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FINAL REPORT

Testing Facility Study No. 2308-123

A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

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2. SUMMARY

The objectives of this study were to determine the potential toxicity effects of mRNA-1273 when administered on Days 1 and 22 via intramuscular bolus injection in Crl:CD(SD) Sprague Dawley rats. The study design was as follows:

Text Table 1
Experimental Design

Group No.	Test Material	Dose Level (µg/dose)	Dose Volume (mL/kg)	Dose Concentration (µg/mL)	Main Study	
					No. of Males	No. of Females
1	Control Article	0	0.2	0	5	5
2	mRNA-1273	30	0.2	150	5	5
3	mRNA-1273	60	0.2	300	5	5
4	mRNA-1273	100	0.2	500	5	5

Crl:CD(SD) Sprague Dawley rats were administered the test or control article formulations by an intramuscular bolus injection on Days 1 and 22. A total of 2 doses were administered to each Crl:CD(SD) Sprague Dawley rat.

The following parameters and endpoints were evaluated in this study: viability, clinical signs, body weights, body weight gains, clinical pathology parameters (hematology and clinical chemistry) and immunogenicity.

There were no mRNA-1273-related mortalities, changes in body weight, or body weight gain.

mRNA-1273 elicited a significant, dose dependent antibody response on Day 35 following dose administrations on Days 1 and 22 at all dose levels.

mRNA-1273-related clinical observations were noted at 24 hours post each dose (i.e. Day 2 and 23) and generally consisted of transient, dose dependent edema with or without hindlimb impairment in all animals at ≥ 30 µg/dose. All edema and/or hindlimb impairment resolved 5 days following dose administration.

mRNA-1273-related hematology changes at Day 23 were consistent with inflammation and were seen at ≥ 30 µg/dose in both sexes. These findings included increases in neutrophil (range: 5.86x to 10.81x of control mean) and eosinophil (range: 2.60x to 4.67x of control mean) counts, decreases in mean albumin (range: 0.90x to 0.85x of control mean) and albumin/globulin ratio (range: 0.86x to 0.75x of control mean) at all dose levels, with increased mean globulin (range: 1.12x to 1.15x of control mean) in males at ≥60 µg/dose.

Other mRNA-1273-related changes observed at 30, 60, and/or 100 µg/dose consisted of decreases in mean reticulocyte (range: 0.80x to 0.65x of control mean), lymphocyte (range: 0.74x to 0.47x of control mean), and/or monocyte (range: 0.58x to 0.52x of control mean) counts. The decreases in reticulocyte counts were associated with mild decreases in red cell mass (erythrocytes, hemoglobin, and/or hematocrit) in the males at ≥30 µg/dose (hemoglobin range: 0.93x to 0.91x of control mean), and increases in RDW (red cell distribution width; range: 1.05x to 1.10x of control mean) at all doses.

Additional minor mRNA-1273-related changes most likely related to alterations in metabolic state and/or hydration status were also seen at 30, 60, and/or 100 µg/dose and included increases in mean creatinine (range: 1.26x to 1.43x of control mean), triglyceride (range: 1.66x to 2.30x of control mean), and/or cholesterol (range: 1.57x to 1.62x of control mean) concentrations. Mean glucose was also mildly increased (1.26x of control mean) in males at 100 µg/dose.

Administration of mRNA-1273 by intramuscular bolus injection on Days 1 and 22 to Crl:CD(SD) Sprague-Dawley rats was well tolerated up to 100 µg/dose.

3. INTRODUCTION

The objective of this study was to characterize the immunogenic response and potential toxicity of mRNA-1273 when administered via intramuscular injection on Days 1 and 22 to Sprague Dawley rats.

The design of this study was based on the following guidelines.

- ICH Harmonised Tripartite Guideline M3 (R2). *Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals.*
- ICH Harmonised Tripartite Guideline S6 (R1). *Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.*
- Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines. The European Agency for the Evaluation of Medicinal Products, CPMP/SWP/465/95: Dec. 17, 1997.
- WHO guidelines on nonclinical evaluation of vaccines. World Health Organization, WHO Technical Report Series, No. 927, 2005.
- Final Rules of the Animal Welfare Act regulations (Code of Federal Regulations, Title 9)
- *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (Office of Laboratory Animal Welfare, Current edition)
- *Guide for the Care and Use of Laboratory Animals* (National Research Council, Current edition)

The study protocol, last protocol amendment, and a deviation are presented in Appendix 1.

Study Initiation Date:	07 Feb 2020
Initiation of Dosing:	13 Feb 2020
Completion of In-life:	18 Mar 2020
Experimental Start Date:	10 Feb 2020
Experimental Completion Date:	23 Apr 2020

4. MATERIALS AND METHODS

4.1. Test Materials

4.1.1. Test and Control Article Characterization

The Sponsor provided to the Testing Facility documentation of the identity, strength, purity, composition, and stability for the test article. A Certificate of Analysis was provided to the Testing Facility and is presented in Appendix 2.

Documentation of the strength, composition, stability, and other pertinent information for the control article was provided.

4.1.2. Test Material Identification

Text Table 2
Test Article Identification

Identification	mRNA-1273
Lot No.	8520100101
Expiration/Retest Date	07 Aug 2020
Purity	80%
Storage Conditions	Frozen at -60°C to -90°C, protected from light
Provided by	Moderna Therapeutics Inc.

Text Table 3
Control Article Identification

Identification	nCoV Formulation Buffer
Alternate Identification	Tris/Sucrose Buffer
Lot No.	DH-02271
Expiration/Retest Date	31 Jan 2021
Storage Conditions	Refrigerated at 2°C to 8°C, protected from light
Provided by	Moderna Therapeutics Inc.

4.2. Reserve Samples

Due to the study duration, a reserve sample was not collected.

4.3. Test Article Inventory and Disposition

The test materials (e.g., test and control articles) were received by the Testing Facility for distribution as needed. Records of the receipt, distribution, storage, and disposition of test materials (including empty containers of Sponsor-provided materials) were maintained. The remaining test article was discarded appropriately after completion of the study.

4.4. Dose Formulation and Analysis

4.4.1. Preparation of Formulations

Text Table 4
Formulation Frequency of Preparation

Dose Formulation	Administration Dose Form	Frequency of Preparation	Storage Conditions
Control	Solution	On each day of dosing	Controlled room temperature[a]
Test Article	Solution	On each day of dosing[b]	Controlled room temperature [a]

[a] Formulations were prepared fresh on each day of dosing and dispensed at room temperature to be used within 4 hours of release.
[b] Test article formulation preparation was based on the actual test article stock concentration presented in Appendix 2.

