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Toxicology Review of mRNA-1283 Vaccine (Final Report)

From: (b) (6)

Through: (b) (6)

To: (b) (6)

File: BLA 125835, original submission

Product: mRNA-1283 Vaccine

Reviewer: (b) (6)
 BLA sections reviewed:
 4.2.3.2 Repeat dose toxicity studies
 4.2.3.3. Genotoxicity studies
 4.2.3.5 Reproductive and developmental toxicity studies

Type and date of submission: Original, September 30th, 2024.

Sponsor: Moderna Tx, Inc. 325 Binney Street, Cambridge MA 02142

Proposed indication: Active immunization to prevent COVID-19 caused by SARS-CoV-2 in individuals 12 years of age and older.

Division name: OVRR/(b) (6)

Pre-BLA telecon was held with Moderna on 10 July 2024. No questions related to toxicology were discussed.

Proprietary name: (b) (4) as their primary name, and “mNEXSPIKE” as the alternative.

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Introduction:

(b) (4) of the same 4 lipids used in Moderna's approved SPIKEVAX™, mRNA-1283 is a lipid-encapsulated, mRNA-based vaccine encoding the NTD and RBD of the S glycoprotein of the SARS-CoV-2 virus. SM-102, a custom manufactured, ionizable lipid, PEG2000-DMG, DSPC, and cholesterol to form RNA-LNP complexes. mRNA-1273 (SPIKEVAX) encodes the full-length Spike of SARS-CoV-2; after translation, 3 protomers combine into an S trimer that is membrane-bound. mRNA-1283 encodes for the 2 key subdomains of the S glycoprotein, the SARS-CoV-2 NTD and RBD, which are linked with a 7-amino acid flexible linker. The linked NTD-RBD polypeptide is attached via a (b) (4) linker to a 23 amino acid transmembrane domain from influenza (HA-TM). This anchors the linked NTD-RBD polypeptide into the cell membrane of antigen-expressing cells. Due to its shorter RNA length, the mRNA-1283 vaccine design has the potential for a longer shelf life at refrigerated conditions.

Known to elicit protective immune responses, mRNA-1283 focuses the immune response to the key neutralization and T-cell epitopes of the NTD and RBD regions of the Spike protein. Specifically, there is a body of evidence that the RBD and NTD are the immuno-dominant epitopes for protective immune responses. The antibody-mediated protection against SARS-CoV-2 is mainly based on anti-RBD neutralizing antibodies (Piccoli et al 2020; Dejnirattisai et al 2021) and to a lesser extent on antibodies targeting the NTD domain (Cerutti et. al 2021). Does not contain the NTD and RBD, the S2 spike region is largely a target for non-neutralizing antibodies which are not considered to be protective (Crowley et al 2022; Amanat et al 2021). mRNA-1283 is an antigen that does not contain the S2 region and would focus the immune response to epitopes that confer protection against COVID-19. Animal challenge studies have indicated protective responses elicited by RBD-based vaccines (Chen et al 2022; Yang et al 2020; Stewart-Jones et al 2023). The RBD and NTD also contain immunodominant T-cell epitopes (Karsten et al 2022), which elicit cellular immunity.

In March 2021, the sponsor initiated the clinical evaluation of mRNA-1283 with the original SARS-CoV-2 sequence RBD and NTD in a 2-dose (primary series) dose-ranging phase 1 study (mRNA-1283-P101) in vaccine-naïve adults.

The sponsor initiated a global, randomized, observer-blind, active-controlled, single-dose pivotal phase 3 study (mRNA-1283-P301) in participants ≥ 12 years of age and older in March 2023. Participants were randomized to receive a single 10 μg dose of mRNA-1283 or a single 50 μg dose of mRNA-1273. The study vaccines aligned with the 2022/2023 variant formulation recommended by the United States FDA and WHO (original: Omicron BA.4/BA.5 bivalent) as study enrollment occurred March through August 2023.

Proposed clinical study:

The clinical study is a randomized, observer-blind, active-controlled phase 3 study to investigate the safety, immunogenicity, and relative vaccine efficacy of mRNA-1283 compared with mRNA-1273 in participants aged ≥ 12 years for the prevention of COVID-19.

The study design supported the primary efficacy and immunogenicity noninferiority objectives, comparing mRNA-1283 with the standard-of-care COVID-19 vaccine, mRNA-1273. Participants were randomized 1:1 to receive mRNA-1283.222 10 μg (original SARS-CoV-2: Omicron BA.4/BA5) or mRNA-1273.222 50 μg (original SARS-CoV-2: Omicron BA.4/BA5) as a single dose. Randomization was stratified by age groups (adolescents, 12 to <18 , 18 to <65 , and ≥ 65 years), with the goal to enroll approximately 1000 adolescents (12 to <18 years) and approximately 30% of participants to be in the ≥ 65 age group.

The follow-up time for all participants is 12 months and the primary study analysis includes at least 6 months of follow-up time for all participants. To qualify for the study, participants were to have previously received a primary series of an authorized/approved COVID-19 vaccine and those ≥ 18 years of age were to have also received at least 1 booster dose, but no more than 5 vaccine doses. Participants 12 to <18 years had no booster dose requirement prior to study entry. Prior SARS-CoV-2 infection and COVID-19 vaccination was allowed unless an infection or vaccination had occurred within 90 days of enrollment. Vaccine administration was at the baseline visit (day 1) and 4 in-person follow-up visits after day 1 were planned (days 29, 91, 181, and 365) as well as 3 scheduled safety follow-up/safety phone calls.

Using electronic diary prompts, COVID-19 symptom surveillance was conducted biweekly. The participants were requested to present for an unscheduled visit for clinical evaluation and collection of respiratory samples for SARS-CoV-2 PCR if the participant had a qualifying symptom. Based on qualifying symptoms, SARS-CoV-2 PCR testing will be, also, conducted in routine clinic visits. Any rapid antigen testing information for SARS-CoV-2 infection (ie, testing at home or in an urgent care setting) was used for descriptive analyses. Rapid antigen testing results did not support the primary rVE objective.

Studies reviewed in this BLA:

Repro Tox Study

- 1- A GLP Combined Perinatal/Postnatal Developmental and Reproductive Toxicity Study of mRNA-1283 by the Intramuscular Route in the (b) (4) Rat. Test Facility Study No. 20346748

General Tox Studies

- 1- A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1283, mRNA-1284, and mRNA-1285 by Intramuscular Injection in (b) (4) Rats. Test Facility Study No. 2308-161
- 2- A 1-Month (3 doses) Study of (b) (4) by Intramuscular Injection in (b) (4) Rat with a 2-Week Recovery Period. Test Facility Study No. 5002033
- 3- A 6-Week (4 Doses) Intramuscular Injection Toxicity Study of (b) (4) in (b) (4) Rats Followed by a 2-Week Recovery Period. Test Facility Study No. 5002034
- 4- (b) (4): A 1-Month (3 Doses) Intramuscular Injection Toxicity Study of (b) (4) in (b) (4) Rats with a 2-Week Recovery Period. Test Facility Study No. 5002045
- 5- A 6-Week (4 doses) Intramuscular Injection Toxicity Study of (b) (4) in (b) (4) Rats Followed by a 2-Week Recovery Period. Test Facility Study No. 5002158
- 6- A 1 Month (3 doses) Intramuscular Injection Vaccine Study of (b) (4) in (b) (4) Rats with a 2-Week Recovery Period. Test Facility Study No. 5002231
- 7- A 1-Month (3 Doses) Intramuscular Injection Toxicity Study of (b) (4) in (b) (4) Rats Followed by a 2-Week Recovery Period. Test Facility Study No. 5002400
- 8- A 4-Week GLP Repeat Dose Toxicity Study of mRNA-1283.222 by Intramuscular Injection Administration in (b) (4) Rats with a 2-Week Recovery Period. Test Facility Study No. 20462697

In Vivo Gene Tox Studies

- 1- (b) (4) mRNA: (b) (4) Test in Rat. Test Facility Study No. 9800399
- 2- (b) (4) Assay in the Rat. Test Facility Study Number AF87FU.125012NGLPICH.BTL

In Vitro Gene Tox Studies

- 1- (b) (4) Bacterial Reverse Mutation Test in (b) (4). Test Facility Study No. 9601035
- 2- (b) (4) Test in Human Peripheral Blood Lymphocytes. Test Facility Study No. 9601036

- 3- (b) (4) Bacterial Reverse Mutation Test in (b) (4)
 . Test Facility Study No. 9601567
- 4- (b) (4) Test in Human Peripheral Blood
 Lymphocytes. Test Facility Study No. 9601568

Toxicology study reviews

Study number 1: A GLP Combined Perinatal/Postnatal Developmental and Reproductive Toxicity Study of mRNA-1283 by the Intramuscular Route in the (b) (4) Rat.
Study number 20346748.

Performing laboratory: (b) (4)

Study initiation date: June 14, 2022

Final report date: May 06, 2024

Test article batch/lot:

Table of test article identification

	Test Article
Identification:	mRNA-1283
Alternate Identification:	mRNA Lipid Nanoparticle Drug Product
Batch/Lot No.:	DH-16459
Expiration Date:	09 Feb 2023
Physical Description:	Off-white
Supplied Stock Concentration:	0.4 mg/mL ^a
Density:	(b) (4)
Storage Conditions^b:	(b) (4) °C, protected from light

^a Test Article was prepared using actual concentration of 0.4 mg/mL.

^b Temperature set to maintain.

Table of control article identification

	Control Article
Identification:	(b) (4) mM Tris, (b) (4) g/L Sucrose pH (b) (4)
Alternate Identification:	(b) (4)
Batch/Lot No.:	(b) (4)
Expiration Date:	12 Apr 2023
Physical Description:	Clear, colorless liquid
Density:	(b) (4)
Storage Conditions^a:	(b) (4) °C

^a Temperature set to maintain.

Animal species and strain: (b) (4) rat

Breeder/supplier: (b) (4)

Number of animals per group and sex: 22 females/group

Age: 69 days

Body weight range: 207-257 grams

Route and site of administration: Intramuscular (IM) injection

Volume of injection: 0.2 mL

Frequency of administration and study duration: Administered by intramuscular (IM) injection during the pre-mating period (28 and 14 days prior to mating) and on gestation days (GDs) 1 and 13.

Dose: 0 or 80 µg/dose

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND. Concentration stability data were generated by the department of Analytical Chemistry, (b) (4) for 1 day and 21 days, for (b) (4) samples stored at ambient temperature and in a freezer set to maintain (b) (4) °C, respectively, over the concentration range of (b) (4), under study no. 2101241. Stability analyses were reported in appendix 3.

Means of administration: Intramuscular (IM) injection.

Report status: Final report.

Experimental design:

Animals were randomized and assigned to 2 different groups. Animals, 22 females/group, were dosed by intramuscular (IM) injection during the pre-mating period (28 and 14 days prior to mating) and on gestation days (GDs) 1 and 13. The dose levels were 0 and 80 µg/dose and administered at a dose volume of 0.2 mL/dose. The details of the study design are listed in the following table:

Table of experimental design

Group No.	Test Material	Dose Level (µg/dose)	Dose Concentration (mg/mL)	Fixed Dose Volume (µL/dose)	Female Numbers ^a	
					Cohort 1 (Cesarean-sectioning)	Cohort 2 (Natural Delivery)
1	Control Article	0	0	200	1601-1622	1623-1644
2	mRNA-1283	80	0.4	200	2601-2622	2623-2637, 2639-2644, 11

^a Cohort 1 females were allocated to the Cesarean-sectioning phase. Cohort 2 females were assigned to the natural delivery (parturition) phase.

Table 1: Experimental design, sponsor provided (Study no. 20346748).

F0 generation females assigned to cohort 1 were Cesarean sectioned on GD 21 and those assigned to cohort 2 were allowed to naturally deliver their litters and euthanized after completion of the 21-day postpartum period. All F0 generation rats were euthanized on GD 21 (cohort 1) or lactation day (LD) 21 (cohort 2). All F1 generation pups assigned to cohort 2 were euthanized on day 13 or day 21 postpartum.

Methods:

Randomization procedure: Yes

Statistical analysis plan: Yes.

The following parameters were evaluated:

Parameter	Population	Frequency (minimum required)	Comments
Viability	Cohort 1 and 2	a. At least twice daily	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings.
Clinical Observations: General	Cohort 1 and 2	- At least once weekly during the acclimation period - Before each dose is administered - Daily on each non-dose administration days (except for the postdose observation timepoints) - Daily during the postdose period (including on the day of scheduled euthanasia)	-
Clinical Observations: Postdose Observations	Cohort 1 and 2	Approximately 6 and 24 hours following each dose	Time intervals for postdose observations may be adjusted if deemed appropriate by the study director during the course of the study. Such adjustments will be documented in the raw data.
Maternal Observations	Cohort 2	Daily during the postpartum period	-
Individual Body Weights	Cohort 1 and 2	- The day of or day after arrival - At least once weekly during the acclimation period - SDs 1, 8, 15, 22, and 28 - GDs 0, 3, 6, 9, 12, 15, 18, 21, and 25 (if necessary) - LDs 1, 4, 7, 10, 14, 17, and 21	-
Food Consumption	Cohort 1 and 2	- At least once weekly until the cohabitation period (including SDs 1, 8, 15, 22, and 28) - GDs 0, 3, 6, 9, 12, 15, 18, and 21 - LDs 1, 4, 7, 10, and 14	Food consumption will be quantitatively measured per cage, where appropriate. For Cohort 2, food consumption will not be tabulated after day 14 postpartum, when it is expected that pups will begin to consume maternal food. Values may be recorded more frequently if it is necessary to replenish the food. These intervals will not be tabulated.
Estrous Cycle Evaluations	Cohort 1 and 2	14 consecutive days before the initiation of cohabitation and then until spermatozoa are observed in a smear of the vaginal contents and/or a copulatory plug is observed <i>in situ</i> during the cohabitation period	Estrous cycles will be evaluated by examining the vaginal cytology of samples obtained by vaginal lavage.

