.5.5 [Module 2.6.5] and Report 5002121 Amendment 1) support the development of mRNA-1273. mRNA-1647 is a novel mRNA-based CMV vaccine that contains 6 distinct mRNA sequences (1 that encodes the full-length CMV glycoprotein B [gB] and 5 that encode the pentameric glycoprotein H [gH]/glycoprotein L [gL]/UL128/UL130/UL131A glycoprotein complex) combined at a target mass ratio of 1:1:1:1:1:1 in the Sponsor's standard proprietary SM-102–containing LNPs.

After a single IM dose of mRNA-1647 in male rats, concentrations of the 6 mRNA constructs of mRNA-1647 (ie, gB, gH, gL, UL128, UL130, and UL131A) were detectable in plasma and tissues in a 1:1:1:1:1:1 ratio. The time after dosing at which the maximum concentration was observed in plasma (T_{max}) was 2 hours for all constructs and was followed by a rapid elimination phase with a half-life ($T_{1/2}$) estimated to range from 2.7 to 3.8 hours. The maximum plasma concentration (C_{max}) ranged from 1.60 to 2.30 ng/mL, and the area under the concentration versus time curve (AUC) from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed ($AUC_{[0\text{-}t]}$) ranged from 22.7 to 25.5 ng·h/mL.

Concentrations of the 6 mRNA constructs of mRNA-1647 were detected at levels above the lower limit of quantitation (LLOQ) in most tissues analyzed, except for the kidney, where all levels were below the LLOQ. For highly exposed tissues (injection site [muscle], lymph nodes

[proximal and distal], and spleen), the C_{max} was observed between 2 and 24 hours post dose. The $T_{1/2}$ was calculated using the average tissue $T_{1/2}$ values for the 6 mRNA constructs; the results were 14.9 hours for injection site (muscle), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

As observed with other IM-delivered vaccines, the highest mRNA concentrations were observed at the injection site followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0. Only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for all constructs ranged from 14.9 to 63.0 hours.

2.4.4. TOXICOLOGY

Table 3 summarizes the nonclinical toxicology program used in support of the development of mRNA-1273. Toxicology results are fully summarized in Module 2.6.6.

Table 3: Summary of Toxicology Program for mRNA-1273

Study Type	Test Article	Species, Strain	Method of Administration; Dose	GLP	Report Number
Repeat-Dose Toxicity					
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706[a]	Rat, Sprague Dawley	IM; 0, 13, 65, 129 µg/dose[b] (Days 1, 15, 29)	Yes	5002045
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706[a]	Rat, Sprague Dawley	IM; 0, 10, 50, 100 µg/dose (Days 1, 15, 29)	Yes	5002231
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1653[c]	Rat, Sprague Dawley	IM; 0, 10, 50, 150 µg/dose (Days 1, 15, 29)	Yes	5002033
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1893[d]	Rat, Sprague Dawley	IM; 0, 10, 30, 96 µg/dose (Days 1, 15, 29)	Yes	5002400
6-week (4 doses) repeat-dose study with 2-week recovery	mRNA-1647[e]	Rat, Sprague Dawley	IM; 0, 8.9, 27, 89 µg/dose[f] (Days 1, 15, 29, 43)	Yes	5002034
6-week (4 doses) repeat-dose study with 2-week recovery	mRNA-1443[g]	Rat, Sprague Dawley	IM; 0, 9.6, 29, 96 µg/dose[h] (Days 1, 15, 29, 43)	Yes	5002158
In Vitro Genotoxicity					
Bacterial reverse mutation test	SM-102	*Salmonella typhimurium, Escherichia coli*	Incubation for 67 hours 29 minutes with 0, 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000 µg/plate SM-102 with or without supplemented rat liver fraction	Yes	9601567
Mammalian cell micronucleus test	SM-102	Human peripheral blood lymphocytes	Incubation for 4 and 24 hours with 0, 163, 286, 500 µg/mL SM-102 with or without supplemented rat liver fraction	Yes	9601568
In Vivo Genotoxicity					
In vivo mammalian erythrocyte micronucleus test	mRNA-1706[a]	Rat, Sprague Dawley	Single IV; 0, 0.6/6.2 (F), 1.3/13.5, 2.6/27.0, 5.2/54.1 (M) mg/kg mRNA-1706/SM-102[i, j]	Yes	9800399
In vivo mammalian erythrocyte micronucleus test	NPI luciferase mRNA[k]	Rat, Sprague Dawley	Single IV; 0, 0.32/6.0, 1.07/20, 3.21/60 mg/kg NPI luciferase mRNA/SM-102	No	AF87FU.125012 NGLPICH.BTL
Other Toxicology					
5-week (2 doses) repeat-dose immunogenicity and toxicity study	mRNA-1273[l]	Rat, Sprague Dawley	IM; 0, 30, 60, 100 µg/dose (Days 1 and 22)	No	2308-123

Abbreviations: CMV = cytomegalovirus; CoV = coronavirus; F = female; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; h = hour; IM = intramuscular; IV = intravenous; M = male; min = minute; mRNA = messenger RNA; NPI = nascent peptide imaging; pp65 = phosphoprotein 65; prME = pre-membrane and envelope; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus; SoA = summary of analysis.

[a] mRNA-1706 contains a single mRNA sequence that encodes the prME structural proteins of Zika virus combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 20 mM Tris, 8% sucrose, pH 7.4.

[b] The original dose levels selected were 0, 10, 50, and 100 µg/dose, respectively (SoA issued on 11 October 2016). The calculated dose levels were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064 (SoA issued on 03 May 2017). The change in the reported mRNA content for mRNA-1706 was 29%.

[c] mRNA-1653 contains 2 distinct mRNA sequences that encode the full-length membrane-bound fusion proteins of human metapneumovirus and parainfluenza virus type 3. The 2 mRNAs are combined at a target mass ratio of 1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 7% PG, 1 mM DTPA, pH 7.4.

[d] mRNA-1893 contains a single mRNA sequence that encodes the prME structural proteins of Zika virus in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 100 mM Tris, 7% PG, 1 mM DTPA, pH 7.5.

[e] mRNA-1647 contains 6 mRNAs that encode the full-length CMV gB and the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex. The 6 mRNAs are combined at a target mass ratio of 1:1:1:1:1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

[f] The original dose levels selected were 0, 10, 30, and 100 µg/dose, respectively (SoA issued on 16 Mar 2017). The calculated dose levels were revised based on the updated concentration reported for mRNA-1647 Lot No. MTDP17015 (SoA issued on 31 May 2017). The change in the reported mRNA content for mRNA-1647 was –11%.

[g] mRNA-1443 contains a single mRNA sequence that encodes a phosphorylation mutant of the CMV phosphoprotein 65 protein (ie, deletion of amino acids 435-438) combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

[h] The original dose levels selected were 0, 10, 30, and 100 µg/dose, respectively (SoA issued on 16 Mar 2017). The calculated dose levels were revised based on the updated concentration reported for mRNA-1443 Lot No. MTDP17017 (SoA issued on 30 May 2017). The change in the reported mRNA content for mRNA-1443 was 4%.

[i] A dose-range finding test was performed prior to the main phase of the study, wherein male and female rats (3 animals/sex) were given a single IV injection (doses 2.6/27.0, 3.9/40.6, and 5.2/54.1 mg/kg mRNA-1706/SM-102 for females, and 2.6/27.0, 5.2/54.1, and 10.3/107.1 mg/kg mRNA-1706/SM-102 for males).

[j] The original dose levels selected were 0, 1.0, 2.0, 4.0, 0.5, 1.0, and 2.0 mg/kg mRNA-1706, respectively (SoA issued on 11 October 2016). The calculated dose levels were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064 (SoA issued on 03 May 2017). The change in the reported mRNA content for mRNA-1706 was 29%.

[k] The NPI luciferase mRNA is combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 25 mM Tris, 123 g/L sucrose, 1 mM DTPA, pH 7.5.

[l] mRNA-1273 contains a single mRNA sequence that encodes the full-length SARS-CoV-2 S-2P combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 20 mM Tris, 87 mg/mL sucrose, 10.7 mM sodium acetate, pH 7.5.

2.4.4.1 Repeat-Dose Toxicity

The safety and tolerability of mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs have been evaluated in multiple GLP-compliant, repeat-dose toxicity studies in Sprague Dawley rats at doses up to 150 µg administered every 2 weeks for up to 6 weeks followed by a 2-week recovery period. Additionally, the Sponsor completed a repeat-dose non-GLP study in Sprague Dawley rats to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses (Section 2.4.4.3).

Rats were administered mRNA-based vaccines (mRNA-1706, mRNA-1653, or mRNA-1893) IM once every 2 weeks for 1 month (3 doses) at doses up to 150 µg followed by a 2-week recovery period (Table 2.6.7.7A [Module 2.6.7] and Report 5002045; Table 2.6.7.7B [Module 2.6.7] and Report 5002231; Table 2.6.7.7C [Module 2.6.7] and Report 5002033; Table 2.6.7.7D [Module 2.6.7] and Report 5002400). In addition, rats were administered mRNA-based vaccines (mRNA-1647 or mRNA-1443) IM once every 2 weeks for 6 weeks (4 doses) at doses up to 96 µg followed by a 2-week recovery period (Table 2.6.7.7E [Module 2.6.7] and Report 5002034; Table 2.6.7.7F [Module 2.6.7] and Report 5002158).

The aggregate rat repeat-dose toxicity profile from the GLP studies for mRNA-based vaccines formulated in SM-102-containing LNPs consisted of IM doses ranging from 8.9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks. All doses administered were tolerated. Test article-related in-life observations at ≥ 8.9 µg/dose included reversible or reversing erythema and edema at the injection site and transient increases in body temperature at 6 hours post-dose returning to baseline 24 hours post-dose.

Test article-related, generally dose-dependent clinical pathology changes were observed at ≥ 8.9 µg/dose. Hematology changes included increases in white blood cells, neutrophils, and eosinophils and decreased lymphocytes; coagulation changes included increases in fibrinogen and activated partial thromboplastin time; and clinical chemistry changes included decreases in albumin, increases in globulin, and a corresponding decrease in albumin/globulin ratio. Clinical pathology changes generally reversed or were reversing by the end of the 2-week recovery period. Test article-related, transient cytokine increases were observed at ≥ 8.9 µg/dose at 6 hours post-dose, including in IP-10, MCP-1, and MIP-1-α. Cytokine changes were generally reversing by the end of the 2-week recovery period.

Post-mortem test article-related and generally dose-dependent changes in organ weights and macroscopic and microscopic findings were observed at ≥ 8.9 µg/dose. Organ weight increases

were observed in the spleen, liver, and adrenal gland. Organ weight changes were generally reversing by the end of the 2-week recovery period. Macroscopic changes included skin thickening at the injection site and enlarged lymph nodes. Injection site changes completely recovered, and lymph node changes were recovering by the end of the 2-week recovery period. Microscopic changes included mixed cell inflammation at the injection site; increased cellularity and mixed cell inflammation in the inguinal, iliac, and popliteal lymph nodes; decreased cellularity in the splenic periarteriolar lymphoid sheath; increased myeloid cellularity in the bone marrow; and hepatocyte vacuolation and Kupffer cell hypertrophy in the liver. Microscopic changes were generally reversing by the end of the 2-week recovery period.

2.4.4.2 Genotoxicity

SM-102, the novel lipid used in mRNA-1273, was evaluated in genotoxicity studies as an individual agent using a standard ICH S2 (R1) approach (ICH 2011), including a GLP-compliant in vitro bacterial reverse mutation (Ames) test in *S. typhimurium* and *E. coli* (Table 2.6.7.8A [Module 2.6.7] and Report 9601567) and a GLP-compliant in vitro micronucleus test in human peripheral blood lymphocytes (Table 2.6.7.8B [Module 2.6.7] and Report 9601568).

In addition, SM-102 was evaluated for in vivo genotoxicity risk in a GLP-compliant in vivo rat micronucleus test using a similar mRNA-based vaccine formulated in SM-102 LNPs (Table 2.6.7.9A [Module 2.6.7] and Report 9800399) and in a non-GLP-compliant in vivo rat micronucleus test using a reporter mRNA (NPI luciferase mRNA) formulated in SM-102 LNPs (Table 2.6.7.9B [Module 2.6.7] and Report AF87FU.125012NGLPICH.BTL).

Genotoxicity assessments of the SM-102 lipid concluded that the lipid is not genotoxic in the bacterial mutagenicity and human peripheral blood lymphocytes chromosome aberration assays. Two intravenous in vivo micronucleus assays were conducted with mRNA-based vaccines formulated in the SM-102-containing LNPs. Results from Report AF87FU.125012NGLPICH.BTL were negative up to 3.21/60 mg/kg NPI luciferase mRNA/SM-102, while results from Report 9800399 were positive at 2.6/27.0 mg/kg mRNA-1706/SM-102 in females and at 5.2/54.1 mg/kg mRNA-1706/SM-102 in males, indicating that there was minimal bone marrow toxicity. The equivocal results are likely driven by micronuclei formation secondary to elevated body temperature induced by LNP-driven systemic inflammation at high systemic (intravenous) doses. Overall, the genotoxic risk to humans is considered to be low due to minimal systemic exposure following IM administration, limited duration of exposure, and negative in vitro results.

2.4.4.3 Other Toxicity

A non-GLP study in Sprague Dawley rats was conducted to characterize the immunogenic response and potential toxicity of mRNA-1273 at IM doses levels of 30, 60, and 100 µg/dose administered on Days 1 and 22 (Section 2.6.2.2.6 [Module 2.6.2], Table 2.6.7.17 [Module 2.6.7], and Report 2308-123).

A strong immunogenic response against SARS-CoV-2 S-2P was observed on Day 35, with measured IgG antibody titers above 10^6 at all dose levels. mRNA-1273 had no effect on body weights and limited, transient clinical signs starting at 30 µg/dose consisting of transient dose-dependent injection site edema with or without hindlimb impairment. Clinical pathology findings consisted, in part, of changes associated with inflammation starting at 30 µg/dose. In general, the changes observed are consistent with the results from the previous GLP rat toxicity studies conducted with other mRNA-based vaccines formulated with SM-102-containing LNPs.

2.4.4.4 Summary of Nonclinical Safety Margins

Pending the outcome of the Phase 3 clinical trial with mRNA-1273, a human dose of 100 µg/dose is anticipated to be safe and to provide protective immunization against SARS-CoV-2 infection.

In the rat repeat-dose toxicity studies in which up to 100 µg/dose of mRNA-1273 administered on Day 1 and Day 22, up to 150 µg/dose of mRNA-1706, mRNA-1653, or mRNA-1893 administered once every 2 weeks for 1 month (3 doses), or up to 96 µg/dose of mRNA-1647 and mRNA-1443 administered once every 2 weeks for 6 weeks (4 doses) were evaluated, the administered mRNA/LNP vaccines were well tolerated. Typical vaccine-associated findings included increases in body temperature and spleen weight, changes in cytokine profile reflecting an inflammatory pattern, and injection site reaction characteristics for vaccines, with all findings showing reversibility. In addition, no exaggerated immune reactions were observed in the rat toxicity studies or in the completed immunogenicity studies in mice (young and old), rats, hamsters, and NHPs.

If a 100 µg/dose of mRNA-1273 is well tolerated in a rat with a conservative body weight estimate of 0.30 kg as compared to a human subject with a conservative body weight of 60.0 kg, there is a 200-fold safety margin for the human dose as compared to the rat dose based on body weight. The efficacy and safety profile of the mRNA-1273 vaccine in the Phase 3 clinical trial will be the ultimate determinant in identifying the approved dose for human subjects.

2.4.5. INTEGRATED OVERVIEW AND CONCLUSIONS

In support of the development of mRNA-1273 against SARS-CoV-2, nonclinical pharmacology, biodistribution, and toxicology studies have been completed using mRNA-1273 or other mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs.

Data from the nonclinical testing program presented in this submission support the clinical efficacy and safety of mRNA-1273 at doses up to 100 µg administered twice IM 28 days apart.

- mRNA-1273 induced high levels of binding and neutralizing antibodies in young and aged mice, rats, hamsters, and NHPs; protected against viral replication in the upper (nasal turbinates) and lower (lung) airways; and did not promote vaccine-associated ERD in these nonclinical models.

- The biodistribution of mRNA-based vaccines formulated in LNPs is predicted to be driven by the characteristics of the LNPs. mRNAs that are within similar LNPs (eg, mRNA-1273 and mRNA-1647) are therefore expected to distribute similarly, and the biodistribution study of mRNA-1647 supports the clinical development of mRNA-1273. This study demonstrated that mRNA constructs do not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for the 6 mRNA constructs of mRNA-1647 ranged from 14.9 to 63.0 hours.

- The aggregate repeat-dose toxicity profile of mRNA vaccines that were developed with the Sponsor's mRNA-based platform in rats at IM doses ranging from 8.9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks was similar and consistent despite the fact that the different mRNA constructs encode different antigens. Therefore, the Sponsor proposes that the toxicity associated with mRNA vaccines formulated in similar LNPs is driven primarily by the LNP composition and, to a lesser extent, by the biologic activity of the antigens encoded by the mRNA; therefore, the aggregate GLP repeat-dose rat data are considered to be representative of mRNA vaccines formulated in the same SM-102 LNPs and support the clinical development of mRNA-1273.

Overall, the nonclinical studies demonstrates that mRNA-1273 is safe and well tolerated, is immunogenic, fully protects animals from viral challenge, and does not promote ERD at either optimal or suboptimal dose levels.

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List of Abbreviations

Abbreviation	Definition
AUC	area under the concentration versus time curve
$AUC_{[0-t]}$	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
CDC	Centers for Disease Control and Prevention
CoV	coronavirus
COVID-19	coronavirus disease
C_{max}	maximum observed concentration
CMV	cytomegalovirus
DMID	Division of Microbiology and Infectious Diseases
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
ELISA	enzyme-linked immunosorbent assay
ERD	enhanced respiratory disease
FDA	Food and Drug Administration
FI	formalin inactivated
gH	glycoprotein H
gL	glycoprotein L
GLP	Good Laboratory Practice
H&E	hematoxylin and eosin
hMPV	human metapneumovirus
ICH	International Council for Harmonisation
IFN	interferon
IgG	immunoglobulin G
IL	interleukin
IM	intramuscular(ly)
IN	intranasal(ly)
IND	investigational new drug
LLOQ	lower limit of quantitation
LNP	lipid nanoparticle
MERS	Middle East Respiratory Syndrome
MN	microneutralization
mRNA	messenger RNA
NIAID	National Institute of Allergy and Infectious Diseases

NOAEL	no observed adverse effect level
NTFIX	non-translated human factor IX
PBS	phospate buffered saline
PEG2000-DMG	1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000
Pentamer	glycoprotein H/glycoprotein L/ UL128/UL130/UL131A glycoprotein complex
PFU	plaque-forming units
PIV3	parainfluenza virus type 3
PK	pharmacokinetic(s)
qRT-PCR	quantitative reverse transcription polymerase chain reaction
RSV	respiratory syncytial virus
S	spike
S-2P	spike protein with 2 proline residues introduced for stability in a prefusion conformation
SARS	severe acute respiratory syndrome
SARS-CoV-2	2019 novel coronavirus
SM-102	heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate
$T_{1/2}$	half-life
Th	T helper
T_{max}	time after dosing at which the maximum concentration was observed
Tris	trometamol
US	United States
VRC	Vaccine Research Center
WHO	World Health Organization

2.4.1 Overview of Nonclinical Testing Strategy

2.4.1.1 mRNA-1273 for the Prevention of Coronavirus Infection

ModernaTX, Inc. (Sponsor) is developing mRNA-1273, a novel lipid nanoparticle (LNP)-encapsulated messenger RNA (mRNA)-based vaccine against the 2019 novel coronavirus (CoV; SARS-CoV-2). mRNA-1273 encodes for the full-length spike (S) protein of SARS-CoV-2, modified to introduce 2 proline residues to stabilize the S protein into a prefusion conformation (S-2P). mRNA-1273 consists of an mRNA Drug Substance that is formulated with LNPs composed of 4 lipids: heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy) hexyl)amino)octanoate (SM-102); cholesterol; 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); and 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 (PEG2000-DMG). mRNA-1273 is provided as a sterile liquid for injection at a concentration of 0.5 mg/mL in 20 mM trometamol (Tris) buffer containing 87 mg/mL sucrose and 10.7 mM sodium acetate, at pH 7.5.

2.4.1.2 Background and Rationale for mRNA-1273 Development

Coronaviruses are a large family of viruses that cause illness ranging from the common cold to more severe diseases, such as Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). Coronaviruses are zoonotic, meaning that they can be transmitted from animals to people.

An outbreak of the CoV disease (COVID-19) caused by SARS-CoV-2 began in Wuhan, Hubei Province, China in December 2019 and has spread throughout China and to over 200 other countries and territories, including the United States (WHO 2020). A CoV RNA was quickly identified in some of these patients.

As of 20 Apr 2020, the World Health Organization (WHO) reports more than 2,314,621 confirmed cases and 157,847 deaths globally and have therefore made the assessment that COVID-19 can be characterized as a pandemic (WHO 2020). As of 20 Apr 2020, the US Centers for Disease Control and Prevention (CDC) reported 746,625 confirmed and probable cases of COVID-19 in all 50 states and 5 jurisdictions, with 39,083 attributed and probable deaths (CDC 2020a). The CDC have reported that the highest risk of disease burden is in older adults and populations with certain underlying comorbid conditions such as heart disease, diabetes, and lung disease (CDC 2020b).

There is currently no vaccine against SARS-CoV-2. Global efforts to evaluate novel antivirals and therapeutic strategies to treat severe SARS-CoV-2 infections have intensified, but no proven therapeutic currently exists. Therefore, there is an urgent public health need for the rapid development of novel interventions to prevent the spread of this disease.

The Sponsor has developed a rapid-response proprietary vaccine platform based on an mRNA delivery system. The platform is based on the principle and observation that cells in vivo can take up mRNA, translate it, and then express protein viral antigen(s) on the cell surface. The delivered mRNA does not enter the cellular nucleus or interact with the genome, is nonreplicating, and is expressed transiently. mRNA vaccines have been used to induce immune responses against infectious pathogens such as cytomegalovirus (CMV) (NCT03382405), human metapneumovirus (hMPV) and parainfluenza virus type 3 (PIV3) (NCT03392389), and influenza virus (NCT03076385 and NCT03345043).

The Sponsor is using its mRNA-based platform to develop mRNA-1273, a novel LNP-encapsulated mRNA-based vaccine against SARS-CoV-2. mRNA-1273 encodes for SARS-CoV-2 S protein stabilized in the prefusion conformation with 2 proline substitutions within the heptad repeat 1 domain (S-2P). The CoV S protein mediates attachment and entry of the virus into host cells (by fusion), making it a primary target for neutralizing antibodies that prevent infection (Corti et al 2015; Wang et al 2015; Yu et al 2015; Johnson et al 2016; Chen et al 2017; Wang et al 2018; Kim et al 2019; Widjaja et al 2019). It has been confirmed that the stabilized SARS-CoV-2 S-2P expresses well and is in the prefusion conformation (Wrapp et al 2020).

Nonclinical studies have demonstrated that CoV S proteins are immunogenic and that S protein-based vaccines, including those based on mRNA delivery platforms, are protective in animals. Prior clinical studies of vaccines targeting related CoVs and other viruses have demonstrated that mRNA-based vaccines are safe and immunogenic. It is therefore anticipated that mRNA-1273 will generate robust immune responses against SARS-CoV-2.

The mRNA-1273 vaccine is currently being evaluated for safety and immunogenicity in a dose-ranging Phase 1 study (NCT04283461) sponsored by the Division of Microbiology and Infectious Diseases (DMID) of the National Institute of Allergy and Infectious Diseases (NIAID). The development of this vaccine is being accelerated as, if it is demonstrated to be safe and immunogenic, it may be used to address the current COVID-19 outbreak, as a result of the uniquely rapid and scalable manufacturing process for mRNA-1273.

2.4.1.3 Nonclinical Testing Strategy for mRNA-1273

The mRNA-1273 vaccine is currently being evaluated for safety and immunogenicity in a dose-ranging Phase 1 study (NCT04283461) sponsored by DMID under US investigational new drug (IND) application #19635. A letter from DMID authorizing the US Food and Drug Administration (FDA) to refer to IND #19635 to support review of this IND is provided in Module 1.4. In addition, nonclinical evaluations in mice are being conducted by the Vaccine Research Center (VRC) in parallel with the Phase 1 study. The interim data for these nonclinical studies are summarized in this document and are on file with the VRC.

In a non-Good Laboratory Practice (GLP) study conducted by the VRC, the immunogenicity and efficacy of mRNA-1273 administered via intramuscular (IM) injection on a prime/boost or prime-only schedule were evaluated in BALB/c mice. The study included assessment of antibody binding and neutralization activity as well as protection monitoring from a homologous virus strain challenge after mRNA-1273 vaccination. The in-life portion of this study is complete and endpoint assay analysis is ongoing. The interim data for this study are briefly summarized in this document and are on file with the VRC.

Concern has been raised over the potential for vaccine-associated disease enhancement associated with vaccines against SARS-CoV-2. This concern has been triggered by preclinical work on SARS-CoV and MERS-CoV vaccines, the experience with feline infectious peritonitis virus and vaccines in cats, and enhanced disease seen with respiratory syncytial virus (RSV), measles, and dengue vaccines in humans. The concern is that a SARS-CoV-2 vaccine could cause enhanced disease and specifically enhanced respiratory disease (ERD) in vaccinees that are subsequently exposed to wild-type SARS-CoV-2. Nonclinical studies focused on the evaluation of enhanced disease with in vivo animal models and analysis of clinical samples will be used to make judgements about the likelihood of vaccine-associated ERD. Ongoing in vivo studies in animal models include immunization with limiting doses of mRNA-1273 to allow breakthrough SARS-CoV-2 infection in immunized mice. Signs of enhancement would be increased illness compared to unvaccinated mice, higher viral titers in lung, and lung pathology showing eosinophilia, mucus production, or neutrophilic alveolitis. In addition, antibody and T-cell responses are being analyzed. Binding antibody titer and neutralizing titer are being measured, as a high ratio of binding to neutralization may indicate the possibility of immune complex deposition and complement activation. However, ratios such as these are highly dependent upon the performance of the assays in question. As SARS-CoV-2 neutralization assays are, to this point, still highly variable and in the process of being further developed, optimized, and validated, study measurement should not be considered a strong predictor of clinical outcomes, especially in the absence of results from a positive control that has demonstrated disease enhancement.

To address the theoretical risk of ERD, the nonclinical safety, immunogenicity, and efficacy of mRNA-1653, a similar mRNA-based vaccine formulated in SM-102-containing LNPs was evaluated in the hMPV cotton rat model. The study included a comparison to a formalin-inactivated (FI) virus control and an evaluation of the potential for vaccine ERD at mRNA-1653 dose levels inducing suboptimal neutralizing antibody and permitting detectable virus replication after challenge. The in-life portion of this study is complete and endpoint assay analysis is ongoing. These data are summarized in this document and have also been submitted in Module 4.

In support of development of mRNA-1273, nonclinical biodistribution and safety studies have been completed by the Sponsor with similar mRNA-based vaccines formulated in SM-102-containing LNPs. The results of these studies are briefly summarized in this document. Detailed information, including the reports for these studies of mRNA-based vaccines developed using the Sponsor's standard proprietary SM-102-containing LNP matrix, are provided in CBER MF# 19622. A letter authorizing FDA to review all relevant nonclinical information contained in this master file to support the review of this IND is provided in Module 1.4.

The biodistribution of mRNA-1647, a similar mRNA-based vaccine formulated in SM-102-containing LNPs, was evaluated in a non-GLP, single-dose, IM injection study in Sprague Dawley rats. The objectives of this study were to determine the tissue distribution and pharmacokinetic (PK) characteristics of mRNA-1647 following IM administration. The biodistribution of mRNA-based vaccines formulated in LNPs is predicted to be driven by the LNP characteristics. Therefore, mRNAs that are within an LNP of the same composition (eg, mRNA-1273 and mRNA-1647) are expected to distribute similarly.

The safety and tolerability of similar mRNA-based vaccines formulated in an SM-102-containing LNP matrix encapsulating mRNA constructs that encode for various antigens have been evaluated in multiple GLP-compliant repeat-dose toxicity studies in Sprague Dawley rats with a 2-week recovery period. A 2-week recovery period was selected based on previous studies in this animal model and is anticipated to demonstrate the reversibility of the findings. The Sponsor proposes that the toxicity associated with mRNA vaccines formulated in LNP formulations are driven primarily by the LNP composition and, to a lesser extent, the biologic activity of the expressed antigens of the mRNA vaccine. This is supported by the consistency of the aggregate rat repeat-dose toxicity profile observed in these GLP studies at IM doses ranging from 9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks and is considered to be representative of mRNA vaccines formulated in the same SM-102 LNP matrix, differing only by the encapsulated mRNA sequence(s). Thus, the aggregate toxicity results from these studies support the development of mRNA-1273.

Additionally, the Sponsor conducted a non-GLP study in Sprague Dawley rats to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses (Study 2308-123). The study is currently ongoing, and preliminary results are included in this document (data on file).

The Sprague Dawley rat was chosen as the animal model for these studies because it is an accepted rodent species for preclinical testing by regulatory agencies and is a relevant species to assess the toxicity of mRNA vaccines, as evidenced by immunogenic response. The IM route of administration was selected in these studies because this is the intended route of administration in humans.

SM-102, the novel lipid used in the mRNA-1273 LNP formulation, was evaluated in genotoxicity studies as an individual agent using a standard International Council for Harmonisation (ICH) S2 (R1) approach (ICH 2011), including a GLP-compliant bacterial reverse mutation (Ames) test and a GLP-compliant in vitro micronucleus test in human peripheral blood lymphocytes. In addition, SM-102 was evaluated for in vivo genotoxicity risk in a GLP-compliant in vivo rat micronucleus test using a similar mRNA-based vaccine formulated in SM-102.

The nonclinical studies supporting the development of mRNA-1273 are listed in Table 1, and the completed and preliminary results are summarized in the sections that follow.

Table 1: Nonclinical Studies Supporting the Development of mRNA-1273

Study Title	Study Number	Test System	eCTD Reference
In Vivo Pharmacology Studies			
Protection monitoring from homologous virus strain challenge after mRNA-1273 vaccination in BALB/c mice	VRC01	BALB/c, F only	Data on file with VRC (ongoing)
Safety Pharmacology Studies			
Safety, immunogenicity, and efficacy of mRNA-1653 in the cotton rat hMPV model[a]	XV-216	hMPV cotton rat F only	4.2.1.3 (ongoing)
Biodistribution Studies			
A single dose intramuscular injection tissue distribution study of mRNA-1647 in male Sprague-Dawley rats[b]	5002121 Amendment 1	Sprague Dawley rat, M only	CBER MF #19622
Repeat-dose Toxicity Studies			
Zika: A 1-month (3 doses) intramuscular injection toxicity study of mRNA-1706 in Sprague Dawley rats with a 2-week recovery period[c, d]	5002045	Sprague Dawley rat, M and F	CBER MF #19622
A 6-week (4 doses) intramuscular injection toxicity study of mRNA-1647 in Sprague-Dawley rats followed by a 2-week recovery period[c, b]	5002034	Sprague Dawley rat, M and F	
A 6-week (4 doses) intramuscular injection toxicity study of mRNA-1443 in Sprague-Dawley rats followed by a 2-week recovery period[c, e]	5002158	Sprague Dawley rat, M and F	
A 1-month (3 doses) study of mRNA-1653 by intramuscular injection in Sprague Dawley Rat with a 2-week recovery period[a, c]	5002033	Sprague Dawley rat, M and F	
A 1-month (3 doses) intramuscular injection toxicity study of mRNA-1893 in Sprague-Dawley rats followed by a 2-week recovery period[a, f]	5002400	Sprague Dawley rat, M and F	
Genotoxicity Studies			
SM-102 bacterial reverse mutation test in *Salmonella typhimurium* and *Escherichia coli*[a]	9601567	*S typhimurium* and *E coli* strains, in vitro	
SM-102 in vitro mammalian cell micronucleus test in human peripheral blood lymphocytes[a]	9601568	Human peripheral blood lymphocytes	
Zika mRNA: mammalian erythrocyte micronucleus test in rat[c, d]	9800399	Sprague Dawley rat, M and F	
Other Toxicity Studies			
A non-GLP repeat dose immunogenicity and toxicity study of mRNA-1273 by intramuscular injection in Sprague Dawley rats	2308-123	Sprague Dawley rat, M and F	Data on file (ongoing)

Abbreviations: CMV = cytomegalovirus; eCTD = electronic common technical document; F = female; GLP = Good Laboratory Practice; hMPV = human metapneumovirus; M = male; mRNA = messenger RNA; PIV3 = parainfluenza virus type 3; SM-102 = heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate; VRC = Vaccine Research Center.