Any residual volumes from each dosing occasion were retained and stored frozen at -60ºC to -90ºC, and were discarded appropriately prior to report finalization.

4.4.2. Preparation Details

Dosing formulations were prepared prior to each dose administration according to the procedures described in the Protocol at appropriate concentrations to meet dose level requirements.

4.4.3. Sample Collection and Analysis

The test and control articles were used as received from the Sponsor; therefore, samples for dose formulation analysis were not collected by the Testing Facility.

4.5. Test System

4.5.1. Receipt

On 10 Feb 2020, Sprague Dawley rats (22/sex) were received from Charles River Laboratories, Raleigh, North Carolina. The animals were approximately 7 weeks old and weighed between 171 and 228 g at initiation of dosing.

4.5.2. Justification for Test System and Number of Animals

The current state of scientific knowledge and the applicable guidelines cited did not provide acceptable alternatives, in vitro or otherwise, to the use of live animals to accomplish the purpose of this study. "The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires in vivo experimentation with a wide variety of animal species" (Federal Register, 1985). "Whole animals are essential in research and testing because they best reflect the dynamic interactions between the various cells, tissues, and organs comprising the human body" (NIH Guide, 1993).

The rat is the usual rodent model used for evaluating the immunogenicity and toxicity of various classes of chemicals and for which there is a large historical database (US FDA CDER, 2006).

The total number of animals used in this study was considered to be the minimum required to properly characterize the effects of the test article and was designed such that it did not require an unnecessary number of animals to accomplish its objectives.

4.5.3. Animal Identification

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

4.5.4. Environmental Acclimation

During the 3-day acclimation period, the animals were observed daily with respect to general health and any signs of disease. These examinations are not reported but are maintained in the study file.

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

Animals were randomly assigned to groups upon receipt. Before the initiation of dosing, any assigned animals considered unsuitable for use in the study were replaced by alternate animals obtained from the same shipment and maintained under the same environmental conditions.

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

4.5.6. Husbandry

4.5.6.1. Housing

The animals were pair- or group-housed in solid bottom cages with nonaromatic bedding. The housing was equipped with an automatic watering valve as specified in the *USDA Animal Welfare Act* (9 Code of Federal Regulations [CFR], Parts 1, 2 and 3) and as described in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, Current edition). Each cage was clearly labeled with study, group, animal number, and sex.

4.5.6.2. Animal Enrichment

Psychological/environmental enrichment was provided according to SOP.

4.5.6.3. Environmental Conditions

Target temperatures of 68°F to 79°F with a target relative humidity of 30% to 70% were maintained. A 12-hour light/12-hour dark cycle was maintained.

4.5.6.4. Food

Block Lab Diet® (Certified Rodent Diet #5002, PMI Nutrition International, Inc.) was provided ad libitum except during designated procedures. Results of analysis for nutritional components and environmental contaminants are provided by the supplier and are on file at the Testing Facility.

There are no known contaminants in the food that would interfere with this study.

4.5.6.5. Water

Tap water was available ad libitum to each animal via an automatic watering system.

There are no known contaminants in the water that would interfere with this study. The drinking water used was monitored for specified contaminants at periodic intervals according to Testing Facility SOP.

4.5.6.6. Veterinary Care

Veterinary care was available throughout the course of the study, and animals were examined by the veterinary staff as warranted by clinical signs or other changes. All veterinary examinations and recommended therapeutic treatments, if any, were documented in the study records and reviewed by the Study Director. The veterinary treatments and observations recorded after initiation of dosing are presented in Appendix 6.

4.6. Experimental Design

Text Table 5
Experimental Design

Group No.	Test Material	Dose Level (µg/dose)	Dose Concentration (µg /mL)	Dose Volume (mL/dose)	Animal Numbers	
					Male	Female
1	Control Article	0	0	0.2	1001, 1102, 1003-1005	1501-1505
2	mRNA-1273	30	150	0.2	2001-2005	2501-2505
3	mRNA-1273	60	300	0.2	3001-3005	3501-3505
4	mRNA-1273	100	500	0.2	4001-4005	4501-4504, 4605

4.6.1. Administration of Test Materials

The control and test article were administered once on Days 1 and 22 via intramuscular injection. The dose levels were 30, 60, and 100 µg/dose and administered at a dose volume of 0.2 mL/dose. The injection site areas and surrounding skin were shaved free of hair at least 48 hours prior to dose administration and as needed for evaluation of the injection site(s). Doses were administered via bolus intramuscular injection into one of the quadriceps (hind leg, thigh). A unique site was used for each injection (left quadricep on Day 1, right quadricep on Day 22). Care was taken to ensure that injection(s) were in the appropriate part of the muscle. The needle was inserted perpendicular to the skin surface. The location of the injection site was documented

for each dose. In addition, each injection site was marked with a large circle for the purposes of erythema and swelling evaluation. Each injection site was remarked at least once weekly. The control group received the control article in the same manner as the treated groups.

Under no circumstances were dose formulations subjected to vortexing or vigorous shaking to avoid disruption of the test article. Before withdrawing a dose formulation into syringes, the dose formulation container was gently swirled to achieve homogeneity. The dosing was conducted in a group number sequence order from Group 1 through Group 4, to minimize any potential risk of test article cross-contamination. Personal protective equipment (PPE) used during dose administration was changed between groups. Dose formulations were dispensed at room temperature shortly before dosing.

4.6.2. Justification of Route and Dose Levels

The intramuscular route is the intended route of administration of this test article in humans.

Doses were selected based on the aggregate toxicity data from various rat toxicity studies conducted using this lipid nanoparticle formulation. The high dose of 100 µg/dose was selected because it was the maximum feasible dose based on the concentration of the test article and the maximum intramuscular dose volume permitted in a rat. This dose was expected to elicit minor clinical observations including transient erythema and edema at the injection site. The mid- (60 µg/dose) and low- (30 µg/dose) doses were expected to produce minimal effects.

4.7. In-life Procedures, Observations, and Measurements

Text Table 6
General In-life Assessments

Parameter	Population(s)	Frequency (minimum required)	Comments
Mortality/Cageside Observations	All Animals	At least twice daily[a,b] (morning and afternoon) beginning upon arrival through termination/release.	Animals were observed within their cage unless necessary for identification or confirmation of possible findings
Detailed Clinical Observations	All Animals	Once daily from the day of receipt (Week -1) and throughout the study.[c]	Animals were removed from the cage. Observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior.
Injection Site Observations	All Animals	Immediately post each dose, 6 hours post each dose, and 24 hours post each dose	The injection site was evaluated for the presence/absence of erythema and/or edema.
Individual Body Weights	All Animals	At receipt, Day -1, and once weekly during the study.	The body weights recorded at receipt are not reported but are maintained in the study file.