Parameter	Population	Frequency (minimum required)	Comments
Cohabitation/ Mating	Cohort 1 and 2	Maximum of 7 days	Rats will be assigned to cohabitation (i.e., pairing), one breeder male per one female. Females observed with spermatozoa in a smear of the vaginal contents and/or a copulatory plug observed <i>in situ</i> will be considered to be at GD 0 and assigned to individual housing. Females not mated after completion of the cohabitation period will be considered to be at GD 0 on the last day of cohabitation, assigned to individual housing and will be euthanized 25 days after the end of the cohabitation period (for rats that do not deliver a litter) or continued on study to be assigned to cohort 2 as needed (for rats that do deliver a litter) or at the discretion of the study director.
Natural Delivery Observations	Cohort 2	Beginning on GD 20, the dams will be observed at least twice daily for signs of delivery	Natural delivery observations will be recorded. The number and vital status of the pups will be recorded.

- = Not applicable; SD = Study Day; GD = Gestation Day; LD = Lactation Day.

Table 2: In-life procedures, observations, and measurements for F0 generation, sponsor provided (Study no. 20346748).

Parameter	Frequency (minimum required)	Comments
Viability	At least twice daily Counted at least once daily (pups in each litter)	Pups will be observed within their cage unless necessary for identification or confirmation of possible findings
Clinical Observations: General	At least once daily starting on day 1 postpartum	-
Individual Body Weights	Days 1, 4, 7, 10, 14, 18, and 21 postpartum	-
Prewaning Reflex and Physical Development:	Surface righting reflex beginning on day 1 postpartum Pinna detached beginning on day 2 postpartum (until both pinna detached) Hair growth beginning on day 7 postpartum Eye opening beginning on day 12 postpartum Auditory startle reflex beginning on day 13 postpartum	Pups will be evaluated daily until criterion is achieved or until the day of weaning. A pup that reaches the criteria will no longer be tested.
	Pupil constriction on day 21 postpartum	Evaluated once.

- = Not applicable.

Table 3: In-life procedures, observations, and measurements for F1 generation (cohort 2), sponsor provided (Study no. 20346748).

Group No.	Subgroup ^a	Timepoint Study Days						
		Prestudy ^{b,c}	SD 15 ^c	GD 1 ^c	GD 13 ^c	GD 21 ^d	LD 13	LD 21 ^d
1-2	Cohort 1	X	X	X	X	X	-	-
1-2	Cohort 2	X	X	X	X	-	X	X
Unscheduled euthanasia (when possible) and dams with no surviving pups		X						

X = Sample to be collected; - = Not applicable; SD = Study Day; GD = Gestation Day; LD = Lactation Day.

^a Samples will be collected from all animals in the cohort, where possible.

^b Samples will be collected from all animals, including animals not assigned to study to account for any replacements. Samples from animals replaced on study and/or not assigned to study after the replacement period will be discarded.

^c Sample will be collected before dose administration.

^d Terminal blood sample.

Table 4: Antibody serum blood sample collection, sponsor provided (Study no. 20346748).

Group No.	Postpartum Period	
	LD 13	LD 21 (If Possible ^a)
1-2	X	X

X = Sample to be collected

^a If possible, milk samples will be obtained on LD 21. Note: the full sample volume may not be able to be obtained on LD 21 due to the expected decline in milk production later in the postpartum period.

Table 5: Milk sample collection, sponsor provided (Study no. 20346748).

Group No.	Scheduled Euthanasia Day	Necropsy Procedures				Fetal Evaluation
		Ovarian/Uterine Examination	Necropsy	Tissue Collection ^a	Organ Weights	
1	GD 21	X	X	X	X ^b	X
2						
Unscheduled Deaths		X	X	X	-	X

X = Procedure to be conducted; - = Not applicable; GD = Gestation Day

^a See Tissue Weighing, Collection, Processing and Evaluation table, attachment A or attachment B, for list of tissues applicable to each procedure.

^b The gravid uterus and placentae will be weighed for all pregnant females that survive to scheduled euthanasia.

Table 6: Terminal procedure-Cohort 1, sponsor provided (Study no. 20346748).

Group No.	Scheduled Euthanasia Day	Necropsy Procedures				Histology Processing and Microscopic Evaluation
		Ovarian/Uterine Examination	Necropsy	Tissue Collection ^a	Organ Weights	
1	Day 21 Postpartum	X	X	X	-	-
2						
Dams that do not deliver	GD 25	X	X	X	-	-
Dams with no surviving pups	^b	X	X	X	-	-
Unscheduled Deaths		X	X	X	-	-

X = Procedure to be conducted; - = Not applicable; GD = Gestation Day

^a See Tissue Weighing, Collection, Processing and Evaluation table, attachment A or attachment B, for list of tissues applicable to each procedure.

^b On the day the observation is made.

Table 7: Terminal procedure-Cohort 2, sponsor provided (Study no. 20346748).

Examination	Procedure
Aborted/Conceptuses <i>in utero</i> /Delivered Conceptuses	Examined for external, visceral, and/or skeletal abnormalities to the extent possible
Late Resorptions	Examined for external abnormalities to the extent possible, and discarded without further examination
Dead Fetuses	Examined for external, visceral, and/or skeletal abnormalities to the extent possible
Body Weights	Recorded for each live fetus.
Sex	Each fetus will be externally sexed.
External	All fetuses will be examined for external abnormalities.

Examination	Procedure
Visceral	Approximately one-half of the fetuses in each litter will be examined for visceral abnormalities by using a modification of the microdissection technique of Staples ¹ . Each fetus will be fixed in Bouin's solution, and the heads will subsequently be examined by free-hand sectioning ² . The head slices of normal fetuses will be discarded following examination; head slices from fetuses with observations will be retained in 70% ethanol. The decapitated carcasses will not be retained.
Skeletal	The remaining fetuses (approximately one-half of the fetuses in each litter) will be retained in alcohol and examined for skeletal abnormalities after staining with alizarin red S ³ . Skeletal preparations will be retained in glycerin with thymol added as a preservative.

Table 8: Fetal examinations (cohort 1), sponsor provided (Study no. 20346748).

Postmortem procedures:

Tissue	Weigh	Collect	Comment
Administration site	-	X	Lateral proximal thigh musculature with overlying skin.
Cervix	-	X	Collect with uterus and ovaries. All nonpregnant animals.
Gravid uterus	X	-	All pregnant animals at scheduled euthanasia for cohort 1.
Gross lesions/masses	-	X	All animals
Ovaries	-	X	Collect with cervix and uterus. All nonpregnant animals.
Placentae	X	X	All pregnant animals at scheduled euthanasia for cohort 1. Placentae will be retained with individual identification.
Uterus	-	X	Collect with cervix and ovaries. All nonpregnant animals.

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

Table 9: Tissue collection and preservation-F0 generation, sponsor provided (Study no. 20346748).

Tissue weighing, collection, and evaluation table - Unscheduled euthanasia F0 generation (cohorts 1 and 2) and F1 generation (cohort 2)

Tissue	Weigh	Collect	Histology	Microscopic Evaluation	Comment
Administration site	-	X	-	-	
Artery, aorta	-	X	-	-	
Body cavity, nasal	-	X	-	-	
Bone marrow	-	X	-	-	Two bone marrow smears will be collected from the femur. Smears will not be collected from animals that are found dead or from animals that were euthanized moribund and then stored in the refrigerator prior to necropsy. Bone marrow smears are allowed to air dry and are not fixed in formalin.
Bone, femur	-	X	-	-	Only 1 required for collection.

¹ Staples RE. Detection of visceral alterations in mammalian fetuses. *Teratology* 1974;9(3):A37-A38.² Wilson JG. Methods for administering agents and detecting malformations in experimental animals. In: Wilson JG, Warkany J, editors. *Teratology: principles and techniques*. Chicago (IL): University of Chicago Press; 1965. p. 262-77.³ Staples RE, Schnell VL. Refinements in rapid clearing technic in the KOH-alizarin red S method for fetal bone. *Stain Technol* 1964;39:61-3.

Tissue	Weigh	Collect	Histology	Microscopic Evaluation	Comment
Bone, sternum	-	X	-	-	-
Brain	-	X	-	-	Seven brain levels ^a to be examined to include olfactory bulb (examine in body cavity, nasal section level 4 ^b).
Cervix	-	X			Collect with uterus and ovaries.
Epididymis	-	X	-	-	Paired examination.
Esophagus	-	X	-	-	-
Eye	-	X	-	-	Paired examination; Preserve in (b) (4) fixative.
Ganglion, dorsal root, lumbar	-	X	-	-	Collect with spinal column.
Gland, adrenal	-	X	-	-	Paired examination.
Gland, clitoral	-	X	-	-	-
Gland, Harderian	-	X	-	-	-
Gland, lacrimal	-	X	-	-	-
Gland, mammary	-	X	-	-	For males, examine only if present in routine section of skin. Collect with inguinal skin.
Gland, parathyroid	-	X	-	-	Examine only if present in the routine section of thyroid.
Gland, pituitary	-	X	-	-	-
Gland, preputial	-	-	-	-	-
Gland, prostate	-	X	-	-	-
Gland, salivary, submandibular	-	X	-	-	-
Gland, salivary, sublingual	-	X	-	-	-
Gland salivary, parotid	-	X	-	-	-
Gland, seminal vesicle	-	X	-	-	Paired examination.
Gland, thyroid	-	X	-	-	Paired examination
Gland, Zymbal's	-	X	-	-	-
Gut-associated lymphoid tissue	-	X	-	-	Examine only if present in routine section of intestine.
Heart	-	X	-	-	-
Joint, femorotibial	-	X	-	-	Only 1 required for collection.
Kidney	-	X	-	-	Paired examination.
Large intestine, cecum	-	X	-	-	-
Large intestine, colon	-	X	-	-	-
Large intestine, rectum	-	X	-	-	-
Larynx	-	X	-	-	Examine level ^c
Liver	-	X	-	-	-
Lung	-	X	-	-	-
Lymph node(s) draining administration site	-	X	-	-	Collect lymph nodes that would be expected to receive primary exposure to the test article (i.e. lymph node draining the administration site [iliac and inguinal]).
Lymph node, mandibular	-	X	-	-	-

Tissue	Weigh	Collect	Histology	Microscopic Evaluation	Comment
Lymph node, mesenteric	-	X	-	-	-
Muscle, skeletal	-	X	-	-	-
Nerve, optic	-	X	-	-	Examine only if present in the routine section of the eye. Preserve in (b) (4) fixative.
Nerve, sciatic	-	X	-	-	-
Nerve, tibial	-	X	-	-	-
Ovary	-	X	-	-	Collect with cervix and uterus.
Oviduct	-	X	-	-	-
Pancreas	-	X	-	-	-
Site(s), administration	-	X	-	-	Right and left quadriceps.
Skin	-	X	-	-	-
Small intestine, duodenum	-	X	-	-	-
Small intestine, ileum	-	X	-	-	-
Small intestine, jejunum	-	X	-	-	-
Spinal cord	-	X	-	-	Examine one transverse and one longitudinal section from each of the following areas cranial cervical, mid-thoracic, lumbar (intumescence)
Spleen	-	X	-	-	-
Stomach	-	X	-	-	-
Testis	-	X	-	-	Paired examination; Preserve in modified (b) (4) fixative.
Thymus	-	X	-	-	-
Tongue	-	X	-	-	-
Trachea	-	X	-	-	-
Ureter	-	X	-	-	-
Urinary bladder	-	X	-	-	-
Uterus	-	X	-	-	Collect with cervix and ovaries.
Vagina	-	X	-	-	-

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

^a Bolon, B., Garman, R. H., Pardo, I. D., Jensen, K., Sills, R., Roulois, A., Radovsky, A. E., Bradley, A., Andrews-Jones, L., Butt, M., Guimprecht, L. STP Position Paper: Recommended Practices for Sampling and Processing the Nervous System (Brain, Spinal Cord, Nerve and Eye) During Nonclinical General Toxicity Studies. *Toxicol Pathol.* 41, 2013. 1028-1048.

^b Young, J. Histopathologic Examination of the Rat Nasal Cavity, *Fundamental and Applied Toxicology*, 1:309-312 (July/August 1981).

^c Roger A. Renne, Katherine M. Gideon, Rodney A. Miller, Paul W. Mellick, and Sandra L. Grumbein. Histologic Methods and Interspecies Variations in the Laryngeal Histology of F344/N Rats and B6G3F1 Mice, *Toxicologic Pathology*, Vol 20, Number 1, 1992 pp 44-51.

Table 10: Tissue weighing, collection, and evaluation, sponsor provided (Study no. 20346748).

Results:

All F0 generation rats assigned to cohorts 1 and 2 survived to scheduled euthanasia.

No mRNA-1283 related effects on mean body weight or mean body weight gain during the pre-mating, gestation, and lactation periods were reported.

Maternal clinical observations

Premating (Cohorts 1 & 2)

Clinical observations (injection site reactions) during the premating period (28 and 14 days prior to cohabitation) were reported.

Limited usage of the hindlimb and swollen hindlimbs were reported in group 2. Swollen hindlimbs (left or right) were reported in all 44 rats across cohorts 1 and 2 and the timing correlated with the schedule of premating dose administration. Swollen hindlimbs were first reported as early as study day (SD) 2 and continued intermittently until SD 48 but occurred primarily after dosing occasions and generally resolved within 2 to 6 days post each dose. Limited usage of the left or right hindlimb was reported in 44 rats across cohort 1 and 2 on one or more occasion between SD 2 and SD 18, following the same pattern of the dose administration schedule. Limited usage of the left hindlimb was most frequently reported between SD 16 and SD 18 and had resolved by SD 19 in majority of the females. Additionally, clinical signs of skin scabs and skin flaking (hindlimbs) at the injection site were reported at a higher incidence in group 2 (6 and 10 females, respectively), compared to group 1 (0 and 0, respectively).