[a] mRNA-1653 consists of 2 distinct mRNA sequences that encode the full -length membrane-bound F proteins of hMPV and PIV3.

[b] mRNA-1647 consists of 6 distinct mRNA sequences that encode the full length CMV glycoprotein B, and the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex (Pentamer).

[c] A GLP-compliant study.

[d] mRNA-1706 encodes a specific Zika virus pre-membrane and envelope polypeptide.

[e] mRNA-1443 encodes a phosphorylation mutant of the CMV pp65 protein.

[f] mRNA-1893 encodes a specific Zika virus pre-membrane and envelope polypeptide.

2.4.2 Pharmacology

2.4.2.1 In Vivo Pharmacology

The immunogenicity and efficacy of mRNA-1273 were evaluated by the VRC in BALB/c mice (Study VRC01). The study included assessment of antibody binding and neutralization activity as well as protection monitoring from a homologous virus strain challenge after mRNA-1273 vaccination. The study design is presented in Table 2. The in-life portion of this study is complete, and endpoint assay analysis is ongoing.

Groups of female BALB/c mice were administered mRNA-1273 (1, 0.1, and 0.01 µg) or phosphate buffered saline (PBS) in a volume of 0.05 mL via IM injection in the right hind leg. Mice were vaccinated on a Week 0 (prime) and Week 3 (boost) or Week 0 (prime) and Week 4 (boost) administration schedule. Sera were collected at Week 2 for all animals, at Week 5 for animals who received a boost injection at Week 3 and at Week 4 (pre-dose) for animals who received a boost injection at Week 4. Samples were assessed for antibody binding to SARS-CoV-2 stabilized prefusion S protein via enzyme-linked immunosorbent assay (ELISA). Positive serum was tested for neutralizing activity (for the 1 and 0.1 µg dose group only) against homologous SARS-CoV-2 via a pseudotyped lentivirus reporter neutralization assay. Additional groups of mice were administered a single prime-only IM injection of mRNA-1273 (10, 1, and 0.1 µg) or PBS on Week 0. Sera were collected from these mice every 2 weeks to assess antibody binding to SARS-CoV-2 stabilized prefusion S protein via ELISA.

To evaluate protection mediated by mRNA-1273, mice were challenged with a mouse-adapted SARS-CoV-2 virus strain, which contains 2 targeted amino acid mutations in the receptor-binding domain to facilitate binding to the mouse ACE-2 receptor. Using this virus strain, mice vaccinated with 1, 0.1, or 0.01 µg mRNA-1273 or PBS were inoculated intranasally (IN) with 10^5 plaque-forming units (PFU) per mouse. Mice on the Week 0 and Week 3 vaccination schedule were challenged 5 weeks after boost (Week 8), and mice on the Week 0 and Week 4 vaccination schedule were challenged 3 weeks after boost (Week 7). Mice vaccinated with a single prime-only injection of 10 µg, 1, or 0.1 µg mRNA-1273 or PBS at Week 0 were challenged 7 weeks post-prime. Animals were followed for 4 days post-challenge for endpoint monitoring, including assessment of weight loss, plaque assays on tissue from lungs and nasal turbinates, RNA quantification for cytokines and viral genome copies, and pathology. Day 2 results of viral load in the nose and lung

are included; data from Day 4 post-challenge are pending. Assessment of viral genome quantification, RNA cytokine levels, and lung pathology are ongoing. Additional measurements relating to the quality of antibody response and the T-helper (Th) 1 and Th2 response balance are also underway. These results will be submitted to IND #19745 when available.

Table 2: Study Design of Vaccine Research Center Study VRC01

BALB/c Mice Treatment Groups[a]	Vaccination Schedule[b]	Sera Collection[c]	Challenge Schedule[d]	Endpoint Monitoring Post-challenge
1, 0.1, and 0.01 µg mRNA-1273 or PBS	Week 0 (prime) Week 3 (boost)	Week 2 Week 5	Week 8	Weight loss, plaque assays on tissue from lungs and nasal turbinates, RNA quantification for cytokines and viral genome copies, and pathology
1, 0.1, and 0.01 µg mRNA-1273 or PBS	Week 0 (prime) Week 4 (boost)	Week 2 Week 4 (pre-dose)	Week 7	
10, 1, and 0.1 µg mRNA-1273 or PBS	Week 0 (prime)	Week 2 Week 4	Week 7	

Abbreviations: ELISA = enzyme-linked immunosorbent assay; PBS = phosphate-buffered saline; PFU = plaque-forming units; SARS-CoV-2 = severe acute respiratory syndrome coronavirus; S protein = spike protein.

[a] Doses were administered at an injection volume of 0.05 mL.

[b] Mice were vaccinated via intramuscular injection in the right hind leg.

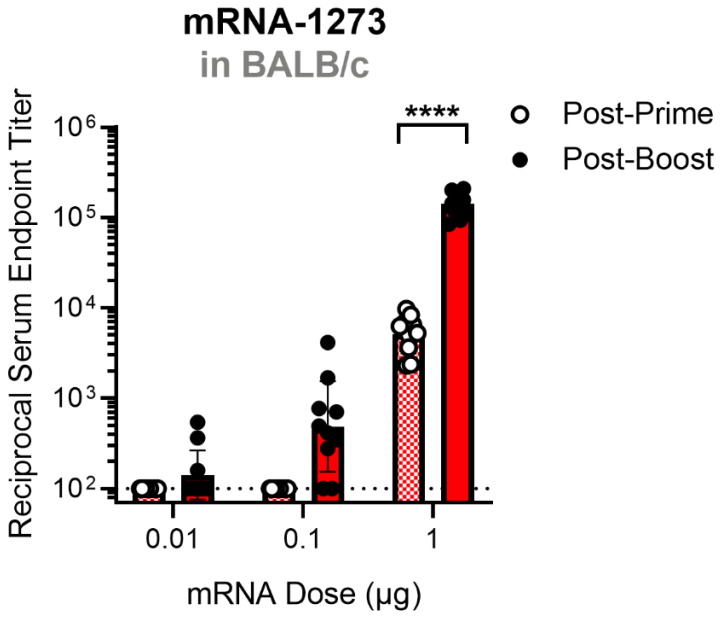
[c] Samples were assessed for antibody binding to SARS-CoV-2 stabilized prefusion S protein via ELISA. Positive serum was tested for neutralizing activity (for the 1 and 0.1 µg dose groups only) against homologous SARS-CoV-2 via a pseudotyped lentivirus reporter neutralization assay.

[d] Mice were inoculated intranasally with 10^5 PFU of a mouse-adapted SARS-CoV-2 virus strain.

Source Vaccine Research Center Study VRC01 data on file.

mRNA-1273 induced a dose-dependent antibody response in BALB/c mice. For mice that received 1 µg mRNA-1273. there was a significantly higher antibody response (p-value < 0.0001) post-boost (Figure 1). In addition, mice immunized with 1 µg of mRNA-1273 had significantly higher neutralizing antibody responses than mice immunized with 0.1 µg mRNA-1273 (p-value < 0.0001); mice immunized with 0.1 µg dose did not have detectable neutralizing activity (Figure 2).

Figure 1: Immunogenicity of mRNA-1273 in BALB/c Mice

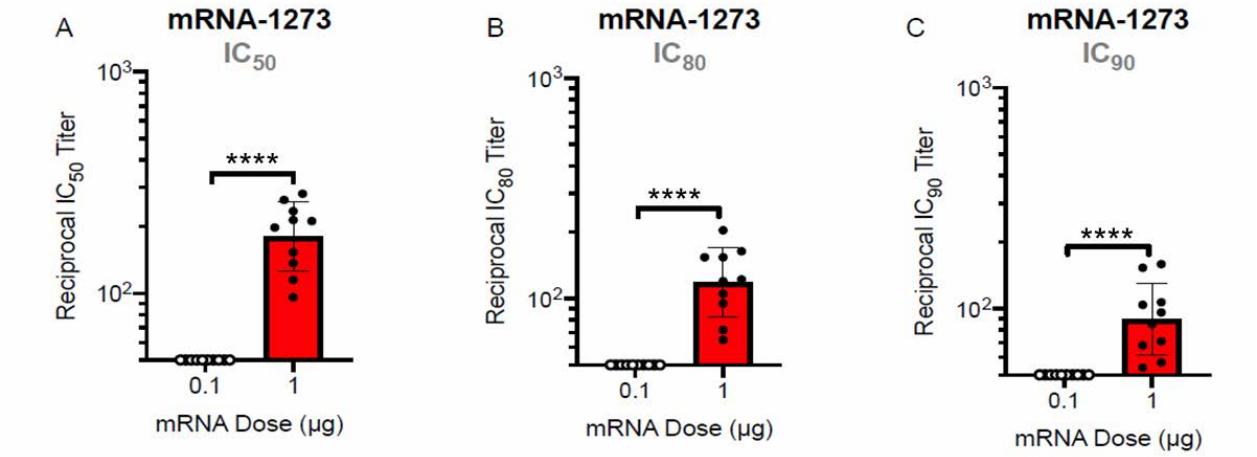


Abbreviations: mRNA = messenger RNA.

Note: Each symbol represents an individual mouse, bars represent geometric mean titers, and error bars indicate geometric SD. Two-way analysis of variance was used to compare post-prime and post-boost responses (* = p-value <0.05, ** = p-value < 0.01, *** = p-value < 0.001, **** = p-value < 0.0001).

Source Vaccine Research Center Study VRC01 data on file.

Figure 2: Neutralizing Antibody Responses After mRNA-1273 Immunization



Abbreviations: IC = inhibitory concentration; mRNA = messenger RNA.

Note: Sigmoidal curves, taking averages of duplicates at each serum dilution, were generated from relative light unit readings. The 50% (IC_{50}; A), 80% (IC_{80}; B), and 90% neutralization (IC_{90}; C) titers were calculated considering uninfected cells as 100% neutralization and cells transduced with only virus as 0% neutralization. Each symbol represents an individual mouse, bars represent geometric mean titers, and error bars indicate geometric SD. Unpaired T-tests were used to compare 0.1 µg and 1 µg doses (* = p-value <0.05, ** = p-value < 0.01, *** = p-value < 0.001, **** = p-value < 0.0001).

Source: Vaccine Research Center Study VRC01 data on file.

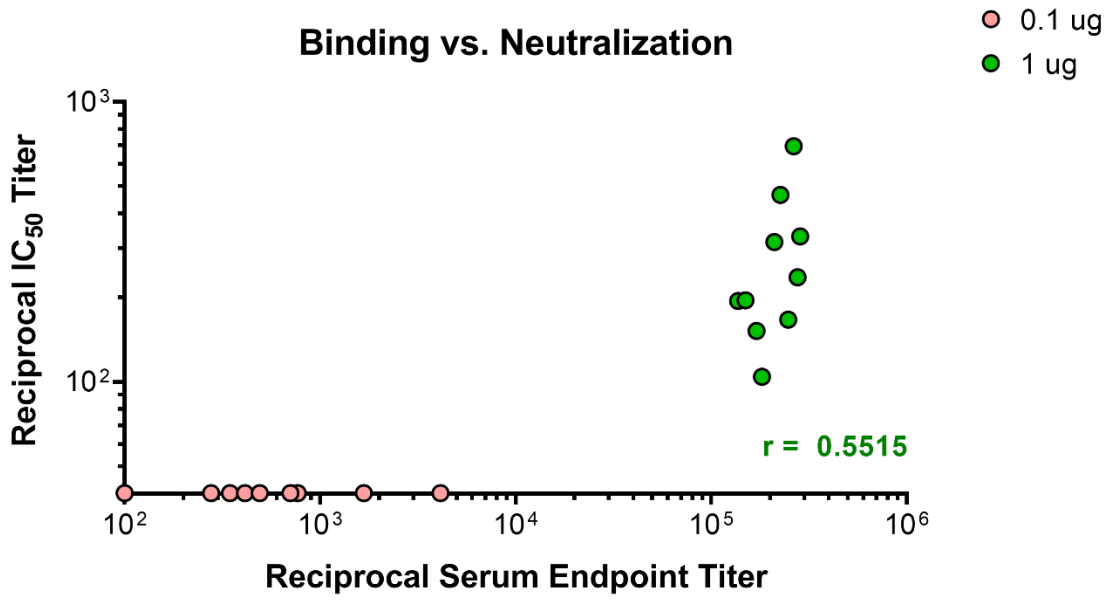
As observed in Figure 1 and Figure 2, at the 0.1 µg mRNA-1273 dose level, binding antibody to the S protein was detected but no neutralization was measured. At the 1 µg mRNA-1273 dose level, both binding antibody and neutralization antibody titers were measured, and a correlation was observed between the 2 values (Figure 3).

In 1966, an FI-RSV vaccine was evaluated in infants and young children. Serum samples from these individuals were found to have moderately high levels of antibodies but were deficient in neutralizing activity, and the vaccine failed to afford protection against infection or disease caused by RSV. Moreover, those who were immunized with the FI-RSV vaccine developed more serious lower respiratory tract disease (Murphy and Walsh 1988). In the youngest cohort of children who received the FI-RSV vaccine, 80% of those infected required hospitalization compared to 5% of placebo recipients, and 2 infants died. In the evaluation of ERD driven by FI-RSV, one mechanism of disease enhancement has been associated with high levels of binding antibody with correspondingly low levels of neutralizing antibody, driving immune complex deposition and complement activation, as was observed in lungs from babies who died from the FI-RSV vaccination trial. This association is thought to result from the improper folding of the RSV fusion protein, caused by the chemical inactivation process. In studies where conformationally stabilized fusion protein was used as the vaccine antigen, binding antibody and neutralization titers showed a strong correlation and a ratio that was significantly higher than that observed from studies with

FI-RSV. Of note, there is not a specified neutralizing antibody to binding antibody ratio that is associated with immune complex deposition and complement activation, and an assessment of a theoretical risk for ERD requires a comparison of the neutralizing antibody to binding antibody ratios between the vaccine candidate and a positive control for which evidence of ERD has been captured.

Although the ratio of binding to neutralization titers may be used to evaluate the theoretical possibility of immune complex deposition and complement activation, such an analysis would not be predictive from the study results available, as a comparator was not included in the completed mouse studies. Ongoing studies in mice include comparators, such as double-inactivated SARS-CoV from which evidence of ERD has been observed in past investigations.

Figure 3: Neutralizing Antibody Titers Correlate to Binding Antibody Titers in Mice Immunized With mRNA-1273



Abbreviations: IC = inhibitory concentration; r = Spearman coefficient.

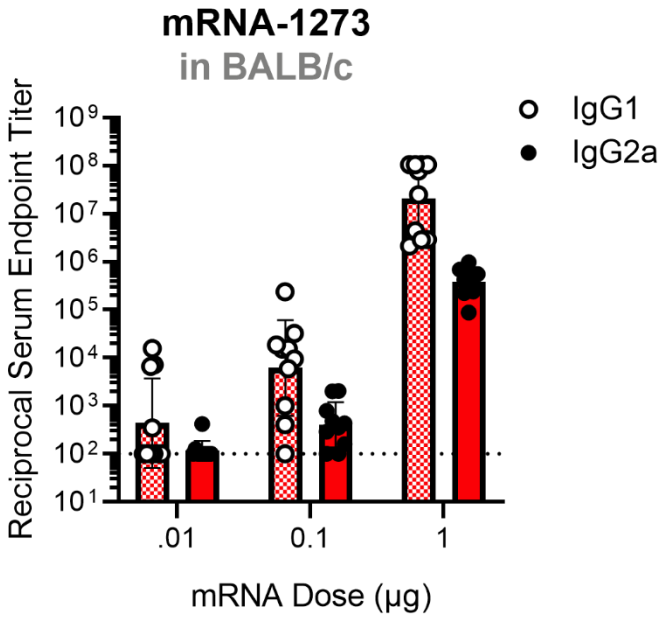
Note: Results are from BALB/c mice immunized with 0.1 µg and 1 µg doses of mRNA-1273 on Week 0 (prime) and Week 3 (boost). Antibody binding and neutralization titers were compared by Spearman correlation at both doses.

Source: Vaccine Research Center Study VRC01 data on file.

In mice, the ratio of immunoglobulin G (IgG) antibody subclasses can be considered a surrogate to indicate Th1/Th2 response. The IgG1 subclass is associated with Th2 response, whereas IgG2a is associated with a Th1 response given that IFN gamma signaling drives this class switching. If this Th1 T-cell signaling was not present (ie a strong Th2-skewed response profile) then IgG2a levels would likely be low.

Two weeks post-boost, sera were analyzed for IgG subclasses from BALB/c mice vaccinated with increasing doses of mRNA-1273. High titers of both IgG1 and IgG2a were detected. A Th2-focused response (predominantly IgG1), was not observed (Figure 4).

Figure 4: Antibody Subclass Responses Following mRNA-1273 Immunization



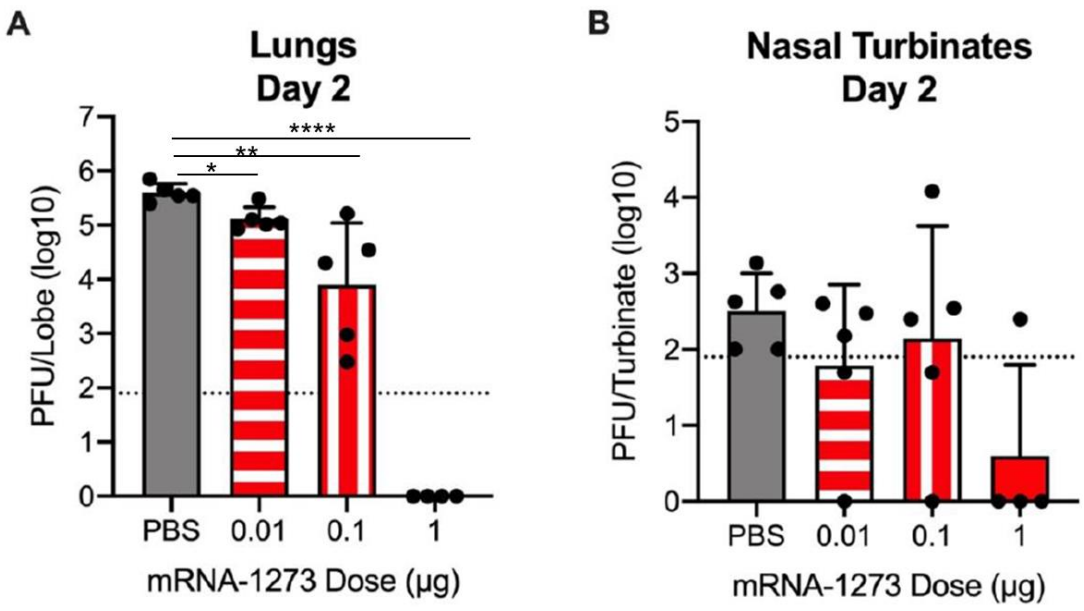
Abbreviations: IgG = immunoglobulin G; mRNA = messenger RNA; Th = T-helper.

Note: Results are from BALB/c mice immunized with 0.1 µg and 1 µg doses of mRNA-1273 on Week 0 (prime) and Week 3 (boost). Antibody binding and neutralization titers were compared by Spearman correlation at both doses. The IgG1 subclass IgG subclass indicative of Th2 response. The IgG2a subclass is indicative of Th1 response.

Source: Vaccine Research Center Study VRC01 data on file.

BALB/c mice immunized with mRNA-1273 at Week 0 (prime) and Week 3 (boost) were challenged IN 5 weeks post-boost (Week 8) with 10^5 PFU of mouse-adapted SARS-CoV-2. Two days after challenge, mice immunized with 1 µg of mRNA-1273 were protected from viral replication in the lungs and nasal turbinates (Figure 5). A dose effect was observed, with 0.1 µg mRNA-1273 dose reducing lung viral load by approximately 2 logs and 0.01 µg mRNA-1273 reducing lung viral load by approximately 0.5 log. Unimmunized mice challenged with a mouse-adapted SARS-CoV-2 had viral loads of about 10^6 PFU per lung lobe. Data from Day 4 post-challenge and measurement of RNA and pathology are still pending.

Figure 5: Efficacy of mRNA-1273 Immunization Using a Three-Week Prime/Boost Interval



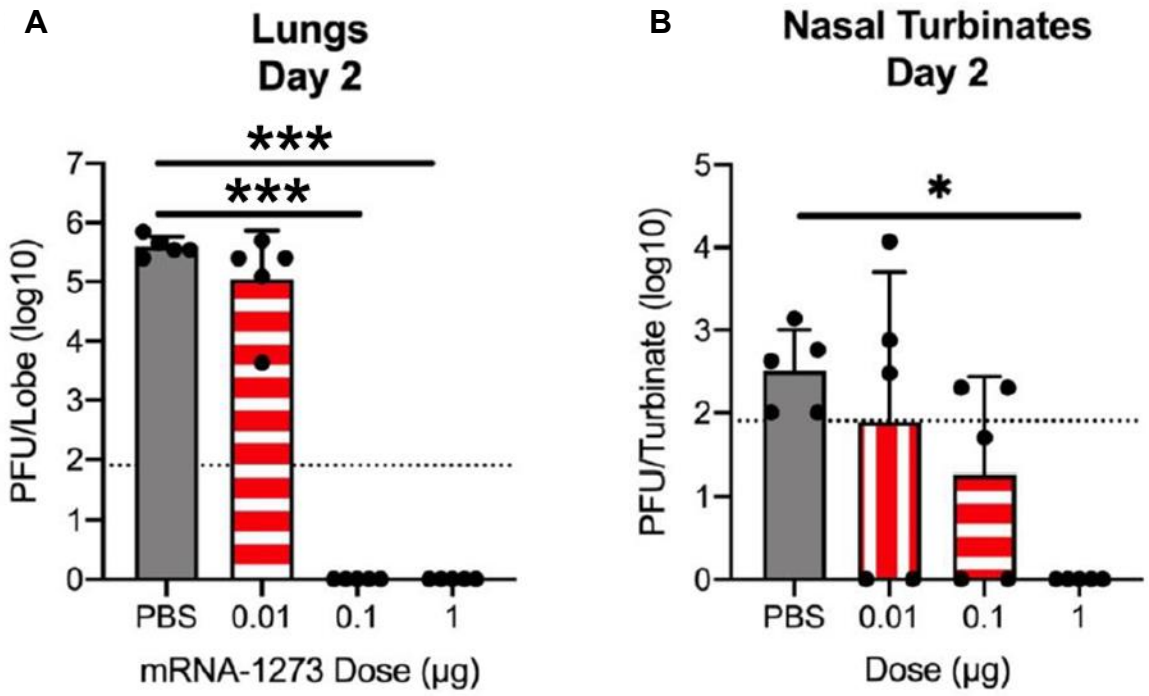
Abbreviations: PFU = plaque-forming units.

Note: On Day 2 post-challenge, mouse lungs (A) and noses (B) were homogenized and assessed for viral load by plaque assay (* = p-value <0.05, ** = p-value < 0.01, *** = p-value < 0.001, **** = p-value < 0.0001). The dotted lines represent the assay limits of detection.

Source: Vaccine Research Center Study VRC01 data on file.

BALB/c mice immunized with mRNA-1273 at Week 0 (prime) and Week 4 (boost) were challenged IN 3 weeks post-boost (Week 7) with 10^5 PFU of mouse-adapted SARS-CoV-2. Two days after challenge, mice immunized with 1 and 0.1 µg of mRNA-1273 were protected from viral replication in the lungs (Figure 6A). Mice immunized with 1 µg of mRNA-1273 were protected from viral replication in the nose (Figure 6B). Data from Day 4 post-challenge and measurement of RNA and pathology are still pending.

Figure 6: Efficacy of mRNA-1273 Using a Four-Week Prime/Boost Interval



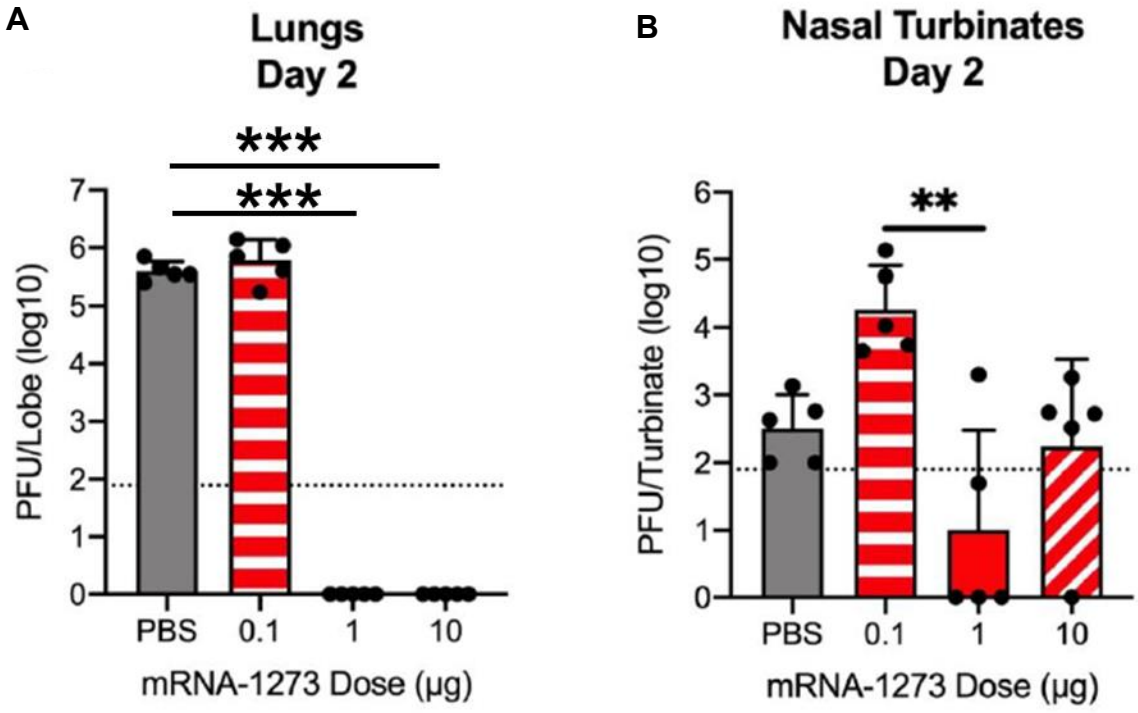
Abbreviations: PFU = plaque-forming units.

Note: On Day 2 post-challenge, mouse lungs (A) and noses (B) were homogenized and assessed for viral load by plaque assay (* = p-value <0.05, ** = p-value < 0.01, *** = p-value < 0.001, **** = p-value < 0.0001). The dotted lines represent the assay limits of detection.

Source: Vaccine Research Center Study VRC01 data on file.

BALB/c mice immunized with a single dose of mRNA-1273 at Week 0 (prime) were challenged IN 7 weeks post-immunization (Week 7) with 10^5 PFU of mouse-adapted SARS-CoV-2. Mice immunized with 1 dose of 10 µg or 1 µg of mRNA-1273 were protected from viral replication in the lung following challenge 7 weeks later (Figure 7). A single dose did not generate detectable serum neutralizing activity, suggesting a potential contribution of T cells or Fc-mediated antibody effector functions to immunity. Data from Day 4 post-challenge and measurement of RNA and pathology are still pending.

Figure 7: Efficacy of a Single Dose of mRNA-1273



Abbreviations: PFU = plaque-forming units.

Note: On Day 2 post-challenge, mouse lungs (A) and noses (B) were homogenized and assessed for viral load by plaque assay (* = p-value <0.05, ** = p-value < 0.01, *** = p-value < 0.001, **** = p-value < 0.0001). The dotted lines represent assay limits of detection.

Source: Vaccine Research Center Study VRC01 data on file.

In conclusion, mRNA-1273 is immunogenic in BALB/c mice, demonstrating a dose-dependent antibody response as measured in both binding and neutralizing antibody titers. A strong correlation was measured between binding antibody and neutralization, and low binding titers at the 0.1 µg dose did not elicit measurable neutralization titers. In addition, a single dose of mRNA-1273 elicited binding antibody titers, with no neutralization titers observed. Mice from this study were challenged with mouse-adapted SARS-CoV-2 virus, and preliminary results indicate that mRNA-1273-induced immunity is protective after challenge. Mice immunized with a single dose of 10 or 1 µg mRNA-1273 were protected from viral replication in the lung following challenge 7 weeks after vaccination, despite the absence of measurable neutralization titers (data not shown). Mice immunized with 2 doses of either 1 or 0.1 µg mRNA-1273 were also protected, dependent upon the dosing schedule. Other results from challenge study, including cytokine measurements and pathology, are ongoing.

2.4.2.2 Safety Pharmacology

The immunogenicity, efficacy, and safety of mRNA-1653, a similar mRNA-based vaccine formulated in SM-102-containing LNP, was evaluated by the Sponsor in a non-GLP study in the hMPV cotton rat challenge model (Study XV-216). The in-life portion of this study is complete, and endpoint assay analysis is ongoing. mRNA-1653 is a novel mRNA-based vaccine being developed for vaccination to induce protective immunity against respiratory diseases associated with hMPV and PIV3. mRNA-1653 consists of 2 distinct mRNA sequences that encode the full-length membrane-bound F proteins of hMPV and PIV3. The 2 mRNA Drug Substances are formulated at a target mass ratio of 1:1 in a mixture of 4 lipids to form a drug lipid complex (LNP). The 4 lipids are SM-102; PEG2000-DMG; DSPC; and cholesterol.