			Body weight changes were calculated for animals between each weighing interval.

[a] Included alternate animals until released from study.
[b] Except on days of receipt and necropsy where frequency was at least once daily.
[c] For observations that could not be attributed to an individual animal due to social housing (e.g., watery feces), the observation was noted to each animal in the socialized group.

4.8. Laboratory Evaluations

4.8.1. Clinical Pathology

Clinical pathology evaluations (hematology and clinical chemistry) were conducted on all animals on Day 23 (24 hours post the last dose). The materials and methods are described in Appendix 9.

4.8.2. Serum ELISA Assay

Blood samples (approximately 0.5 mL) were collected from all animals via the sublingual vein for ELISA assay of the test article predose on Day 1 and once on Day 35. The animals were not fasted prior to blood collection. After the final blood collection, the animals were euthanized by carbon dioxide inhalation followed by an SOP approved method to ensure death. The carcasses were discarded without further evaluation.

Blood samples were collected in non-additive, barrier-free serum separator tubes and allowed to clot at controlled room temperature until centrifuged at controlled room temperature. The resulting serum was divided into 2 approximately equal aliquots in pre-labeled cryovials. All aliquots were flash frozen on dry ice and stored frozen at -60°C to -90°C within 60 minutes of collection. Samples were shipped on dry ice to the Vaccine Research Center, National Institutes of Health, Bethesda, Maryland, for serum ELISA assay or to the Sponsor for possible future exploratory analysis (for a deviation see Appendix 1). Any possible future exploratory analysis conducted by the Sponsor and/or Vaccine Research Center, National Institutes of Health, will be outside the scope of this study and therefore not included in the report.

5. STATISTICS

All results presented in the tables of the report were calculated using non-rounded values as per the raw data rounding procedure and may not be exactly reproduced from the individual data presented. Text Table 7 defines the set of comparisons used in the statistical analyses described in this section.

Text Table 7
Statistical Comparisons

Control Group	Treatment Group
1	2, 3, 4

The raw data were tabulated within each time interval, and the mean and standard deviation were calculated for each endpoint by sex and group. For each endpoint, treatment groups were compared to the control group using the analysis outlined in Text Table 8.

Text Table 8
Statistical Analysis

Endpoints	Type of Analysis
Body Weights Body Weight Change Hematology Clinical Chemistry	Group Pair-wise Comparisons (General ANOVA)

5.1. Group Pair-wise Comparisons (General ANOVA)

Included below are the details of the statistical routines that were applied to the data, dependent on the data specific assumptions outlined as part of the routine. The actual analysis performed for each endpoint and collection interval is included in the summary tables. The experimental unit for statistical analysis was the individual animal.

If the control group had a sample size less than 3, no inferential statistics were calculated. If a particular endpoint and/or parameter within a given collection interval had the same value across all experimental units, no inferential statistics were calculated.

For endpoints and/or parameters where all groups with sample sizes of 3 or greater were included, the normality of the residuals and homogeneity of variances were tested to determine if the data were approximately normal or if a log transformation or rank transformation was required. Levene's test was used to assess homogeneity of group variances and Shapiro-Wilk's test was used to test the normality of the residuals (Milliken and Johnson, 1992; Royston, 1992).

For the raw data, if Levene's test was not significant ($p \geq 0.01$) and Shapiro-Wilk's test was not significant ($p \geq 0.01$), then a normal distribution was used. If either the Levene's test was significant ($p < 0.01$) or Shapiro-Wilk's test was significant ($p < 0.01$), normality and homogeneity of variances were tested with a log transformation used on the data.

For the log transformed data, if Levene's test was not significant ($p \geq 0.01$) and Shapiro-Wilk's test was not significant ($p \geq 0.01$), then a log normal distribution was used. If either the Levene's test was significant ($p < 0.01$) or Shapiro-Wilk's test was significant ($p < 0.01$), then a rank transformation was used on the data.

For raw or log transformed data, a one-way analysis of variance was used to test each endpoint for the effects of treatment (Zar, 1999). If the treatment effect was significant ($p < 0.05$), linear contrasts were constructed for a Dunnett's pair-wise comparison of treatment groups as described above.

For rank transformed data, a Kruskal-Wallis test was used to test each endpoint for the effects of treatment. If the treatment effect was significant ($p < 0.05$), a non-parametric Dunn's pair-wise comparison test of each treatment group with the control group was performed.

Results of all pair-wise comparisons are reported at the 0.05 and 0.01 significance levels. All endpoints were analyzed using two-tailed tests.

6. COMPUTER SYSTEMS

Critical computerized systems used in the study are listed below in Text Table 9 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 9
Critical Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
DocuSign®	19	Collection of 21 CFR Part 11 compliant signature
Deviation Information Library	2.1	Deviations
Logbook	5.3	Electronic notebook and data collection system for veterinary communications, observations, and treatments.
ExyLIMS	3.0	A comprehensive laboratory information management system used to manage data, including but not limited to: instrumentation, test articles, standards, and samples.
NextDocs®	6.1	Electronic documentation management of Deviation Events and Corrective and Preventative Actions (CAPA).
Provantis™	9.4	Client-server, Oracle-based system used for electronic documentation and data management from compound receipt through reporting.
SAS®	9.2	The SAS® System is an integrated system of software products that enables a user to perform data entry, retrieval, data management, reporting, graphics, statistical analysis, and applications development.
Siemens Environmental Monitoring	3.11	Environmental monitoring, alarming, and reporting applications.
Niagara Framework® Software System	2.3	

7. RETENTION AND DISPOSITION OF RECORDS AND SAMPLES

All study-specific raw data, documentation, protocol, samples, and final reports from this study were archived at a Charles River archival facility unless otherwise specified in the protocol. At least one year after issue of the draft report, the Sponsor will be contacted.

All records, retained samples and reports generated from phases or segments performed by the Sponsor or subcontractors for the Sponsor were maintained by that laboratory. Details regarding the retention of the materials were provided to the Study Director.

8. RESULTS

8.1. Mortality

(Appendix 3)

All animals survived to study termination.