Gestation (Cohorts 1 & 2)

Limited usage of the hindlimb and swollen hindlimbs were reported in group 2. Swollen hindlimbs (left and/or right) were reported in 40 rats across cohorts 1 and 2 and the timing correlated with the schedule of premating dose administration. Swollen hindlimbs was reported on GD 1 and continued intermittently during the dosing period. Limited usage of the right hindlimb was reported in 7 rats across cohorts 1 and 2 on one or more occasion starting on GD 2, following the same pattern of the dose administration schedule. Additionally, clinical signs of skin scabs and skin flaking (hindlimbs) at the injection site increased in group 2 (10 and 31 females, respectively), compared to group 1 (2 and 0, females respectively).

Lactation (Cohort 2)

Skin flaking (hindlimbs) was reported in group 2. This is considered secondary to injection of the test article and was reported in 9 rats in cohort 2 intermittently between LD 1 to 21.

Body weights and body weight gains, gravid uterine weight, adjusted body weight and adjusted body weight gains.

No mRNA-1283 related effects on mean body weight or mean body weight gain during the premating, gestation, and lactation periods were reported.

No mRNA-1283 related effects on mean gravid uterine weight, mean adjusted body weight or mean adjusted body weight gain relative to controls (-9%, -1%, and -4%, respectively, relative to controls) were reported.

No mRNA-1283 related effects on mean food consumption during the premating, gestation, and lactation periods were reported.

Estrous cycle evaluation, mating, and fertility

No test article-related effects on estrous cycling (number of estrous cycles and cycle length) and mating performance (days to mating and mating and fertility indices) were reported.

The mean number of estrous cycles pre-cohabitation (1.9) in group 2 was similar to the group 1 mean number of estrous cycles pre-cohabitation (1.9). Similarly, the mating index (95.5%), and fertility index (92.9%) were similar to group 1 values. The pregnancy index (88.6%) was decreased in group 2 compared to the group 1 (97.7%). The mean cycle length pre-cohabitation (4.44 days) was statistically significantly increased ($p \leq 0.05$) in group 2 compared with group 1 mean cycle length (4.15 days). The reduction in the number pregnant is within the historical control range.

Sex: Female		0 ug/dose	80 ug/dose
Day(s) Relative to Pairing (Litter: A)		Group 1	Group 2
Group Size - Females		44	44
Number of Cycles -13/0 [k]	Mean	1.9	1.9
	SD	0.6	0.7
	N	44	44
	%Diff	-	1.2
Mean Cycle Lengths (Days) -13/0 [k]	Mean	4.15	4.44*
	SD	0.40	0.64
	N	42	42
	%Diff	-	7.07

Table 11: Summary of estrous cycles; F0 generation-pre-cohabitation, sponsor provided (Study no. 20346748).

Sex: Female		0 ug/dose	80 ug/dose
Day(s) Relative to Pairing (Litter: A)		Group 1	Group 2
Group Size - Females		44	44
Paired - Females	N+ve	44	44
Mated Females	N+ve	43	41
Pregnant	N+ve	43	39
Pre-coital Interval (Days) [k]	Mean	2.2	2.7
	SD	1.2	1.3
	N	43	41
	%Diff	-	22.9
Pregnant No Confirmed Mating [f]	N+ve	0	1
Confirmed Mating Days 1-7 [f]	N+ve	43	41
	%	100.0	100.0
Female Mating Index [f]	%	97.7	95.5
	ProA	43/44	42/44
Female Fertility Index [f]	%	100.0	92.9
	ProA	43/43	39/42
Female Pregnancy Index [f]	%	97.7	88.6
	ProA	43/44	39/44

Table 12: Summary of reproductive performance; F0 generation, sponsor provided (Study no. 20346748).

Sex: Female		0 ug/dose	80 ug/dose
Day(s) Relative to Mating (Litter: A)		Group 1	Group 2
Group Size - Females		44	44
Number of Females Pregnant [f]	N+ve	43	39
	%	97.7	88.6
Female with Live Fetuses [f]	N+ve	22	17
	%	51.2	43.6
Female with Resorptions [f]	N+ve	12	10
	%	27.9	25.6
Female with all Nonviable [f]	N+ve	0	2
	%	0.0	10.5
Terminal Euthanasia [f]	N+ve	44	43
	%	100.0	97.7
Found Dead [f]	N+ve	0	0
	%	0.0	0.0
Unscheduled Euthanasia [f]	N+ve	0	1
	%	0.0	2.3
Aborted [f]	N+ve	0	0
	%	0.0	0.0

Table 13: Summary of maternal performance and mortality; F0 generation, sponsor provided (Study no. 20346748).

Macroscopic observations

No test article-related macroscopic observations were reported during necropsy examination.

Ovarian and uterine examinations, and litter observations (Cohort 1)

No test article-related effects on any ovarian, uterine, and litter parameter were reported.

Pregnancy was confirmed in 43 and 39 F0 in groups 1 and 2, respectively. Of these F0 groups 1 and 2 (22 and 17, respectively) had live fetuses and were examined for ovarian and uterine contents on GD 21.

There were increased mean pre-and post-implantation loss (13.51% and 6.44%, respectively) in group 2 compared to group 1 (10.95% and 4.34%, respectively). Implantation loss was reflected in total mean number of early resorptions (1.0) in group 2, compared to group 1 (0.6). The mean total number of fetuses was 13.1 in group 2, compared to 14.8 in group 1 (89% of controls).

Reproductive indices summary data presented in the following table:

Reproductive Index	0 µg/dose (Control)	80 µg/dose	Historical Control Data Range
Pre-Implantation Loss	10.95	13.51	1.6 - 15.1
Post-Implantation Loss	4.34	6.44	0.8 - 11.0
Mean – Number of Early Resorptions	0.6	1.0	0.1 - 1.1
Mean – Total Number of Live Fetuses	14.8	13.1	10.9 - 15.2

Table 14: Summary of reproductive indices, sponsor provided (Study no. 20346748).

Enlarged placenta (female 1609) and fused placentas (females 1614 and 1619) were reported in group 1.

The mean fetal body weights (males) were statistically significant ($p \leq 0.05$) in group 2 compared with group 1. The litter means for number of implantations, corpora lutea, live and dead fetuses, late resorptions, fetal sex ratio, fetal body weights (males and females), and placental weights were similar between groups 1 and 2. All numbers were within the historical control data.

Sex: Female		0 ug/dose	80 ug/dose
Day(s) Relative to Mating (Litter: A)		Group 1	Group 2
Female with Live Fetuses	N+ve	22	17
	%	100.0	100.0
Number of Corpora Lutea [k]	Mean	17.7	16.3
	SD	3.7	2.6
	N	21	17
	%Diff	-	-7.8
Number of Implantations [k]	Mean	15.5	14.1
	SD	2.2	3.7
	N	22	17
	%Diff	-	-8.7
Pre-implantation Loss (%) [k]	Mean	10.95	13.51
	SD	11.95	20.45
	N	21	17
	%Diff	-	23.36
Total Number of Resorptions [k]	Mean	0.7	1.0
	SD	0.7	1.9
	N	22	17
	%Diff	-	46.7
Number of Early Resorptions [k]	Mean	0.6	1.0
	SD	0.7	1.9
	N	22	17
	%Diff	-	69.2
Number of Late Resorptions [k]	Mean	0.1	0.0
	SD	0.3	0.0
	N	22	17
	%Diff	-	-100.0
Total Number of Fetuses [k]	Mean	14.8	13.1
	SD	2.0	3.8
	N	22	17
	%Diff	-	-11.2
Number of Live Fetuses [k]	Mean	14.8	13.1
	SD	2.0	3.8
	N	22	17
	%Diff	-	-11.2
Number of Live Male Fetuses [k]	Mean	7.7	6.4
	SD	2.8	2.6
	N	22	17
	%Diff	-	-16.5

Sex: Female		0 ug/dose	80 ug/dose
Day(s) Relative to Mating (Litter: A)		Group 1	Group 2
Number of Live Female Fetuses [k]	Mean	7.1	6.7
	SD	2.1	2.6
	N	22	17
	%Diff	-	-5.4
Number of Dead Fetuses [k]	Mean	0.0	0.0
	SD	0.0	0.0
	N	22	17
	%Diff	-	-
Post-implantation Loss (%) [k]	Mean	4.34	6.44
	SD	4.45	12.86
	N	22	17
	%Diff	-	48.46
Live Male Fetus/Litter (%) [k]	Mean	51.41	48.54
	SD	15.78	13.66
	N	22	17
	%Diff	-	-5.58
Mean Fetal Weight all (g) [G]	Mean	5.622	5.933
	SD	0.469	0.528
	N	22	17
	%Diff	-	5.529
Mean Fetal Weight males (g) [G]	Mean	5.735	6.065 *
	SD	0.487	0.499
	N	22	17
	%Diff	-	5.761
Mean Fetal Weight females (g) [G]	Mean	5.482	5.801
	SD	0.490	0.580
	N	22	17
	%Diff	-	5.823
Live Mean Placental Weight (g) [G]	Mean	0.519	0.562
	SD	0.062	0.103
	N	22	17
	%Diff	-	8.386

Table 15: Summary of ovarian and uterine examinations and litter observations; F0 generation, sponsor provided (Study no. 20346748).

Fetal examinations (Cohort 1)

Fetal observations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development).

No test article-related effects on fetal external, visceral, or skeletal parameters were reported.

Exam Type: Skeletal	0 ug/dose Group 1	80 ug/dose Group 2
Number of Fetuses Examined:	166	115
Number of Fetuses Evaluated:	325	223
Number of Litters Examined:	22	17
Number of Litters Evaluated:	22	17
Variation	20	20
Number of Fetuses	12.16	16.83
Litter % of Fetuses [k]	11	12
Number of Litters		
All classifications	20	20
Number of Fetuses	12.16	16.83
Litter % of Fetuses [k]	11	12
Number of Litters		

Table 16: Summary of fetal abnormalities by classification, sponsor provided (Study no. 20346748).

Exam Type: Skeletal		0 ug/dose Group 1	80 ug/dose Group 2
Number of Fetuses Examined:		166	115
Number of Fetuses Evaluated:		325	223
Number of Litters Examined:		22	17
Number of Litters Evaluated:		22	17
Forelimb			
Metacarpal, 1 or more, Unossified - Variation	Fetuses N(%)	2(1.07)	0(0.00)
	Litters N(%)	2(9.1)	0(0.0)
Skull			
Frontal, Both, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.84)
	Litters N(%)	0(0.0)	1(5.9)
Hyoid body, Unossified - Variation	Fetuses N(%)	1(0.65)	4(3.15)
	Litters N(%)	1(4.5)	3(17.6)
Parietal, Both, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.84)
	Litters N(%)	0(0.0)	1(5.9)
Squamosal, Both, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.84)
	Litters N(%)	0(0.0)	1(5.9)
Zygomatic arch, Both, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	4(3.36)*
	Litters N(%)	0(0.0)	3(17.6)
Sternebra			
Sternebra, 1 or more, Fused - Variation	Fetuses N(%)	1(0.51)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)
Sternebra, 1 or more, Misshapen - Variation	Fetuses N(%)	1(0.65)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)
Sternebra, 1 or more, Unossified - Variation	Fetuses N(%)	1(0.51)	2(1.49)
	Litters N(%)	1(4.5)	2(11.8)
Sternebra, 1 or more, Incomplete ossification - Variation	Fetuses N(%)	4(2.27)	0(0.00)
Sternebra (Continued...)			
Sternebra, 1 or more, Incomplete ossification - Variation	Litters N(%)	2(9.1)	0(0.0)

Exam Type: Skeletal		0 ug/dose Group 1	80 ug/dose Group 2
Number of Fetuses Examined:		166	115
Number of Fetuses Evaluated:		325	223
Number of Litters Examined:		22	17
Number of Litters Evaluated:		22	17
Supernumerary rib			
Cervical, 1 or more, Short - Variation	Fetuses N(%)	2(1.14)	3(2.33)
	Litters N(%)	1(4.5)	3(17.6)
Thoracolumbar, 1 or more, Short - Variation	Fetuses N(%)	10(6.44)	6(5.88)
	Litters N(%)	7(31.8)	4(23.5)
Vertebra			
Cervical arch, 1 or more, Misshapen - Variation	Fetuses N(%)	1(0.57)	2(1.31)
	Litters N(%)	1(4.5)	2(11.8)
Cervical arch, 1 or more, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.84)
	Litters N(%)	0(0.0)	1(5.9)
Thoracic centrum, 1 or more, Incomplete ossification - Variation	Fetuses N(%)	1(0.57)	2(1.63)
	Litters N(%)	1(4.5)	2(11.8)

[Fetuses %] - Dunn: * = $p \leq 0.05$

Fetuses (%) N=Group Fetal Incidence; (N)=Mean Litter % of Fetuses with the Abnormality

Table 17: Summary of fetal abnormalities by classification, sponsor provided (Study no. 20346748).

Unossified Hyoid body in fetuses' skull were higher in group 2 (3.15%) when compared to group 1 (0.65%). Unossified Hyoid body in litters skull were higher in group 2 (17.6%) when compared to group 1 (4.5%).

Incomplete ossification in Zygomatic arch in fetuses' skull were significantly higher in group 2 (3.36%) when compared to group 1 (0.00%). Incomplete ossification in Zygomatic arch in litters skull were significantly higher in group 2 (17.6%) when compared to group 1 (0.00%). These variations were within the range of the historical control data.

SKULL	ABNORMALITIES	N	RANGE/STUDY	
			N	%
Zygomatic Arch	Frontals			
	: Incomplete ossification, both	L 82	0-7	(0-36.8)
		F 121	0-11	(0-10.0)
	: Incomplete ossification, left	L 27	0-4	(0-16.0)
		F 29	0-5	(0-3.3)
	: Incomplete ossification, right	L 33	0-3	(0-15.0)
		F 34	0-4	(0-3.1)
	: Misshapen, both	L 2	0-1	(0-5.3)
		F 2	0-1	(0-0.7)

Table 18: Historical control data, sponsor provided (Study no. 20346748).