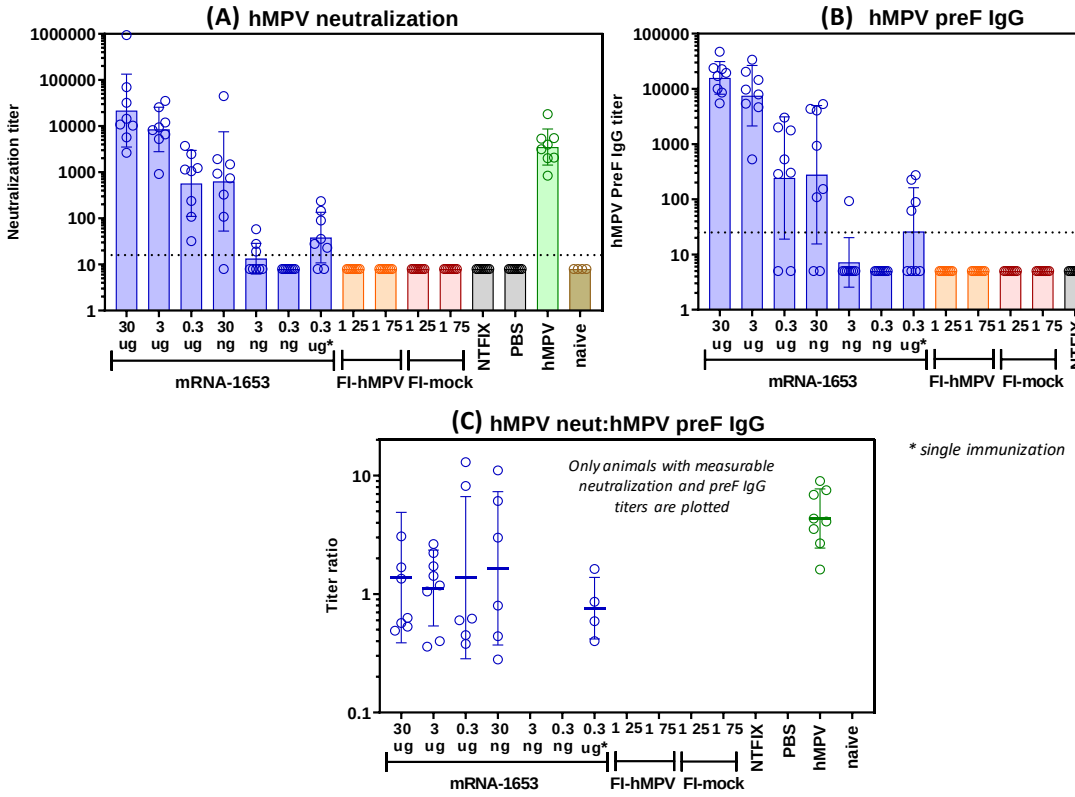
Groups of female cotton rats (n= 8 per group) were administered mRNA-1653 (30 µg; 3 µg; 0.3 µg, 30 ng, 3 ng, or 0.3 ng), FI-hMPV (1:25 or 1:75 dilution), mock FI-hMPV (1:25 or 1:75 dilution), a high dose of an LNP-formulated irrelevant mRNA (non-translated human factor IX, [NTFIX]), or PBS via IM injection on Days 0 and 28. Additional groups of rats were administered IM injections of 0.3 ng mRNA-1653 or IN infection with wild-type hMPV on Day 0 only. All vaccinated and control animals were challenged IN on Day 56 with 10^5 PFU of hMPV. On Day 61, 5 days after challenge, nasal tissue and lungs were collected. Four naïve animals were not vaccinated or challenged.

Endpoints included assessment of serum hMPV neutralization titer via microneutralization (MN) assay, serum hMPV prefusion F-specific IgG titer via ELISA, and calculation of the hMPV neutralizing to prefusion F IgG titer ratio on Day 56; measurement of hMPV viral load in the nose and lung via plaque assay on Day 61; lung histopathology (analyst 1) via hematoxylin and eosin (H&E) staining on Day 61; and measurement of cytokines (interleukin [IL]-4, IL-5, IL-13, interferon [IFN] gamma, IL-2) via quantitative reverse transcription polymerase chain reaction (qRT-PCR) on Day 61. Additional immune response analyses for the assessment of serum hMPV postfusion F-specific IgG titer, hMPV neutralization to postfusion F IgG titer ratio, and hMPV prefusion F IgG to postfusion F IgG titer ratio, and serum PIV3 neutralization titer via MN assay on Day 56 and lung histopathology, including eosinophils (analyst 2) via H&E staining are ongoing. These results will be submitted for the IND when available.

mRNA-1653 induced hMPV neutralizing (Figure 8A) and binding (prefusion F-specific IgG) (Figure 8B) antibodies in a dose-dependent manner. Intranasal infection with hMPV also induced a robust hMPV-specific antibody response. Unexpectedly, FI-hMPV did not induce a measurable antibody response, although it is possible antibody was present but below the limit of quantification or that binding antibodies were directed to other non-F viral antigens. Injections with non-hMPV materials (FI-mock, NTFIX, or PBS) did not elicit hMPV-specific responses, as expected. The neutralizing to binding antibody titer ratio was calculated for each animal that had

a titer above the limit of quantification in both assays (Figure 8C). The titer ratio was consistent across mRNA-1653 dose levels and approximately 3-fold lower than that in the IN hMPV infection group. Given that FI-hMPV did not induce a measurable antibody response, a titer ratio for these groups could not be calculated for comparison.

Figure 8: hMPV Neutralization and Binding Antibody Titers in Serum on Day 56

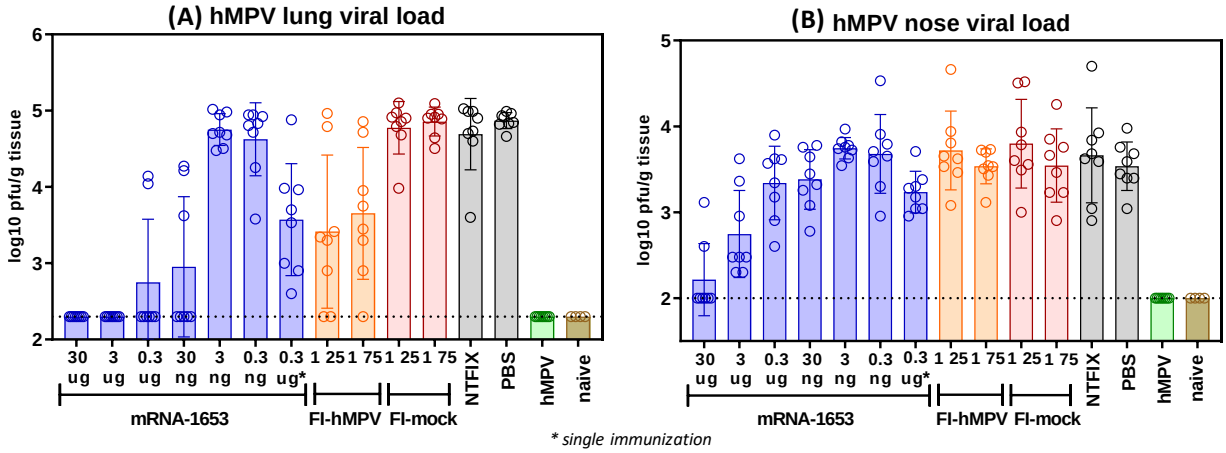


Abbreviations: FI = formalin inactivated; hMPV = human metapneumovirus; IgG = immunoglobulin G; NTFIX = non-translated human factor IX; neut = neutralization; PBS = phosphate-buffered saline; preF = prefusion F-specific.

Source: Study XV-216.

mRNA-1653 vaccination protected cotton rats from IN hMPV challenge on Day 56 in a dose-dependent manner, relative to PBS control animals (Figure 9). Viral load was inversely correlated with serum neutralizing antibody titer before challenge (Figure 8). Animals in the mRNA-1653 groups dosed twice with 0.3 µg, 30 ng, or 3 ng or dosed once with 0.3 µg, demonstrated suboptimal immunity and breakthrough infection, suggesting that this study meets the validity criteria to assess for vaccine ERD. Although hMPV-specific antibody was not detected in the FI-hMPV groups (Figure 8), some incomplete protection from viral challenge was observed, suggesting that mechanisms other than antibody could be contributing to protection. Intranasal hMPV infection at Day 0 completely blocked replication of a secondary hMPV infection at Day 56, whereas injections with non-hMPV materials (FI-mock and NTFIX) afforded no protection.

Figure 9: hMPV Lung and Nose Viral Load 5 Days After hMPV Challenge

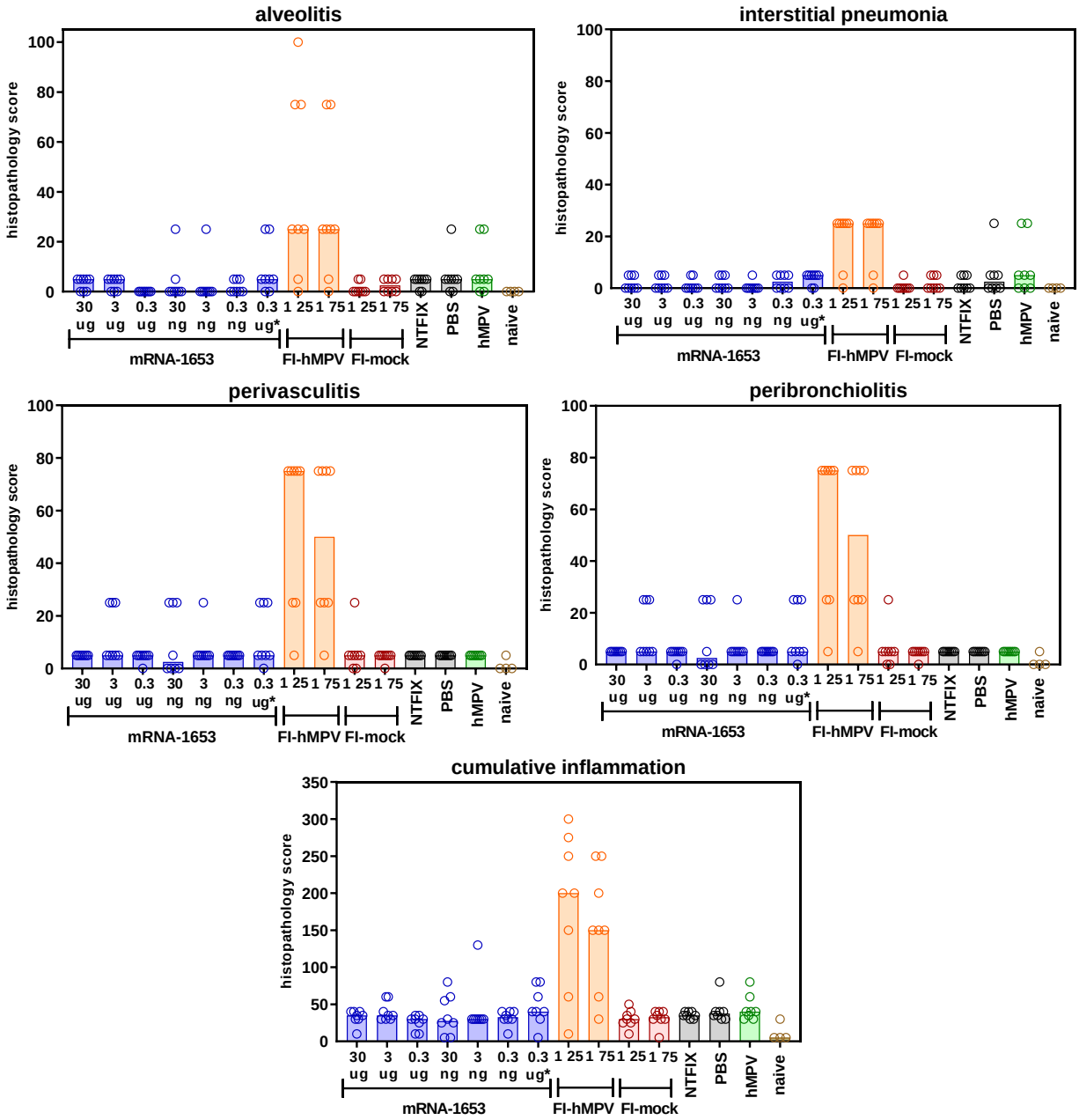


Abbreviations: FI = formalin inactivated; hMPV = human metapneumovirus; NTFIX = non-translated human factor IX; PBS = phosphate-buffered saline; PFU = plaque-forming units.

Source: Study XV-216.

FI-hMPV induced the characteristic features of enhanced lung inflammation after challenge (elevated histopathology scores for all 5 parameters evaluated), relative to primary or secondary hMPV infection. In contrast, mRNA-1653 did not show enhanced lung inflammation after challenge, as the histopathology scores in were in line with those from primary or secondary hMPV infection (Figure 10). This was true even at mRNA-1653 dose levels that induced suboptimal immunity (Figure 8) and permitted breakthrough virus replication (Figure 9).

Figure 10: Lung Histopathology 5 Days After hMPV Challenge



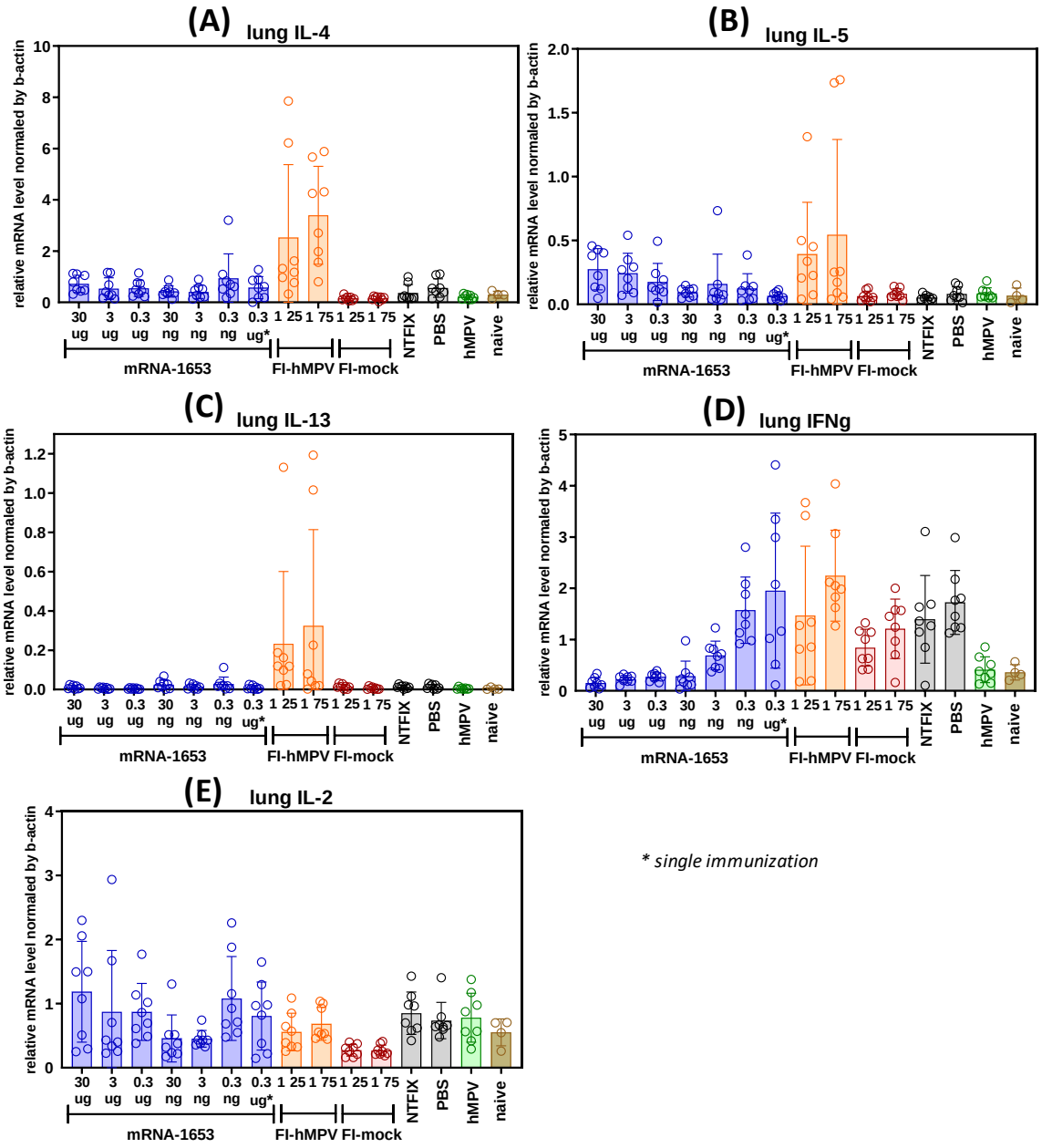
Abbreviations: FI = formalin inactivated; hMPV = human metapneumovirus; NTFIX = non-translated human factor IX; PBS = phosphate-buffered saline.

Source: Study XV-216.

FI-hMPV-vaccinated animals had elevated levels of mRNA for the Th 2 cytokines IL-4, IL-5, and IL-13 in their lungs after hMPV challenge, compared to PBS controls. In contrast, mRNA for Th2 cytokines was not elevated in the mRNA-1653 groups, at any dose level tested. mRNA for the Th1 cytokine IFN gamma (Figure 11D) seemed to be a marker of active hMPV infection, as it was

detected primarily in the groups with high virus load in the lungs (Figure 9A). The Th0/Th1 cytokine IL-2 was detected at similar levels in all study groups (Figure 11E).

Figure 11: Cytokine RNA in Lung 5 Days After hMPV Challenge



Abbreviations: FI = formalin inactivated; hMPV = human metapneumovirus; IFNg = interferon gamma; IL = interleukin; mRNA = messenger RNA; NTFIX = non-translated human factor IX; PBS = phosphate-buffered saline.

Source: Study XV-216.

In conclusion, the immunogenicity, efficacy, and safety of mRNA-1653 in the hMPV cotton rat challenge model showed that mRNA-1653 induced a dose-dependent increase in hMPV

neutralizing antibody that correlated with protection from hMPV challenge. In addition, mRNA-1653 vaccination did not induce enhanced lung inflammation or Th2 cytokine levels after hMPV challenge, even at dose levels that induced suboptimal immunity and breakthrough virus replication; therefore, mRNA-1653 did not prime for ERD.

2.4.3 Pharmacokinetics

2.4.3.1 Distribution

To evaluate the generalized tissue distribution and tissue half-life ($T_{1/2}$) of mRNA-1273, the biodistribution of mRNA-1647, a similar mRNA-based vaccine formulated in SM-102-containing LNPs, was evaluated in male rats. mRNA-1647 is a novel mRNA-based cytomegalovirus vaccine that contains 6 mRNA Drug Substances, which encode the pentameric glycoprotein H (gH)/glycoprotein L (gL)/ UL128/UL130/UL131A glycoprotein complex (Pentamer). The 6 mRNA Drug Substances are formulated at a target mass ratio of 1:1:1:1:1:1 in a mixture of the same 4 lipids as mRNA-1273. The biodistribution of mRNA-based vaccines formulated in LNPs is predicted to be driven by the LNP characteristics. Therefore, mRNAs that are within an LNP of the same composition (eg, mRNA-1273 and mRNA-1647) are expected to distribute similarly. Thus, the biodistribution study performed using mRNA-1647 supports the development of mRNA-1273.

This non-GLP biodistribution study was performed in male Sprague Dawley rats given a single IM injection of 100 µg of mRNA-1647 and included the determination of mRNA-1647 PK (Study 5002121 Amendment 1 in CBER MF# 19622). Blood and tissues were collected, and samples were analyzed for the concentration of mRNA-1647 using a qualified branched DNA multiplex method.

Following a single IM dose in male rats, concentrations for all 6 mRNA-1647 constructs were detectable in plasma and tissues in a 1:1:1:1:1:1 ratio. The time after dosing at which the maximum concentration was observed (T_{max}) in plasma was 2 hours for all constructs and was followed by a rapid elimination phase with a $T_{1/2}$ estimated to range from 2.7 to 3.8 hours. The maximum observed concentration (C_{max}) and area under the concentration versus time curve (AUC) from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed ($AUC_{[0\text{-}t]}$) in plasma were from 1.60 to 2.30 ng/mL and from 22.7 to 25.5 ng·h/mL, respectively.

Concentrations for all 6 mRNA-1647 constructs were detected at levels above the lower limit of quantitation (LLOQ) or were similar to plasma levels in most tissues analyzed, except for the kidney, where all levels were below the LLOQ. For highly exposed tissues (injection site [muscle], lymph nodes [proximal and distal], spleen), C_{max} was observed between 2 and 24 hours post-dose.

The $T_{1/2}$ was calculated using the average tissue $T_{1/2}$ values for all 6 constructs. The results were as follows: 14.9 hours for muscle (site of injection), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

As observed with other IM-delivered vaccines, the highest mRNA concentrations were observed at the injection site followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0.

Overall, only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than the injection site, lymph nodes, and spleen.

2.4.4 Toxicology

2.4.4.1 Repeat-Dose Toxicity

The safety and tolerability of similar mRNA-based vaccines formulated in an SM-102-containing LNP matrix encapsulating mRNA constructs that encode for various antigens have been evaluated in multiple GLP-compliant repeat-dose toxicity studies in Sprague Dawley rats followed by a 2-week recovery period. The Sponsor proposes that the toxicity associated with mRNA vaccines formulated in LNP formulations are driven primarily by the LNP composition and, to a lesser extent, the biologic activity of the expressed antigens of the mRNA vaccine. This is supported by the consistency of the aggregate rat repeat-dose toxicity profile observed in these GLP studies at IM doses ranging from 9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks and is considered to be representative of mRNA vaccines formulated in the same SM-102 LNP matrix, differing only by the encapsulated mRNA sequence(s). Thus, the aggregate toxicity results from these studies support the development of mRNA-1273.

All doses administered in these GLP-compliant repeat-dose toxicity studies in rats were tolerated. Test article-related in-life observations observed at ≥ 9 µg/dose included reversible or reversing erythema and edema at the injection site and transient increases in body temperature at 6 hours post-dose returning to baseline 24 hours post-dose. The lowest no observed adverse effect level (NOAEL) determined across the aggregate of the completed studies was 89 µg/dose.

Test article-related, generally dose-dependent clinical pathology changes were observed at ≥ 9 µg/dose. Hematology changes included increases in white blood cells, neutrophils, and eosinophils and decreased lymphocytes; coagulation changes included increases in fibrinogen and activated partial thromboplastin time; and clinical chemistry changes included decreases in albumin, increases in globulin, and a corresponding decrease in albumin/globulin ratio. Clinical pathology changes generally reversed or were reversing by the end of the 2-week recovery period.

Test article-related transient cytokine increases were observed at ≥ 9 µg/dose at 6 hours post-dose including IFN gamma protein, monocyte chemoattractant protein and macrophage inflammatory protein alpha. Cytokine changes were generally reversing by the end of the 2-week recovery period.

Post-mortem test article-related and generally dose-dependent changes in organ weights and macroscopic and microscopic findings were observed at ≥ 9 µg/dose. Organ weight increases were observed in the spleen, liver, and adrenal gland. Organ weight changes were generally reversing by the end of the 2-week recovery period. Macroscopic changes included skin thickening at the injection site and enlarged lymph nodes. Injection site changes completely recovered, and lymph node changes were recovering by the end of the 2-week recovery period. Microscopic changes included mixed cell inflammation at the injection site; increased cellularity and mixed cell inflammation in the inguinal, iliac, and popliteal lymph nodes; decreased cellularity in the splenic periarteriolar lymphoid sheath; increased myeloid cellularity in the bone marrow; and hepatocyte vacuolation and Kupffer cell hypertrophy in the liver. Microscopic changes were generally reversing by the end of the 2-week recovery period.

Detailed information including the reports for these studies are provided in CBER MF# 19622.

2.4.4.2 Genotoxicity

2.4.4.2.1 In Vitro

In the bacterial reverse mutation (Ames) test (Study 9601567 in CBER MF# 19622) that evaluated SM-102 for mutagenicity, 4 strains of *Salmonella typhimurium* [TA98, TA100, TA1535, TA1537] and the *Escherichia coli* WP2 uvrA strain were tested with SM-102 at a range of concentrations up to the standard limit dose for the assay. The results for this study were negative. In addition, an in vitro micronucleus test in human peripheral blood lymphocytes (Study 9601568 in CBER MF# 19622) evaluated SM-102 for clastogenic activity. The results for this study were also negative.

2.4.4.2.2 In Vivo

An in vivo micronucleus study was conducted with mRNA-1706, a representative SM-102-containing LNP investigational product (Study 9800399 in CBER MF# 19622). The study was conducted in rats using an intravenous route of administration. The intravenous route was selected to maximize exposure to SM-102 and the other lipids incorporated into the mRNA-based investigational product LNPs. SM-102 induced statistically significant increases in micronucleated immature erythrocytes in male rats at both 24 and 48 hours and in female rats at 48 hours only; however, there was no clear dose response, and the increases were generally weak

and associated with minimal bone marrow toxicity. These observations indicate that the risk to humans after IM administration is low, due to minimal systemic exposure.

2.4.4.3 Other Toxicity Studies

A non-GLP study in Sprague Dawley rats was conducted by the Sponsor to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses (Study 2308-123). mRNA-1273 was administered to male and female Sprague Dawley rats as an IM injection on Day 1 and Day 22 at dose levels of 30, 60, and 100 µg/dose. Safety endpoints included body weight changes, clinical observations, and assessments of hematology, and clinical chemistry. In addition, SARS-CoV-2 S protein binding titers were measured for all animals in the study at baseline and Day 35 by ELISA. The study is currently ongoing, and preliminary results are included below (data on file).

All animals had no measurable IgG titers at baseline and displayed a strong response to mRNA-1273, with measured IgG titers above 10^6 at all dose levels on Day 35, after the second injection (data not shown).

There were no mRNA-1273-related effects on body weight. mRNA-1273-related clinical signs were observed on Day 1 and Day 22 starting at the 30 µg/dose. Clinical signs consisting of transient dose-dependent injection site edema with or without hindlimb impairment were observed at approximately 24 hours post-dose and generally resolved within 7 days after dose administration.

mRNA-1273-related clinical pathology changes associated with inflammation, including increased neutrophils, eosinophils, and/or globulin, were observed starting at the 30 µg/dose. Other mild mRNA-1273-related changes observed at 30, 60, and/or 100 µg/dose consisted of decreased red cell mass, reticulocytes, and lymphocytes and increased creatinine, triglyceride, cholesterol, and/or glucose.

In general, these changes are consistent with the results from the previous GLP rat toxicity studies conducted with the Sponsor's SM-102 LNP.

2.4.5 Integrated Overview and Conclusions

mRNA-1273 is a novel LNP-encapsulated mRNA-based vaccine that encodes for the full-length S protein of 2019-nCoV. mRNA-1273 encodes for the full-length S protein of SARS-CoV-2, modified to introduce 2 proline residues to stabilize the S protein into a prefusion conformation (S-2P). mRNA-1273 consists of an mRNA Drug Substance that is formulated with LNPs composed of 4 lipids: SM-102; cholesterol; DSPC; and PEG2000-DMG. mRNA-1273 is provided as a sterile liquid for injection at a concentration of 0.5 mg/mL in Tris buffer containing 87 mg/mL sucrose and 10.7 mM sodium acetate, at pH 7.5.

The mRNA-1273 vaccine is currently being evaluated for safety and immunogenicity in a dose-ranging Phase 1 study (NCT04283461) sponsored by DMID. In addition, nonclinical evaluations in mice and rats are being conducted by the VRC and the Sponsor in parallel with the Phase 1 study. In support of development of mRNA-1273 against SARS-CoV-2, nonclinical immunogenicity, biodistribution, and safety studies have been completed by the Sponsor with similar mRNA-based vaccines formulated in SM-102-containing LNPs.

The immunogenicity and efficacy of low doses of mRNA-1273 (10, 1, 0.1, and 0.01 µg) were evaluated by the VRC in BALB/c mice (Study VRC01). mRNA-1273 is immunogenic in BALB/c mice, demonstrating a dose-dependent antibody response as measured in both binding and neutralizing antibody titers. A strong correlation was measured between binding antibody and neutralization, and low binding titers at the 0.1 µg dose did not elicit measurable neutralization titers. In addition, a single dose of mRNA-1273 elicited binding antibody titers, with no neutralization titers observed. Mice from this study were challenged with mouse-adapted SARS-CoV-2 virus, and preliminary results indicate that mRNA-1273-induced immunity is protective after challenge. Mice immunized with a single dose of 10 or 1 µg mRNA-1273 were protected from viral replication in the lung following challenge 7 weeks after vaccination, despite the absence of measurable neutralization titers. Mice immunized with 2 doses of either 1 or 0.1 µg mRNA-1273 were also protected, dependent upon the dosing schedule. Other results from challenge study, including cytokine measurements and pathology, are ongoing.

To address the theoretical risk of ERD, the immunogenicity, efficacy, and safety of mRNA-1653, a similar mRNA-based vaccine formulated in SM-102-containing LNPs, was evaluated in a non-GLP study in the hMPV cotton rat challenge model (Study XV-216). The in-life portion of this study is complete, and endpoint assay analysis is ongoing. Results showed that mRNA-1653 induced a dose-dependent increase in hMPV neutralizing antibody that correlated with protection from hMPV challenge. In addition, mRNA-1653 vaccination did not induce enhanced lung inflammation or Th2 cytokine levels after hMPV challenge, even at dose levels that induced suboptimal immunity and breakthrough virus replication; therefore, mRNA-1653 did not prime for ERD.

A non-GLP biodistribution study was completed with mRNA-1647, a similar mRNA-based vaccine formulated in SM-102-containing LNPs, in male Sprague Dawley rats and is provided to support the development of mRNA-1273 (Study 5002121 Amendment 1 in CBER MF# 19622). All the mRNA constructs present in mRNA-1647 demonstrated nearly identical PK behavior, and all 6 constructs were detectable in a 1:1:1:1:1:1 ratio following a single IM dose. The plasma T_{max} was achieved at 2 hours post-dosing for all constructs and was followed by a rapid elimination phase with a $T_{1/2}$ estimated to range from 2.7 to 3.8 hours. Concentrations for all 6 mRNA-1647 constructs were detected at levels above the LLOQ or were similar to plasma levels in most tissues

analyzed, except for the kidney, where all levels were below the LLOQ. For highly exposed tissues (injection site [muscle], lymph nodes [proximal and distal], spleen), C_{max} was observed between 2 and 24 hours post-dose. The $T_{1/2}$ was calculated using the average tissue $T_{1/2}$ values for all 6 constructs. The results were as follows: 14.9 hours for muscle (site of injection), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen. As observed with other IM-delivered vaccines, the highest mRNA concentrations were observed at the injection site followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0. Overall, only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than the injection site, lymph nodes, and spleen.

The safety and tolerability of similar mRNA-based vaccines formulated in an SM-102-containing LNP matrix encapsulating mRNA constructs encoding for various antigens have been evaluated in multiple GLP-compliant repeat-dose toxicity studies in Sprague Dawley rats followed by a 2-week recovery period. The Sponsor proposes that the toxicity associated with mRNA vaccines formulated in LNP formulations are driven primarily by the LNP composition and, to a lesser extent, the biologic activity of the expressed antigens of the mRNA vaccine. This is supported by the consistency of the aggregate rat repeat-dose toxicity profile observed in these GLP studies at IM doses ranging from 9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks and is considered to be representative of mRNA vaccines formulated in the same SM-102 LNP matrix, differing only by the encapsulated mRNA sequence(s). Thus, the aggregate toxicity results from these studies support the development of mRNA-1273. The lowest NOAEL determined across the aggregate of the completed studies was 89 µg/dose.

Additionally, the Sponsor conducted a non-GLP study in Sprague Dawley rats to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses. mRNA-1273 was administered via IM injection at dose levels of 30, 60, and 100 µg/dose on Day 1 and Day 22 (Study 2308-123). The study is currently ongoing and preliminary results are described here. All animals had no measurable IgG titers at baseline and displayed a strong response to mRNA-1273, with measured IgG titers above 10^6 at all dose levels on Day 35, after the second injection. mRNA-1273 had no effect on body weights. Clinical signs starting at 30 µg/dose consisted of transient dose-dependent injection site edema with or without hindlimb impairment. These changes were observed at approximately 24 hours post-dose and generally resolved within 7 days after dose administration. Clinical pathology findings consisted, in part, of changes associated with inflammation starting at 30 µg/dose. In general, the changes observed are consistent with the results from the previous GLP rat toxicity studies conducted with the Sponsor's SM-102 LNP.