8.2. Detailed Clinical, Veterinary, and Injection Site Observations

(Table 1, Appendix 4, Appendix 5 and Appendix 6)

There were mRNA-1273-related clinical and veterinary observations noted starting at around 24 hours post the Day 1 dose. These findings included edema with or without hindlimb impairment noted in all treated animals. There appeared to be a dose dependent trend to the hindlimb impairment caused by the edema noted within the quadriceps muscle. All hindlimb impairment and edema resolved by the end of the first study week (Day 3 and 6, respectively). The mRNA-1273-related clinical and veterinary observations noted following the Day 22 dose were similar to the Day 1 dose. Edema with or without hindlimb impairment was observed starting ~24 hours postdose; seemed dose dependent; was observed in animals at all treatment levels; and had completely resolved by Day 27.

Other clinical/veterinary observations were transient, not dose-responsive, occurred sporadically, noted for an animal in the control group, and/or are commonly seen within this strain, age and species.

8.3. Body Weight and Body Weight Gains

(Body weight - Figure 1, Table 2, and Appendix 7)

(Body weight gains - Figure 2, Table 3, and Appendix 8)

There were no mRNA-1273-related effects on body weights.

All fluctuations among individual and mean body weight values, regardless of statistical significance, were considered not test article-related due to the lack of dose response and/or negligible magnitude.

8.4. Hematology

(Appendix 9)

Administration of mRNA-1273 to rats was associated with hematology changes at 30, 60, and 100 µg/dose. These changes, presented in Text Table 4, occurred in red cell mass (red blood cell count, hemoglobin, and hematocrit), reticulocyte, neutrophil, lymphocyte, monocyte, eosinophil counts, and/or RDW.

Text Table 4
mRNA-1273-Related Hematology Changes

Group	1*		2		3		4	
Dose Level (µg/dose)	0		30		60		100	
Sex	M	F	M	F	M	F	M	F
Hemoglobin (g/dL)								
Day 23 (24 hr post)	17.42	16.44	**0.92x**	–	**0.91x**	–	**0.93x**	–
Erythrocytes (10^6x cells/µL)								
Day 23 (24 hr post)	8.600	8.618	**0.94x**	–	**0.94x**	–	**0.96x**	–
Hematocrit (%)								
Day 23 (24 hr post)	53.46	49.48	**0.93x**	–	**0.91x**	–	**0.93x**	–
Reticulocytes (10^3x cells/µL)								
Day 23 (24 hr post)	223.48	179.28	**0.78x**	**0.79x**	**0.77x**	**0.80x**	**0.77x**	**0.65x**
Neutrophils (10^3x cells/µL)								
Day 23 (24 hr post)	0.946	1.178	**9.79x**	**7.67x**	**10.81x**	**6.58x**	**8.26x**	**5.86x**
Lymphocytes (10^3x cells/µL)								
Day 23 (24 hr post)	7.978	6.004	**0.65x**	–	**0.58x**	0.74x	**0.47x**	0.61x
Monocytes (10^3x cells/µL)								
Day 23 (24 hr post)	0.220	0.124	–	–	**0.58x**	–	**0.52x**	–
Eosinophils (10^3x cells/µL)								
Day 23 (24 hr post)	0.040	0.054	**3.30x**	**4.67x**	**4.00x**	**3.26x**	**2.60x**	**3.48x**
RDW (%)								
Day 23 (24 hr post)	12.52	11.28	1.06x	1.05x	**1.08x**	**1.07x**	**1.10x**	**1.07x**

M = Males; F = Females
hr = hour; post = postdose
RDW = red cell distribution width
A dash (—) indicates absence of a mRNA-1273-related change. Numerical values indicate fold change of the treated group mean value relative to the control group mean value. **Bolded** values indicate the mean value was statistically different from controls ($p < 0.05$ or $p < 0.01$).
* Control group values are reported for comparison.

mRNA-1273-related changes consistent with inflammation were seen at ≥ 30 µg/dose in both sexes and included moderate increases in neutrophil (range: 5.86x to 10.81x of control mean) and eosinophil (range: 2.60x to 4.67x of control mean) counts. These effects correlated to other signals of inflammation described below (see Section 3.2).

mRNA-1273-related changes at 30, 60, and/or 100 µg/dose in both sexes consisted of mild to moderate decreases in mean reticulocyte (range: 0.80x to 0.65x of control mean), lymphocyte (range: 0.74x to 0.47x of control mean), and/or monocyte (range: 0.58x to 0.52x of control mean) counts. The decreases in reticulocyte counts were associated with mild decreases in red cell mass (red blood cell count, hemoglobin, and hematocrit) in the males at 30, 60, and 100 µg/dose (range: 0.91x to 0.96x of control mean), and mild increases in RDW (range: 1.05x to 1.10x) in both sexes at all doses.

All other fluctuations among individual and mean hematology values, regardless of statistical significance, were considered sporadic, consistent with biologic variation and/or negligible in magnitude, and not related to mRNA-1273 administration.

8.5. Clinical Chemistry

(Appendix 9)

Administration of mRNA-1273 to rats was associated with clinical chemistry changes at 30, 60, and 100 µg/dose. These changes, presented in Text Table 5, occurred in mean creatinine, albumin, globulin, albumin/globulin ratio, triglyceride, cholesterol, and/or glucose concentrations.

Text Table 5
mRNA-1273-Related Clinical Chemistry Changes

Group	1*		2		3		4	
Dose Level (µg/dose)	0		30		60		100	
Sex	M	F	M	F	M	F	M	F
Creatinine (mg/dL)								
Day 23 (24 hr post)	0.28	0.40	**1.36x**	**1.32x**	–	**1.26x**	**1.43x**	**1.37x**
Albumin (g/dL)								
Day 23 (24 hr post)	3.53	3.86	**0.90x**	**0.90x**	**0.87x**	**0.88x**	**0.88x**	**0.85x**
Globulin (g/dL)								
Day 23 (24 hr post)	3.12	3.47	–	–	1.12x	–	**1.15x**	–
Albumin/Globulin Ratio								
Day 23 (24 hr post)	1.13	1.12	**0.83x**	0.87x	**0.78x**	0.87x	**0.75x**	0.86x
Triglycerides (mg/dL)								
Day 23 (24 hr post)	40.0	35.5	**1.88x**	–	**2.30x**	2.02x	1.66x	–
Cholesterol (mg/dL)								
Day 23 (24 hr post)	55.6	76.9	**1.62x**	–	**1.57x**	–	**1.58x**	–
Glucose (mg/dL)								
Day 23 (24 hr post)	69.0	84.5	–	–	–	–	**1.26x**	–

M = Males F = Females
hr = hour; post = postdose
A dash (—) indicates absence of a mRNA-1273-related change. Numerical values indicate fold change of the treated group mean value relative to the control group mean value. **Bolded** values indicate the mean value was statistically different from controls ($p < 0.05$ or $p < 0.01$).
* Control group values are reported for comparison.

mRNA-1273-related changes consistent with inflammation were seen at 30, 60, and/or 100 µg/dose in both sexes, and included mild to moderate decreases in mean albumin (range: 0.90x to 0.85x of control mean) and albumin/globulin ratio (range: 0.86x to 0.75x of control mean), with increased mean globulin (range: 1.12x to 1.15x of control mean) in males at 60 and 100 µg/dose. These effects correlated to other signals of inflammation described above (see Section 8.4).