External examinations

No test article-related external malformations or variations were reported. All fetal external abnormalities (malformations) occurred in group 1, domed head reported in 2 fetuses (1602-5, -17) and umbilical hernia reported in 1 fetus (1615-15), respectively.

Visceral examinations

No test article-related visceral malformations or variations were reported. All fetal visceral abnormalities, absent right kidney and ureter (malformations) were reported in a single group 1 fetus (1607-2).

Skeletal examinations

No test article-related skeletal malformations or variations were reported. All fetal skeletal abnormalities were variations that were 1) considered reversible delays in growth or development; 2) similarly reported in group 1; and/or 3) within the historical control data range.

Skeletal variations in group 2 consisted of hyoid body and sternebra (unossified), frontal, parietal, squamosal, zygomatic arch, cervical arch and thoracic centrum (incomplete ossification), cervical arch (misshapen) and supernumerary short ribs (cervical and thoracolumbar). Of these observations, although zygomatic arch, incomplete ossification was considered statistically significant ($p \leq 0.05$), it was limited to 4 fetuses. These findings were not considered test article-related because they were either 1) present in group 1; 2) occurred at higher incidence in group 1; and/or 3) within the historical control data range.

Natural delivery and litter observations (Cohort 2)

Natural delivery

No test article-related effects on any natural delivery parameters were reported. Pregnancy was confirmed in 21 and 22 F0 females in groups 1 and 2, respectively. Of these pregnant females, 21 and 20 rats delivered a litter in groups 1 and 2, respectively.

All natural delivery parameters were similar between groups 1 and 2 or were within the historical control range, including gestation length (mean 21.5 and 21.6 days in groups 1 and 2, respectively), gestation index (number of rats with live offspring/number of pregnant rats was 21/21 and 20/22 in groups 1 and 2, respectively), mean implantation sites per delivered litter (15.6 in groups 1 and 2), percentage of dams with no liveborn pups (0% in groups 1 and 2), mean percentage of stillborn pups/litter (0.3% in group 1), live birth index (99.7% and 100%, in groups 1 and 2, respectively), and percentage of post-implantation loss per litter (5.21% and 6.12%, in groups 1 and 2, respectively). All maternal grooming and nesting/nursing activities were normal.

Litter observations

No test article-related effects on any litter parameter including the viability index (percentage of pups born that survived 4 days postpartum), lactation index (percentage of pups born that survived 21 days postpartum), and sex ratio on day 21 postpartum were reported.

Sex: Female		0 ug/dose	80 ug/dose
Day(s) Relative to Littering (Litter: A)		Group 1	Group 2
Group Size - Females		22	22
Number of Females Pregnant [f]	N+ve	21	22
	%	95.5	100.0
Gestation Index [f]	%	100.0	90.9
	ProA	21/21	20/22
Females Completing Delivery [f]	N+ve	21	20
Females with Liveborn [f]	N+ve	21	20
Female with no Liveborn Pups [f]	N+ve	0	0
Fem w/ Stillborn Pups [f]	N+ve	1	0
Stillborn Pups/Litter (%) [k]	Mean	0.30	0.00
	SD	1.36	0.00
	N	21	20
	%Diff	-	-100.00
Number Pups Stillborn	Sum	1	0
Number Live Newborn Pups [k]	Mean	14.7	14.7
	SD	1.9	2.0
	N	21	20
	%Diff	-	-0.4
	Sum	309	293
Live Birth Index (%) [k]	Mean	99.70	100.00
	SD	1.36	0.00
	N	21	20
	%Diff	-	0.30
Post-implant Loss/Litter (%) [k]	Mean	5.21	6.12
	SD	7.04	6.68
	N	20	19
	%Diff	-	17.56
Live Male Pups/Litter (%) Birth [G]	Mean	49.61	45.13
	SD	16.03	16.58
	N	21	20
	%Diff	-	-9.02
Implantation Sites - Total	Mean	15.6	15.6
	SD	2.0	2.3
	N	20	19
	%Diff	-	0.2
Gestation Length (Days)	Mean	21.5	21.6
	SD	0.5	0.5
	N	21	20
	%Diff	-	0.4

Table 19: Natural delivery observations; F0 generation, sponsor provided (Study no. 20346748).

Summary of litter observations: F0 generation

Sex: Female		0 ug/dose	80 ug/dose
Day(s) Relative to Littering (Litter: A)		Group 1	Group 2
Group Size - Females		22	22
Females with Liveborn	N+ve	21	20
Viability Index Birth-4 (%) [k]	Mean	98.38	93.12
	SD	2.99	15.90
	N	21	20
	%Diff	-	-5.35
Lactation Index (%) [k]	Mean	79.52	78.50
	SD	2.18	3.28
	N	21	20
	%Diff	-	-1.29
Live Male Pups/Litter (%) 21 [G]	Mean	50.34	45.42
	SD	14.34	13.97
	N	21	20
	%Diff	-	-9.78

Table 20: Litter observations; F0 generation, sponsor provided (Study no. 20346748).

F1 generation pups (Cohort 2)

Clinical observations

No test article-related effects on clinical observations in the F1 pups were reported. Clinical signs that were reported in the F1 pups were unrelated to mRNA-1283 because 1) the findings were limited to group 1; 2) the number of pups/litters affected in group 2 was similar to group 1; or 3) the observation was limited to a single pup/litter.

Observation Type: Pup Observations From Day 0 to 21 (Litter Date)		0 µg/dose Group 1	80 µg/dose Group 2
LITTERS EXAMINED	N	21	20
Dehydrated Suspected			
Number of Times Recorded	N	1	2
Number of Pups Affected	N	1	1
Number of Litters Affected	N	1	1
Fur, Thin Cover			
Number of Times Recorded	N	154	0
Number of Pups Affected	N	28	0
Number of Litters Affected	N	3	0
Skin, Pallor			
Number of Times Recorded	N	1	2
Number of Pups Affected	N	1	1
Number of Litters Affected	N	1	1
Skin, Discolored			
Number of Times Recorded	N	0	1
Number of Pups Affected	N	0	1
Number of Litters Affected	N	0	1
Thin			
Number of Times Recorded	N	0	2
Number of Pups Affected	N	0	1

Observation Type: Pup Observations From Day 0 to 21 (Litter Date)		0 µg/dose Group 1	80 µg/dose Group 2
Number of Litters Affected	N	0	1
Cold to Touch			
Number of Times Recorded	N	1	0
Number of Pups Affected	N	1	0
Number of Litters Affected	N	1	0
Tip of tail black			
Number of Times Recorded	N	0	7
Number of Pups Affected	N	0	1
Number of Litters Affected	N	0	1

Table 21: Pup clinical observations: F1 generation, sponsor provided (Study no. 20346748).

Pup body weights

No test article-related effects on mean body weights in the preweaning F1 generation male and female pups were reported. Mean combined pup body weights were 97% of controls on day 21 postpartum and within the historical control range.

Reflex and physical development

No test article-related effects on pinna detachment, surface righting, hair growth, eye opening, auditory startle, or pupil constriction in F1 generation preweaning male and female pups were reported.

Sex: Female		0 ug/dose Group 1	80 ug/dose Group 2
Day(s) Relative to Littering (Litter: A)			
LMean Surface Righting [k]	Mean	1.8	2.2
	SD	0.5	0.8
	N	21	20
	%Diff	-	23.3
LMean Pinna Detached [k]	Mean	2.7	2.8
	SD	0.7	0.6
	N	21	20
	%Diff	-	2.7
LMean Hair Growth [k]	Mean	7.0	7.0
	SD	0.0	0.0
	N	21	20
	%Diff	-	0.0
LMean Eyes Opened [k]	Mean	14.0	13.7
	SD	0.8	0.8
	N	21	20
	%Diff	-	-1.7
LMean Auditory Startle [k]	Mean	13.0	13.0
	SD	0.0	0.0
	N	21	20
	%Diff	-	0.0

Table 22: Litter mean day pup reflex and development; F1 generation, sponsor provided (Study no. 20346748).

Pup necropsy

No test article-related macroscopic observations detected in preweaning F1 generation male and female pups during necropsy examination.

Prior to scheduled euthanasia on day 21 postpartum, there were necropsy examinations performed on 5 and 9 F1 generation pup in groups 1 and 2, respectively, that did not survive to scheduled euthanasia. Of these pups, 6 out of 9 pups in group 2 did not have milk present in the stomach at necropsy, which suggests that the pups did not nurse.

Litter: A

Exam Type: Pup Necropsy		0 ug/dose Group 1	80 ug/dose Group 2
Number of Pups Examined:		94	80
Number of Litters Examined:		20	18
Stomach Stomach content, Absent	Pups N(%)	1(1.1)	0(0.0)
	Litters N(%)	1(5.0)	0(0.0)
Stomach Stomach content, Absent	Pups N(%)	1(20.0)	6(66.7)
	Litters N(%)	1(20.0)	4(66.7)

Table 23: Pup gross pathology; F1 generation, sponsor provided (Study no. 20346748).

No test article-related macroscopic observations in surviving F1 generation male and female pups at scheduled euthanasia on day 21 postpartum were reported.

Antibody evaluation (Cohort 1 & 2)

A robust IgG SARS-CoV-2 NTD and RBD protein antibody response was reported in group's 2, F0 females, on SD 15 continuing through LD 21, in F1 fetuses on GD 21, and in F1 pups on LDs 13 and 21, and in F0 maternal milk on LDs 13 and 21.

Maternal serum antibody evaluation

The SARS-CoV-2 NTD and RBD protein IgG antibody mean results calculated from the maternal serum concentration data in rats on SD 15, GDs 1, 13, and 21, and LDs 13 and 21 are presented in the following table.

Day of Collection	Cohort	SARS-CoV-2 NTD protein IgG antibodies	SARS-CoV-2 RBD protein IgG antibodies
SD 1 (pre-dose)	1 and 2	<6.5 AU/mL ^a	<12.5 AU/mL ^b
SD 15	1 and 2	1366 AU/mL	7554 AU/mL
GD 1	1 and 2	21950 AU/mL	68691 AU/mL
GD 13	1 and 2	54098 AU/mL	89586 AU/mL
GD 21	1	23431 AU/mL	36635 AU/mL
LD 13	2	28713 AU/mL	46655 AU/mL
LD 21	2	22050 AU/mL	34319 AU/mL

SD = Study Day; GD = Gestation Day; LD = Lactation Day; AU= Antibody Units/mL

a = Less than the Lower Limit of Quantitation (0.013 AU/mL) multiplied by the lowest dilution factor < 0.013 AU/mL * 500 or < 6.5 AU/mL

b = Less than the Lower Limit of Quantitation (0.025 AU/mL) multiplied by the lowest dilution factor < 0.025 AU/mL * 500 or < 12.5 AU/mL

Table 24: Mean SARS-CoV-2 maternal serum antibody titers (antibody units/mL), sponsor provided (Study no. 20346748).

Fetal and pup antibody serum evaluation

The SARS-CoV-2 NTD and RBD protein IgG antibody mean titer results calculated from the fetal serum on GD 21 and pup serum on PNDs 13 and 21 are presented in the following table:

Day of Collection	Cohort	SARS-CoV-2 NTD protein IgG antibodies	SARS-CoV-2 RBD protein IgG antibodies
GD 21 (fetal)	1	2730 AU/mL	4197 AU/mL
PND 13 (pup)	2	28215 AU/mL	41225 AU/mL
PND 21 (pup)	2	19318 AU/mL	32713 AU/mL

GD = Gestation Day; PND = Post-Natal Day; AU= Antibody Units/mL.

Table 25: Mean fetal and pup SARS-CoV-2 serum antibody titers (antibody Units/mL), sponsor provided (Study no. 20346748).

Milk sample evaluation

The SARS-CoV-2 NTD and RBD protein IgG antibody mean titer results calculated from the mean milk concentration data in rats on LDs 13 and 21 are presented in the following table:

Day of Collection	Cohort	SARS-CoV-2 NTD protein IgG antibodies	SARS-CoV-2 RBD protein IgG antibodies
LD 13	2	2995 AU/mL	4441 AU/mL
LD 21	2	2830 AU/mL	4347 AU/mL

LD = Lactation Day; AU= Antibody Units/ mL.

Table 26: Mean maternal SARS-CoV-2 milk antibody titers (antibody Units/mL), sponsor provided (Study no. 20346748).

Conclusion

Injection site reactions were limited to limited usage of and/or swollen hindlimbs and skin scabbing/flaking, which occurred following dosing occasions and generally resolved within 2 to 6 days post each dose. No test article-related effects on reproduction or pre- and postnatal developmental endpoints in F0 or F1 generation were reported. A robust IgG antibody response was detected in F0 females on SD 15 continuing through LD 21, and there were also antibodies detected in F1 fetuses on GD 21, in F1 pups on PNDs 13 and 21, and in the milk samples of F0 females on LDs 13 and 21.

GLP study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

Investigators Brochure: Having read and evaluated the Investigators Brochure, is it a fair, objective and reasonable summary of the toxicology data – yes (X) or no ().

Study number 2: A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1283, mRNA-1284, and mRNA-1285 by Intramuscular Injection in (b) (4) Rats.
Study number: 2308-161.

Performing laboratory: (b) (4)

Study initiation date: July 20th, 2020

Final report date: December 11th, 2020

Test article batch/lot:

Table of test article identification

Identification	mRNA-1283	mRNA-1284	mRNA-1285
Lot No.	8520700101	(b) (4)	(b) (4)
Expiration/Retest Date	13 Oct 2020	(b) (4)	(b) (4)
Purity	(b) (4)	(b) (4)	(b) (4)
Storage Conditions	Frozen at (b) (4) °C, protected from light, and (b) (4)	(b) (4)	(b) (4)
Provided by	Moderna Therapeutics Inc.	Moderna Therapeutics Inc.	Moderna Therapeutics Inc.