In both a bacterial reverse mutation (Ames) test (Study 9601567 in CBER MF# 17532) and an in vitro micronucleus test in human peripheral blood lymphocytes (Study 9601568 in CBER MF# 17532), the results for SM-102, the novel lipid used in the mRNA-1273 LNP formulation, were negative. An in vivo micronucleus study (Study 9800399 in CBER MF# 17532) showed that a similar SM-102-containing vaccine, mRNA-1706, induced statistically significant increases in micronucleated immature erythrocytes in male rats at both 24 and 48 hours and in female rats at 48 hours only; however, there was no clear dose response and the increases were generally weak and associated with minimal bone marrow toxicity. These observations indicate that the risk to humans after IM administration is low due to minimal systemic exposure.

2.4.6 References

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charles river

NON-GLP FINAL REPORT AMENDMENT NO. 01

Test Facility Study No. 5002121

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

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

TEST FACILITY:
Charles River Laboratories Montreal ULC
Sherbrooke Site (CR SHB)
1580 Ida-Metivier
Sherbrooke, QC J1E 0B5
Canada

SUMMARY OF CHANGES AND JUSTIFICATIONS

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

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

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1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Study Director (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	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			

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.

Appendix 1

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.

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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, 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).

Appendix 1

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

Appendix 1

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.

Appendix 1

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

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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

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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).

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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messenger therapeutics

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

Summary of Analysis

(b) (4)

Appendix 2

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

(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)

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.

Appendix 3

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.

Appendix 3

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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Analytical Procedure (AP.5002121.SP.01)

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Analytical Procedure (AP.5002121.SP.01)

(b) (4)

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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)

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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Phone 617-714-6500 • fax 617-583-1998

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)

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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

TABLE OF CONTENTS

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.

Appendix 7

Appendix 2
AP.5002121.bDNAp.03

Appendix 7

ANALYTICAL PROCEDURE

charles river | every step of the way.

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

Appendix 7

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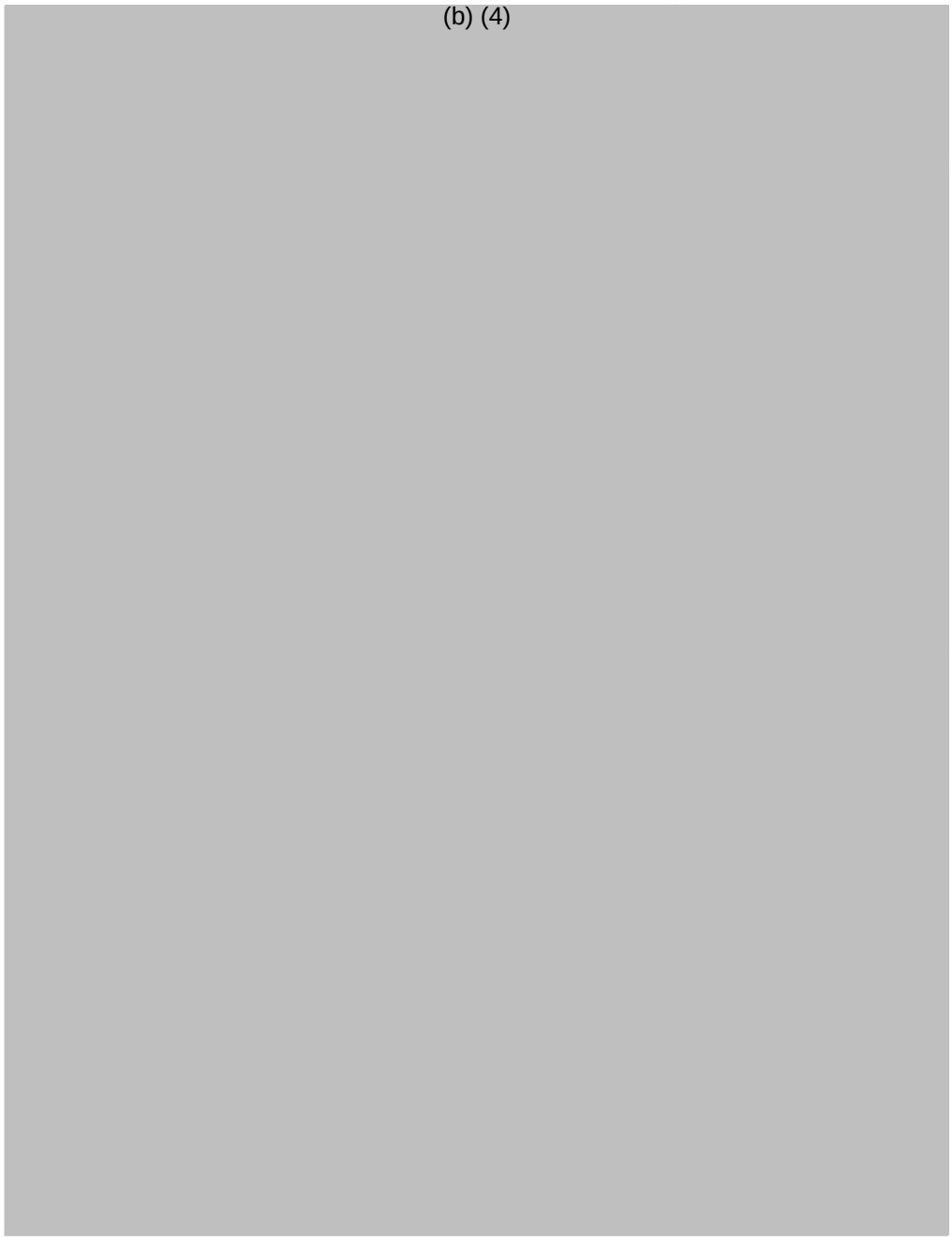
Appendix 7



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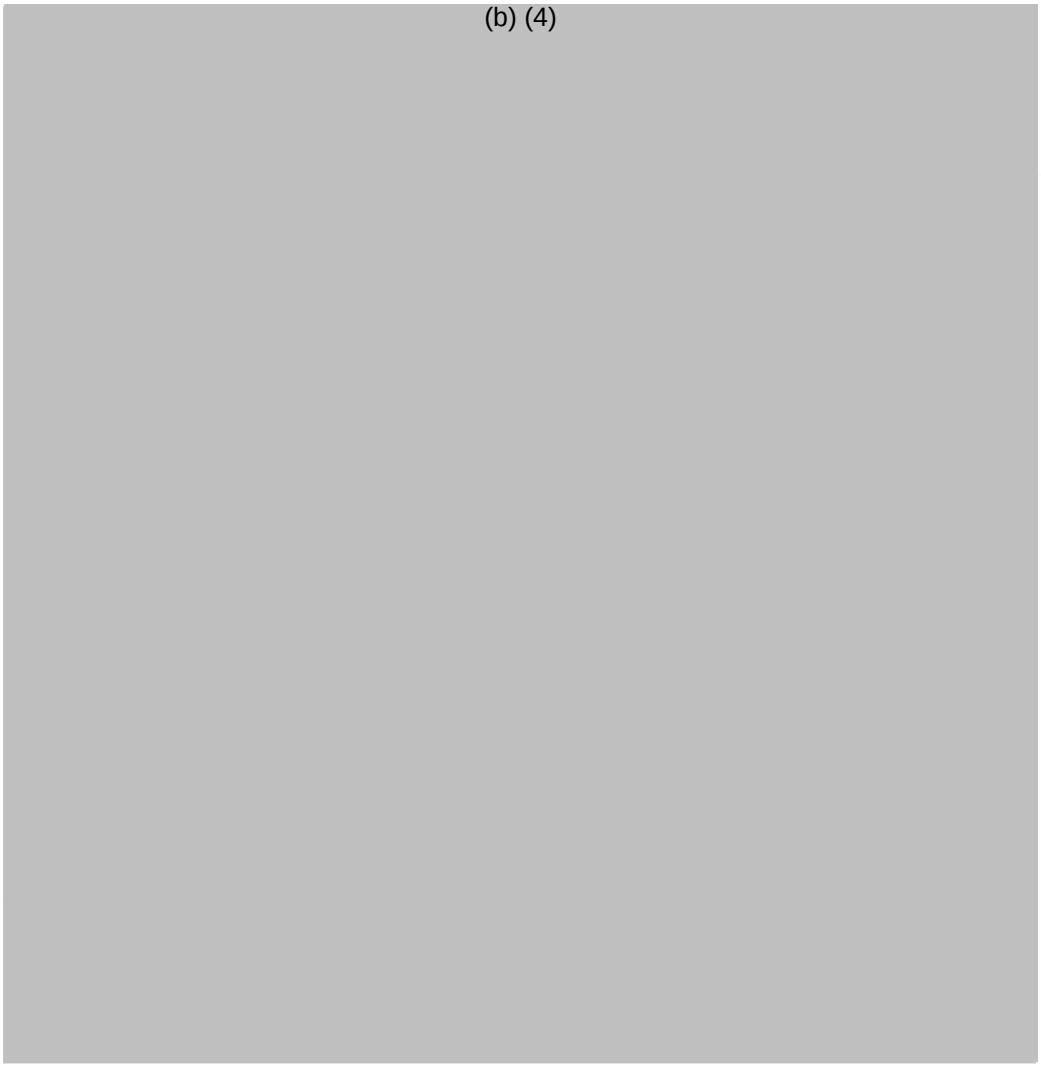

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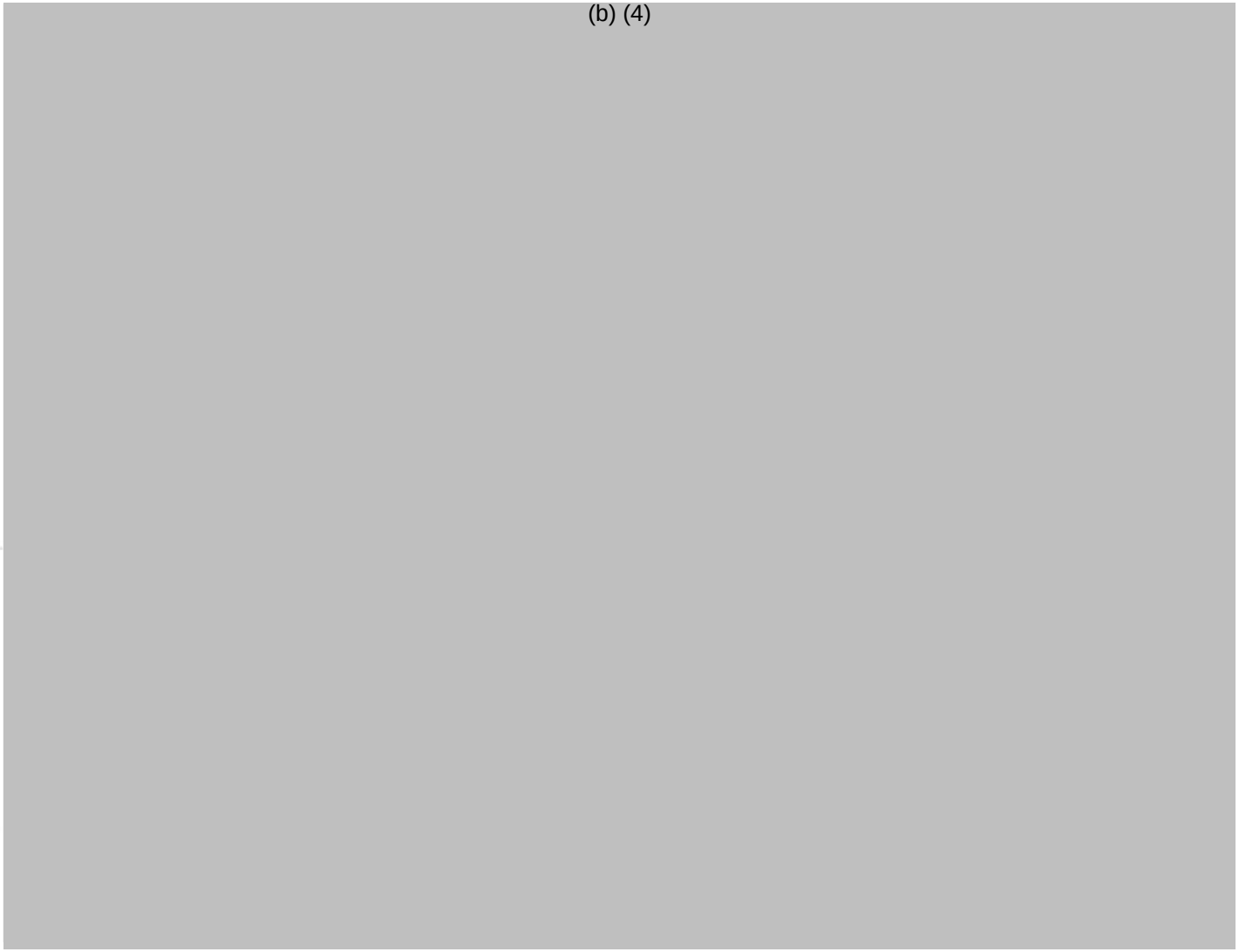

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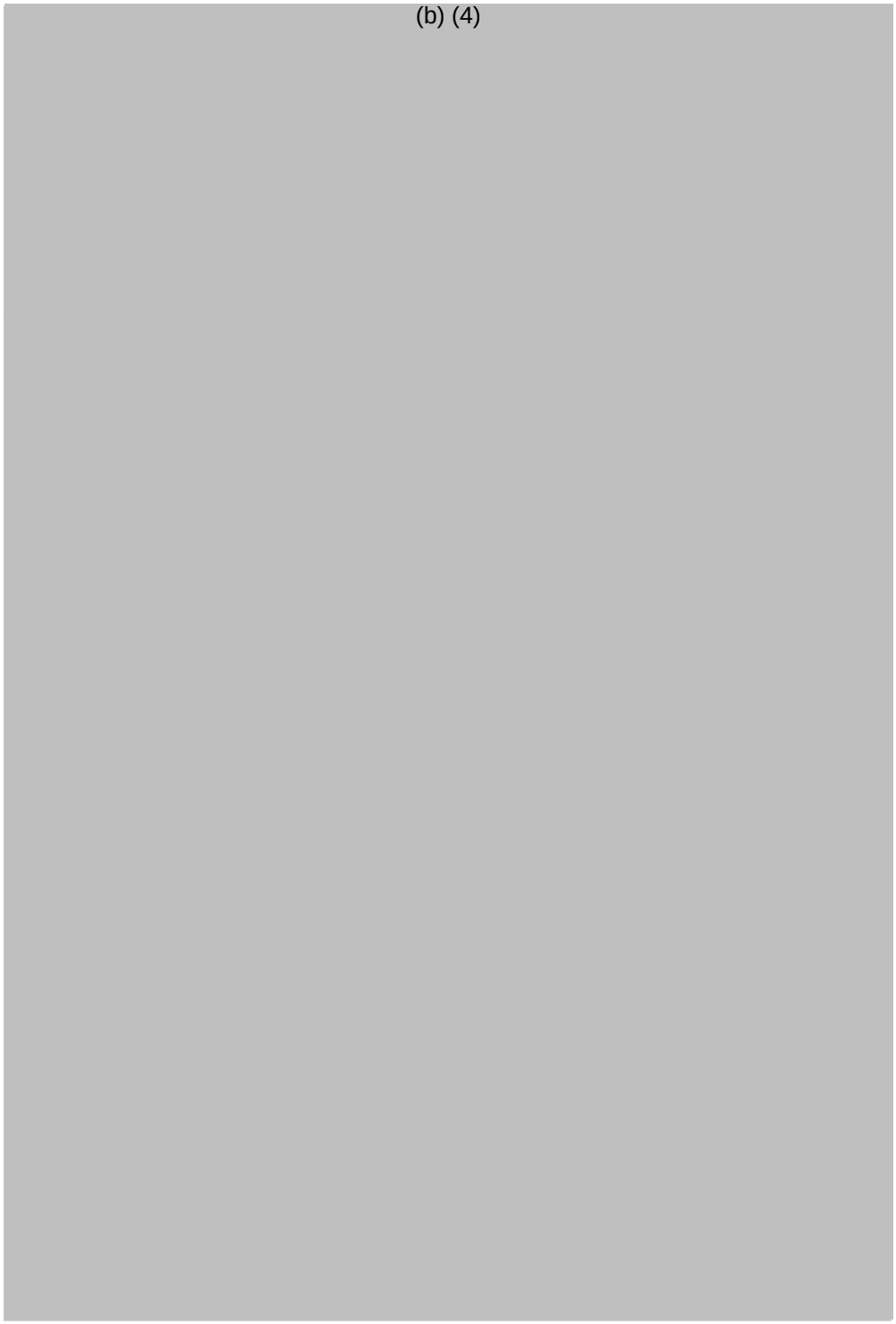

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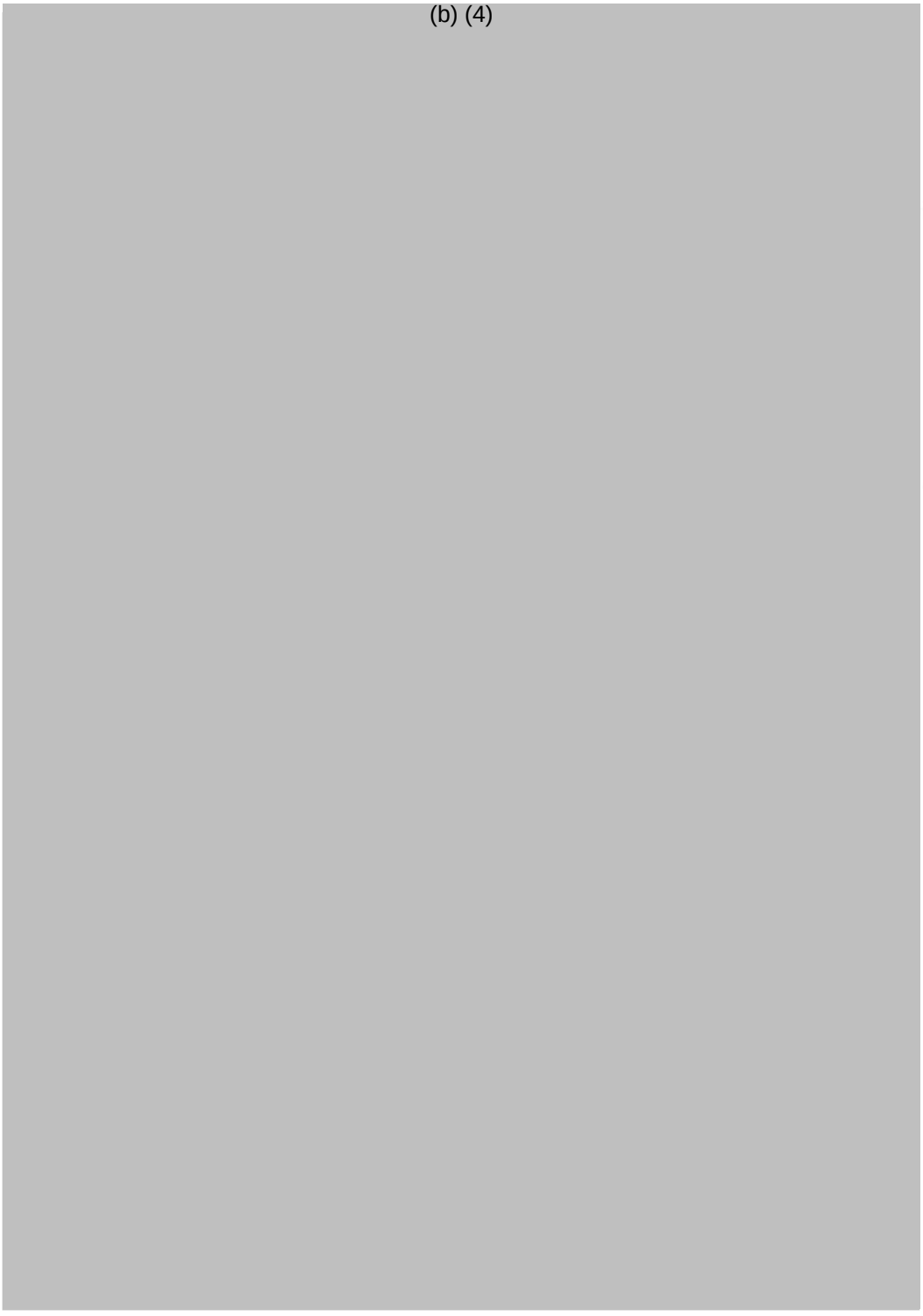

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Appendix 7

ANALYTICAL PROCEDURE

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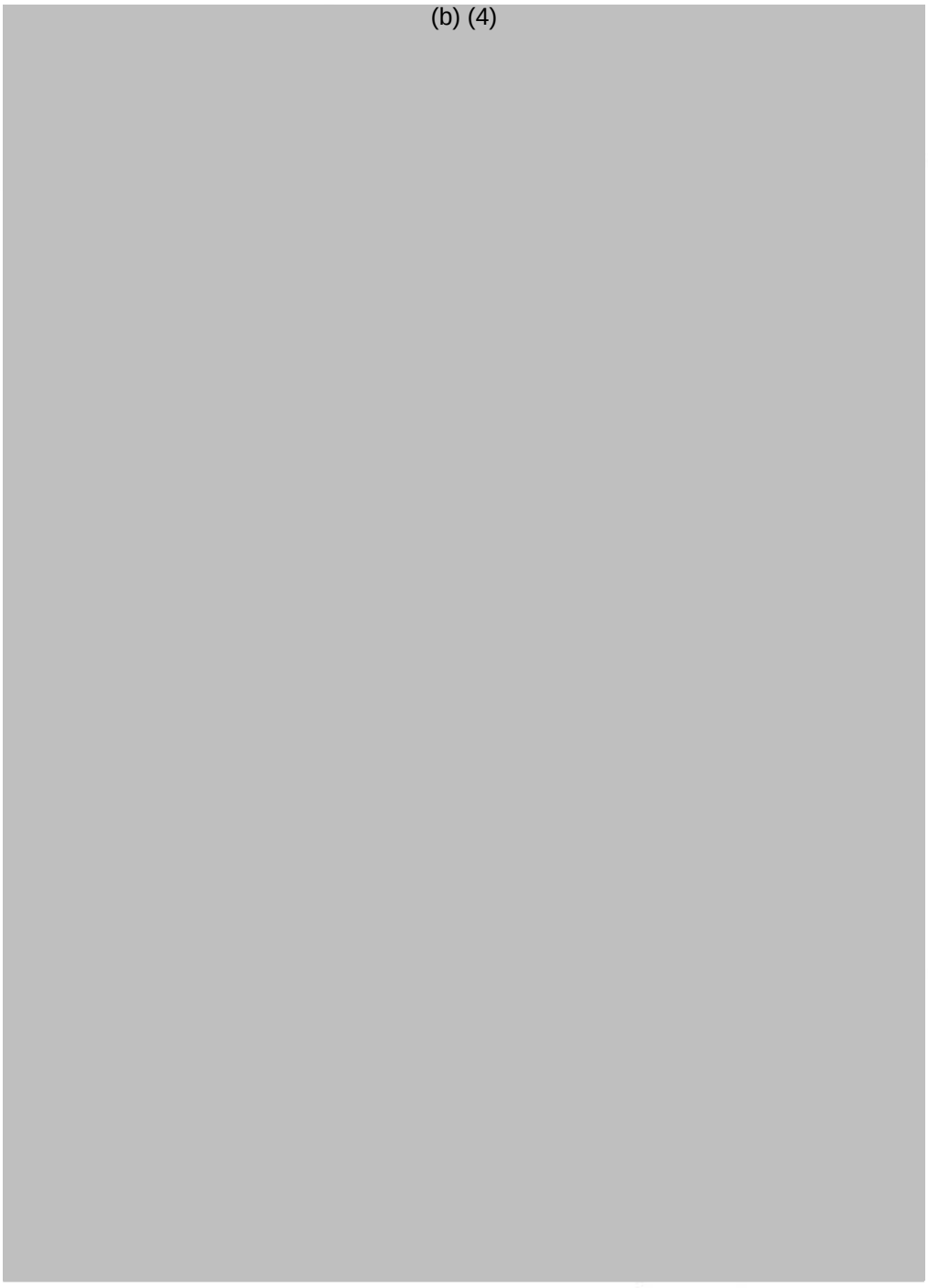

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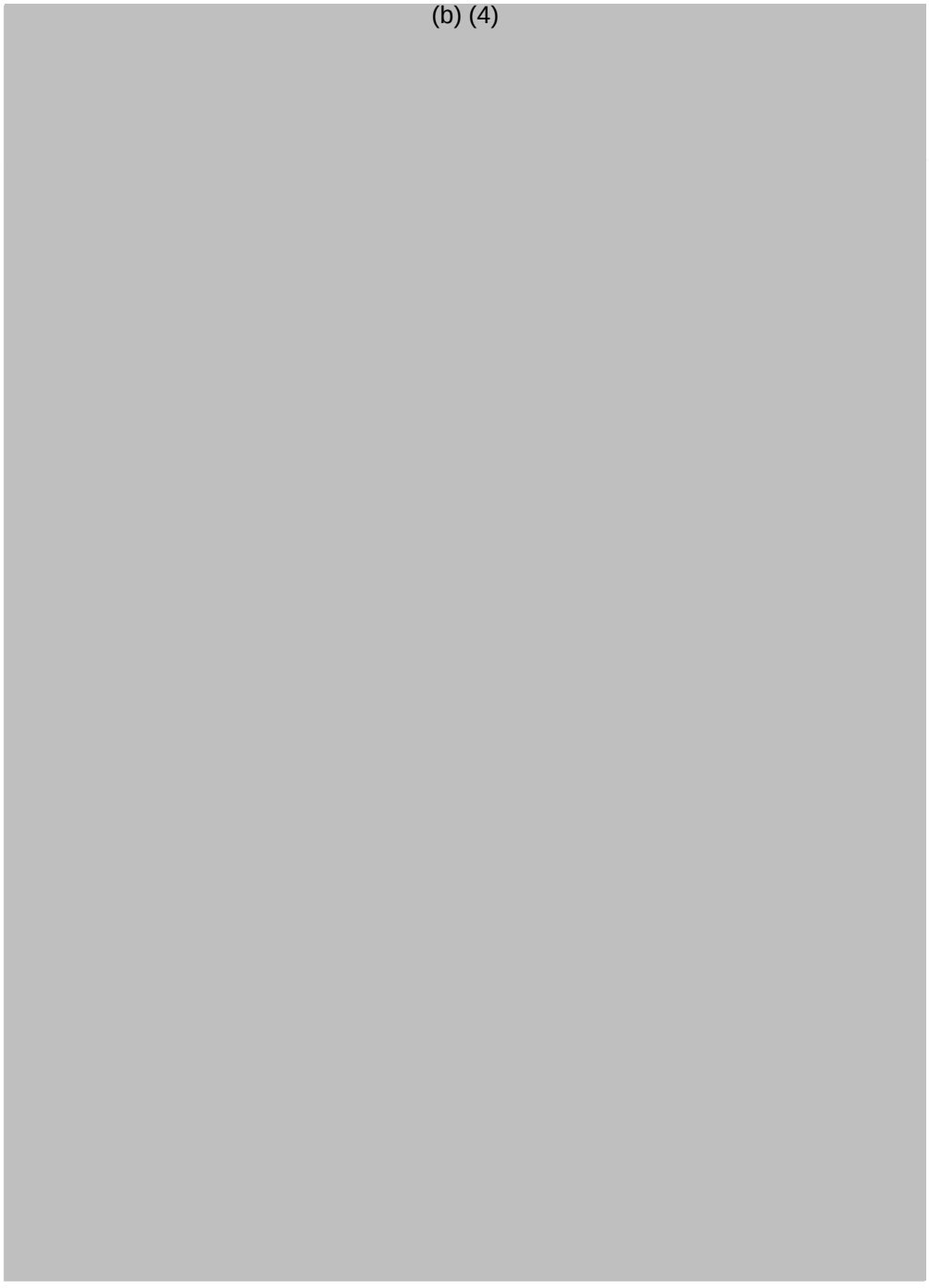

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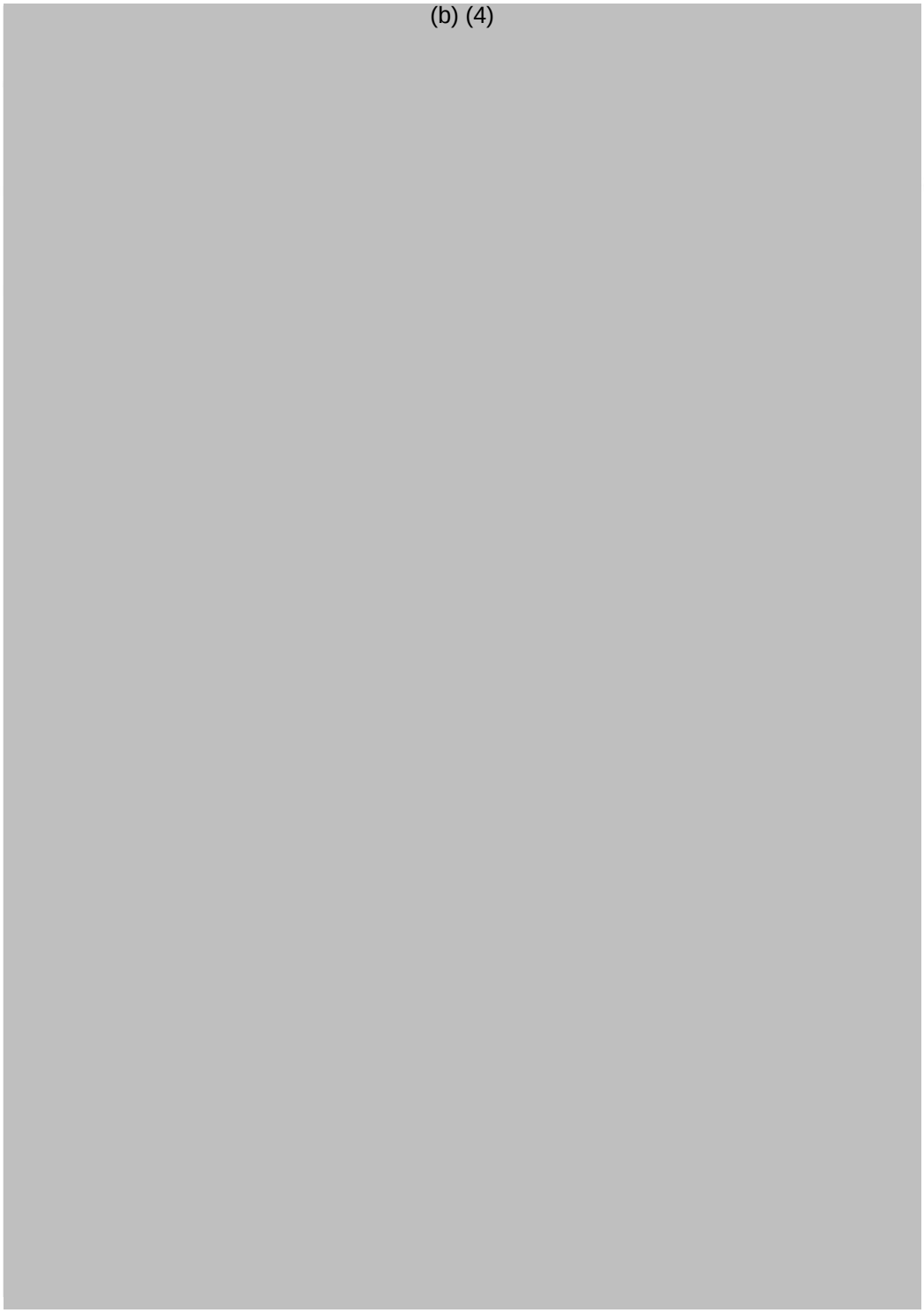