Other mRNA-1273-related changes noted at 30, 60, and/or 100 µg/dose in both sexes consisted of mild increases in mean creatinine (range: 1.26x to 1.43x of control mean), triglyceride (range: 1.66x to 2.30x of control mean), and/or cholesterol (range: 1.57x to 1.62x of control mean) concentrations. Mean glucose was also mildly increased (1.26x of control mean) in males at 100 µg/dose. This collection of changes was most likely related to mild alterations in metabolic state and hydration status.

All other fluctuations among individual and mean clinical chemistry values, regardless of statistical significance, were considered sporadic, consistent with biologic variation and/or negligible in magnitude, and not related to mRNA-1273 administration.

8.6. Serum ELISA Assay

(Appendix 10)

30, 60, and 100 µg doses of mRNA-1273 elicited significant antibody concentrations in rats by 34 days post initial and 13 days post 2nd immunization in a dose-independent manner. A summary of the group mean antibody titer levels on Day 35 are present below in Text Tables 6 and 7. Pretest antibody titer levels were not detectable and therefore are not presented with the tables below.

Text Table 6
mRNA-1273 Antibody Titer Levels in Males

Group	**1**	**2**	**3**	**4**
Dose Level (µg/dose)	**0**	**30**	**60**	**100**
Sex	**M**	**M**	**M**	**M**
Day 35	LOQ	2,486,970.54	3,571,545.26	2,361,125.79

LOQ is ≤ 100.00

Text Table 7
mRNA-1273 Antibody Titer Levels in Females

Group	**1**	**2**	**3**	**4**
Dose Level (µg/dose)	**0**	**30**	**60**	**100**
Sex	**F**	**F**	**F**	**F**
Day 35	LOQ	4,492,100.43	3,221,503.68	4,949,493.90

LOQ is ≤ 100.00

9. CONCLUSION

Administration of mRNA-1273 by intramuscular bolus injection on Days 1 and 22 to Crl:CD(SD) Sprague-Dawley rats was well tolerated up to 100 µg/dose.

10. REFERENCES

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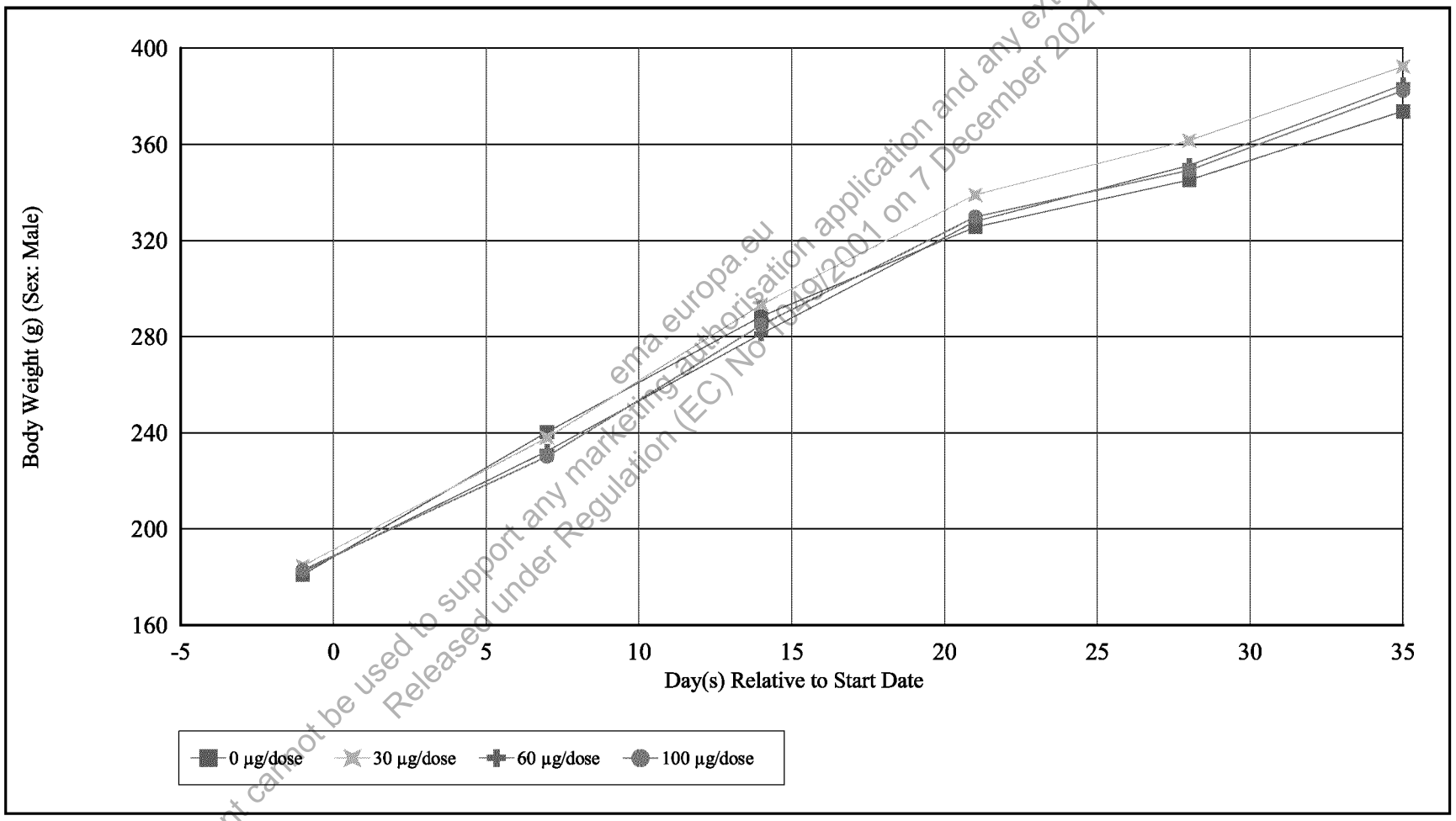
Figure 1
Mean Body Weight Values