Table of control article identification

Identification	(b) (4) mM Tris, (b) (4) Sucrose
Alternate Identification	Tris/Sucrose Buffer
Batch (Lot) No.	(b) (4)
Expiration/Retest Date	Not Available
Storage Conditions	Refrigerated at (b) (4) protected from light
Provided by	Moderna Therapeutics Inc.

Animal species and strain: (b) (4)

Breeder/supplier: (b) (4)

Number of animal per group and sex: 10/sex/dose

Age: 8 weeks

Body weight range: Between 144 and 273 g and between 149 and 230 g

Route and site of administration: Intramuscular injection

Volume of injection: 200 µL/dose

Frequency of administration and study duration: Days 1 and 22. Study duration was 40 days.

Dose: See study design below

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND. No stability results were submitted.

Means of administration: Intramuscular injection

Report status: Final

Experimental design:

Animals were randomized and assigned to 13 different groups. Each group consisted of 10/sex/group. Animals were dosed by IM injection on study days 1 and 22. The details of the study design are listed in the following table:

Group No.	Test Material	Dose Level (µg/dose)	Dose Volume (mL/dose)	Dose Concentration (µg/mL)	Main Study	
					No. of Males	No. of Females
1	Control Article	0	0.2	0	5	5
2	mRNA-1283	30		150	5	5
3		60		300	5	5
4		100		500	5	5
(b) (4)						

No. = Number

Table 27: Experimental design, sponsor provided (Study no. 2308-161).

Methods:

Randomization procedure: Yes

Statistical analysis plan: Yes.

The following parameters were evaluated:

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	Daily throughout the study. ^c	Animals were removed from the cage. On occasion, clinical observations were recorded at unscheduled intervals. 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. The examinations performed prior to day 1 are not reported but are maintained in the study file.

Parameter	Population(s)	Frequency (minimum required)	Comments
Injection Site Observations	All Animals	Immediately postdose, 6 hours and 24 hours post dose	The injection site was evaluated for the presence/absence or 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 and for the entire dosing period.
Clinical Pathology	All Animals	Day 23 (24 hours post the 2 nd dose) and day 36	
Serum ELISA Assay ^d	All Animals	Pre-dose on day 1 and once on day 35.	

^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.

^d Blood collected via the sublingual vein for ELISA assay

Table 28: General in-life assessments, sponsor provided (Study no. 2308-161).

Terminal Procedures

Group No.	Scheduled Euthanasia Day	Necropsy Procedures			Histology Processing	Microscopic Evaluation
		Necropsy	Tissue Collection	Organ Weights		
Group 1, 5-10 Females	36	X	Select Tissues ^a	NA	Select Tissues ^a	Select Tissues ^a
Group 1 Males, Group 2-4 Females	37	X	Select Tissues ^a	NA	Select Tissues ^a	Select Tissues ^a
Group 8-10 Males	38	X	Select Tissues ^a	NA	Select Tissues ^a	Select Tissues ^a
Group 5-7 Males	39	X	Select Tissues ^a	NA	Select Tissues ^a	Select Tissues ^a
Group 2-4 Males	40	X	Select Tissues ^a	NA	Select Tissues ^a	Select Tissues ^a
Group 11-13	36	X	Select Tissues ^a	NA	Select Tissues ^a	Select Tissues ^a

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

“Histology processing”= embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.

^a Liver and spleen tissues were collected and evaluated.

Table 29: Terminal procedure, sponsor provided (Study no. 2308-161).

Group Nos.	Time Point(s)	Hematology	Clinical Chemistry
All Animals	Day 23 (24 hours post the last dose) day 36	X	X
Unscheduled Euthanasia (when possible)	See the unscheduled euthanasia section of this protocol.		
Volume (mL)^a:	NA	0.5 mL	0.8 mL
Fasting Required:	Free access to drinking water but will be fasted overnight (at least 8 hours) prior to blood collection.		
Anticoagulant:	NA	K ₂ EDTA	Serum gel separator
Special Requirements:	NA	NA	NA
Processing:	NA	None	Serum

X = Sample to be collected; NA = Not applicable

^a Additional blood samples may be obtained (e.g. due to sample quality) if permissible sampling frequency and blood volume are not exceeded. Blood sample collection method: Sublingual, or another suitable vein

Table 30: Clinical pathology sample collection, sponsor provided (Study no. 2308-161).

Postmortem procedures:

Organ	Macroscopic Evaluation and Collection
Animal ID	X
Artery, aorta	X
Body cavity, nasal	X
Bone marrow, sternum	X
Bone marrow smear	X ^a
Bone, femur	X (1)
Bone, sternum	X
Brain	X
Epididymis	X (2) ^b
Esophagus	X
Eye	X (2) ^b
Ganglion, dorsal root, lumbar	X
Gland, adrenal	X (2)
Gland, clitoral	X (2)
Gland, lacrimal	X (2) (extra-orbital)
Gland, Harderian	X (2)
Gland, mammary	X
Gland, parathyroid	X (2)
Gland, pituitary	X
Gland, preputial	X (2)
Gland, prostate	X
Gland, salivary, submandibular	X (2)
Gland, salivary, sublingual	X (2)
Gland salivary, parotid	X (2)
Gland, seminal vesicle	X (2)
Gland, thyroid	X (2)
Gland, Zymbal's	X (2)
Gut-associated lymphoid tissue ^c	X
Heart	X
Joint, femorotibial	X (1)
Kidney	X (2)

Organ	Macroscopic Evaluation and Collection
Large intestine, cecum	X
Large intestine, colon	X
Large intestine, rectum	X
Larynx	X
Liver	X
Lung	X
Lymph node(s) draining administration site(s): Inguinal	X
Lymph node, mandibular	X (2)
Lymph node, mesenteric	X
Muscle, skeletal	X (2)
Nerve, optic	X (2) ^b
Nerve, sciatic	X (2)
Nerve, tibial	X (2)
Ovary	X (2)
Oviduct	X (2)
Pancreas	X
Site(s), administration	X
Skin	X
Small intestine, duodenum	X
Small intestine, ileum	X
Small intestine, jejunum	X
Spinal cord	X
Spleen	X
Stomach	X
Testis	X (2) ^b
Thymus	X
Tongue	X
Trachea	X
Ureter	X (2)
Urinary bladder	X
Uterus/Cervix	X
Vagina	X
X = Procedure to be conducted. - = Not applicable. (1) = one side. (2) = both sides. Macroscopic abnormalities in the organs listed and in other organs will be sampled at necropsy, processed for histology and examined microscopically. ^a Two bone marrow smears will be collected from the femur at scheduled and unscheduled necropsies (for possible examination). Smears will not be collected from animals that are found dead or from animals that were euthanized moribund and then stored in the refrigerator prior to necropsy. Bone marrow smears are allowed to air dry and are not fixed in formalin. ^b Eyes and optic nerves are preserved in (b) (4) fixative. Testes and epididymides are preserved in modified (b) (4) fixative.	

Table 31: Tissue collection and preservation, sponsor provided (Study no. 2308-161).

Results:

No test article-related mortality was reported.

Clinical chemistry, hematology, and coagulation:Clinical chemistry

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤ 1.5)	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, potassium, sodium, phosphorus
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Alanine aminotransferase (ALT or SGPT)* Aspartate aminotransferase (AST or SGOT)*	
B) HEPATOBILIARY	Alkaline phosphatase (ALP) SD36 M $\uparrow$ = 1.7 G12 Total bilirubin*	
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation)
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen (BUN)
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Fasting triglycerides* Albumin (A)* Globulin* A/G ratio* Total protein*	Carbon dioxide Lactate dehydrogenase (LDH) Total Cholesterol GGT Creatine kinase (CK)

* See results and tables below

Table 32: Clinical chemistry results. (Study no. 2308-161).

Clinical chemistry results showed an (b) (4).

In both sexes, mRNA-1283-related changes consistent with inflammation were seen at ≥ 30 $\mu\text{g}/\text{dose}$. These changes included mild to moderate decreases in mean total protein (range: 0.92x to 0.94x of control mean), albumin (range: 0.81x to 0.88x of control mean) and/or albumin/globulin ratio (range: 0.77x to 0.82x of control mean). In recovery (day 36) groups, there was evidence of resolution of these effects in all affected groups.

At ≥ 60 $\mu\text{g}/\text{dose}$, mRNA-1283-related changes consisted of mild increases in triglyceride (range: 1.41x to 1.76x of control mean) levels. This finding might be related to mild alterations in metabolic status.

mRNA-1284, mRNA-1285 or mRNA-1284/mRNA-1285

(b) (4)

Group	1*		2		3		4	
Dose Level (µg/dose)	0		30 µg/dose mRNA-1283		60 µg/dose mRNA-1283		100 µg/dose mRNA-1283	
Sex	M	F	M	F	M	F	M	F
AST (U/L)								
Day 23 (24 hr PD)	139.4	161.4	—	—	—	—	—	—
Day 36	115.8	128.8	—	—	—	—	—	—
ALT (U/L)								
Day 23 (24 hr PD)	32.6	32.0	—	—	—	—	—	—
Day 36	39.0	32.0	—	—	—	—	—	—
Total Bilirubin (mg/dL)								
Day 23 (24 hr PD)	0.14	0.15	—	—	—	—	—	—
Day 36	0.12	0.11	—	—	—	—	—	—
Total Protein (g/dL)								
Day 23 (24 hr PD)	6.86	7.12	—	—	0.94x	—	0.92x	—
Day 36	6.94	7.06	—	—	—	—	—	—
Albumin (g/dL)								
Day 23 (24 hr PD)	3.62	4.00	0.88x	0.88x	0.84x	0.85x	0.81x	0.88x
Day 36	3.64	4.06	—	—	—	—	0.93x	—
Albumin/Globulin Ratio								
Day 23 (24 hr PD)	1.12	1.30	0.82x	0.80x	0.82x	0.77x	0.77x	0.77x
Day 36	1.10	1.38	—	0.81x	—	0.81x	0.95x	0.84x
Triglyceride (mg/dL)								
Day 23 (24 hr PD)	61.8	43.0	—	—	—	1.41x	1.76x	—
Day 36	64.0	36.4	—	—	—	—	—	—
M = Males F = Females. 24 hr PD = 24 hours postdose. AST = Aspartate aminotransferase; ALT = Alanine aminotransferase. A dash (—) indicates absence of a test article-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.								

Table 33: mRNA-1283-related clinical chemistry changes in males and females, sponsor provided (Study no. 2308-161).

(b) (4)

2 pages have been determined to be not releasable: (b)(4)

(b) (4)

(b) (4)

Hematology

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.54, ie, ≥ 1.6 or ≤ 1.6	Not of NOTE
Red blood cells	Reticulocytes* Mean Corp. Hb. Conc. (MCHC)* Mean Corp. Volume (MCV)* RDW%*	Hematocrit (Hct) Hemoglobin Conc. (Hb) Total Erythrocyte Count (RBC) Mean Corp. Hb. (MCH)
White blood cells	White Blood Cells (WBC)	Macrophage Leukocytes

⁴ With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

	SD23 F ↓ = 0.6 G4 (b) (4) Monocyte count SD23 F ↑ = 2.3 G2 (b) (4) Basophils (b) (4) SD23 F ↑ = 1.7 G2 SD23 F ↓ = 0.5 G4 SD23 F ↑ = 1.6 G5 (b) (4) SD36 F ↑ = 1.6 G2 SD36 F ↑ = 2.1 G4 (b) (4) Large Unstained Cells (LUC) SD23 M ↑ = 1.7 G3 (b) (4) SD23 F ↑ = 1.7 G2 (b) (4)	
--	---	--

	Neutrophil count* Lymphocyte count* Eosinophils count*	
Clotting potential	Platelet count*	Activated partial-thromboplastin time clotting time Prothrombin time Fibrinogen
Others		Bone marrow cytology

* See results and tables below

Table 37: Hematology results. (Study no. 2308-161).

Hematology results showed a decrease in WBC levels in groups 4, (b) (4) females at study day 23. WBC levels were increased in groups (b) (4) females at study day 23. Monocyte levels were increased in groups 2, (b) (4) females at study day 23. (b) (4)

Basophil levels were increased in groups 2, (b) (4) females at study day 23. Basophil levels were decreased in groups 4, (b) (4) females at study day 23. Basophil levels were increased in groups 2, 4, (b) (4) females at study day 36. LUC levels were increased in groups 3 and (b) (4) males at study day 23. LUC levels were increased in groups 2, (b) (4) females at study day 23. (b) (4)

mRNA-1283

At ≥ 30 $\mu\text{g}/\text{dose}$ and in both sexes, mRNA-1283-related changes were consistent with inflammation and included increases in neutrophil counts (range: 4.7x to 10.0x of control mean), and/or eosinophil (range: 2.0x to 3.4x of control mean) counts.

At ≥ 30 $\mu\text{g}/\text{dose}$ and in both sexes, mRNA-1283-related changes consisted of decreases in mean reticulocyte (0.81x of control mean), and/or platelet (range: 0.68x to 0.78x of control mean) counts. These findings might be associated with mild effects on mean MCV (1.04x of control mean), MCHC (range: 0.96x to 0.97x of control mean) and/or RDW (range: 1.05x to 1.13x of control mean). In recovery (day 36) groups there was evidence of resolution of these effects in all groups.

At ≥ 30 $\mu\text{g}/\text{dose}$ and in both sexes, mRNA-1283-related changes included decreased lymphocyte counts (range: 0.18x to 0.56x of control mean). These effects were resolved by day 36 in all groups.