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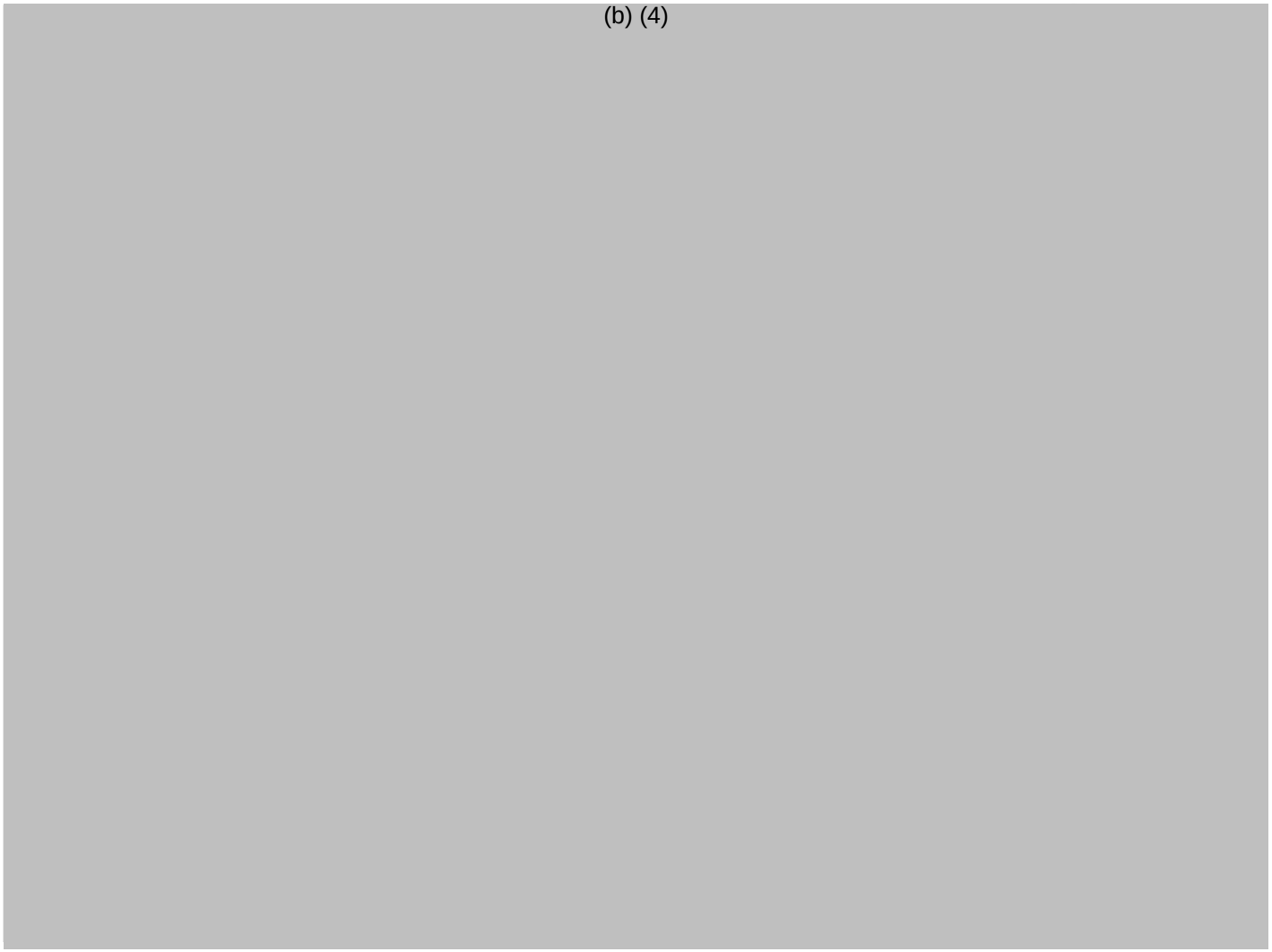

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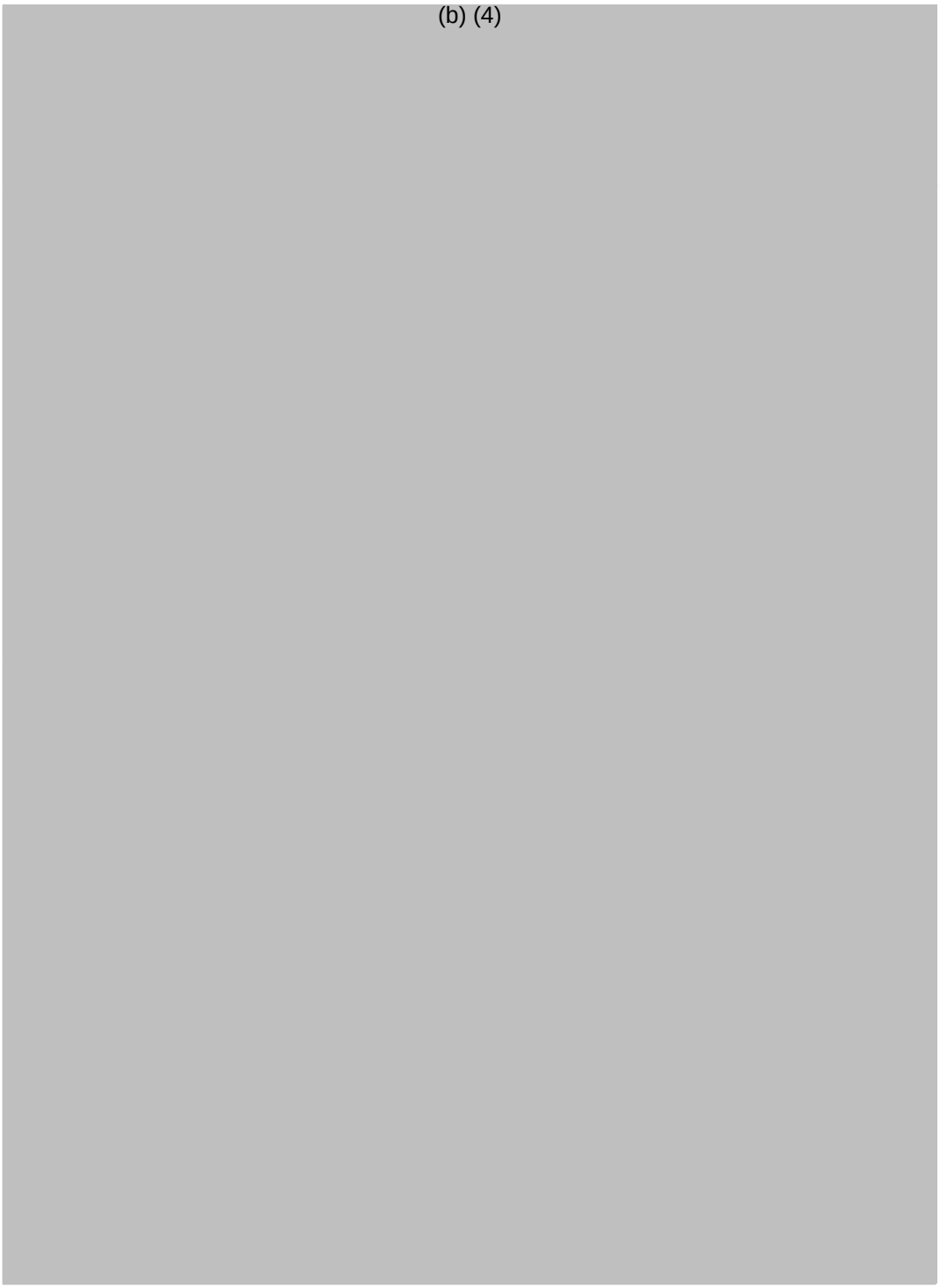

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Appendix 7

ANALYTICAL PROCEDURE

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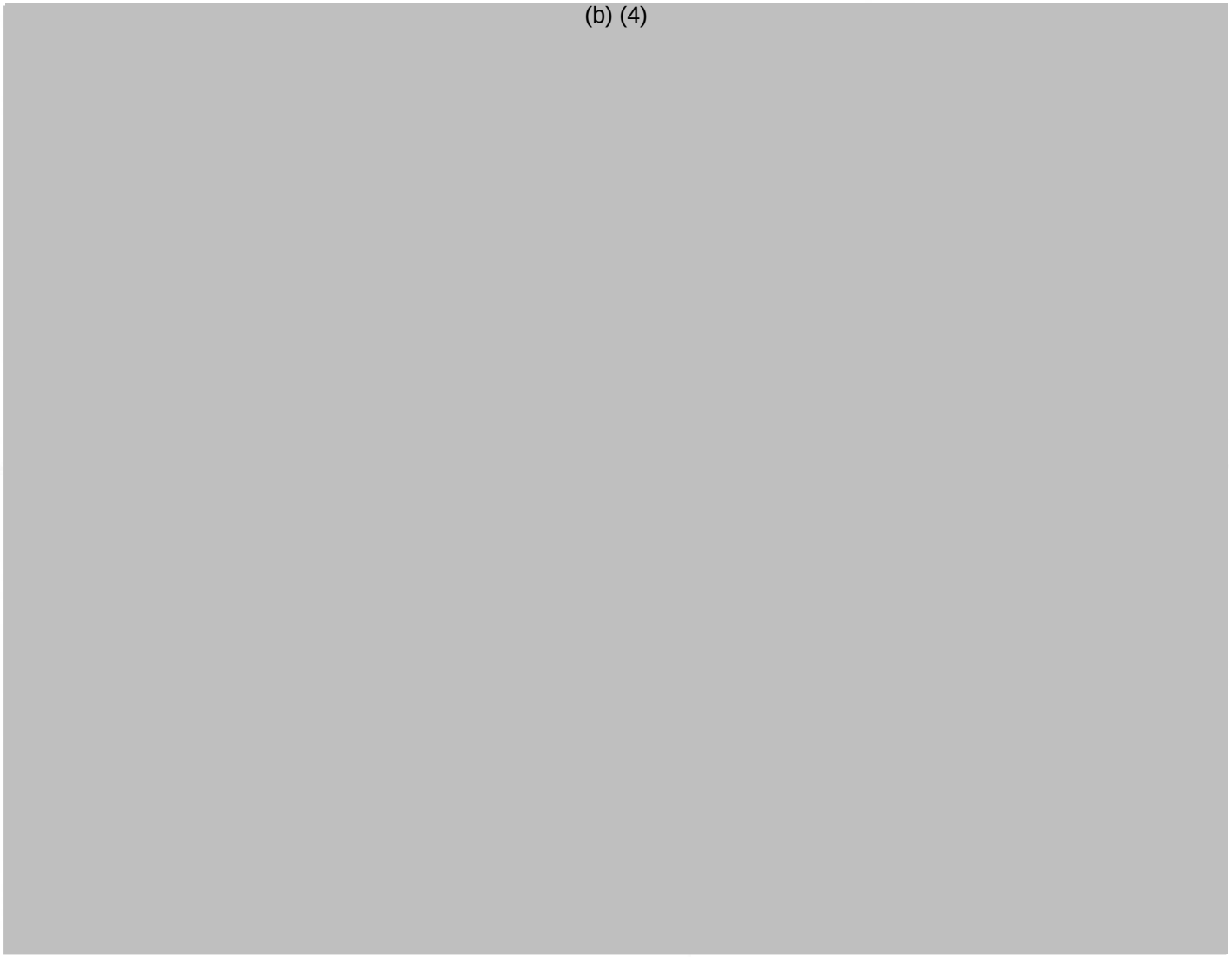

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Appendix 5
Certificate of Analysis

Appendix 7

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messenger therapeutics

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

Summary of Analysis

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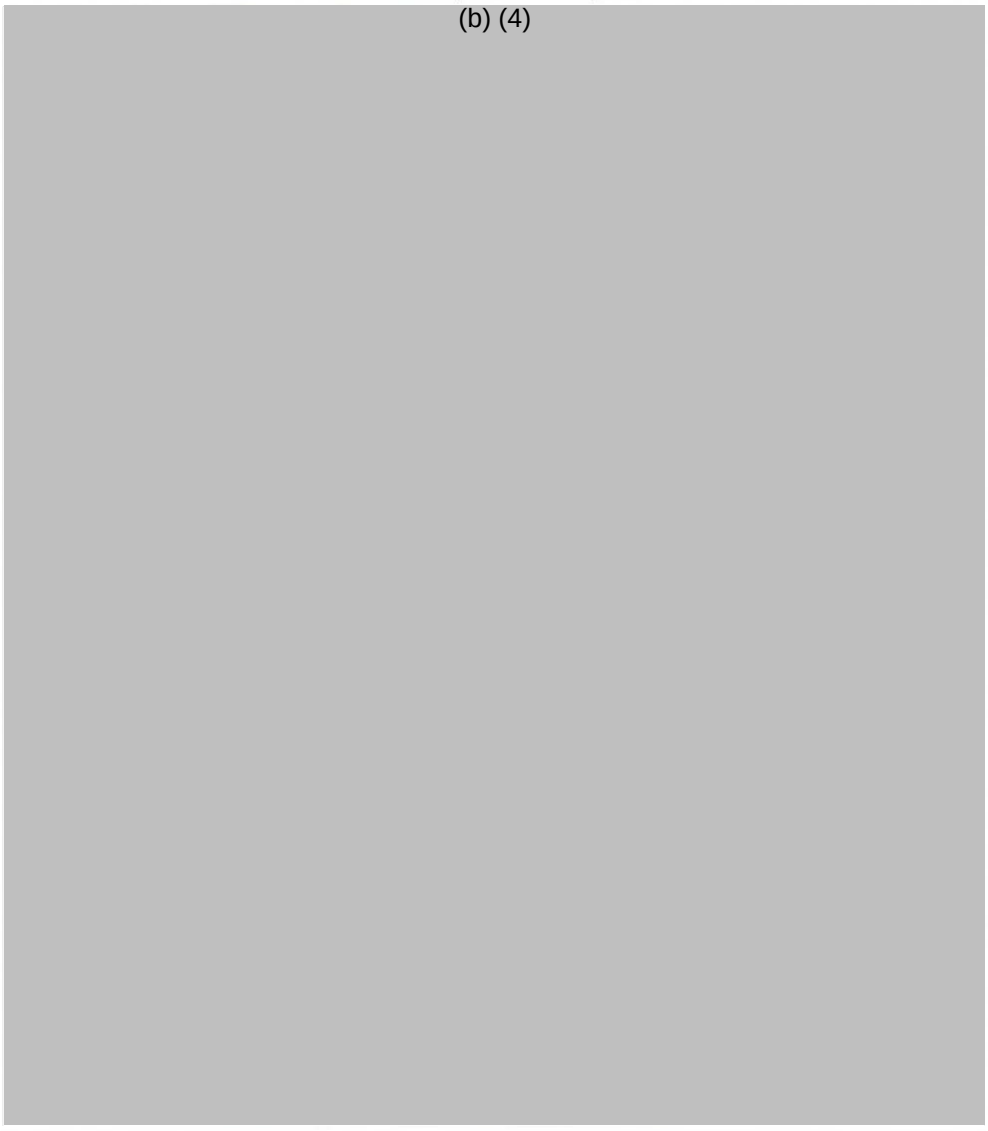

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(b) (4)

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

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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 **14.9** ~~**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 **14.9** ~~**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.

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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

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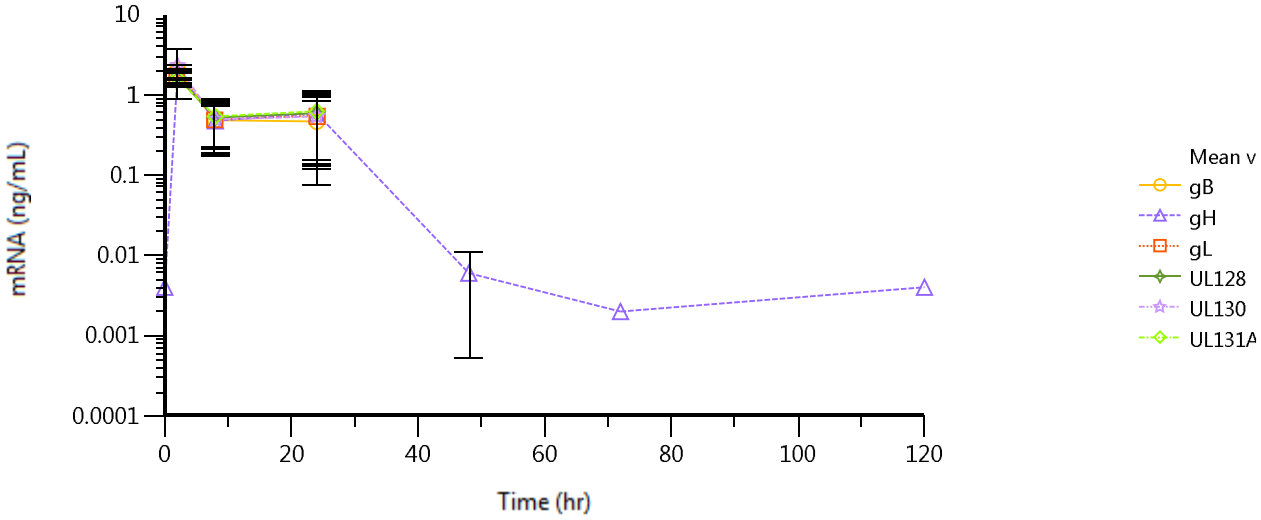
Figure 1.1: Summary (± SD) Male Sprague-Dawley Rat Plasma mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1



Appendix 8

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

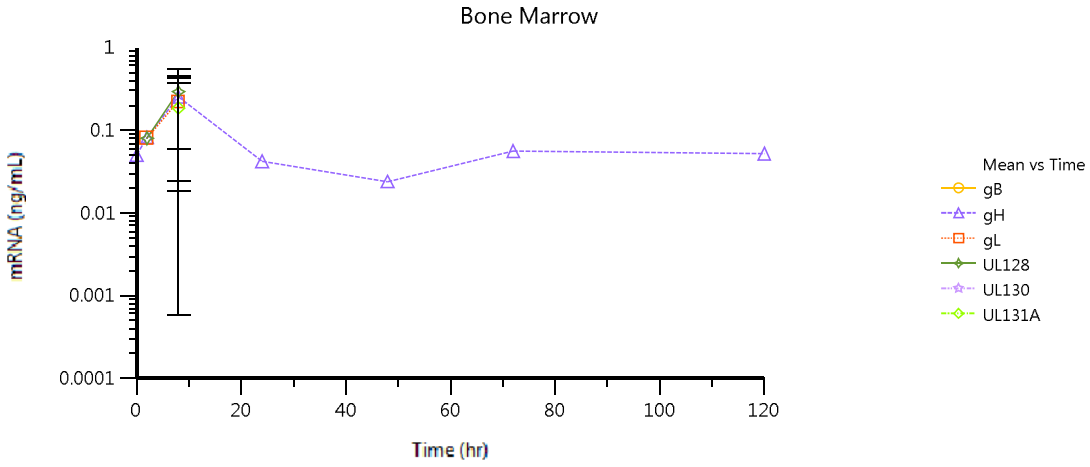


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Figure 1.3: Summary (± SD) Male Sprague-Dawley Rat Brain mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

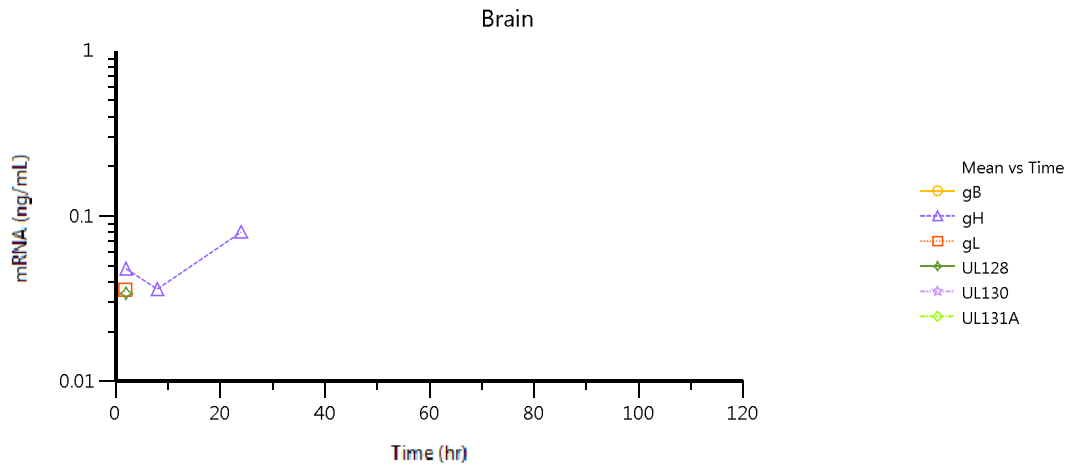

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Figure 1.4: Summary (± SD) Male Sprague-Dawley Rat Distal Lymph Nodes mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

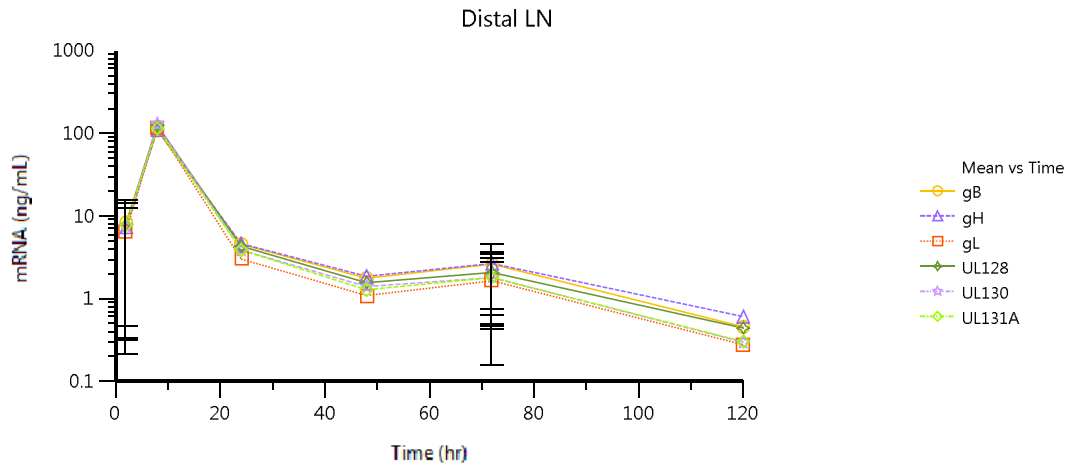

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Figure 1.5: Summary (± SD) Male Sprague-Dawley Rat Eye mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

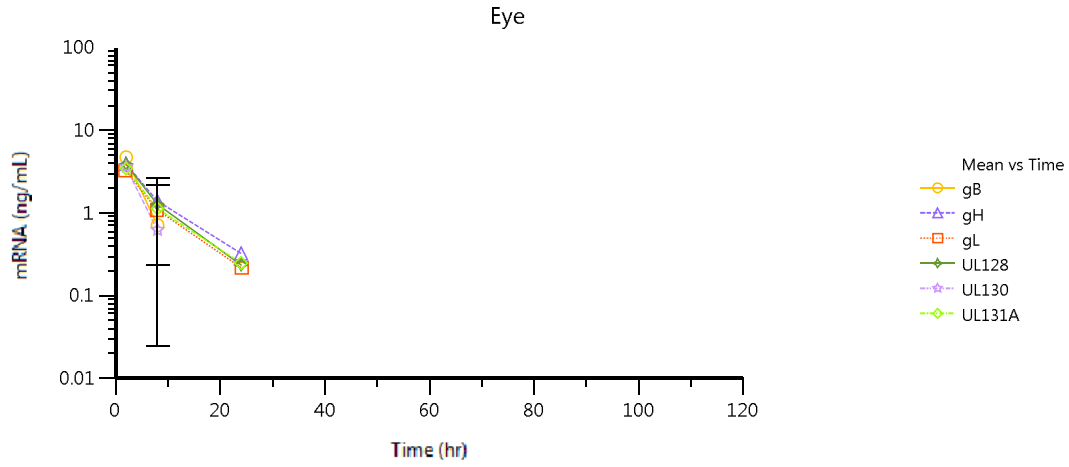

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Figure 1.6: Summary (± SD) Male Sprague-Dawley Rat Heart mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

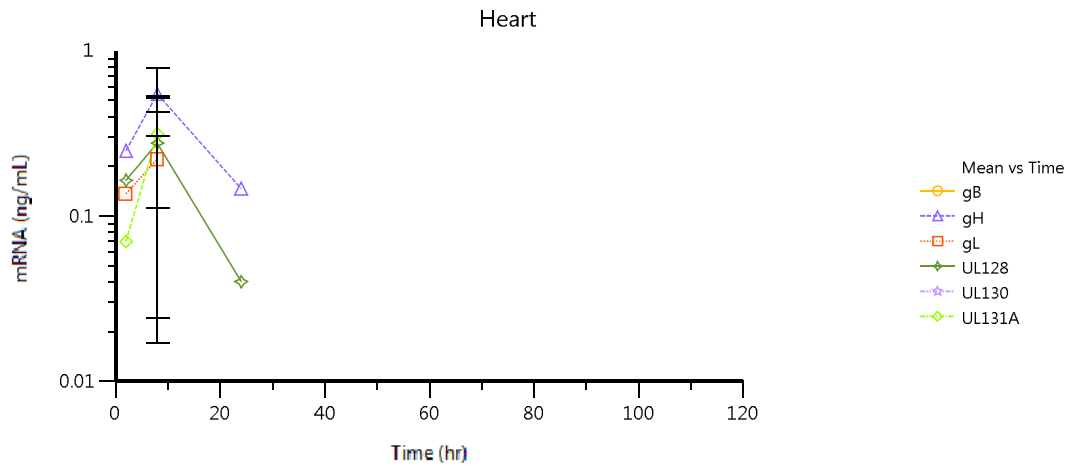

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Figure 1.7: Summary (± SD) Male Sprague-Dawley Rat Injection Site Muscle mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

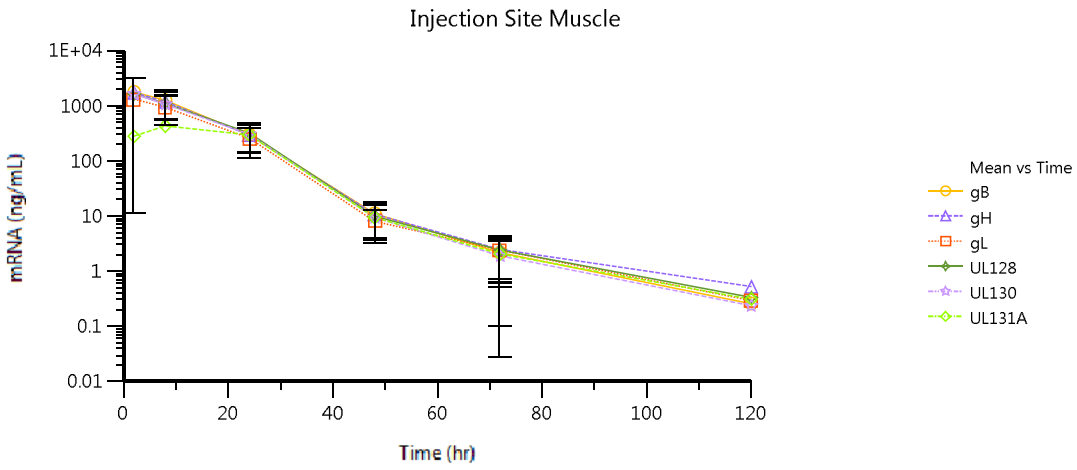

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Figure 1.8: Summary (± SD) Male Sprague-Dawley Rat Jejunum mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

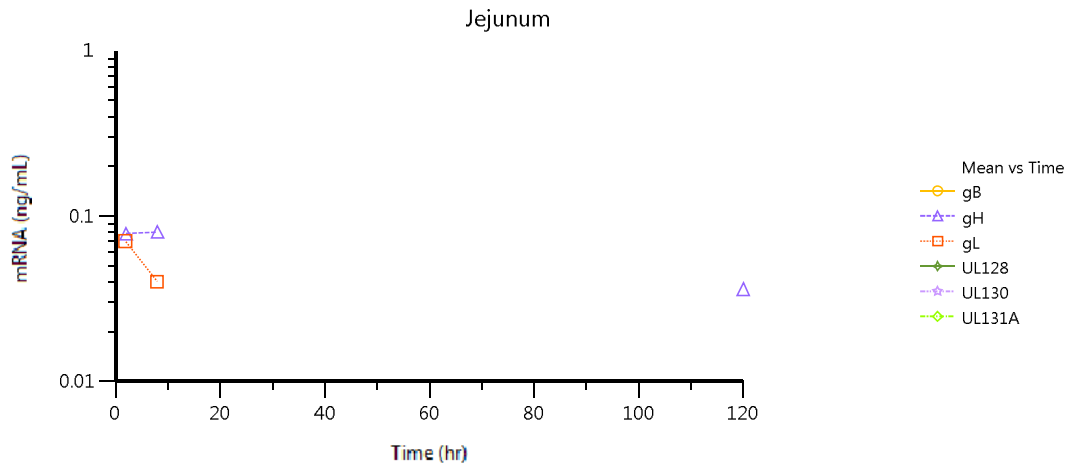

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Figure 1.9: Summary (± SD) Male Sprague-Dawley Rat Kidney mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

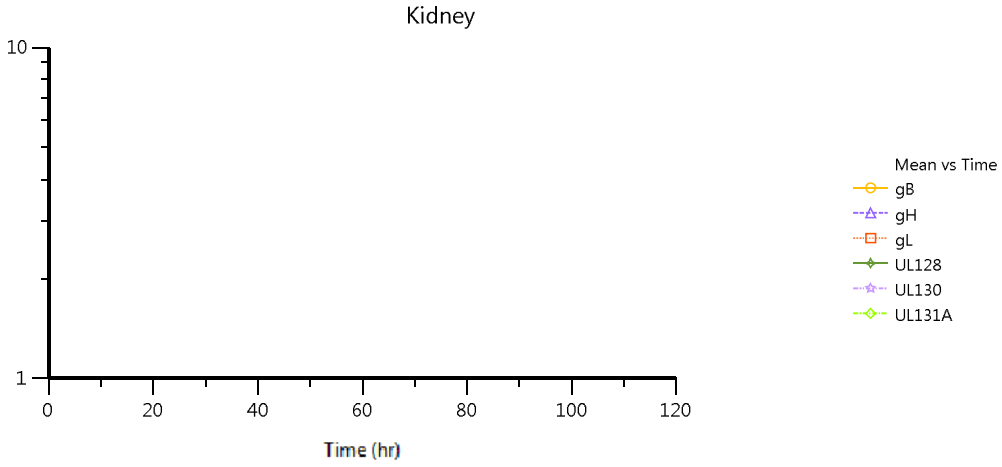

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Figure 1.10: Summary (± SD) Male Sprague-Dawley Rat Liver mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