2308-123
A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

Mean Body Weight Values



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A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

Mean Body Weight Values

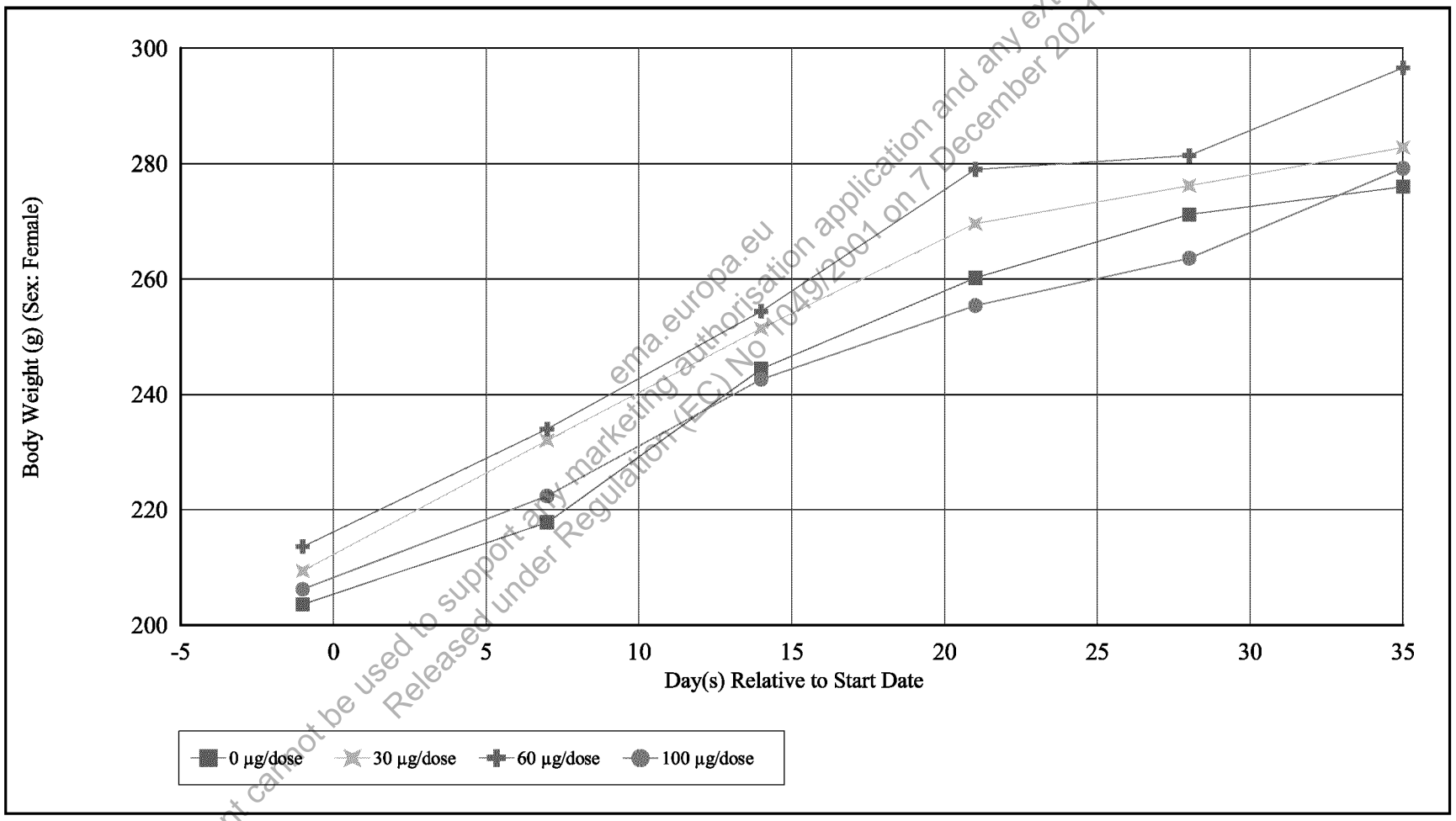
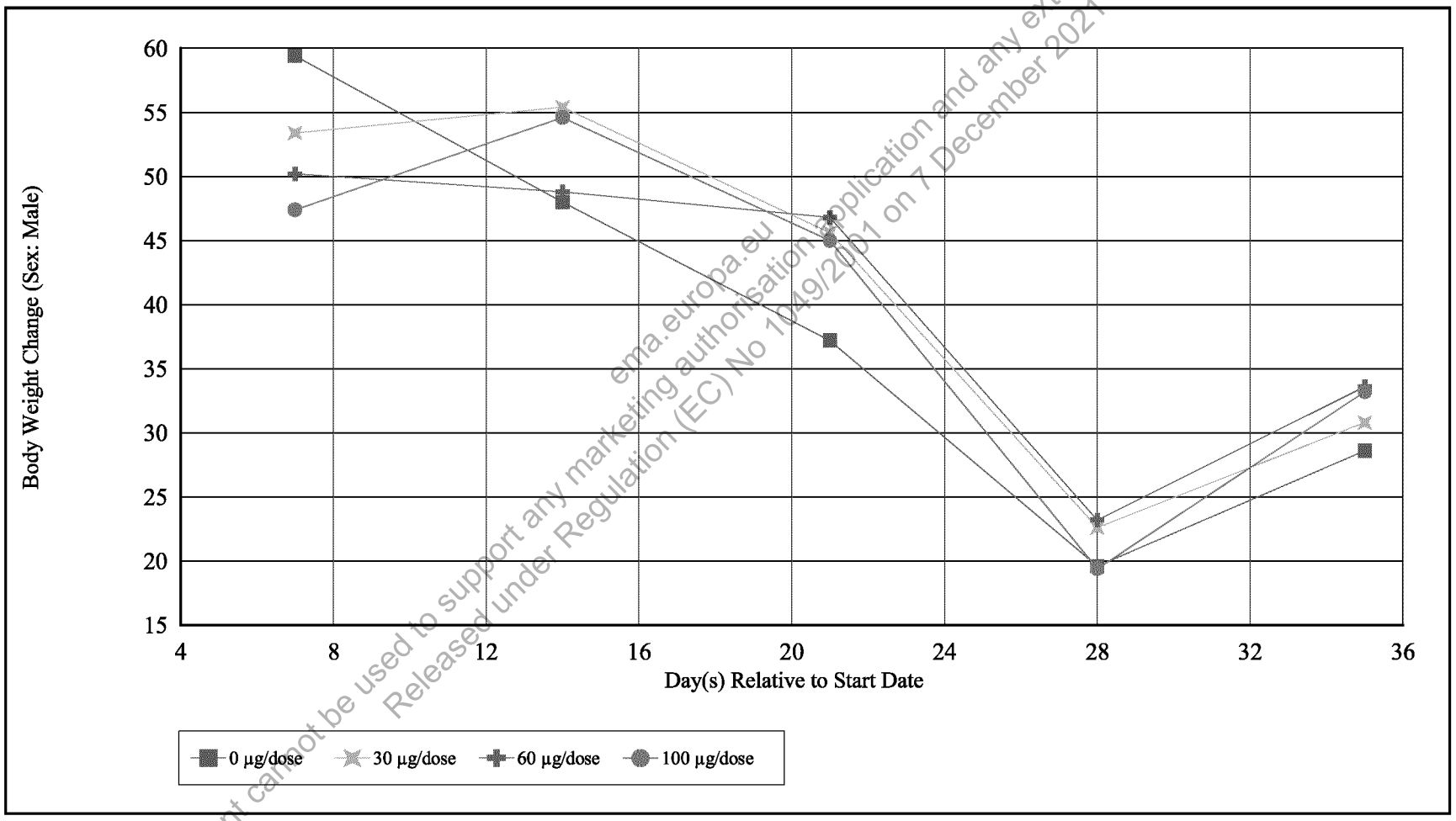