Regardless of statistical significance, all other fluctuations among individual and mean hematology values were considered sporadic, consistent with biologic variation and/or negligible in magnitude, and not related to test article administration.

mRNA-1284, mRNA-1285 or mRNA-1284/mRNA-1285

At 30, 60, and 100 µg/dose for both test articles and in combination groups, administration of mRNA-1284, mRNA-1285 or mRNA-1284/mRNA-1285 to rats was associated with hematology changes (MCV, MCHC, platelet, reticulocyte, neutrophil, lymphocyte, eosinophil counts, and/or RDW) on days 23 and/or 36 (see tables below).

Group	1*		2		3		4	
Dose Level (µg/dose)	0		30 µg/dose mRNA-1283		60 µg/dose mRNA-1283		100 µg/dose mRNA-1283	
Sex	M	F	M	F	M	F	M	F
Platelets (10³x cells/µL)								
Day 23 (24 hr PD)	1061.2	1140.4	—	0.78x	0.83x	0.69x	0.74x	0.68x
Day 36	1053.8	1155.0	—	—	—	—	—	—
Reticulocytes (10³x cells/µL)								
Day 23 (24 hr PD)	211.16	146.98	—	—	0.81x	—	—	—
Day 36	228.74	248.40	—	—	—	—	—	—
Neutrophils (10³x cells/µL)								
Day 23 (24 hr PD)	1.604	0.806	6.1x	10.0x	7.1x	7.4x	5.7x	4.7x
Day 36	1.416	0.862	—	—	—	—	—	—
Lymphocytes (10³x cells/µL)								
Day 23 (24 hr PD)	11.434	8.422	0.50x	0.56x	0.43x	0.30x	0.34x	0.18x
Day 36	10.542	6.008	—	—	—	—	—	—
Eosinophils (10³x cells/µL)								
Day 23 (24 hr PD)	0.072	0.080	3.4x	2.7x	3.1x	3.2x	3.4x	2.0x
Day 36	0.096	0.120	—	—	—	—	—	—
RDW (%)								
Day 23 (24 hr PD)	12.40	10.82	1.06x	1.08x	1.05x	1.07x	1.08x	1.09x
Day 36	12.02	12.82	1.12x	1.07x	1.13x	1.05x	1.13x	1.06x
MCV (fL)								
Day 23 (24 hr PD)	57.72	55.80	—	—	—	—	1.04x	—
Day 36	56.12	57.82	—	—	—	—	1.04x	—
MCHC (g/dL)								
Day 23 (24 hr PD)	33.78	34.76	—	—	—	0.96x	0.97x	0.97x
Day 36	32.98	33.42	—	—	—	—	—	—

M = Males F = Females.

24 hr PD = 24 hours postdose.

MCV = Mean corpuscular volume; MCHC = Mean corpuscular hemoglobin concentration.

RDW = Red blood cell distribution width.

A dash (—) indicates absence of a test article-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.

Table 38: mRNA-1283-related hematology changes in males and females, sponsor provided (Study no. 2308-161).

3 pages have been determined to be not releasable: (b)(4)

mRNA-1284 and mRNA-1285:

mRNA-1284 and mRNA-1285-related observations reported (b) (4)

mRNA-1284/mRNA-1285:

(b) (4)

Organ weight:

Not collected.

Gross pathology:

At necropsy, the spleen and liver were collected from the designated animals and processed according to the protocol. Other tissues besides the spleen and liver were not intended to be evaluated or collected.

Microscopic findings:

At doses of ≥ 30 $\mu\text{g}/\text{dose}$, a minimal or mild mRNA-1283 related increase in extramedullary hematopoiesis was reported in the spleen.

(b) (4)

Other microscopic findings were considered unrelated to test article [mRNAs (1283- (b) (4))] administration because they were considered incidental, of the nature commonly reported in this strain and age of (b) (4) rats, and/or were of similar incidence and severity in control and treated animals.

In conclusion, intramuscular administration of mRNA-1283, (b) (4) caused changes in the spleen (increased extramedullary hematopoiesis). (b) (4)

Groups	1		2		3		4	
TA	Control(PBS)		mRNA-1283		mRNA-1283		mRNA-1283	
Dose ($\mu\text{g}/\text{dose}$)	0		30		60		100	
Sex	M	F	M	F	M	F	M	F
Animal per Group	5	5	5	5	5	5	5	5
	5	5	5	5	5	5	5	5

Groups	1		2		3		4	
TA	Control(PBS)		mRNA-1283		mRNA-1283		mRNA-1283	
Dose (µg/dose)	0		30		60		100	
Sex	M	F	M	F	M	F	M	F
Animal per Group	5	5	5	5	5	5	5	5
Spleen (No. examined)	0	1	0	3	5	4	5	5
Extramedullary hematopoiesis	0	1	0	3	4	2	1	1
Minimal	0	0	0	0	1	2	4	4

Table 42: Summary of microscopic findings-mRNA-1283, sponsor provided (Study no. 2308-161).

Groups	1		2		3		4	
TA	Control(PBS)		mRNA-1283		mRNA-1283		mRNA-1283	
Dose (µg/dose)	0		30		60		100	
Sex	M	F	M	F	M	F	M	F
Animal per Group	5	5	5	5	5	5	5	5
Spleen (No. examined)	5	5	5	5	5	5	5	5
Extramedullary hematopoiesis	0	1	5	2	5	4	5	4
Minimal	0	1	1	1	0	1	1	2
Mild	0	0	4	1	5	3	4	2

Table 43: Summary of microscopic findings-mRNA-1284, sponsor provided (Study no. 2308-161).

Groups	1		2		3		4	
TA	Control(PBS)		mRNA-1283		mRNA-1283		mRNA-1283	
Dose (µg/dose)	0		30		60		100	
Sex	M	F	M	F	M	F	M	F
Animal per Group	5	5	5	5	5	5	5	5
Spleen (No. examined)	5	5	5	5	5	5	5	5
Extramedullary hematopoiesis	0	1	5	4	5	3	5	5
Minimal	0	1	2	3	1	2	3	4
Mild	0	0	3	1	4	1	2	1

Table 44: Summary of microscopic findings-mRNA-1285, sponsor provided (Study no. 2308-161).

(b) (4)

Body temperature:

Not collected.

Serology:

For ELISA assay, blood samples (approximately 0.5 mL) were collected from all animals via the sublingual vein at pre-dose on day 1 and once on day 35. Animals were not fasted prior to blood collection.

mRNA-1283:

Thirteen days post 2nd immunization, mRNA-1283 doses of 30, 60, and 100 µg/dose elicited significant neutralizing antibodies (S2P, RBD and/or NTD) in a dose-independent manner. A summary of the group mean binding antibody titer levels is present in the tables below.

Group	1		2		3		4	
Dose Level (µg/dose)	0		30		60		100	
Sex	M	F	M	F	M	F	M	F
Pretest	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Day 35	LOQ	LOQ	151473	286783	118854	210709	212440	261659
LOQ = ≤ 25								

Table 46: mRNA-1283 S2P binding antibody titer levels, sponsor provided (Study no. 2308-161).

Group	1		2		3		4	
Dose Level (µg/dose)	0		30		60		100	
Sex	M	F	M	F	M	F	M	F
Pretest	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Day 35	LOQ	LOQ	130868	324420	139356	289742	205348	434105
LOQ = ≤ 25								

Table 47: mRNA-1283 RBD binding antibody titer levels, sponsor provided (Study no. 2308-161).

Group	1		2		3		4	
Dose Level (µg/dose)	0		30		60		100	
Sex	M	F	M	F	M	F	M	F
Pretest	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Day 35	LOQ	LOQ	100464	218215	114220	219762	193254	326014
LOQ = ≤ 25								

Table 48: mRNA-1283 NTD binding antibody titer levels, sponsor provided (Study no. 2308-161).

mRNA-1284 and mRNA-1285:

(b) (4)

One page has been determined to be not releasable: (b)(4)

(b) (4)

Test article related effects are listed in the table below:

Test article related effects	
	(b) (4)
↑ Monocyte	
↑ Basophil at study day 36	
↑ LUC at study day 23	
↑ Neutrophils at study day 23	
↓ Lymphocyte at study day 23	
↑ Eosinophils at study day 23	
Edema	
Extramedullary hematopoiesis in spleen	
Immune responses	

Table 56: Test article related effect (Study no. 2308-161).

Assessment:

No treatment-related mortality or body weight were reported.

The hepatocellular leakage enzymes (AST and ALT) are useful in detecting injury to liver parenchymal cells. Generally, increased serum activity represents enzyme leakage from cells through damaged cell membranes. AST is useful as an indicator of liver and/or muscle injury in large and small animals. The increases in AST and ALT levels in groups (b) (4) are test article related.

Bilirubin occurs in the normal catabolic pathway that breaks down heme in vertebrates and is a yellow compound. Through this catabolism process the body clears the waste products that arise from the destruction of aged or abnormal red blood cells⁵. This process includes the followings: hemoglobin gets stripped of the heme molecule which thereafter passes through various processes of porphyrin catabolism, depending on the part of the body in which the breakdown occurs. As an example, the molecules excreted in the urine differ from those in the feces⁶. The production of biliverdin from heme is the first major step in the catabolic pathway. This is after which the enzyme biliverdin reductase performs the second step, producing bilirubin from biliverdin^{7,8}. The metabolites of bilirubin are excreted through bile and urine. Elevated levels of bilirubin may indicate certain diseases⁹. Also, the elevated levels are responsible for the yellow color of bruises and the yellow discoloration in jaundice.

A triglyceride is an ester derived from glycerol and three fatty acids.¹⁰ Triglycerides are the main constituents of body fat in humans and animals, as well as vegetable fat.¹¹ They are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver, and are a major component of human skin oils.¹² In the human body, high levels of

⁵ Braunstein E (3 May 2019). "Overview of Hemolytic Anemia – Hematology and Oncology". *Merck Manuals Professional Edition* (in Latin). Retrieved 5 May 2019.

⁶ "Bilirubin blood test", *U.S. National Library of Medicine*.

⁷ Boron W, Boulpaep E. *Medical Physiology: a cellular and molecular approach*, 2005. 984–986. Elsevier Saunders, United States. [ISBN 1-4160-2328-3](#)

⁸ Mosqueda L, Burnight K, Liao S (August 2005). "The life cycle of bruises in older adults". *Journal of the American Geriatrics Society*. **53** (8): 1339–43. [doi:10.1111/j.1532-5415.2005.53406.x](#). [PMID 16078959](#). [S2CID 12394659](#).

⁹ Smith ME, Morton DG (2010). "[LIVER AND BILIARY SYSTEM](#)". *The Digestive System*. Elsevier. pp. [85–105](#). [doi:10.1016/b978-0-7020-3367-4.00006-2](#). [ISBN 978-0-7020-3367-4](#).

¹⁰ "[Nomenclature of Lipids](#)". IUPAC-IUB Commission on Biochemical Nomenclature (CBN). Retrieved 2007-03-08.

¹¹ Nelson, D. L.; Cox, M. M. (2000). *Lehninger, Principles of Biochemistry* (3rd ed.). New York: Worth Publishing. [ISBN 1-57259-153-6](#).

¹² Lampe, M. A.; Burlingame, A. L.; Whitney, J.; Williams, M. L.; Brown, B. E.; Roitman, E.; Elias, M. (1983). "Human stratum corneum lipids: characterization and regional variations". *J. Lipid Res*. **24**: 120–130. [PMID 6833889](#)

triglycerides in the bloodstream have been linked to atherosclerosis and, by extension, the risk of heart disease¹³ and stroke.¹⁴

Monocytosis could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair. The increases in the monocyte count might be related to test article treatment.

Basophils play a role in both parasitic infections and allergies. Basopenia has been reported in association with autoimmune urticaria.

LUC is a measurement of the large, peroxidase-negative cells which cannot be further characterized (i.e. as large lymphocytes, virocytes, or stem cells) present in a biological specimen. In LUC are found large lymphoid cells, more immature lymphocytes and other cells. If the value is higher than normal, blood counts should be checked under a microscope slide.

Neutrophils are key components in the system of defense against infection. An individual with absence or scarcity of neutrophils (neutropenia) is vulnerable to infection. The increase in neutrophils might be related to the immune responses initiated by the test article treatment.

A lymphocyte is any of 3 types of white blood cell (all 3 are agranulocytes) in a vertebrate's immune system. They include natural killer cells (NK cells) (which function in cell-mediated, cytotoxic innate immunity), T cells (for cell-mediated, cytotoxic adaptive immunity), and B cells (for humoral, antibody-driven adaptive immunity). Thus, any decrease, in one or all of these cell types, might affect the immune responses.

Eosinophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. They are granulocytes that develop during haematopoiesis in the bone marrow before migrating into blood.

Test article-related observations were reported on days 1 and 22 included edema with or without hindlimb impairment. These findings were in dose-dependent trend to the hindlimb impairment caused by the edema reported within the hindlimb. All hindlimb impairment and edema were resolved at approximately 2-5 days post dose.

The spleen plays important roles in regard to red blood cells and the immune system¹⁵. It removes old red blood cells and holds a reserve of blood in case of hemorrhagic shock while also recycling iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes. The globin portion of hemoglobin is degraded to its constitutive amino acids, and the heme portion is metabolized to bilirubin, which is subsequently shuttled to the liver for removal¹⁶. It synthesizes antibodies in its white pulp and removes

¹³ [*"Boston scientists say triglycerides play key role in heart health"*](#). The Boston Globe. Retrieved 2014-06-18.

¹⁴ Drummond, K. E.; Brefere, L. M. (2014). Nutrition for Foodservice and Culinary Professionals (8th ed.). John Wiley & Sons. [ISBN 978-0-470-05242-6](#).

¹⁵ Spleen, Internet Encyclopedia of Science.

¹⁶ Mebius RE, Kraal G. (2005). Structure and function of the spleen. Nat Rev Immunol. 5(8):606-16.

antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation.

Test article-related changes in the spleen (increased extramedullary hematopoiesis) were reported. Also, test article-related changes in the liver (hypertrophy/hyperplasia of sinusoidal cells) were reported and these changes were reported in all doses.