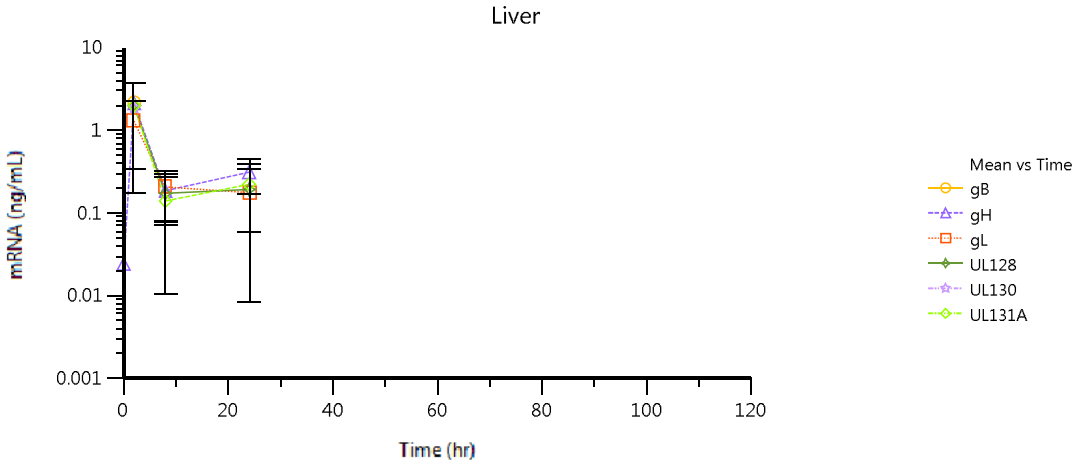

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Figure 1.11: Summary (± SD) Male Sprague-Dawley Rat Lung mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

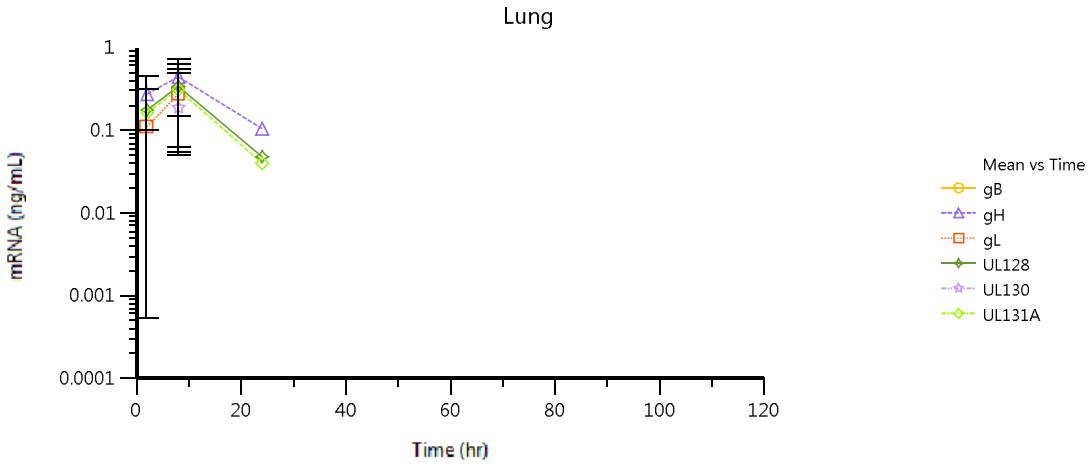

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Figure 1.12: Summary (± SD) Male Sprague-Dawley Rat Proximal Lymph node mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

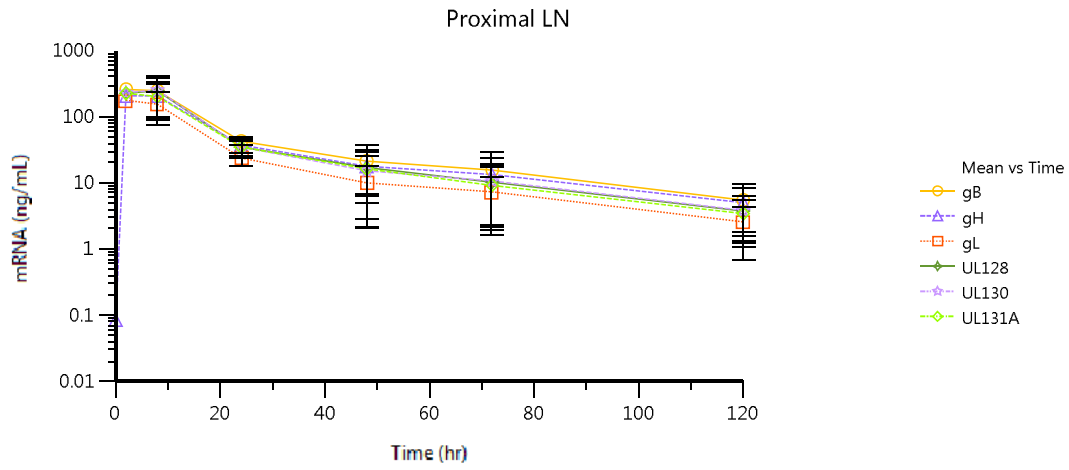

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Figure 1.13: Summary (± SD) Male Sprague-Dawley Rat Spleen mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

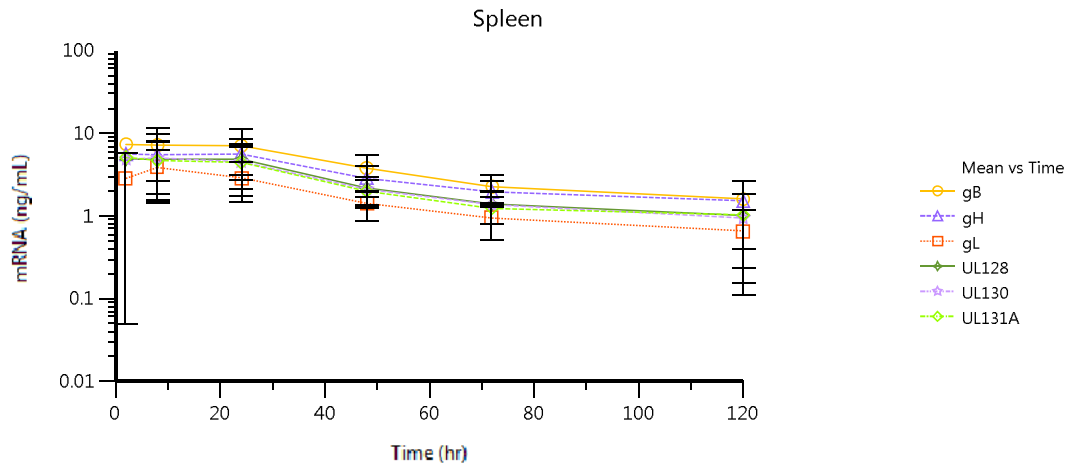

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Figure 1.14: Summary (± SD) Male Sprague-Dawley Rat Stomach mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

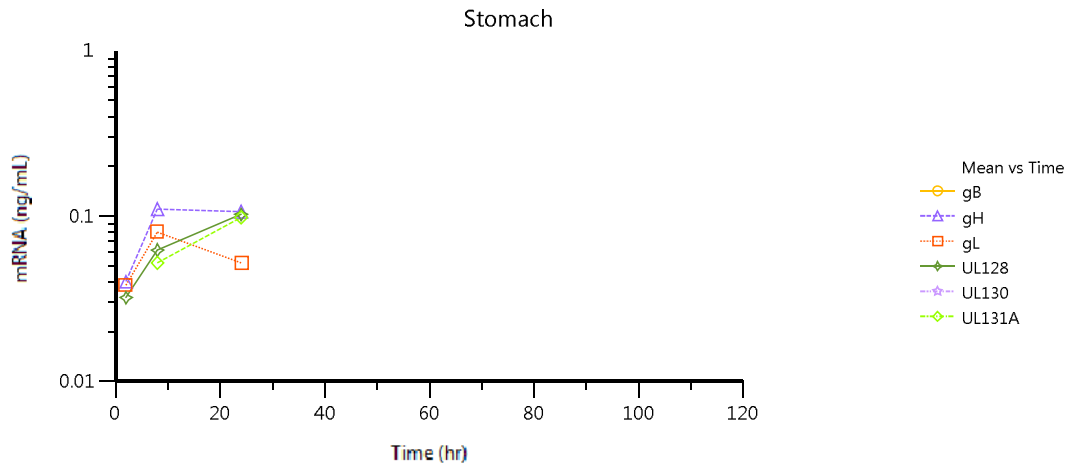

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Figure 1.15: Summary (± SD) Male Sprague-Dawley Rat Testes mRNA-1647 Concentrations Following Intramuscular Administration of mRNA-1647 on Day 1

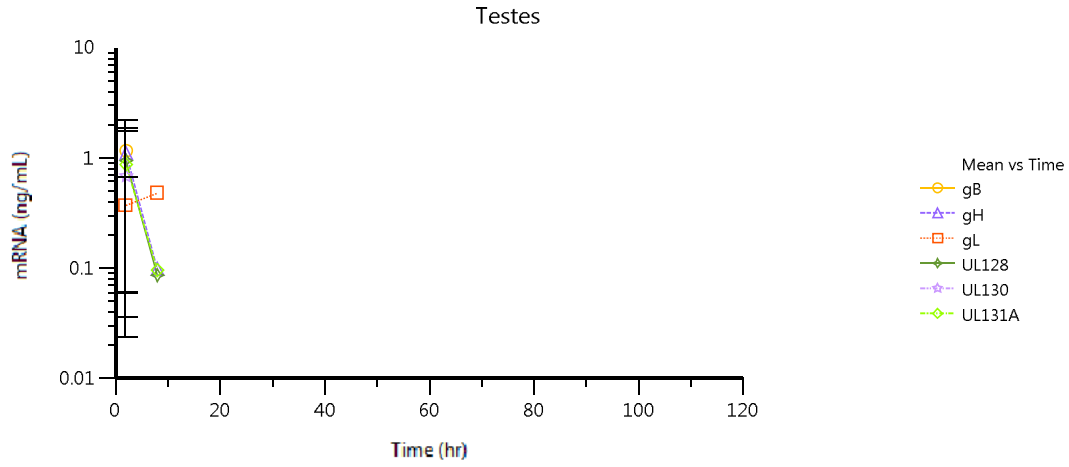

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Table 1
Concentrations of mRNA-1647 in Male Crl:CD(SD) Sprague-Dawley Rat Plasma and Tissues

Appendix 8

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.

Appendix 8

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

Appendix 8

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
	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
Jejunum	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
	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
Kidney	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
	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
Liver	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
	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
Lung	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
	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
Proximal LN	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

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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

Table of Contents

2.6.5.1 PHARMACOKINETICS: OVERVIEW

Type of Study	Test Article	Test System	Method of Administration	Testing Facility	Report Number	Location in eCTD
Distribution						
Single Dose IM tissue distribution study in male Sprague Dawley rats	mRNA-1647[a]	Rat, Sprague Dawley	Single IM Dose	Charles River Laboratories, Sherbrooke, QC, Canada	5002121 Amendment 1	4.2.2.3

Abbreviations: CMV = cytomegalovirus; gB = glycoprotein B; gH = glycoprotein; gL = glycoprotein L; eCTD = electronic common technical document; IM = intramuscular; mRNA = messenger RNA.

[a] mRNA-1647 contains 6 mRNAs that encode the full-length CMV gB and the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex. The 6 mRNAs are combined at a target mass ratio of 1:1:1:1:1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

2.6.5.2 ANALYTICAL METHODS AND VALIDATION REPORTS

Species/ Sample Matrix	Analyte	Calibration Range	Type of Assay	Method Utilized	Noteworthy Details (Qualification[a])	GLP Compliance	Report Number
Rat/ plasma and tissues	mRNA-1647	The LLOQs for plasma and tissues were set at 0.05 ng/mL for the gB and UL130 constructs and 0.01 ng/mL for the gH, gL, UL128, and UL131A constructs.	bDNA multiplex assay	QuantiGene 2.0 Plex assay	A multiplex bDNA assay is a hybridization-based method that combines multi-analyte profiling beads and bDNA signal amplification to enable the detection and quantitation of multiple RNA targets simultaneously. After preparation, a sample is combined with an array of fluorescent microspheres (capture beads) and probe sets specific for each RNA molecule of interest and allowed to incubate overnight. The capture beads are used as a support to capture RNA molecules, and the probe sets are used to quantify multiple target-specific RNA molecules within a single sample. Signal amplification is mediated by DNA amplification molecules that hybridize to one of the synthetic probes within each RNA-specific probe set. The capture beads are hybridized with pre-amplifier, amplifier, and label probe solutions. The label probes bind to streptavidin-conjugated R-phycoerythrin, and the resulting fluorescence signal associated with individual capture beads is read on a Luminex® flow cytometer. The signal is reported as the median fluorescence intensity and is proportional to the number of target RNA molecules present in the sample.	No	5002121 Amendment 1

Abbreviations: bDNA = branched DNA; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; LLOQ = lower limit of quantification.

[a] The method was not formally validated.

Source: Report 5002121 Amendment 1.

2.6.5.5 PHARMACOKINETICS: ORGAN DISTRIBUTION

Test Article: mRNA-1647

Study Title: A single dose intramuscular injection tissue distribution study of mRNA-1647 in male Sprague-Dawley rats

Species/Strain/Sex: Rat/Sprague Dawley/male **Location in eCTD:** 4.2.2.3

Number per Group: 5 animals terminated per time point (35 total) **Report Number:** 5002121 Amendment 1 **GLP Study:** No

Feeding Condition: Ad libitum **Dose Level:** 100 µg **Vehicle/Formulation:** PBS, pH 7.2 **Sample Matrix:** Plasma and tissues

Plasma and Tissue Collection Time Points: 0 (pre-dose), 2, 8, 24, 48, 72, and 120 h post-dose **Analyte:** mRNA-1647 (6 mRNA constructs)

Pharmacokinetic Parameters							
Sample Matrix	**mRNA Construct**	**T_{max} (h)[a]**	**C_{max} (ng/mL)[a]**	**$AUC_{(0-t)}$ (ng × h/mL)[a,b]**	**$T_{1/2}$ (h)[c]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[d]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average**
Plasma	gB	2.0	2.02 ± 0.181	22.7 ± 3.77	NC	NA	NA
	gH	2.0	1.91 ± 0.187	24.9 ± 4.49	NC	NA	
	gL	2.0	1.74 ± 0.177	23.4 ± 4.07	NC	NA	
	UL128	2.0	1.66 ± 0.151	24.1 ± 4.44	NC	NA	
	UL130	2.0	2.30 ± 0.621	25.5 ± 4.65	NC	NA	
	UL131A	2.0	1.60 ± 0.153	24.8 ± 4.59	NC	NA	
Tissue							
Bone marrow	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.254 ± 0.0871	7.85 ± 2.03	NC	0.316	
	gL	8.0	0.224 ± 0.0920	2.78 ± 1.03	NC	0.119	
	UL128	8.0	0.292 ± 0.120	3.53 ± 1.33	NC	0.147	
	UL130	NC	NC	NC	NC	NC	
	UL131A	8.0	0.186 ± 0.0829	2.05 ± 0.912	NC	0.0825	

Pharmacokinetic Parameters							
Sample Matrix	**mRNA Construct**	**T_{max} (h)[a]**	**C_{max} (ng/mL)[a]**	**$AUC_{(0-t)}$ (ng × h/mL)[a,b]**	**$T_{1/2}$ (h)[c]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[d]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average**
Brain	gB	NC	NC	NC	NC	NC	NR
	gH	24.0	0.0800 ± 0.0491	2.19 ± 1.08	NC	0.0880	
	gL	2.0	0.0360 ± 0.0360	0.144 ± 0.144	NC	0.00615	
	UL128	2.0	0.0340 ± 0.0340	0.136 ± 0.136	NC	0.00564	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Distal lymph node	gB	8.0	108 ± 101	1,460 ± 1,110	31.6	64.1	62.8
	gH	8.0	110 ± 102	1,490 ± 1,130	36.2	59.8	
	gL	8.0	117 ± 109	1,460 ± 1,200	30.6	62.6	
	UL128	8.0	125 ± 117	1,620 ± 1,290	32.1	67.1	
	UL130	8.0	129 ± 121	1,630 ± 1,330	27.9	64	
	UL131A	8.0	114 ± 108	1,470 ± 1,190	28.5	59.2	
Eye	gB	2.0	4.72 ± 2.77	26.7 ± 13.6	NC	1.18	1.24
	gH	2.0	3.92 ± 2.19	37.6 ± 11.0	NC	1.51	
	gL	2.0	3.23 ± 1.84	29.2 ± 9.75	NC	1.25	
	UL128	2.0	3.91 ± 2.19	34.5 ± 12.2	NC	1.43	
	UL130	2.0	3.61 ± 2.14	21.3 ± 11.0	NC	0.838	
	UL131A	2.0	3.43 ± 1.96	31.1 ± 10.2	NC	1.26	
Heart	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.548 ± 0.107	9.94 ± 1.85	NC	0.400	
	gL	8.0	0.220 ± 0.0907	2.96 ± 1.05	NC	0.127	
	UL128	8.0	0.276 ± 0.113	4.49 ± 1.51	NC	0.186	
	UL130	NC	NC	NC	NC	NC	
	UL131A	8.0	0.312 ± 0.0896	3.71 ± 1.02	NC	0.150	

Pharmacokinetic Parameters							
Sample Matrix	**mRNA Construct**	**T_{max} (h)[a]**	**C_{max} (ng/mL)[a]**	**$AUC_{(0-t)}$ (ng × h/mL)[a,b]**	**$T_{1/2}$ (h)[c]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[d]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average**
Injection site, muscle	gB	2.0	1,770 ± 803	27,100 ± 4,880	13.5	1190	939
	gH	2.0	1,720 ± 828	26,100 ± 4,700	17.1	1050	
	gL	2.0	1,310 ± 638	20,900 ± 3,720	15.2	893	
	UL128	2.0	1,620 ± 720	25,300 ± 4,090	14.9	1050	
	UL130	2.0	1,630 ± 777	24,500 ± 4,240	13.8	961	
	UL131A	8.0	427 ± 210	12,100 ± 2,830	15.0	487	
Jejunum	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.0800 ± 0.0490	2.06 ± 1.04	NC	0.0827	
	gL	2.0	0.0700 ± 0.0429	0.720 ± 0.472	NC	0.0308	
	UL128	NC	NC	NC	NC	NC	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Kidney	gB	NC	NC	NC	NC	NC	NR
	gH	NC	NC	NC	NC	NC	
	gL	NC	NC	NC	NC	NC	
	UL128	NC	NC	NC	NC	NC	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Liver	gB	2.0	2.16 ± 1.21	8.65 ± 4.83	NC	0.381	0.499
	gH	2.0	2.12 ± 0.982	16.8 ± 4.15	NC	0.674	
	gL	2.0	1.30 ± 0.432	11.0 ± 2.37	NC	0.470	
	UL128	2.0	2.00 ± 0.814	13.7 ± 3.72	NC	0.570	
	UL130	2.0	1.87 ± 1.01	7.46 ± 4.04	NC	0.293	
	UL131A	2.0	1.99 ± 0.928	13.9 ± 4.04	NC	0.562	

Pharmacokinetic Parameters							
Sample Matrix	**mRNA Construct**	**T_{max} (h)[a]**	**C_{max} (ng/mL)[a]**	**$AUC_{(0-t)}$ (ng × h/mL)[a,b]**	**$T_{1/2}$ (h)[c]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[d]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average**
Lung	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.442 ± 0.130	8.04 ± 1.96	NC	0.323	
	gL	8.0	0.274 ± 0.0984	3.45 ± 1.12	NC	0.148	
	UL128	8.0	0.340 ± 0.129	5.40 ± 1.74	NC	0.224	
	UL130	8.0	0.188 ± 0.188	2.07 ± 2.07	NC	0.0812	
	UL131A	8.0	0.310 ± 0.111	4.86 ± 1.49	NC	0.196	
Proximal lymph node	gB	2.0	260 ± 121	5,850 ± 949	33.5	257	201
	gH	8.0	206 ± 51.6	4,860 ± 722	38.2	195	
	gL	2.0	175 ± 81.9	3,460 ± 538	36.3	148	
	UL128	8.0	246 ± 66.6	5,190 ± 875	32.8	215	
	UL130	8.0	252 ± 67.2	5,240 ± 881	35.7	206	
	UL131A	2.0	225 ± 106	4,600 ± 719	32.2	185	
Spleen	gB	2.0	7.36 ± 3.81	460 ± 52.9	46.9	20.2	13.4
	gH	24.0	5.63 ± 1.28	371 ± 39.5	83.0	14.9	
	gL	8.0	3.83 ± 1.04	196 ± 21.0	68.2	8.36	
	UL128	24.0	4.87 ± 1.22	297 ± 34.8	68.8	12.3	
	UL130	8.0	5.03 ± 1.41	288 ± 33.0	64.9	11.3	
	UL131A	2.0	5.10 ± 2.64	277 ± 33.1	46.2	11.2	
Stomach	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.110 ± 0.0696	3.49 ± 1.59	NC	0.140	
	gL	8.0	0.0800 ± 0.0499	2.07 ± 1.19	NC	0.0886	
	UL128	24.0	0.102 ± 0.0648	2.85 ± 1.47	NC	0.118	
	UL130	NC	NC	NC	NC	NC	
	UL131A	24.0	0.0980 ± 0.0634	2.53 ± 1.39	NC	0.102	

Pharmacokinetic Parameters							
Sample Matrix	**mRNA Construct**	**T_{max} (h)[a]**	**C_{max} (ng/mL)[a]**	**$AUC_{(0-t)}$ (ng × h/mL)[a,b]**	**$T_{1/2}$ (h)[c]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[d]**	**$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average**
Testes	gB	2.0	1.16 ± 0.719	4.64 ± 2.88	NC	0.204	0.209
	gH	2.0	1.11 ± 0.480	5.52 ± 2.20	NC	0.222	
	gL	8.0	0.420 ± 0.335	6.08 ± 3.73	NC	0.260	
	UL128	2.0	0.946 ± 0.397	4.73 ± 1.85	NC	0.196	
	UL130	2.0	0.682 ± 0.442	2.73 ± 1.77	NC	0.107	
	UL131A	2.0	0.872 ± 0.380	4.54 ± 1.85	NC	0.183	

Abbreviations: eCTD = electronic common technical document; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; mRNA = messenger RNA; NA = not applicable; NC = not calculable (insufficient data points above lower limit of quantitation); NR = not reported (some constructs measured all samples as below limit of quantitation); PBS = phosphate-buffered saline.

[a] T_{max} and $T_{1/2}$ data reported as the mean; C_{max} and $AUC_{(0-t)}$ data reported as the mean ± standard error.

[b] 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.

[c] Due to the lack of a distinct elimination phase in plasma, the $T_{1/2}$ of the mRNA constructs could not be calculated; however, the $T_{1/2}$ was estimated to range from 2.7 to 3.8 hours.

[d] For $AUC_{(0-t)}$ Ratio, samples listed as NC were not calculable because all samples were below limit of quantitation.

Source: Report 5002121 Amendment 1 (Appendix 8, Table 2 and Table 3).

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List of Abbreviations

Abbreviation	Definition
AUC	area under the concentration versus time curve
$AUC_{(0-t)}$	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
bDNA	branched DNA
C_{max}	maximum plasma concentration
CoV	coronavirus
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
gB	glycoprotein B
gH	glycoprotein H
gL	glycoprotein L
GLP	Good Laboratory Practice
IM	intramuscular(ly)
LLOQ	lower limit of quantitation
LNP	lipid nanoparticle
mRNA	messenger RNA
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PG	propylene glycol
PK	pharmacokinetic
S	spike
S-2P	spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
SARS-CoV-2	2019 novel coronavirus
SM-102	heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate
$T_{1/2}$	half-life
Tris	tris(hydroxymethyl)aminomethane
T_{max}	time to peak (maximum) plasma concentration

2.6.4.1 BRIEF SUMMARY

ModernaTX, Inc. (Sponsor) has developed mRNA-1273, a novel lipid nanoparticle (LNP)-encapsulated messenger RNA (mRNA)-based vaccine against the 2019 novel coronavirus (CoV; SARS-CoV-2). mRNA-1273 contains a single mRNA that encodes the full-length spike (S) protein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilize the S protein into the prefusion conformation. The mRNA is combined in a mixture of 4 lipids common to the Sponsor's mRNA vaccine platform: SM-102, cholesterol, DSPC, and PEG2000-DMG.

The results of a biodistribution study of mRNA-1647 support the development of mRNA-1273. mRNA-1647 is a novel mRNA-based cytomegalovirus vaccine that contains 6 distinct mRNA sequences (1 that encodes the full-length cytomegalovirus glycoprotein B [gB], and 5 that encode the pentameric glycoprotein H [gH]/glycoprotein L [gL]/UL128/UL130/UL131A glycoprotein complex) combined at a target mass ratio of 1:1:1:1:1:1 in the Sponsor's standard proprietary SM-102–containing LNPs.

The biodistribution of mRNA-1647 was evaluated in a non-Good Laboratory Practice (GLP), single-dose, intramuscular (IM) injection study in Sprague Dawley rats. The objectives of this study were to determine the tissue distribution and pharmacokinetic (PK) characteristics of mRNA-1647 following IM administration. The biodistribution of mRNA-based vaccines in LNPs is predicted to be driven by the LNP characteristics. Therefore, mRNAs that are within an LNP of the same composition (eg, mRNA-1273 and mRNA-1647) are expected to distribute similarly.

Concentrations for all 6 mRNA-1647 constructs, gB, gH, gL, UL128, UL130, and UL131A, were detectable in plasma and tissues in a 1:1:1:1:1:1 ratio. After a single IM dose in male rats, the time after dosing at which the maximum concentration was observed in plasma (T_{max}) was 2 hours for all constructs and was followed by a rapid elimination phase, with a half-life ($T_{1/2}$) estimated to range from 2.7 to 3.8 hours. The maximum plasma concentration (C_{max}) ranged from 1.60 to 2.30 ng/mL, and the area under the concentration versus time curve (AUC) from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed ($AUC_{[0\text{-}t]}$) ranged from 22.7 to 25.5 ng × h/mL.

Concentrations for all 6 mRNA-1647 constructs were detected at levels above the lower limit of quantitation (LLOQ) in most tissues analyzed, except for the kidney, where all levels were below the LLOQ. For highly exposed tissues (injection site [muscle], lymph nodes [proximal and

distal], and spleen), the C_{max} was observed between 2 and 24 hours post-dose. The $T_{1/2}$ was calculated using the average tissue $T_{1/2}$ values for all 6 constructs. The results were 14.9 hours for injection site (muscle), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

As observed with other IM delivered vaccines, the highest mRNA concentrations were observed at the injection site followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0.

Overall, only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for all constructs ranged from 14.9 to 63.0 hours.. The completed nonclinical PK and biodistribution study is presented in Table 1.

Table 1: Nonclinical Biodistribution Study Supporting Development of mRNA-1273

Study Type	Test Article	Species, Strain	Method of Administration, Dose	GLP	Report Number
Single-dose tissue distribution study	mRNA-1647	Rat, Sprague Dawley	IM injection, dose of 100 µg on Day 1	No	5002121 Amendment 1

Abbreviations: CMV = cytomegalovirus; gB = glycoprotein B; gH = glycoprotein; gL = glycoprotein L; GLP = Good Laboratory Practice; IM = intramuscular; mRNA = messenger RNA; .

[a] mRNA-1647 contains 6 mRNAs that encode the full-length CMV gB and the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex. The 6 mRNAs are combined at a target mass ratio of 1:1:1:1:1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

2.6.4.2 METHODS OF ANALYSIS

The procedure followed during the course of this study, along with the assay acceptance criteria, was detailed in a bioanalytical protocol. The LLOQs for plasma and tissues was set at 0.05 ng/mL for the gB and UL130 constructs and 0.01 ng/mL for the gH, gL, UL128, and UL131A constructs. Samples were analyzed in duplicate. Details on how biological samples were collected and processed are provided in the study report (Report 5002121 Amendment 1 Section 4.12).

Samples were analyzed for all 6 mRNA constructs (gB, gH, gL, UL128, UL130, and UL131A) present in mRNA-1647. To quantify these multiple constructs in mRNA-1647, a multiplex branched DNA (bDNA) assay was used. This assay is a hybridization-based method that combines multi-analyte profiling beads and bDNA signal amplification to enable the detection and quantitation of multiple RNA targets simultaneously. After preparation, a sample is combined with an array of fluorescent microspheres (capture beads) and probe sets specific for each RNA molecule of interest and allowed to incubate overnight. The capture beads are used as a support to capture RNA molecules, and the probe sets are used to quantify multiple target-specific RNA molecules within a single sample. Signal amplification is mediated by DNA amplification molecules that hybridize to one of the synthetic probes within each RNA-specific probe set. The capture beads are hybridized with pre-amplifier, amplifier, and label probe solutions. The label probes bind to streptavidin-conjugated R-phycoerythrin, and the resulting fluorescence signal associated with individual capture beads is read on a Luminex® flow cytometer. The signal is reported as the median fluorescence intensity and is proportional to the number of target RNA molecules present in the sample.

2.6.4.3 ABSORPTION

No absorption studies with mRNA-1273 have been performed.

2.6.4.4 DISTRIBUTION

2.6.4.4.1 Tissue Distribution Studies

The objective of this non-GLP study was to determine the tissue distribution of mRNA-1647 when given once by IM injection to rats. The PK characteristics of mRNA-1647 were determined in plasma and tissue. A group of 35 male Sprague Dawley rats was given a single IM injection of 100 µg of mRNA-1647 in a dose volume of 200 µL (dose concentration of 0.5 mg/mL) on Day 1. Subgroups of 5 rats each were sacrificed pre-dose and 2, 8, 24, 48, 72, and 120 hours after IM dosing. Blood and tissues were collected and processed for quantitation of the 6 mRNA constructs (gB, gH, gL, UL128, UL130, and UL131A) present in mRNA-1647 using a qualified bDNA multiplex method (Section 2.6.4.2). The overall design of this study is presented in Table 2.

Table 2: A Single-Dose IM Pharmacokinetic and Biodistribution Study of mRNA-1647 in Sprague Dawley Rats

Group Number	Test Article (Method of Administration)	Species/ Strain	Number of Animals/Sex	Dose Level (µg)	Dose Volume (µL)	Dose Concentration (mg/mL)	Sample Collection Time Points (h)
1	mRNA-1647 (single IM injection)	Rats/ Sprague Dawley	35/male	100	200	0.5	0 (pre-dose), 2, 8, 24, 48, 72, and 120

Abbreviations: IM = intramuscular.
Source: Report 5002121 Amendment 1 (Text Table 3 and Text Table 4).

No quantifiable concentrations for any of the mRNA constructs were observed in plasma or tissue in pre-dose samples, with the exception of 2 plasma samples for which the gH construct concentration was slightly above the LLOQ. For all 6 mRNA constructs present in mRNA-1647, post-dose levels were detectable in plasma and tissues in a 1:1:1:1:1:1 ratio. Mean plasma concentrations were quantifiable up to 24 hours with an interanimal coefficient of variation from 21.8% and 79.8%. The only quantifiable plasma samples beyond 24 hours were 6 gH constructs that were slightly above the LLOQ.

After a single IM dose in male rats, the T_{max} for all 6 mRNA constructs was 2 hours, followed by a rapid elimination phase. Mean concentrations became undetectable for all constructs after 24 hours with the exception of gH, which was detectable up to the last time point of 120 hours. Due to the lack of a distinct elimination phase, the $T_{1/2}$ of the mRNA constructs could not be calculated; however, the $T_{1/2}$ was estimated to range from 2.7 to 3.8 hours. The C_{max} and $AUC_{(0\text{-}t)}$ ranged from 1.60 to 2.30 ng/mL and from 22.7 to 25.5 ng × h/mL, respectively (Table 3).