Figure 2
Mean Body Weight Change Values

2308-123
A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

Mean Body Weight Change Values, g



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Mean Body Weight Change Values, g

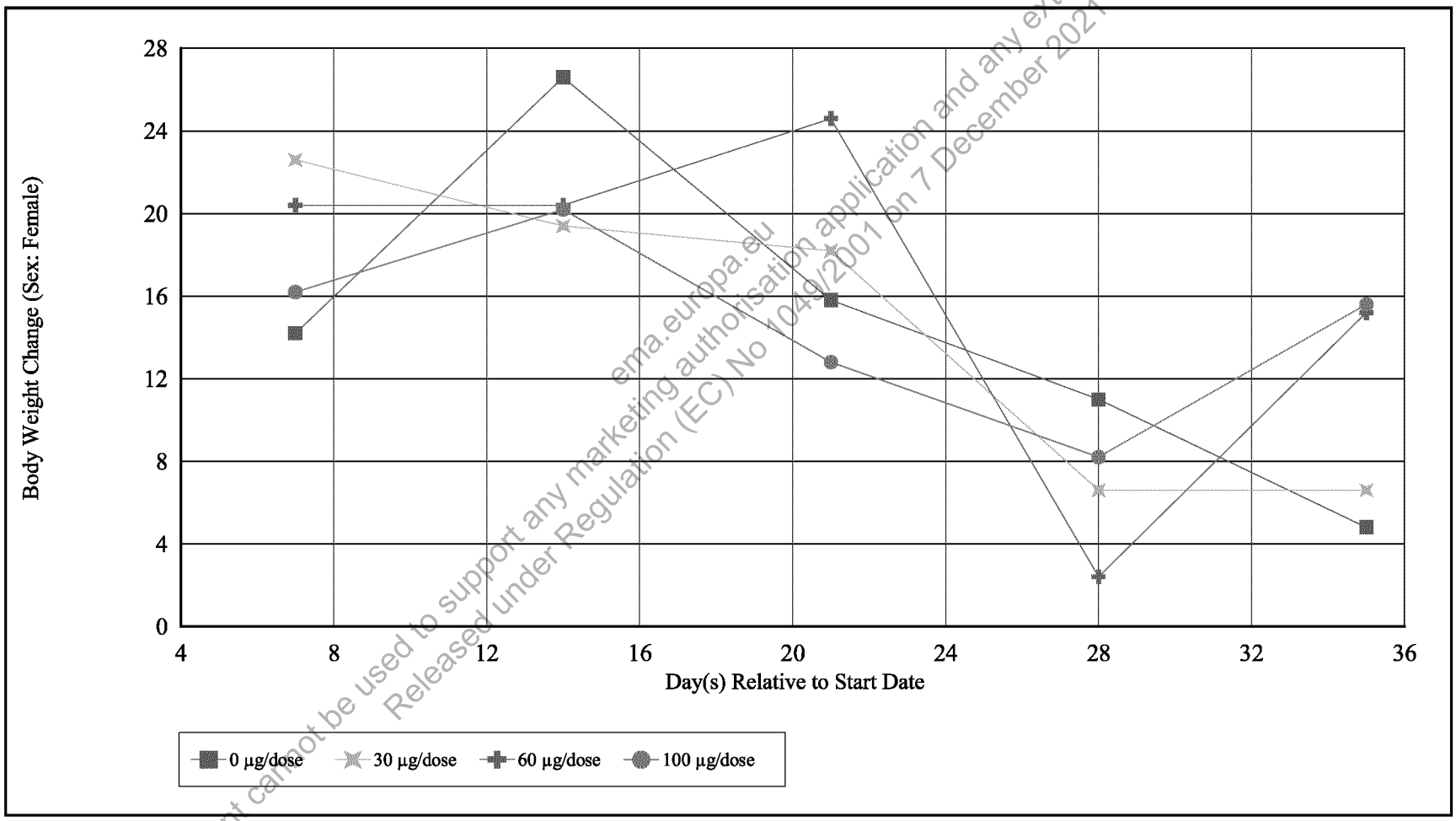

Table 1
Summary of Detailed Clinical Observations

2308-123
A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

Summary of Detailed Clinical Observations

Observation Type: All Types	Male			
From Day 1 (Start Date) to 35 (Start Date)	0 µg/dose	30 µg/dose	60 µg/dose	100 µg/dose
Total Number of Animals:	5	5	5	5
-EXTERNAL APPEARANCE				
Limb function impaired				
Number of Times Recorded	0	1	5	5
Number of Animals Affected	-	1	5	5
Material around eyes, Black				
Number of Times Recorded	0	0	1	0
Number of Animals Affected	-	-	1	-
Material around nose, Red				
Number of Times Recorded	0	0	1	2
Number of Animals Affected	-	-	1	2
Thin				
Number of Times Recorded	0	0	6	0
Number of Animals Affected	-	-	2	-
-PELAGE/SKIN				
Abrasion(s)				
Number of Times Recorded	0	0	2	0
Number of Animals Affected	-	-	2	-
Edema				
Number of Times Recorded	0	10	30	29
Number of Animals Affected	-	5	5	5
Hair sparse				
Number of Times Recorded	0	5	39	0
Number of Animals Affected	-	1	3	-
Scabbed area				
Number of Times Recorded	0	0	20	6
Number of Animals Affected	-	-	3	1
Skin discolored, Brown				
Number of Times Recorded	0	0	2	0
Number of Animals Affected	-	-	1	-
Skin discolored, Red				
Number of Times Recorded	0	0	0	6
Number of Animals Affected	-	-	-	1
Unkempt appearance				
Number of Times Recorded	0	0	0	9
Number of Animals Affected	-	-	-	3