Immune responses were reported in all test article-treated animals.

(b) (4)

Non-observable adverse effect level (NOAEL)

According to the findings above, the NOAEL for the test articles tested should be as follows:

<u>Test article</u>	<u>NOAEL (µg)</u>
mRNA-1283	100

(b) (4)

Study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

Investigators Brochure: Having read and evaluated the Investigators Brochure, is it a fair, objective and reasonable summary of the toxicology data – yes () or no (X). The findings of this study need to be added to the IB.

Internal communications:

(b) (4)

Conclusions:

Based on nonclinical toxicity assessments, liver enzymes should be monitored in the clinical trials.

(b) (4)

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(b) (4)

Study number 9: A 4-Week GLP Repeat Dose Toxicity Study of mRNA-1283.222 by Intramuscular Injection Administration in (b) (4) Rats with a 2-Week Recovery Period. Study number 20462697

Performing laboratory: (b) (4)

Study initiation date: August 30th, 2023

Final report date: April 18th, 2024

Test article batch/lot:

Table of test article identification

	Test Article
Identification:	mRNA-1283.222 Drug Product
Alternate Identification:	mRNA-1283.222
Batch/Lot No.:	8516100102
Expiration Date:	26 Jan 2025
Concentration (Total RNA Content):	0.05 mg/mL
Storage Conditions:	Frozen at (b) (4) °C, protected from light
Provided by:	ModernaTX, Inc.

Vehicle control identification

	Vehicle Control
Identification:	(b) (4) mM Tris, (b) (4) g/L sucrose, pH (b) (4)
Lot No.:	(b) (4)
Expiration Date:	23 Nov 2023
Storage Conditions:	Frozen at (b) (4) °C, protected from light
Provided by:	ModernaTX, Inc.

Animal species and strain: (b) (4)

Breeder/supplier: (b) (4)

Number of animal per group and sex: 10/sex/dose

Age: 8 weeks

Body weight range: 205 – 391 g for males and 187 – 258 g for females

Route and site of administration: Intramuscular injection

Volume of injection: 200 µL/dose

Frequency of administration and study duration: Days 1 and 29. Study duration was 43 days.

Dose: See study design below

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Expiration dates were reported.

Means of administration: Intramuscular injection

Report status: Final

Experimental design:

Animals were randomized and assigned to 4 different groups. Each group consisted of 15 or 10/sex/group. Animals were dosed by IM injection on study days 1 and 29. The details of the study design are listed in the following table:

Group No.	Test Article	Dose Level (µg/dose)	Dose Volume (mL)	Dose Concentration (µg/mL)	Animal Nos.			
					Main Study		Recovery Study	
					Male	Female	Male	Female
1	Vehicle	0	0.2	0	1006 to 1015	1506 to 1515	1001 to 1005	1501 to 1505
2	mRNA-1283.222	2		10	2001 to 2010	2501 to 2510	-	-
3		5		25	3001 to 3010	3501 to 3510	-	-
4		10		20	4006 to 4015	4506 to 4515	4001 to 4005	4501 to 4505

Table 132: Experimental design (Study no. 20462697).

Methods:**Randomization procedure:** Yes**Statistical analysis plan:** Yes.**The following parameters were evaluated:**In-life procedures, observations, and measurements

General in-life assessments – Main study and recovery animals

Parameter	Population(s)	Frequency (minimum required)	Comments
Mortality/Cageside Observations	All surviving animals	At least twice daily (morning and afternoon) beginning upon arrival through termination/release. ^{a,b}	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings. Animals will be observed for morbidity, mortality, injury, and availability of food and water. Any animals in poor health will be identified for further monitoring and possible euthanasia.
Post Dose Observations	All main study and recovery animals	1 and 6 hours post each dose	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings.
Detailed Clinical Observations	All main study and recovery animals	Once pretest (day -1) and then once weekly thereafter throughout the study (to include 24 hours post each dose) ^c	Animals are removed from the cage. Pretest interval will be conducted on all animals received/transferred.
Individual Body Weights	All main study and recovery animals	Within 3 days of arrival, day -1, and once weekly during the terminal and recovery periods.	Not collected from animals found dead.
Food Consumption	All main study and recovery animals	Weekly ^d	Quantitatively measured
Ophthalmic Examinations	All main study and recovery animals	Pretest (all animals) and all survivors examined again during week 4 and at end of recovery (if treatment-related findings exist)	See details in section below.
Injection Site Observations	All animals	On dosing days: 6 hours, and 24 hours post each dose and scored daily until resolution	The presence (present/not present) of erythema and/or edema will be recorded for individual animals at the injection site. Animals will be removed from their cages for these observations.

^a Procedures on alternate animals will be conducted per testing facility SOP.^b Except on days of receipt and necropsy where frequency will be at least once daily.^c For observations that cannot be attributed to an individual animal due to social housing (e.g., watery feces), the observation will be noted to each animal in the socialized group.^d For observations of reduced appetite that cannot be attributed to an individual animal due to social housing, the observations will be noted for each animal in the socialized group.

Table 133: General in-life assessments – Main study and recovery animals, sponsor provided (Study no. 20462697).

Group Nos.	Time Point(s)	Hematology	Coagulation	Clinical Chemistry
All main study animals	Prior to main study necropsy	X	X	X
All recovery animals	Prior to recovery necropsy	X	X	X
Unscheduled euthanasia (when possible)	See the unscheduled euthanasia section of this protocol.			
Target volume:	-	1 mL	1.2 mL	1.3 mL
Fasting:	Free access to drinking water but will be fasted overnight (at least 8 hours) prior to blood collection.			
Anticoagulant:	-	K ₂ EDTA	Sodium Citrate	Serum Gel Separator
Processing:	-	None	Plasma	Serum

X = Sample to be collected; - = Not applicable; hr = Hour; pre = Predose; post= Postdose. Blood sample collection method: Vena cava after isoflurane inhalation.

Table 134: Clinical pathology sample collection, sponsor provided (Study no. 20462697).

Group Nos.	Time Points		
	Pretest (All animals)	Day 29 24 hours post dose (Main study animals)	Day 43 Prior to termination (Recovery animals)
1-4	X	X	X

Table 135: Anti-protein antibody sample collection, sponsor provided (Study no. 20462697).

Terminal procedures are summarized in the following tables:

Unscheduled deaths:

Group No.	Necropsy Procedures			Histology Processing	Microscopic Evaluation
	Necropsy	Tissue Collection	Organ Weights		
Found Dead or Unscheduled Euthanasia	X	Full List ^a	-	Full List ^a	Full List ^a

Table 136: Terminal procedures, unscheduled deaths, sponsor provided (Study no. 20462697).

Main study animals

Group No.	Scheduled Euthanasia Day	Necropsy Procedures			Histology Processing	Microscopic Evaluation
		Necropsy	Tissue Collection	Organ Weights		
1, 4	30	X	Full List ^a	Full List ^a	Full List ^a	Full List ^a
2, 3		X	Full List ^a	Full List ^a	Gross Lesions Target Tissues ^b	Gross Lesions Target Tissues ^b

Table 137: Main study animals, sponsor provided (Study no. 20462697).

Recovery animals

Group No.	Scheduled Euthanasia Day	Necropsy Procedures			Histology Processing	Microscopic Evaluation
		Necropsy	Tissue Collection	Organ Weights		
1,4	43	X	Full List ^a	Full List ^a	Full List ^a	Full List ^a

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

‘Histology processing’= Embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.

^a See tissue weighing, collection, processing, and evaluation table (attachment A) for list of tissues applicable to each procedure.

^b Any target tissues identified by the study pathologist during microscopic evaluation of full list animals will be communicated to the study director, then processed, evaluated and reported in non-full list animals

Table 138: Recovery animals, sponsor provided (Study no. 20462697).

Postmortem procedures:

Tissue weighing, collection, processing, and evaluation table.

Organ	Weigh	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Animal ID	-	X	-	-
Artery, aorta	-	X	X	X
Body cavity, nasal	-	X	-	-
Bone marrow smear	-	X ^a	-	-
Bone marrow, sternum	-	X	X	X
Bone, femur	-	X (1)	X (1)	X (1)
Bone, sternum	-	X	X	X
Brain	X	X	X	X
Epididymis	X (2)	X (2) ^b	X (2)	X (2)
Esophagus	-	X	X	X
Eye	-	X (2) ^b	X (2)	X (2)
Ganglion, dorsal root, lumbar	-	X	-	-
Gland, adrenal	X (2)	X (2)	X (2)	X (2)
Gland, clitoral	-	X (2)	-	-
Gland, coagulating	-	X (2)	-	-
Gland, Harderian	-	X (2)	X (1)	X (1)
Gland, lacrimal	-	X (2)	-	-
Gland, mammary	-	X	X	X
Gland, parathyroid	-	X (2)	X (2)	X (2)
Gland, pituitary	X	X	X	X
Gland, preputial	-	X (2)	-	-
Gland, prostate	X	X	X	X
Gland, salivary, parotid	-	X (2)	-	-
Gland, salivary, sublingual	-	X (2)	-	-
Gland, salivary, mandibular	-	X (2)	X (1)	X (1)
Gland, seminal vesicle	-	X (2)	X (2)	X (2)
Gland, thyroid	-	X (2)	X (2)	X (2)
Gland, thyroid/parathyroid	X (2)	-	-	-
Gland, Zymbal's	-	X (2)	-	-
Gut-associated lymphoid tissue	-	X	X	X
Heart	X	X	X	X
Joint, femorotibial	-	X (1)	X (1)	X (1)
Kidney	X (2)	X (2)	X (2)	X (2)
Large intestine, cecum	-	X	X	X
Large intestine, colon	-	X	X	X
Large intestine, rectum	-	X	X	X
Larynx	-	X	-	-
Liver	X	X	X	X

Organ	Weigh	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Lung	-	X	X	X
Lymph node(s) draining administration site(s): iliac (maintain orientation)	-	X (2)	X (2)	X (2)
Lymph node(s) draining administration site(s): inguinal (orientation maintained)	-	X (2)	X (2)	X (2)
Lymph node, mandibular	-	X (2)	X (1)	X (1)
Lymph node, mesenteric	-	X	X	X
Muscle, skeletal (masseter)	-	X (2)	X (1)	X (1)
Nerve, optic	-	X (2) ^b	X (2)	X (2)
Nerve, sciatic	-	X (2)	X (1)	X (1)
Nerve, tibial	-	X (2)	-	-
Ovary	X (2)	X (2)	X (2)	X (2)
Oviduct	-	X (2)	-	-
Pancreas	-	X	X	X
Site, injection, intramuscular (both)	-	X (2)	X (2)	X (2)
Skin	-	X	X	X
Small intestine, duodenum	-	X	X	X
Small intestine, ileum	-	X	X	X
Small intestine, jejunum	-	X	X	X
Spinal cord, cervical	-	X	X	X
Spinal cord, thoracic	-	X	X	X
Spinal cord, lumbar	-	X	X	X
Spleen	X	X	X	X
Stomach	-	X	X	X
Testis	X (2)	X (2) ^b	X (2)	X (2)
Thymus	X	X	X	X
Tongue	-	X	X	X
Trachea	-	X	X	X
Ureter	-	X (2)	-	-
Urinary bladder	-	X	X	X
Uterus/Cervix	X	X	X	X
Vagina	-	X	X	X
Macroscopic abnormalities ^c	-	X	X	X

X = Procedure to be conducted. - = Not applicable. (1) = One side. (2) = Both sides.

^a Bone marrow smears will be collected from the humerus at scheduled and unscheduled necropsies (for possible examination). Smears will not be collected from animals that are found dead or from animals that were euthanized moribund and then stored in the refrigerator prior to necropsy. Bone marrow smears will be allowed to air dry and are not fixed in formalin.

^b Eyes and optic nerves will be preserved in (b) (4) fixative. Testes and epididymides will be preserved in modified (b) (4) fixative.

^c Macroscopic abnormalities in the tissues listed, and in any other tissues, will be sampled, processed, and evaluated histologically.

Table 139: Tissue weighing, collection, processing, and evaluation, sponsor provided (Study no. 20462697).

Results:

No test article-related mortality was reported.

Clinical chemistry, hematology, and coagulation:Clinical chemistry

Glucose concentrations in control males and females on day 30 tended to be minimally lower than Moderna-specific historic control values, and the potential impact of improper sample handling on this parameter could not be definitively determined. As a result, serum glucose concentrations were deemed unreliable and therefore excluded from the clinical pathology interpretation.

Individual and group mean control values for males and females on day 30 were comparable with Moderna-specific historic control values for the following parameters: ALT, AST, ALP, GGT, CK activity and total bilirubin, urea nitrogen, creatinine, cholesterol, triglyceride, calcium, and phosphorus concentrations, suggesting there were minimal to no effects of improper sample handling on these parameters. There were no apparent differences between vehicle-dosed control groups and mRNA-1283.222-dosed groups at any dose level for any of these parameters that were evaluated.

On day 43, females at 10 µg/dose exhibited a higher mean globulin concentration (1.13x mean control) with resultant lower albumin to globulin ratio (0.93x mean control) which were not clearly mRNA-1283.222-related due to the small magnitude of difference. Remaining fluctuations among individual and mean values on day 43 were considered sporadic, consistent with biologic variation, and/or negligible in magnitude, and not related to mRNA-1283.222 administration.

Coagulation

Group	1		2		3		4	
Dose (µg/dose) ^a	0		2		5		10	
Sex	M	F	M	F	M	F	M	F
FIB (mg/dL)								
Day 30	287.6	217.0	1.24x	1.26x	1.68x	1.61x	2.14x	2.05x
Day 43	280.2	183.4	NE	NE	NE	NE	–	–

M = Males; F = Females.

NE = Not evaluated (no recovery animals were included per protocol). FIB = Fibrinogen.