Table 3: Plasma Pharmacokinetic Parameters for a Single IM Dose of 100 µg of mRNA-1647 in Male Sprague Dawley Rats

Matrix	Construct	T_{max} (h)[a]	C_{max} (ng/mL)[a]	$AUC_{(0-t)}$ (ng × h/mL)[a]	$T_{1/2}$ (h)[b]
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
	UL131A	2.0	1.60 ± 0.153	24.8 ± 4.59	NC

Abbreviations: gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; IM = intramuscular; NC = not calculable (insufficient data points above the lower limit of quantification).

[a] T_{max} data reported as the mean; C_{max} and $AUC_{(0-t)}$ data reported as the mean ± standard error.

[b] Due to the lack of a distinct elimination phase, the $T_{1/2}$ of the mRNA constructs could not be calculated; however, the $T_{1/2}$ was estimated to range from 2.7 to 3.8 hours.

Source: Report 5002121 Amendment 1 (Appendix 8, Table 2).

All constructs of mRNA-1647 were quantifiable in most tissues analyzed, except for the kidney, where all levels were below the LLOQ. For highly exposed tissues (injection site [muscle], lymph nodes [proximal and distal], and spleen), the C_{max} was observed between 2 and 24 hours post-dose. The $T_{1/2}$ was calculated using the average tissue $T_{1/2}$ values for all 6 constructs. The results were 14.9 hours injection site (muscle), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

As observed with other IM delivered vaccines, the highest mRNA concentrations were observed at the injection site (muscle) followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0.

Overall, only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for all constructs ranged from 14.9 to 63.0 hours. (Table 4).

Table 4: Tissue Pharmacokinetic Parameters for a Single IM Dose of 100 µg of mRNA-1647 in Male Sprague Dawley Rats

Matrix	Construct	T_{max} (h)[a]	C_{max} (ng/mL)[a]	$AUC_{(0-t)}$ (ng × h/mL)[a,b]	$T_{1/2}$ (h)[a]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[c]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average
Bone marrow	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.254 ± 0.0871	7.85 ± 2.03	NC	0.316	
	gL	8.0	0.224 ± 0.0920	2.78 ± 1.03	NC	0.119	
	UL128	8.0	0.292 ± 0.120	3.53 ± 1.33	NC	0.147	
	UL130	NC	NC	NC	NC	NC	
	UL131A	8.0	0.186 ± 0.0829	2.05 ± 0.912	NC	0.0825	
Brain	gB	NC	NC	NC	NC	NC	NR
	gH	24.0	0.0800 ± 0.0491	2.19 ± 1.08	NC	0.0880	
	gL	2.0	0.0360 ± 0.0360	0.144 ± 0.144	NC	0.00615	
	UL128	2.0	0.0340 ± 0.0340	0.136 ± 0.136	NC	0.00564	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Distal lymph node	gB	8.0	108 ± 101	1,460 ± 1,110	31.6	64.1	62.8
	gH	8.0	110 ± 102	1,490 ± 1,130	36.2	59.8	
	gL	8.0	117 ± 109	1,460 ± 1,200	30.6	62.6	
	UL128	8.0	125 ± 117	1,620 ± 1,290	32.1	67.1	
	UL130	8.0	129 ± 121	1,630 ± 1,330	27.9	64	
	UL131A	8.0	114 ± 108	1,470 ± 1,190	28.5	59.2	

Matrix	Construct	T_{max} (h)[a]	C_{max} (ng/mL)[a]	$AUC_{(0-t)}$ (ng × h/mL)[a,b]	$T_{1/2}$ (h)[a]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[c]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average
Eye	gB	2.0	4.72 ± 2.77	26.7 ± 13.6	NC	1.18	1.24
	gH	2.0	3.92 ± 2.19	37.6 ± 11.0	NC	1.51	
	gL	2.0	3.23 ± 1.84	29.2 ± 9.75	NC	1.25	
	UL128	2.0	3.91 ± 2.19	34.5 ± 12.2	NC	1.43	
	UL130	2.0	3.61 ± 2.14	21.3 ± 11.0	NC	0.838	
	UL131A	2.0	3.43 ± 1.96	31.1 ± 10.2	NC	1.26	
Heart	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.548 ± 0.107	9.94 ± 1.85	NC	0.400	
	gL	8.0	0.220 ± 0.0907	2.96 ± 1.05	NC	0.127	
	UL128	8.0	0.276 ± 0.113	4.49 ± 1.51	NC	0.186	
	UL130	NC	NC	NC	NC	NC	
	UL131A	8.0	0.312 ± 0.0896	3.71 ± 1.02	NC	0.150	
Injection site muscle	gB	2.0	1,770 ± 803	27,100 ± 4,880	13.5	1190	939
	gH	2.0	1,720 ± 828	26,100 ± 4,700	17.1	1050	
	gL	2.0	1,310 ± 638	20,900 ± 3,720	15.2	893	
	UL128	2.0	1,620 ± 720	25,300 ± 4,090	14.9	1050	
	UL130	2.0	1,630 ± 777	24,500 ± 4,240	13.8	961	
	UL131A	8.0	427 ± 210	12,100 ± 2,830	15.0	487	
Jejunum	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.0800 ± 0.0490	2.06 ± 1.04	NC	0.0827	
	gL	2.0	0.0700 ± 0.0429	0.720 ± 0.472	NC	0.0308	
	UL128	NC	NC	NC	NC	NC	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	

Matrix	Construct	T_{max} (h)[a]	C_{max} (ng/mL)[a]	$AUC_{(0-t)}$ (ng × h/mL)[a,b]	$T_{1/2}$ (h)[a]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[c]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average
Kidney	gB	NC	NC	NC	NC	NC	NR
	gH	NC	NC	NC	NC	NC	
	gL	NC	NC	NC	NC	NC	
	UL128	NC	NC	NC	NC	NC	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Liver	gB	2.0	2.16 ± 1.21	8.65 ± 4.83	NC	0.381	0.499
	gH	2.0	2.12 ± 0.982	16.8 ± 4.15	NC	0.674	
	gL	2.0	1.30 ± 0.432	11.0 ± 2.37	NC	0.470	
	UL128	2.0	2.00 ± 0.814	13.7 ± 3.72	NC	0.570	
	UL130	2.0	1.87 ± 1.01	7.46 ± 4.04	NC	0.293	
	UL131A	2.0	1.99 ± 0.928	13.9 ± 4.04	NC	0.562	
Lung	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.442 ± 0.130	8.04 ± 1.96	NC	0.323	
	gL	8.0	0.274 ± 0.0984	3.45 ± 1.12	NC	0.148	
	UL128	8.0	0.340 ± 0.129	5.40 ± 1.74	NC	0.224	
	UL130	8.0	0.188 ± 0.188	2.07 ± 2.07	NC	0.0812	
	UL131A	8.0	0.310 ± 0.111	4.86 ± 1.49	NC	0.196	
Proximal lymph nodes	gB	2.0	260 ± 121	5,850 ± 949	33.5	257	201
	gH	8.0	206 ± 51.6	4,860 ± 722	38.2	195	
	gL	2.0	175 ± 81.9	3,460 ± 538	36.3	148	
	UL128	8.0	246 ± 66.6	5,190 ± 875	32.8	215	
	UL130	8.0	252 ± 67.2	5,240 ± 881	35.7	206	
	UL131A	2.0	225 ± 106	4,600 ± 719	32.2	185	

Matrix	Construct	T_{max} (h)[a]	C_{max} (ng/mL)[a]	$AUC_{(0-t)}$ (ng × h/mL)[a,b]	$T_{1/2}$ (h)[a]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma)[c]	$AUC_{(0-t)}$ Ratio (Tissue/Plasma) Average
Spleen	gB	2.0	7.36 ± 3.81	460 ± 52.9	46.9	20.2	13.4
	gH	24.0	5.63 ± 1.28	371 ± 39.5	83.0	14.9	
	gL	8.0	3.83 ± 1.04	196 ± 21.0	68.2	8.36	
	UL128	24.0	4.87 ± 1.22	297 ± 34.8	68.8	12.3	
	UL130	8.0	5.03 ± 1.41	288 ± 33.0	64.9	11.3	
	UL131A	2.0	5.10 ± 2.64	277 ± 33.1	46.2	11.2	
Stomach	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.110 ± 0.0696	3.49 ± 1.59	NC	0.140	
	gL	8.0	0.0800 ± 0.0499	2.07 ± 1.19	NC	0.0886	
	UL128	24.0	0.102 ± 0.0648	2.85 ± 1.47	NC	0.118	
	UL130	NC	NC	NC	NC	NC	
	UL131A	24.0	0.0980 ± 0.0634	2.53 ± 1.39	NC	0.102	
Testes	gB	2.0	1.16 ± 0.719	4.64 ± 2.88	NC	0.204	0.209
	gH	2.0	1.11 ± 0.480	5.52 ± 2.20	NC	0.222	
	gL	8.0	0.420 ± 0.335	6.08 ± 3.73	NC	0.260	
	UL128	2.0	0.946 ± 0.397	4.73 ± 1.85	NC	0.196	
	UL130	2.0	0.682 ± 0.442	2.73 ± 1.77	NC	0.107	
	UL131A	2.0	0.872 ± 0.380	4.54 ± 1.85	NC	0.183	

Abbreviations: gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; IM = intramuscular; NC = not calculable (insufficient data points above the lower limit of quantitation); NR = not reported (some constructs measured all samples as below limit of quantitation).

[a] T_{max} and $T_{1/2}$ data reported as the mean; C_{max} and $AUC_{(0-t)}$ data reported as the mean ± standard error.

[b] 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.

[c] For $AUC_{(0-t)}$ Ratio, samples listed as NC were not calculable because all samples were below limit of quantitation.

Source: Report 5002121 Amendment 1 (Appendix 8, Table 2 and Table 3)

2.6.4.5 METABOLISM

No metabolism studies with mRNA-1273 have been performed.

2.6.4.6 EXCRETION

No excretion studies with mRNA-1273 have been performed.

2.6.4.7 PHARMACOKINETIC DRUG INTERACTIONS

No PK drug interaction studies with mRNA-1273 have been performed.

2.6.4.8 OTHER PHARMACOKINETIC STUDIES

No other PK studies with mRNA-1273 have been performed.

2.6.4.9 DISCUSSION AND CONCLUSION

A non-GLP biodistribution study was completed with mRNA-1647, an mRNA-based vaccine combined in SM-102–containing LNPs, in male Sprague Dawley rats and is provided to support the development of mRNA-1273 using the Sponsor's mRNA technology platform. The biodistribution of mRNA-based vaccines in LNPs is predicted to be driven by the LNP characteristics. Therefore, mRNAs that are within an LNP of the same composition (eg, mRNA-1273 and mRNA-1647) are expected to distribute similarly.

- Concentrations for mRNA constructs were detected at levels above the LLOQ in most tissues analyzed, except for the kidney, where all levels were below the LLOQ.

- As observed with other IM-delivered vaccines, the highest mRNA concentrations were observed at the injection site followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0.

- The T_{max} in plasma was achieved at 2 hours post-dose, with an estimated $T_{1/2}$ in plasma ranging from 2.7 to 3.8 hours. For highly exposed tissues, C_{max} was observed between 2 and 24 hours post-dose. The $T_{1/2}$ values, calculated using the average tissue $T_{1/2}$ values for all 6 constructs, were 14.9 hours for site of injection (muscle), 34.8 hours for

proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

Overall, only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for all constructs ranged from 14.9 to 63.0 hours.. The biodistribution of mRNA-based vaccines in LNPs is predicted to be driven by the LNP characteristics. Therefore, mRNAs that are within an LNP of the same composition (eg, mRNA-1273 and mRNA-1647) are expected to distribute similarly.

2.6.4.10 TABLES AND FIGURES

The tables and figures are included in the body of the document.

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List of Abbreviations

Abbreviation	Definition
ACE-2	angiotensin converting enzyme 2
cDC	conventional dendritic cell
CDC	Centers for Disease Control and Prevention
CMV	cytomegalovirus
CoV	coronavirus
COVID-19	coronavirus disease 2019
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
DTPA	diethylenetriamine pentaacetic acid
ERD	enhanced respiratory disease
gB	glycoprotein B
GD	Gestation Day
gH	glycoprotein H
gL	glycoprotein L
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
hMPV	human metapneumovirus
ICH	International Council for Harmonisation
Ig	immunoglobulin
IM	Intramuscular(-ly)
LD	Lactation Day
LLOQ	lower limit of quantitation
LNP	lipid nanoparticle
mAb	monoclonal antibody
mRNA	messenger RNA
NHP	nonhuman primate
NPI	nascent peptide imaging
NTD	N-terminal domain
pDC	plasmacytoid dendritic cell
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PIV3	parainfluenza virus type 3
RBD	receptor binding domain
S	spike

Abbreviation	Definition
S-2P	spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
SARS	severe acute respiratory syndrome
SARS-CoV-1 DIV	double-inactivated severe acute respiratory syndrome coronavirus-1
SARS-CoV-2	2019 novel coronavirus
SM-102	heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate
Th	T helper
Tris	tris(hydroxymethyl)aminomethane
WHO	World Health Organization
WT	wild type

2.4.1. OVERVIEW OF NONCLINICAL TESTING STRATEGY

2.4.1.1 Background

Coronaviruses (CoVs) are part of a large family of viruses that cause illnesses ranging from the common cold to more severe diseases, such as Middle East respiratory syndrome and severe acute respiratory syndrome (SARS).

An outbreak of the CoV disease 2019 (COVID-19) caused by the 2019 novel CoV (2019-nCoV, later designated SARS-CoV-2) began in Wuhan, Hubei Province, China, in Dec 2019 and the disease has since spread globally (WHO 2020a). The WHO declared COVID-19 a pandemic on 11 Mar 2020; however, widespread community transmission was already occurring in many locations. As of Nov 2020, more than 53 million cases and over 1.3 million deaths worldwide have been attributed to the COVID-19 pandemic (WHO 2020a; WHO 2020b). Widespread community transmission of SARS-CoV-2 has been reported in the Americas, Europe, Africa, and Southeast Asia, and clusters of cases continue to be reported throughout Asia and Australia (WHO 2020a). During the winter, the combination of re-opening of schools and increase in indoor activity, because of lower temperatures, is expected to further increases in COVID-19 cases and deaths in some parts of the world.

Current evidence suggests that SARS-CoV-2 is primarily transmitted via direct contact or person-to-person via respiratory droplets by coughing or sneezing from an infected individual (regardless of whether they are symptomatic) (Chen et al 2020; Licciardi et al 2020; Rothan and Byrareddy 2020; Shen et al 2020). Airborne transmission may be possible during certain medical procedures and in indoor, crowded, and poorly ventilated environments (WHO 2020c). Common symptoms of COVID-19 include fever and cough, and other symptoms include shortness of breath or difficulty breathing, muscle aches, chills, sore throat, headache, and loss of taste or smell. Individuals at highest risk of COVID-19 and severe COVID-19 are older adults (≥ 65 years old) and people of any age who have certain underlying medical conditions such as cancer, chronic kidney disease, chronic obstructive pulmonary disease, serious heart conditions, immunocompromised state, obesity, pregnancy, sickle cell disease, and type 2 diabetes mellitus; smokers are also at increased risk for severe COVID-19 disease (CDC 2020).

Currently, there is no FDA-approved vaccine against SARS-CoV-2. Without further advances in the use of nonpharmaceutical interventions, over 2.5 million COVID-19 deaths are projected globally by 01 Mar 2021, with daily deaths peaking at about 15,000/day during this time (IHME 2020). Global efforts to evaluate novel antivirals and therapeutic strategies to treat severe

SARS-CoV-2 infections have intensified, and there is an urgent public health need for rapid development of novel prophylactic therapies, including vaccines, to prevent the spread of this disease.

ModernaTX, Inc. (Sponsor) has developed a rapid-response proprietary vaccine platform based on a messenger RNA (mRNA) delivery system. The platform is based on the principle and observation that cells can take up mRNA, translate it, and then express viral antigen(s) on the cell surface. The delivered mRNA does not enter the nucleus or interact with the genome, is nonreplicating, and is expressed transiently. mRNA vaccines developed with the Sponsor's mRNA-based platform have been used to induce immune responses against infectious pathogens such as cytomegalovirus (CMV; NCT03382405), human metapneumovirus (hMPV) and parainfluenza virus type 3 (PIV3; NCT03392389), Zika virus (NCT04064905), and influenza virus (NCT03076385 and NCT03345043).

The Sponsor has used its mRNA-based platform to develop mRNA-1273, a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine against SARS-CoV-2. mRNA-1273 contains a single mRNA that encodes the full-length SARS-CoV-2 spike (S) protein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilize the S protein into the prefusion conformation. The CoV S protein mediates attachment and entry of the virus into host cells (by binding to the angiotensin converting enzyme 2 [ACE-2] receptor followed by membrane fusion), making it a primary target for neutralizing antibodies that prevent infection (Corti et al 2015; Wang et al 2015; Yu et al 2015; Johnson et al 2016; Chen et al 2017; Wang et al 2018; Kim et al 2019; Widjaja et al 2019). It has been confirmed that the stabilized SARS-CoV-2 S-2P mRNA expresses well in mammalian cells and is in the prefusion conformation (Wrapp et al 2020).

Nonclinical studies have demonstrated that CoV S proteins are immunogenic and that S protein-based vaccines, including those based on mRNA delivery platforms, are protective in animals (Corbett et al 2020a, Corbett et al 2020b, Graham et al 2020, Mercado et al 2020, Tian et al 2020, Tostanoski et al 2020, Vogel et al 2020). Prior clinical studies of vaccines targeting related CoVs and other viruses have assessed the immunogenicity and safety profiles of mRNA-based vaccines (Anderson et al 2020, Folegatti et al 2020, Jackson et al 2020, Keech et al 2020, Mulligan et al 2020, Sadoff et al 2020, Walsh et al 2020).

The clinical development of mRNA-1273 to support its use in the adult population consists of 3 ongoing clinical trials being conducted in the US: a Phase 1, open-label, dose-ranging study (NCT04283461) sponsored by the National Institute of Allergy and Infectious Diseases and a Phase 2a, randomized, observer-blind, placebo-controlled, dose-confirmation study

(NCT04405076) and a Phase 3 randomized, stratified, observer-blind, placebo-controlled study (NCT04470427) conducted by the Sponsor to evaluate the efficacy, safety, and immunogenicity of the vaccine. The development of mRNA-1273 has been accelerated to address the current COVID-19 outbreak, benefiting from the uniquely rapid and scalable manufacturing processes that have been developed for this vaccine.

2.4.1.2 Test Material

mRNA-1273 contains a single mRNA that encodes for SARS-CoV-2 S-2P combined in a mixture of 4 lipids common to the Sponsor's mRNA vaccine platform: SM-102, cholesterol, DSPC, and PEG2000-DMG, respectively. The mRNA-1273 Drug Product is provided as a sterile suspension for injection at a concentration of 0.20 mg/mL in 20 mM Tris buffer containing 87 g/L sucrose and 4.3 mM acetate, at pH 7.5.

The pivotal submission-enabling toxicology studies were conducted with mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs. The development mRNA-1273 lots (Lots AMPDP-200005 and 8520100101) evaluated in the nonclinical pharmacology programs were prepared with a manufacturing process comparable to the GMP mRNA-1273 Drug Product evaluated in the Phase 3 clinical trial (as described in Module 3, Section 3.2.P.2.3 [Scale A process]) and were therefore representative of the clinical presentations. Lots AMPDP-200005 and 8520100101 were manufactured at a concentration of 0.5 mg/mL in 20 mM Tris buffer containing 87 g/L sucrose and 10.7 mM acetate, at pH 7.5.

The distribution, toxicity, and genotoxicity associated with mRNA vaccines formulated in LNPs are driven primarily by the composition of the LNPs and, to a lesser extent, by the biologic activity of the antigen(s) encoded by the mRNA. Therefore, the distribution study, Good Laboratory Practice (GLP)-compliant toxicology studies, and in vivo GLP-compliant genotoxicity study conducted with mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs are considered supportive and BLA-enabling for mRNA-1273. SM-102, the novel lipid used in mRNA-1273, and the commercially available PEG2000-DMG (b) (4) were evaluated as individual agents in GLP-compliant in vitro genotoxicity studies. The potential effects of mRNA-1273 on fertility and pre- and postnatal development in pregnant and lactating female Sprague Dawley rats was assessed in a GLP-compliant perinatal/postnatal reproductive toxicity study. Additionally, the immunogenicity and toxicity profiles of mRNA-1273 were assessed in a non-GLP repeat-dose study.

2.4.1.3 Nonclinical Testing Program

The nonclinical testing program was designed to adhere to international regulatory guidelines, the intended clinical development program, and pharmacology and toxicology principles and was consistent with International Council for Harmonisation (ICH) guidelines for biological drug development, including ICH S6(R1) (Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals) and appropriate GLP regulations that were applicable when studies were conducted. The pivotal nonclinical safety studies were conducted according to the OECD Principles of Good Laboratory Practice (ENV/MC/CHEM[98]17) or GLP regulations in other countries that are signatories to the OECD Mutual Acceptance of Data agreement (eg, US FDA Code of Federal Regulations Title 21, Part 58: Good Laboratory Practice for Nonclinical Laboratory Studies).

The route of administration of mRNA-1273 used in the nonclinical studies was intramuscular (IM), consistent with the clinical route.

Nonclinical primary pharmacology evaluations were conducted in vitro in HEK293T cells and in vivo in young and aged mice (BALB/c, BALB/cJ, C57/BL6J, and B6C3F1/J strains), golden Syrian hamsters, and rhesus macaques (nonhuman primates [NHPs]) animal models to characterize the expression and the immunogenicity of mRNA-1273, as well as its effects on viral replication and disease progression after SARS-CoV-2 challenge, and to evaluate its safety profile and its potential to promote vaccine-associated enhanced respiratory disease (ERD) after viral challenge, which has previously been observed with vaccines against respiratory syncytial virus (Kim et al 1969), measles (Polack 2007), and in animal models of SARS-CoV vaccination (Czub et al 2005; Deming et al 2007; Bolles et al 2011; Corbett et al 2020a). Additionally, the immunogenicity of mRNA-1273 was assessed in a non-GLP repeat-dose pharmacology study in Sprague Dawley rats.

As SARS-CoV-2 is a newly emerged CoV, there were no established animal models for the evaluation of prophylactic vaccines and therapeutics. As a proof of concept, the expression of the mRNA-encoded SARS-CoV-2 S-2P antigen was confirmed in vitro and in vivo, and nonclinical studies were initiated in multiple animal species in order to gain a comprehensive understanding of the effects of mRNA-1273 immunization. Wild-type (WT) mice are a convenient and easy-to-use model to assess vaccine immunogenicity; however, the ACE-2 receptor, the primary route for SARS-CoV-2 binding and entry, differs significantly between mice and humans and, as result, WT SARS-CoV-2 does not infect mice. Therefore, a mouse-adapted SARS-CoV-2 strain, which was developed by the laboratory of Dr. Ralph Baric at the University of North Carolina at Chapel Hill, was used to assess protection of immunized mice from SARS-CoV-2 challenge.

Although this mouse-adapted strain infects young mice and induces mild disease symptoms, more severe symptoms are evident in aged mice (> 12 months) (Dinnon et al 2020). Aged mice were therefore included in the nonclinical pharmacology program to further characterize the immune response and the level of protection from viral challenge. In addition, this model was used to characterize the quality of the immune response to determine if the mRNA-1273-induced immunity would be predicted to promote vaccine-associated ERD. The immunogenicity and protection study in aged mice was designed to directly address this concern through the evaluation of dose levels predicted to drive optimal or suboptimal protection from viral challenge. Golden Syrian hamsters were also selected as a relevant model for evaluation. Wild-type SARS-CoV-2 productively infects hamsters, causing weight loss and moderate to severe lung pathology. This model was selected because it is currently the only animal species in which severe respiratory disease is evident after virus challenge (Chan et al 2020). Nonhuman primates are the species most closely related to humans and have previously recapitulated several important aspects of SARS-CoV infection (Lu et al 2020). Although SARS-CoV-2 infection in NHPs result only in mild clinical symptoms, infection does cause illness with evidence of pneumonia (Johansen et al 2020).

The biodistribution of mRNA-based vaccines formulated in LNPs is predicted to be driven by the LNP characteristics and mRNAs that are within LNPs of the same composition (ie, SM-102-containing LNPs) are expected to distribute similarly to the LNPs. Thus, the distribution of mRNA-1647, an mRNA-based CMV vaccine that contains 6 mRNA sequences combined in SM-102-containing LNPs, assessed in a non-GLP, single IM dose biodistribution study supports the development of mRNA-1273.

The Sprague Dawley rat was selected as the animal model for the toxicity assessments because it is an accepted rodent species for nonclinical toxicology testing by regulatory agencies and is a relevant species to assess the toxicity and immunogenicity of mRNA vaccines, as evidenced by immunogenic responses.

The toxicological profile associated with mRNA-based vaccines formulated in SM-102-containing LNPs, including mRNA-1273, is driven primarily by the LNP composition and, to a lesser extent, by the biologic activity of the antigen(s) encoded by the mRNA. The safety and tolerability of 5 mRNA-based vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs (2 Zika virus vaccines: mRNA-1706 and mRNA-1893; 1 hMPV and PIV3 vaccine: mRNA-1653; and 2 CMV vaccines: mRNA-1647 and mRNA-1443) have been evaluated in 6 GLP-compliant repeat-dose toxicity studies in Sprague Dawley rats. Additionally, the Sponsor completed a non-GLP repeat-dose

study in Sprague Dawley rats to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses.

Rats were administered doses up to the anticipated maximum tolerated dose of 150 µg/dose, where clinical observations including vocalization (at 100 µg/dose) were accompanied by body weight loss and decrease in food consumption. The number of doses selected for the individual GLP studies was 1 more than the intended number of doses proposed for the individual clinical studies. The number of doses ranged from 3 to 4, and doses were administered every 2 weeks.

SM-102, the novel lipid used in mRNA-1273, and the commercially available PEG200-DMG (b) (4) were evaluated in genotoxicity studies as individual agents using a standard ICH S2 (R1) approach (ICH 2011), including GLP-compliant bacterial reverse mutation (Ames) tests in *Salmonella typhimurium* and *Escherichia coli* and GLP-compliant in vitro micronucleus tests in human peripheral blood lymphocytes. In addition, SM-102 was evaluated for in vivo genotoxicity risk in a GLP-compliant in vivo rat micronucleus test using an mRNA-based vaccine formulated in SM-102 LNPs (mRNA-1706) and a non-GLP-compliant in vivo rat micronucleus test using a reporter mRNA (nascent peptide imaging [NPI] luciferase mRNA) formulated in SM-102 LNPs.

Additionally, a GLP-compliant combined developmental and perinatal/postnatal reproductive toxicity study was conducted to assess the potential effects of mRNA-1273 on fertility and pre- and postnatal development in pregnant and lactating female Sprague Dawley rats.

2.4.2. PHARMACOLOGY

Table 1 summarizes the nonclinical pharmacology program for mRNA-1273. Pharmacology results are fully summarized in Module 2.6.2.

Table 1: Summary of Pharmacology Program for mRNA-1273

Study Type/Description	Test Article Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Evaluation of in vitro expression of SARS-CoV-2 mRNA and in vivo expression of mRNA-1273	SARS-CoV-2 S-2P mRNA: 0.003125 through 0.2 µg mRNA-1273: 2 or 10 µg	HEK293T cells BALB/c mice	In vitro transfection In vivo IM; single injection	No	MOD-4117.1273
Evaluation of immunogenicity, protective capacity, and safety in young mice	mRNA-1273: 0.01, 0.1, 1 or 10 µg SARS-CoV-2 S-2P: 0.01, 0.1, or 1 µg (+ SAS-adjuvant)	Mouse (young), BALB/cJ, C57BL/6J, B6C3F1/J	IM; prime only prime/boost (3-week interval) prime/boost (4-week interval)	No	VRC01
Immunization and protein restimulation in young BALB/c mice with enhanced respiratory disease endpoint monitoring	mRNA-1273: 1 or 10 µg SARS-CoV-2 S-2P: 10 µg (+ alum)	Mouse (young), BALB/c	IM; prime/boost (2-week interval)	No	MOD-3937
Immunogenicity and determination of titer dynamic range in young BALB/c mice	mRNA-1273: 0.0025 through 20 µg	Mouse (young), BALB/c	IM; prime/boost (3-week interval)	No	MOD-3938/ MOD-3940
Immunogenicity and characterization of cellular response in young BALB/cJ mice	mRNA-1273: 0.1, 1, or 10 µg SARS-CoV-1 DIV: 0.2 or 1 µg (+ alum) CDS: 0.2 or 1 µg (+ alum)	Mouse (young), BALB/c	IM; prime/boost (3-week interval)	No	VRC05
Efficacy and enhanced respiratory disease in aged BALB/c mice	mRNA-1273: 0.1 or 1 µg SARS-CoV-1 DIV: 0.1 µg (+ alum)	Mouse (aged), BALB/c	IM; prime/boost (3-week interval)	No	VRC02

Study Type/Description	Test Article Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Five-week (2 doses: prime/boost) repeat-dose immunogenicity with safety endpoints	mRNA-1273: 0, 30, 60, or 100 µg	Rat, Sprague Dawley	IM; prime/boost (3-week interval)	No	2308-123
Protection from WT SARS-CoV-2 in hamsters using optimal and suboptimal doses	mRNA-1273: 1, 5, or 25 µg	Hamster, golden Syrian	IM; prime/boost (3-week interval)	No	UTMB01
Immunogenicity and protective efficacy in NHPs	mRNA-1273: 10 or 100 µg	NHP, rhesus macaque (Indian-origin)	IM; prime/boost (4-week interval)	No	VRC04
Evaluation of immunogenicity and efficacy from expanded dose range in NHPs	mRNA-1273: 2.5, 30, or 100 µg	NHP, rhesus macaque (Indian-origin)	IM; prime/boost (4-week interval)	No	VRC07

Abbreviations: alum = aluminum hydroxide; CDS = conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein; GLP = Good Laboratory Practice; IM = intramuscular; mRNA = messenger RNA; NHP = nonhuman primate; SARS-CoV-1 DIV = double-inactivated severe acute respiratory syndrome coronavirus-1; SARS-CoV-2 = 2019 novel coronavirus; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SAS = Sigma Adjuvant System®; WT = wild-type.

2.4.2.1 Primary Pharmacology

Nonclinical primary pharmacology studies were conducted in vitro in HEK293T cells and in vivo in young and aged mice (BALB/c, BALB/cJ, C57BL/6J, and B6C3F1/J strains), golden Syrian hamsters, and rhesus macaques (NHPs) animal models to characterize the expression and the immunogenicity of mRNA-1273, as well as its effects on viral replication and disease progression after SARS-CoV-2 challenge, and to evaluate its safety profile and potential to promote vaccine-associated ERD after viral challenge (Module 2.6.2). Additionally, the immunogenicity of mRNA-1273 was evaluated in a non-GLP repeat-dose pharmacology study in Sprague Dawley rats (Module 2.6.6; Section 2.6.6.9).