2308-123
A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

Summary of Detailed Clinical Observations

Observation Type: All Types	Female			
From Day 1 (Start Date) to 35 (Start Date)	0 µg/dose	30 µg/dose	60 µg/dose	100 µg/dose
Total Number of Animals:	5	5	5	5
-EXTERNAL APPEARANCE				
Limb function impaired				
Number of Times Recorded	0	0	4	5
Number of Animals Affected	-	-	4	5
Material around eyes, Black				
Number of Times Recorded	0	0	0	0
Material around nose, Red				
Number of Times Recorded	0	0	0	0
Thin				
Number of Times Recorded	0	0	0	0
-PELAGE/SKIN				
Abrasion(s)				
Number of Times Recorded	0	0	0	0
Edema				
Number of Times Recorded	0	13	31	30
Number of Animals Affected	-	5	5	5
Hair sparse				
Number of Times Recorded	6	24	72	116
Number of Animals Affected	1	2	3	2
Scabbed area				
Number of Times Recorded	0	0	0	0
Skin discolored, Brown				
Number of Times Recorded	0	0	0	0
Skin discolored, Red				
Number of Times Recorded	0	0	0	0
Unkempt appearance				
Number of Times Recorded	0	0	0	0

Table 2
Summary of Body Weight Values

2308-123
A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

Summary of Body Weight Values

Body Weight (g)

Sex: Male Day(s) Relative to Start Date		0 µg/dose	30 µg/dose	60 µg/dose	100 µg/dose
-1	Mean	181.0	184.6	182.2	182.8
	SD	10.27	6.54	8.17	10.80
	N	5	5	5	5
7	Mean	240.4	238.0	232.4	230.2
	SD	9.50	11.02	10.53	14.87
	N	5	5	5	5
14	Mean	288.4	293.4	281.2	284.8
	SD	10.95	17.62	7.05	20.75
	N	5	5	5	5
21	Mean	325.6	339.0	328.0	329.8
	SD	13.97	22.75	11.18	22.35
	N	5	5	5	5
28	Mean	345.2	361.6	351.2	349.2
	SD	12.77	24.47	12.70	22.54
	N	5	5	5	5

Anova & Dunnett

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A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

Summary of Body Weight Values

Body Weight (g)

Sex: Male Day(s) Relative to Start Date		0 µg/dose	30 µg/dose	60 µg/dose	100 µg/dose
35	Mean	373.8	392.4	384.8	382.4
	SD	15.25	26.54	18.05	21.90
	N	5	5	5	5

Anova & Dunnett

2308-123
A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

Summary of Body Weight Values

Body Weight (g)

Sex: Female Day(s) Relative to Start Date		0 µg/dose	30 µg/dose	60 µg/dose	100 µg/dose
-1	Mean	203.6	209.4	213.6	206.2
	SD	8.73	4.93	8.08	3.49
	N	5	5	5	5
7	Mean	217.8	232.0	234.0	222.4
	SD	12.11	6.16	15.35	6.66
	N	5	5	5	5
14	Mean	244.4	251.4	254.4	242.6
	SD	17.46	8.62	15.71	8.29
	N	5	5	5	5
21	Mean	260.2	269.6	279.0	255.4
	SD	20.72	15.19	22.30	10.14
	N	5	5	5	5
28	Mean	271.2	276.2	281.4	263.6
	SD	26.34	8.93	18.77	14.38
	N	5	5	5	5

Anova & Dunnett

2308-123
A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

Summary of Body Weight Values

Body Weight (g)

Sex: Female Day(s) Relative to Start Date		0 µg/dose	30 µg/dose	60 µg/dose	100 µg/dose
35	Mean	276.0	282.8	296.6	279.2
	SD	20.06	15.93	30.39	21.18
	N	5	5	5	5

Anova & Dunnett

Table 3
Summary of Body Weight Change Values

2308-123
A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

Summary of Body Weight Change Values, g

Body Weight Change

Sex: Male Day(s) Relative to Start Date		0 µg/dose	30 µg/dose	60 µg/dose	100 µg/dose
-1 → 7	Mean	59.4	53.4	50.2 a	47.4 b
	SD	2.19	5.59	2.59	5.86
	N	5	5	5	5
7 → 14	Mean	48.0	55.4	48.8	54.6
	SD	5.48	10.14	8.29	6.07
	N	5	5	5	5
14 → 21	Mean	37.2	45.6	46.8	45.0
	SD	8.01	5.98	12.58	5.24
	N	5	5	5	5
21 → 28	Mean	19.6	22.6	23.2	19.4
	SD	4.83	5.59	3.70	5.81
	N	5	5	5	5
28 → 35	Mean	28.6	30.8	33.6	33.2
	SD	5.50	5.76	8.65	5.26
	N	5	5	5	5

Anova & Dunnett: a = p < 0.05; b = p < 0.01

2308-123
A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

Summary of Body Weight Change Values, g

Body Weight Change

Sex: Female Day(s) Relative to Start Date		0 µg/dose	30 µg/dose	60 µg/dose	100 µg/dose
-1 → 7	Mean	14.2	22.6	20.4	16.2
	SD	4.97	6.02	7.57	5.97
	N	5	5	5	5
7 → 14	Mean	26.6	19.4	20.4	20.2
	SD	9.32	3.44	7.20	5.50
	N	5	5	5	5
14 → 21	Mean	15.8	18.2	24.6	12.8
	SD	3.63	7.79	7.54	9.01
	N	5	5	5	5
21 → 28	Mean	11.0	6.6	2.4	8.2
	SD	9.14	7.80	9.56	6.61
	N	5	5	5	5
28 → 35	Mean	4.8	6.6	15.2	15.6
	SD	11.45	8.79	13.33	7.44
	N	5	5	5	5

Anova & Dunnett