The expression of the mRNA-encoded SARS-CoV-2 S-2P antigen was confirmed in vitro and in vivo. Sustained levels of antigen expression were observed over 24, 48, and 72 hours in HEK293T cells transiently transfected with a dose range (0.003125 through 0.2 µg) of an mRNA construct that encodes the SARS-CoV-2 S-2P antigen, as demonstrated by an overall increase in the frequency of cells expressing the receptor binding domain (RBD) or the N-terminal domain (NTD) epitopes (using the monoclonal antibody [mAb] clones CR3022 and 4A8, respectively) of

the SARS-CoV-2 S protein; a slight dose effect was observed. Immunization of BALB/c mice with 2 or 10 µg mRNA-1273 induced in vivo expression of the SARS-CoV-2 S-2P antigen in spleen and draining lymph node immune cells (conventional dendritic cells [cDCs] and plasmacytoid dendritic cells [pDCs]), as demonstrated by staining with mAbs specific to the RBD (CR3022) or the NTD (4A8) epitopes of the SARS-CoV-2 S protein. The level of antigen expression measured in cDCs was similar to that of pDCs. The higher dose (10 µg mRNA-1273) induced higher levels of antigen expression in these cells.

Immunogenicity was characterized in young and aged mice, rats, hamsters, and NHPs through the evaluation of the humoral (immunoglobulin [Ig] G binding antibodies), cellular (T-cell cytokines and T helper [Th] 1-directed CD4+ and CD8+ responses), and/or neutralizing antibody responses elicited by prime-only or prime/boost immunization schedule with a range of mRNA-1273 dose levels.

Protection by mRNA-1273 immunization was assessed in young and aged mice, hamsters, and NHPs immunized with a prime-only or prime/boost schedule, followed by viral challenge with a high dose of SARS-CoV-2 (mouse-adapted SARS-CoV-2 strain; hamsters and NHPs: WT SARS-CoV-2 strain, Washington state isolate). mRNA-1273 dose levels and immunization schedules predicted to drive optimal and suboptimal protection were included in these studies to identify immune signatures for each regimen and to assess the level of protection mediated by different dose levels. Suboptimal dose levels that confer only partial protection were also included to evaluate the theoretical risk of disease enhancement. Viral load and replication in the upper (nasal turbinates) and lower (lungs) airways, as well as lung pathology and inflammation, were evaluated after viral challenge.

The potential of mRNA-1273 to promote vaccine-associated ERD was assessed in young and aged mice, hamsters, and NHPs through the evaluation of immunogenicity endpoints (IgG1:IgG2a ratio, Th1/Th2 cytokine profiles, and the ratio of binding to neutralizing antibodies) indicative of a protective versus a disease enhancement phenotype, and through monitoring of viral load, viral replication, and histopathological evaluation of lung tissues after viral challenge. The immune signature of mice immunized with mRNA-1273 was compared to that of vaccines that have been associated with ERD (SARS-CoV-1 DIV and conformationally disrupted spike protein [CDS] in alum adjuvant) included as controls in 2 mouse studies.

These studies demonstrated that mRNA-1273 is immunogenic in all the species assessed, showing a dose-dependent response in IgG binding antibody titers and neutralizing antibody activities. Antigen-specific T-cell responses were observed in mice and NHPs. Direct measurement of Th1-directed responses in mice and NHPs, indirect measurement of

Th1-directed responses (IgG2a/c:IgG1 antibody subclasses) in mice, and the high levels of neutralizing antibody in all species lessen the concerns regarding the risk of ERD associated with mRNA-1273 immunization. Additionally, a robust and dose-dependent CD8+ T-cell response in mice and a low CD8+ T-cell response in NHPs were observed after boosting with a second dose of mRNA-1273.

In addition to measurements of the immune response, mice, hamsters, and NHPs were challenged with a high dose of SARS-CoV-2 (mice: mouse-adapted SARS-CoV-2 strain; hamsters and NHPs: WT SARS-CoV-2 strain, Washington state isolate); mice and hamsters were challenged intranasally and NHPs were challenged intranasally and intratracheally. Dose levels predicted to be optimal (fully protective) and suboptimal (subprotective) were included in these studies. At higher doses, mice, hamsters, and NHPs were fully protected from viral replication in both lungs and nasal passages. At suboptimal dose levels, animals were either fully protected in the lungs or had reduced viral burden after challenge compared to control animals. There were no observations of increased viral load in animals immunized with suboptimal dose levels of mRNA-1273, which further supports that mRNA-1273 immunization does not promote ERD. Lung histopathology assessments were performed to verify reduction of inflammation, immune complex deposition, and immune cell invasion in response to viral challenge in animals immunized with mRNA-1273 compared to control (PBS) animals. In animals immunized with either optimal or suboptimal mRNA-1273 dose levels, histopathological evaluation of the lungs of mice and NHPs confirmed the lack of evidence of ERD, as demonstrated by minimal inflammation and no noteworthy neutrophilic-associated alveolar disease or eosinophil-dominant inflammatory response, which have been historically associated with vaccine-associated ERD. In contrast, moderate to severe inflammation involving the small airways and the adjacent alveolar interstitia was elicited by SARS-CoV-2 infection in PBS-control animals.

Overall, nonclinical pharmacology studies demonstrated that mRNA-1273 is well tolerated, is immunogenic, and provides protection from SARS-CoV-2 challenge. In mice, hamsters, and NHPs, a prime-only immunization schedule induced robust SARS-CoV-2-specific binding and neutralizing antibody responses that significantly increased after boosting with a second dose of mRNA-1273. A prime/boost immunization schedule elicited a substantial dose-dependent binding antibody response in rats. In addition, Th1-directed antigen-specific CD4+ and CD8+ T-cell responses were observed in mice and a Th1-directed antigen-specific CD4+ T-cell response was observed in NHPs. mRNA-1273 was fully protective from viral challenge in immunized mice and hamsters when administered as a prime-only or prime/boost schedule at $\geq$ 1 µg/dose and in immunized NHPs when administered as a prime/boost schedule at $\geq$ 30 µg/dose. Furthermore, mRNA-1273 did not promote vaccine-associated ERD in mice,

hamsters, and NHPs as demonstrated by balanced Th1/Th2-directed immune responses to immunization, the absence of increased lung pathology, and controlled viral replication after viral challenge when administered at doses predicted to be fully (optimal dose) or partially (suboptimal dose) protective.

2.4.3. PHARMACOKINETICS AND TISSUE DISTRIBUTION

Table 2 lists the nonclinical pharmacokinetics and tissue distribution study with mRNA-1647 in support of the development of mRNA-1273. Biodistribution results are fully summarized in Module 2.6.4.

Table 2: Summary of Pharmacokinetics Program for mRNA-1273

Study Type	Test Article	Species, Strain	Method of Administration, Dose	GLP	Report Number
Single-dose tissue distribution study	mRNA-1647[a]	Rat, Sprague Dawley	IM injection dose of 100 µg on Day 1	No	5002121 Amendment 1

Abbreviations: CMV = cytomegalovirus; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; IM = intramuscular; mRNA = messenger RNA.

[a] mRNA-1647 contains 6 mRNAs that encode the full-length CMV gB and the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex. The 6 mRNAs are combined at a target mass ratio of 1:1:1:1:1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

2.4.3.1 Pharmacokinetics and Tissue Distribution

The results of a biodistribution, non-GLP, single dose, IM injection study of mRNA-1647 in male Sprague Dawley rats (Table 2.6.5.5 [Module 2.6.5] and Report 5002121 Amendment 1) support the development of mRNA-1273. mRNA-1647 is a novel mRNA-based CMV vaccine that contains 6 distinct mRNA sequences (1 that encodes the full-length CMV glycoprotein B [gB] and 5 that encode the pentameric glycoprotein H [gH]/glycoprotein L [gL]/UL128/UL130/UL131A glycoprotein complex) combined at a target mass ratio of 1:1:1:1:1:1 in the Sponsor's standard proprietary SM-102–containing LNPs.

After a single IM dose of mRNA-1647 in male rats, concentrations of the 6 mRNA constructs of mRNA-1647 (ie, gB, gH, gL, UL128, UL130, and UL131A) were detectable in plasma and tissues in a 1:1:1:1:1:1 ratio. The time after dosing at which the maximum concentration was observed in plasma (T_{max}) was 2 hours for all constructs and was followed by a rapid elimination phase with a half-life ($T_{1/2}$) estimated to range from 2.7 to 3.8 hours. The maximum plasma concentration (C_{max}) ranged from 1.60 to 2.30 ng/mL, and the area under the concentration versus time curve (AUC) from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed ($AUC_{[0\text{-}t]}$) ranged from 22.7 to 25.5 ng·h/mL.

Concentrations of the 6 mRNA constructs of mRNA-1647 were detected at levels above the lower limit of quantitation (LLOQ) in most tissues analyzed, except for the kidney, where all levels were below the LLOQ. For highly exposed tissues (injection site [muscle], lymph nodes

[proximal and distal], and spleen), the C_{max} was observed between 2 and 24 hours post dose. The $T_{1/2}$ was calculated using the average tissue $T_{1/2}$ values for the 6 mRNA constructs; the results were 14.9 hours for injection site (muscle), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

As observed with other IM-delivered vaccines, the highest mRNA concentrations were observed at the injection site followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0. Only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for all constructs ranged from 14.9 to 63.0 hours.

2.4.4. TOXICOLOGY

Table 3 summarizes the nonclinical toxicology program used in support of the development of mRNA-1273. Toxicology results are fully summarized in Module 2.6.6.

Table 3: Summary of Toxicology Program for mRNA-1273

Study Type	Test Article	Species, Strain	Method of Administration; Dose	GLP	Report Number
Repeat-Dose Toxicity					
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706[a]	Rat, Sprague Dawley	IM; 0, 13, 65, 129 µg/dose[b] (Days 1, 15, 29)	Yes	5002045
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706[a]	Rat, Sprague Dawley	IM; 0, 10, 50, 100 µg/dose (Days 1, 15, 29)	Yes	5002231
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1653[c]	Rat, Sprague Dawley	IM; 0, 10, 50, 150 µg/dose (Days 1, 15, 29)	Yes	5002033
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1893[d]	Rat, Sprague Dawley	IM; 0, 10, 30, 96 µg/dose (Days 1, 15, 29)	Yes	5002400
6-week (4 doses) repeat-dose study with 2-week recovery	mRNA-1647[e]	Rat, Sprague Dawley	IM; 0, 8.9, 27, 89 µg/dose[f] (Days 1, 15, 29, 43)	Yes	5002034
6-week (4 doses) repeat-dose study with 2-week recovery	mRNA-1443[g]	Rat, Sprague Dawley	IM; 0, 9.6, 29, 96 µg/dose[h] (Days 1, 15, 29, 43)	Yes	5002158
In Vitro Genotoxicity					
Bacterial reverse mutation test	SM-102	*Salmonella typhimurium, Escherichia coli*	Incubation for 67 hours 29 minutes with 0, 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000 µg/plate SM-102 with or without supplemented rat liver fraction	Yes	9601567
Bacterial reverse mutation test	PEG2000-DMG (b) (4)	*Salmonella typhimurium, Escherichia coli*	Incubation for 67 h and 57 min with 0, 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000 µg/plate PEG2000-DMG with or without supplemented rat liver fraction	Yes	9601035
Mammalian cell micronucleus test	SM-102	Human peripheral blood lymphocytes	Incubation for 4 and 24 hours with 0, 163, 286, 500 µg/mL SM-102 with or without supplemented rat liver fraction	Yes	9601568
Mammalian cell micronucleus test	PEG2000-DMG (b) (4)	Human peripheral blood lymphocytes	Incubation for 4 h and/or 24 hours with 0, 0, 53.3, 93.3, 163, 286, 500 µg/mL PEG2000-DMG with or without supplemented rat liver fraction	Yes	9601036
In Vivo Genotoxicity					

Study Type	Test Article	Species, Strain	Method of Administration; Dose	GLP	Report Number
In vivo mammalian erythrocyte micronucleus test	mRNA-1706[a]	Rat, Sprague Dawley	Single IV; 0, 0.6/6.2 (F), 1.3/13.5, 2.6/27.0, 5.2/54.1 (M) mg/kg mRNA-1706/SM-102[j, k]	Yes	9800399
In vivo mammalian erythrocyte micronucleus test	NPI luciferase mRNA[l]	Rat, Sprague Dawley	Single IV; 0, 0.32/6.0, 1.07/20, 3.21/60 mg/kg NPI luciferase mRNA/SM-102	No	AF87FU.125012 NGLPICH.BTL
Reproductive and Developmental Toxicity					
Combined developmental and perinatal/postnatal developmental and reproductive toxicity study	mRNA-1273[m]	Rat, Sprague Dawley	IM; 100 µg/dose (Study Days 1 and 15 [28 and 14 days prior to mating, respectively] and Gestation Days 1 and 13)	Yes	20248897
Other Toxicology					
5-week (2 doses) repeat-dose immunogenicity and toxicity study	mRNA-1273[n]	Rat, Sprague Dawley	IM; 0, 30, 60, 100 µg/dose (Days 1 and 22)	No	2308-123

Abbreviations: CMV = cytomegalovirus; CoV = coronavirus; F = female; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; h = hour; IM = intramuscular; IV = intravenous; M = male; min = minute; mRNA = messenger RNA; NPI = nascent peptide imaging; pp65 = phosphoprotein 65; prME = pre-membrane and envelope; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus; SoA = summary of analysis.

[a] mRNA-1706 contains a single mRNA sequence that encodes the prME structural proteins of Zika virus combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 20 mM Tris, 8% sucrose, pH 7.4.

[b] The original dose levels selected were 0, 10, 50, and 100 µg/dose, respectively (SoA issued on 11 October 2016). The calculated dose levels were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064 (SoA issued on 03 May 2017). The change in the reported mRNA content for mRNA-1706 was 29%.

[c] mRNA-1653 contains 2 distinct mRNA sequences that encode the full-length membrane-bound fusion proteins of human metapneumovirus and parainfluenza virus type 3. The 2 mRNAs are combined at a target mass ratio of 1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 7% PG, 1 mM DTPA, pH 7.4.

[d] mRNA-1893 contains a single mRNA sequence that encodes the prME structural proteins of Zika virus in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 100 mM Tris, 7% PG, 1 mM DTPA, pH 7.5.

[e] mRNA-1647 contains 6 mRNAs that encode the full-length CMV gB and the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex. The 6 mRNAs are combined at a target mass ratio of 1:1:1:1:1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

[f] The original dose levels selected were 0, 10, 30, and 100 µg/dose, respectively (SoA issued on 16 Mar 2017). The calculated dose levels were revised based on the updated concentration reported for mRNA-1647 Lot No. MTDP17015 (SoA issued on 31 May 2017). The change in the reported mRNA content for mRNA-1647 was –11%.

[g] mRNA-1443 contains a single mRNA sequence that encodes a phosphorylation mutant of the CMV phosphoprotein 65 protein (ie, deletion of amino acids 435-438) combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

[h] The original dose levels selected were 0, 10, 30, and 100 µg/dose, respectively (SoA issued on 16 Mar 2017). The calculated dose levels were revised based on the updated concentration reported for mRNA-1443 Lot No. MTDP17017 (SoA issued on 30 May 2017). The change in the reported mRNA content for mRNA-1443 was 4%.

[i] Multiple test articles (b) (4) were assessed in this study. Only data relevant to the development of mRNA-1273 are discussed in this dossier.

[j] A dose-range finding test was performed prior to the main phase of the study, wherein male and female rats (3 animals/sex) were given a single IV injection (doses 2.6/27.0, 3.9/40.6, and 5.2/54.1 mg/kg mRNA-1706/SM-102 for females, and 2.6/27.0, 5.2/54.1, and 10.3/107.1 mg/kg mRNA-1706/SM-102 for males).

[k] The original dose levels selected were 0, 1.0, 2.0, 4.0, 0.5, 1.0, and 2.0 mg/kg mRNA-1706, respectively (SoA issued on 11 October 2016). The calculated dose levels were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064 (SoA issued on 03 May 2017). The change in the reported mRNA content for mRNA-1706 was 29%.

[l] The NPI luciferase mRNA is combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 25 mM Tris, 123 g/L sucrose, 1 mM DTPA, pH 7.5.

[m] mRNA-1273 contains a single mRNA sequence that encodes the full-length SARS-CoV-2 S-2P combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 20 mM Tris, 87 mg/mL sucrose, 17.5 mM sodium acetate, pH 7.5.

[n] mRNA-1273 contains a single mRNA sequence that encodes the full-length SARS-CoV-2 S-2P combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 20 mM Tris, 87 mg/mL sucrose, 10.7 mM sodium acetate, pH 7.5.

2.4.4.1 Repeat-Dose Toxicity

The safety and tolerability of mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs have been evaluated in multiple GLP-compliant, repeat-dose toxicity studies in Sprague Dawley rats at doses up to 150 µg administered every 2 weeks for up to 6 weeks followed by a 2-week recovery period. Additionally, the Sponsor completed a repeat-dose non-GLP study in Sprague Dawley rats to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses (Section 2.4.4.3).

Rats were administered mRNA-based vaccines (mRNA-1706, mRNA-1653, or mRNA-1893) IM once every 2 weeks for 1 month (3 doses) at doses up to 150 µg followed by a 2-week recovery period (Table 2.6.7.7A [Module 2.6.7] and Report 5002045; Table 2.6.7.7B [Module 2.6.7] and Report 5002231; Table 2.6.7.7C [Module 2.6.7] and Report 5002033; Table 2.6.7.7D [Module 2.6.7] and Report 5002400). In addition, rats were administered mRNA-based vaccines (mRNA-1647 or mRNA-1443) IM once every 2 weeks for 6 weeks (4 doses) at doses up to 96 µg followed by a 2-week recovery period (Table 2.6.7.7E [Module 2.6.7] and Report 5002034; Table 2.6.7.7F [Module 2.6.7] and Report 5002158).

The aggregate rat repeat-dose toxicity profile from the GLP studies for mRNA-based vaccines formulated in SM-102-containing LNPs consisted of IM doses ranging from 8.9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks. All doses administered were tolerated. Test article-related in-life observations at ≥ 8.9 µg/dose included reversible or reversing erythema and edema at the injection site and transient increases in body temperature at 6 hours post-dose returning to baseline 24 hours post-dose.

Test article-related, generally dose-dependent clinical pathology changes were observed at ≥ 8.9 µg/dose. Hematology changes included increases in white blood cells, neutrophils, and eosinophils and decreased lymphocytes; coagulation changes included increases in fibrinogen and activated partial thromboplastin time; and clinical chemistry changes included decreases in albumin, increases in globulin, and a corresponding decrease in albumin/globulin ratio. Clinical pathology changes generally reversed or were reversing by the end of the 2-week recovery period. Test article-related, transient cytokine increases were observed at ≥ 8.9 µg/dose at 6 hours post-dose, including in IP-10, MCP-1, and MIP-1-α. Cytokine changes were generally reversing by the end of the 2-week recovery period.

Post-mortem test article-related and generally dose-dependent changes in organ weights and macroscopic and microscopic findings were observed at ≥ 8.9 µg/dose. Organ weight increases were observed in the spleen, liver, and adrenal gland. Organ weight changes were generally reversing by the end of the 2-week recovery period. Macroscopic changes included skin thickening at the injection site and enlarged lymph nodes. Injection site changes completely recovered, and lymph node changes were recovering by the end of the 2-week recovery period. Microscopic changes included mixed cell inflammation at the injection site; increased cellularity and mixed cell inflammation in the inguinal, iliac, and popliteal lymph nodes; decreased cellularity in the splenic periarteriolar lymphoid sheath; increased myeloid cellularity in the bone marrow; and hepatocyte vacuolation and Kupffer cell hypertrophy in the liver. Microscopic changes were generally reversing by the end of the 2-week recovery period.

2.4.4.2 Genotoxicity

SM-102, the novel lipid used in mRNA-1273, and the commercially available PEG2000-DMG (b) (4) were evaluated in genotoxicity studies as individual agents using a standard ICH S2 (R1) approach (ICH 2011), including GLP-compliant in vitro bacterial reverse mutation (Ames) tests in *S typhimurium* and *E coli* (Table 2.6.7.8A [Module 2.6.7] and Report 9601567; Table 2.6.7.8B [Module 2.6.7] and Report 9601035) and GLP-compliant in vitro micronucleus tests in human peripheral blood lymphocytes

(Table 2.6.7.8C [Module 2.6.7] and Report 9601568; Table 2.6.7.8D [Module 2.6.7] and Report 9601036).

In addition, SM-102 was evaluated for in vivo genotoxicity risk in a GLP-compliant in vivo rat micronucleus test using a similar mRNA-based vaccine formulated in SM-102 LNPs (Table 2.6.7.9A [Module 2.6.7] and Report 9800399) and in a non-GLP-compliant in vivo rat micronucleus test using a reporter mRNA (NPI luciferase mRNA) formulated in SM-102 LNPs (Table 2.6.7.9B [Module 2.6.7] and Report AF87FU.125012NGLPICH.BTL).

Genotoxicity assessments of the SM-102 lipid concluded that the lipid is not genotoxic in the bacterial mutagenicity and human peripheral blood lymphocytes chromosome aberration assays. Two intravenous in vivo micronucleus assays were conducted with mRNA-based vaccines formulated in the SM-102-containing LNPs. Results from Report AF87FU.125012NGLPICH.BTL were negative up to 3.21/60 mg/kg NPI luciferase mRNA/SM-102, while results from Report 9800399 were positive at 2.6/27.0 mg/kg mRNA-1706/SM-102 in females and at 5.2/54.1 mg/kg mRNA-1706/SM-102 in males. The equivocal results are likely driven by micronuclei formation secondary to increased cytokines and/or body temperature induced by LNP-driven systemic inflammation at high systemic (intravenous) doses. Overall, the genotoxic risk to humans is considered to be low due to minimal systemic exposure following IM administration, limited duration of exposure, and negative in vitro results.

Genotoxicity assessments of the PEG2000-DMG lipid concluded that the lipid is not genotoxic in the bacterial mutagenicity and the human peripheral blood lymphocytes chromosome aberration assays.

2.4.4.3 Reproductive and Developmental Toxicity

A GLP-compliant combined developmental and perinatal/postnatal developmental and reproductive toxicity study was conducted to assess the potential effects of mRNA-1273 on fertility and pre- and postnatal development in pregnant and lactating female Sprague Dawley rats following IM administration of the human dose of 100 µg to dams on Study Days 1 and 15 (28 and 14 days prior to mating, respectively) and Gestation Days (GDs) 1 and 13 (Table 2.6.7.11 [Module 2.6.7], and Report 20248897).

Female rats assigned to Cohort 1 were euthanized on GD 21 for Caesarean-sectioning and fetal examinations. Female rats assigned to Cohort 2 were allowed to deliver their litters naturally and dams and pups were followed through Lactation Day (LD) 21.

Robust IgG response to S-2P antigen was observed in both the F_0 rats and F_1 generation rats following immunization of F_0 rats with mRNA-1273. In the F_0 rats, peak titer of 442,138 antibody units/mL was reached on GD 13. Titers subsequently plateaued at parturition (GD 21) and stayed relatively constant through LD 21. High IgG antibodies to SARS-CoV-2 S-2P were also observed in GD 21 F_1 fetuses and LD 21 F_1 pups, indicating strong transfer of antibodies from dam to fetus and from dam to pup.

In the F_0 generation, there were no mRNA-1273-related effects or changes in the following parameters: mortality, body weight, body weight gain, food consumption, macroscopic observations, estrous cycling during pre-cohabitation, mating and fertility, ovarian/uterine examinations, or natural delivery or litter observations. mRNA-1273-related clinical observations included transient thin fur cover, swollen hindlimbs, and limited usage of the hindlimb during the premating, gestation, and/or lactation phases of the study, with the most observations noted following dose administration on GD 13. These mRNA-1273–related observations were not considered adverse as effects did not significantly impair the animal's mobility, access to food, or ability to thrive. Only thin fur cover was still present during the lactation phase and was resolved by LD 18.

In the F_1 generation, there were no mRNA-1273-related effects or changes in the following parameters: mortality, body weight, clinical observations, macroscopic observations, gross pathology, external or visceral malformations or variations, skeletal malformations, and mean number of ossification sites per fetus per litter. mRNA-1273-related variations in skeletal examination included statistically significant increases in the number of F_1 rats with 1 or more wavy ribs and 1 or more rib nodules. Wavy ribs appeared in 6 fetuses and 4 litters with a fetal prevalence of 4.03% and a litter prevalence of 18.2%. Rib nodules appeared in 5 of those 6 fetuses. Skeletal variations are structural changes that do not impact development or function of a developing embryo, are considered reversible, and often correlate with maternal toxicity and/or lack of other indicators of developmental toxicity (Carney and Kimmel 2007). Maternal toxicity in the form of clinical observations was observed for 5 days following the last dose (GD 13), correlating with the most sensitive period for rib development in rats (GDs 14 to 17). Furthermore, there were no other indicators of mRNA-1273-related developmental toxicity observed, including delayed ossification; therefore, these common skeletal variations were not considered adverse.

Overall, maternal administration of mRNA-1273 on Study Day 1 (28 days prior to mating), Study Day 15 (14 days prior to mating) and GD 1, and GD 13 did not have any adverse effects on the F_0 or F_1 generations. The mRNA-1273-related, non-adverse effects were limited to an increase in the number of fetuses with common skeletal variations of 1 or more rib nodules and 1

or more wavy ribs with no effect on viability or growth and development of the F_1 generation. Robust IgG titers were observed in the rats following 4 immunizations of mRNA-1273 vaccine. Peak titer was reached on GD 13 and plateaued at the time of parturition GD 21 and stayed constant through LD 21. Strong maternal-to-fetal and maternal-to-pup transfer of antibodies was observed with mRNA-1273.

2.4.4.4 Other Toxicity

A non-GLP study in Sprague Dawley rats was conducted to characterize the immunogenic response and potential toxicity of mRNA-1273 at IM doses levels of 30, 60, and 100 µg/dose administered on Days 1 and 22 (Section 2.6.2.2.6 [Module 2.6.2], Table 2.6.7.17 [Module 2.6.7], and Report 2308-123).

A strong immunogenic response against SARS-CoV-2 S-2P was observed on Day 35, with measured IgG antibody titers above 10^6 at all dose levels. mRNA-1273 had no effect on body weights and limited, transient clinical signs starting at 30 µg/dose consisting of transient dose-dependent injection site edema with or without hindlimb impairment. Clinical pathology findings consisted, in part, of changes associated with inflammation starting at 30 µg/dose. In general, the changes observed are consistent with the results from the previous GLP rat toxicity studies conducted with other mRNA-based vaccines formulated with SM-102-containing LNPs.

2.4.4.5 Summary of Nonclinical Safety Margins

Pending the outcome of the Phase 3 clinical trial with mRNA-1273, a human dose of 100 µg/dose is anticipated to be safe and to provide protective immunization against SARS-CoV-2 infection.

In the rat repeat-dose toxicity studies in which up to 100 µg/dose of mRNA-1273 administered on Day 1 and Day 22, up to 150 µg/dose of mRNA-1706, mRNA-1653, or mRNA-1893 administered once every 2 weeks for 1 month (3 doses), or up to 96 µg/dose of mRNA-1647 and mRNA-1443 administered once every 2 weeks for 6 weeks (4 doses) were evaluated, the administered mRNA/LNP vaccines were well tolerated. In addition, results from the combined developmental and perinatal/postnatal reproductive toxicity study showed that a 100 µg/dose of mRNA-1273 administered once every 2 weeks for 6 weeks (4 doses) to Sprague Dawley rats did not result in any adverse effects on the F_0 or F_1 generations. Typical vaccine-associated findings included increases in body temperature and spleen weight, changes in cytokine profile reflecting an inflammatory pattern, and injection site reaction characteristics for vaccines, with all findings

showing reversibility. In addition, no exaggerated immune reactions were observed in the rat toxicity studies or in the completed immunogenicity studies in mice (young and old), rats, hamsters, and NHPs.

If a 100 µg/dose of mRNA-1273 is well tolerated in a rat with a conservative body weight estimate of 0.30 kg as compared to a human subject with a conservative body weight of 60.0 kg, there is a 200-fold safety margin for the human dose as compared to the rat dose based on body weight. The efficacy and safety profile of the mRNA-1273 vaccine in the Phase 3 clinical trial will be the ultimate determinant in identifying the approved dose for human subjects.

2.4.5. INTEGRATED OVERVIEW AND CONCLUSIONS

In support of the development of mRNA-1273 against SARS-CoV-2, nonclinical pharmacology, biodistribution, and toxicology studies have been completed using mRNA-1273 or other mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs.

Data from the nonclinical testing program presented in this submission support the clinical efficacy and safety of mRNA-1273 at doses up to 100 µg administered twice IM 28 days apart.

- The expression of the mRNA-encoded SARS-CoV-2 S-2P antigen was confirmed in vitro and in vivo. HEK293T cells transfected with a dose range (0.003125 through 0.2 µg) of mRNA expressed the encoded SARS-CoV-2 S-2P antigen, as demonstrated by surface-protein staining with mAbs specific to the RBD (CR3022) or NTD (4A8) epitopes of the SARS-CoV-2 S protein. The expression of these epitopes was similarly confirmed in spleen and draining lymph node immune cells (cDCs and pDCs) in BALB/c mice administered a single IM dose (2 or 10 µg) of mRNA-1273.

- Nonclinical pharmacology studies of mRNA-1273 vaccination were performed in young and aged mice, golden Syrian hamsters, and rhesus macaques (NHPs). These studies demonstrate that mRNA-1273 is well tolerated, is immunogenic, and drives robust SARS-CoV-2-specific antibody and T-cell responses. Nonclinical viral challenge studies in animal models demonstrated that mRNA-1273 fully protects immunized animals from viral challenge when administered on a prime only or prime/boost schedule at dose levels ≥ 1 µg/dose in mice and hamsters and when administered on a prime/boost schedule at ≥ 30 µg/dose in nonhuman primates. In addition, these studies have shown that mRNA-1273 does not promote vaccine-associated ERD at any dose level evaluated, including those that only provided partial protection from challenge.

- The biodistribution of mRNA-based vaccines formulated in LNPs is predicted to be driven by the characteristics of the LNPs. mRNAs that are within similar LNPs (eg, mRNA-1273 and mRNA-1647) are therefore expected to distribute similarly, and the biodistribution study of mRNA-1647 supports the clinical development of mRNA-1273. This study demonstrated that mRNA constructs do not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for the 6 mRNA constructs of mRNA-1647 ranged from 14.9 to 63.0 hours.

- The aggregate GLP repeat-dose toxicity profile of mRNA vaccines that were developed with the Sponsor's mRNA-based platform in rats at IM doses ranging from 8.9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks was similar and consistent despite the fact that the different mRNA constructs encode different antigens. Therefore, the Sponsor proposes that the toxicity associated with mRNA vaccines formulated in similar LNPs is driven primarily by the LNP composition and, to a lesser extent, by the biologic activity of the antigens encoded by the mRNA and the aggregate GLP repeat-dose rat data, together with results from a mRNA-1273 non-GLP repeat dose rat study at clinically relevant doses, supports the clinical development of mRNA-1273.
- Results of a developmental and reproductive toxicity study showed that administration of a 100 µg/dose mRNA-1273 to Sprague Dawley rats at 28 and 14 days prior to mating and again at GDs 1 and 13 did not result in any adverse effects on the F_0 and F_1 generations; a small increase in the common non-adverse malformation of wavy ribs/nodules was observed in mRNA-1273-treated animals at this dose. Additionally, a robust IgG response to the S-2P antigen was observed in the dams, fetuses, and pups suggesting a strong transfer of antibodies from dam to fetus and from dam to pup.

Overall, the nonclinical studies demonstrate that mRNA-1273 is safe and well tolerated, is immunogenic, fully protects animals from viral challenge, and does not promote ERD at either optimal or suboptimal dose levels.

2.4.6. REFERENCES

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