A dash (–) indicates absence of an mRNA-1283.222-related change. Numerical values (groups 2-4) indicate fold change (x) of the mRNA-1283.222-dosed group mean value relative to the sex-matched vehicle control group (group 1) mean value, provided for reference.

Bolded values indicate the mRNA-1283.222-dosed group mean value was statistically different from the vehicle-dosed control group mean value at $p \leq 0.01$.

^a Dose administered via intramuscular injection on days 1 and 29.

Table 140: mRNA-1283.222-related coagulation changes, sponsor provided (Study no. 20462697).

Test article-related and dose-related increases in mean fibrinogen concentrations (range: 1.24x – 2.14x mean control) were reported in males and females at ≥ 2 µg/dose on day 30 and were most consistent with an acute phase and/or inflammatory response.

Following a 2-week recovery period, increases in fibrinogen concentrations reported at 10 µg/dose at the terminal necropsy were no longer apparent, suggesting full recovery of these changes.

Remaining fluctuations among individual and mean values were considered sporadic, consistent with biologic variation, and/or negligible in magnitude, and not related to test article administration.

Hematology

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.553, ie, ≥ 1.6 or ≤ 1.6)	Not of NOTE
Red blood cells	Reticulocytes*	Hematocrit (Hct) Hemoglobin Conc. (Hb) Total Erythrocyte Count (RBC) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC) Mean Corp. Volume (MCV) RDW%
White blood cells	Neutrophil count* Lymphocyte count* Eosinophils count SD30 M $\uparrow$ = 1.6 G4 SD30 F $\uparrow$ = 1.6 G2 SD43 F $\uparrow$ = 1.9 G4 Large Unstained Cells (LUC) SD30 M $\uparrow$ = 1.8 G4 SD43 F $\uparrow$ = 1.9 G4 Monocyte count SD43 F $\uparrow$ = 2.0 G4 Basophils SD43 F $\uparrow$ = 2.0 G4	Macrophage Leukocytes White Blood Cells (WBC)
Clotting potential	Fibrinogen** SD30 M $\uparrow$ = 1.7 G3 SD30 M $\uparrow$ = 2.0 G4 SD30 F $\uparrow$ = 1.6 G3 SD30 F $\uparrow$ = 2.1 G4	Activated partial-thromboplastin time clotting time Prothrombin time Platelet count
Others		Bone marrow cytology

* See results and tables below. ** Discussed on page 147.

Table 141: Hematology results. (Study no. 20462697).

⁵³ With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

Neutrophil counts increase (range: 1.52x - 2.37x mean control) were reported in group 4 males and groups 2, 3, and 4 females on day 30.

Decreases in mean reticulocyte counts (range: 0.83x – 0.79x mean control) were reported in groups 2, 3, and 4 males on day 30 and were likely secondary to the acute phase and/or inflammatory response.

Decreases in mean lymphocyte counts (range: 0.82x - 0.68x mean control) were reported in groups 2, 3, and 4 females on day 30. On day 43, these changes were recovered.

On day 43, increase in mean lymphocyte (1.70x mean control), monocyte (1.98x mean control), eosinophil (1.85x mean control), and resultant total white blood cell counts (1.59x mean control) were reported in group 4. Higher mean parameters of erythrocyte mass comprising red blood cell count, hemoglobin concentration, and hematocrit (up to 1.10x mean control) were also reported in group 4.

Eosinophil levels were increased in group 4 males and in group 2 females on day 30. Eosinophil levels were increased in group 4 females on day 43. LUC levels were increased in group 4 males and females on study days 30 and 43, respectively. Monocyte levels were increased in group 4 females on day 43. Basophil levels were increased in group 4 females on day 43.

Remaining fluctuations among individual and mean values, regardless of statistical significance, were considered sporadic, consistent with biologic variation, and/or negligible in magnitude, and not test article related.

Group	1		2		3		4	
Dose (µg/dose) ^a	0		2		5		10	
Sex	M	F	M	F	M	F	M	F
NEUT (10 ³ /µL)								
Day 30	2.319	1.024	–	1.52x	–	2.07x	1.76x	2.37x
Day 43	2.674	1.322	NE	NE	NE	NE	–	–
RETIC (10 ⁹ /L)								
Day 30	226.72	162.22	0.83x	–	0.80x	–	0.79x	–
Day 43	184.08	149.00	NE	NE	NE	NE	–	–
LYMPH (10 ³ /µL)								
Day 30	7.923	7.838	–	0.82x	–	0.75x	–	0.68x
Day 43	8.502	4.754	NE	NE	NE	NE	–	–

M = Males; F = Females. NE = Not evaluated (no recovery animals were included per protocol).

NEUT = Neutrophil; RETIC = Reticulocyte; LYMPH = Lymphocyte.

A dash (–) indicates absence of an mRNA-1283.222-related change. Numerical values (groups 2-4) indicate fold change (x) of the mRNA-1283.222-dosed group mean value relative to the sex-matched vehicle control group (group 1) mean value, provided for reference.

Bolded values indicate the mRNA-1283.222-dosed group mean value was statistically different from the vehicle-dosed control group mean value at $p \leq 0.05$ or $p \leq 0.01$.

^a Dose administered via intramuscular injection on days 1 and 29.

Table 142: mRNA-1283.222-related hematology changes, sponsor provided (Study no. 20462697).

Systemic toxicity:

No treatment-related mortality, clinical signs, body weight, food consumption, ophthalmology, or macroscopic findings were reported.

Organ weight:

No treatment-related organ weight findings were reported.

Gross pathology:

No treatment-related gross pathology findings were reported.

Microscopic findings:Main Study

Mixed cell inflammation in injection site 2 were reported. The inflammation ranged from minimal to moderate in severity; moderate severity occurring more in groups 3 and 4 compared with group 2. Mixed cell inflammation was characterized by an infiltrate of mononuclear cells (predominantly macrophages) with varying numbers of neutrophils accompanied by fluid accumulation, generally limited to tissue spaces in fascial planes and subcutis. Cellular infiltrate sporadically extended slightly around (perimysium) and into muscle bundles (endomysium), with occasional minimal degeneration of muscle fibers. Minimal to mild mononuclear cell infiltrate diagnosed in group 1 was due to the intramuscular injection procedure. Mononuclear cell infiltration, similarly, prevalent in test article-dosed sites, was therefore due to the injection procedure.

Neutrophil infiltrates were reported in the right iliac lymph node (draining lymph node of injection site 2) in groups 3 and 4. This finding occurred in rats with mixed cell inflammation in the injection site and was considered part of or secondary to the inflammatory process in the injection site.

Minimal fibrosis and mixed cell inflammation in a subset of animals were reported at injection site 1 and considered test article related. Fibrosis was characterized by pale loose material in the loose connective tissue of the subcutaneous area. Minimal fibrosis of the same character was reported in injection site 1 of one control female, indicating that this was due to the injection procedure. Mixed cell inflammation was reported in injection site 1 of 3 males and one female in groups 2 and 3. Mixed cell inflammation was of the same character as that reported in injection site 2 of other animals but was not reported in injection site 2 of the animals affected at injection site 1.

Sex	Male				Female			
Group Number	1	2	3	4	1	2	3	4
Dose (µg/dose)	0	2	5	10	0	2	5	10
Number of Animals Per Dose Group	10	10	10	10	10	10	10	10
SITE, INJECTION, INTRAMUSCULAR, 2 Number Examined	10	10	10	10	10	10	10	10
Inflammation, mixed cell								
Number affected	-	5	7	8	-	8	6	5
minimal	-	1	1	-	-	-	-	1
mild	-	3	2	4	-	8	2	2
moderate	-	1	4	4	-	-	4	2

Sex	Male				Female			
Group Number	1	2	3	4	1	2	3	4
Dose (µg/dose)	0	2	5	10	0	2	5	10
Number of Animals Per Dose Group	10	10	10	10	10	10	10	10
SITE, INJECTION, INTRAMUSCULAR, 2 Number Examined	10	10	10	10	10	10	10	10
LYMPH NODE, ILIAC, RIGHT Number Examined	10	10	10	10	9	10	10	10
Infiltrate, neutrophil								
Number affected	-	-	-	1	-	-	1	2
minimal	-	-	-	1	-	-	1	-
mild	-	-	-	-	-	-	-	1
moderate	-	-	-	-	-	-	-	1

- = absence of a finding.

Table 143: mRNA-1283.222-related findings in intramuscular injection site 2 and right iliac lymph node– Main study (sampled 24 hours post dose), sponsor provided (Study no. 20462697).

Sex	Male				Female			
Group Number	1	2	3	4	1	2	3	4
Dose (µg/dose)	0	2	5	10	0	2	5	10
Number of Animals Per Dose Group	10	10	10	10	10	10	10	10
SITE, INJECTION, INTRAMUSCULAR 1 Number Examined	10	10	10	10	10	10	10	10
Fibrosis								
minimal	-	-	1	-	1	-	1	4
Inflammation, mixed cell								
mild	-	2	-	-	-	1	-	-
moderate	-	-	1	-	-	-	-	-

- = absence of a finding.

Table 144: mRNA-1283.222-related findings in intramuscular injection site 1 – Main study (sampled 29 days post dose), sponsor provided (Study no. 20462697).

Recovery study

Minimal fibrosis and a slightly increased incidence of mononuclear cell infiltrate in group 4 were reported. The minimal fibrosis at injection site 2 in group 4 was considered secondary to the prior inflammation. The mononuclear cell infiltration at injection site 2 may be attributed to the prior mixed cell inflammation and indicated recovery from that process.

There were no findings in injection site 1 in the recovery animals that could be attributed directly to the test article. The only potential difference was minimal fibrosis in two group 4 males compared to no fibrosis in group 1 males.

Sex	Male		Female	
Group Number	1	4	1	4
Dose (µg/dose)	0	10	0	10
Number of Animals Per Dose Group	5	5	5	5
SITE, INJECTION, INTRAMUSCULAR, 2 Number Examined	5	5	5	5
Fibrosis				
minimal	-	3	-	2
Infiltrate, mononuclear cell				

Sex	Male		Female	
Group Number	1	4	1	4
Dose (µg/dose)	0	10	0	10
Number of Animals Per Dose Group	5	5	5	5
minimal	2	3	-	3
mild	-	1	-	-

- = absence of a finding.

Table 145: mRNA-1283.222-related findings in intramuscular injection site 2– Recovery study (sampled 14 days post last dose), sponsor provided (Study no. 20462697).

Sex	Male		Female	
Group Number	1	4	1	4
Dose (µg/dose)	0	10	0	10
Number of Animals Per Dose Group	5	5	5	5
SITE, INJECTION, INTRAMUSCULAR 1	5	5	5	5
Number Examined				
Fibrosis				
minimal	-	2	1	1

- = absence of a finding.

Table 146: Microscopic changes in intramuscular injection site 1 – Recovery study (sampled 42 days post dose), sponsor provided (Study no. 20462697).

Other findings in intramuscular injection sites that occurred with low sporadic incidence (e.g., granuloma, foreign material) were reported in groups 1, 2, 3, and 4. These findings might be associated with the administration procedure.

Findings of hemorrhage and inflammation in the tongue and erythrocytosis in the mandibular lymph nodes did not differ substantively between vehicle-dosed and test article-dosed rats and were secondary to the sublingual blood collection procedure. All other microscopic findings did not differ substantively between vehicle-dosed rats and test article-dosed rats, occurred in single individuals, and/or were similar to findings common to this species/strain and, therefore, were not considered related to test article administration.

Body temperature:

Not collected.

Serology:

To assess IgG antibodies against SARS-CoV-2 S2P wild-type and BA.4/5 S2P proteins, ELISAs against S2P wild-type and BA.4/5 S2P proteins (according to (b) (4) protocols BS-4466 version v3 and BS-4507 version v1, respectively) were used. A total of 200 serum samples were evaluated to detect antibodies against SARSCoV-2 S2P wild-type and 200 serum samples were evaluated to detect antibodies against SARS-CoV-2 BA.4/5 S2P proteins.

No detectable IgG titers were reported in group 1 at all timepoints and for groups 2-3 (mRNA-1283.222) at pretest timepoint. For group 4, sample ID 4012 showed a detectable IgG titer at pretest timepoint against S2P wild-type protein only.

In groups 2, 3, and 4 (following day 29 immunizations of mRNA-1283.222), a robust SARS-CoV-2 S2P wild-type and SARS-CoV-2 BA.4/5 S2P IgG titers were reported which were generally dose-independent. These responses persisted in all dose groups following the 2-week recovery period. There was an increase in IgG titers in group 4 on day 43 when compared to day 29 time point.

Group		1	2	3	4
Dose Level (µg/dose)		0	2	5	10
Sex		Combined (M and F)	Combined (M and F)	Combined (M and F)	Combined (M and F)
SARS-CoV-2 S2P wild-type	Pretest	<30	<30	<30	<30
	Day 29 (Predose)	<30	6,357	9,701	11,543
	Day 43	<30	N/A	N/A	101,226
SARS-CoV-2 BA.4/5 S2P	Pretest	<21	<21	<21	<21
	Day 29 (Predose)	<21	7,582	11,051	13,915
	Day 43	<21	N/A	N/A	135,805

M = Males; F = Females. Note: Lower Limit of Quantitation for SARS-CoV-2 S2P wild-type is 0.059 antibody Units/mL (AU/mL). Lower Limit of Quantitation for SARS-CoV-2 BA.4/5 S2P is 0.042 antibody Units/mL (AU/mL). Dilution factor 1:500 is the lowest dilution factor used for these samples.

Note: A “not-detected” antibody response for SARS-CoV-2 S2P wild-type is reported as “<30 AU/mL which was derived from calculation “< 0.059 antibody Units/mL * 500”. A “not-detected” antibody response for SARS-CoV-2 BA.4/5 S2P was reported as “<21 AU/mL which is derived from the calculation “< 0.042 antibody Units/mL * 500”.

Table 147: Mean IgG antibodies against SARS-CoV-2 S2P wild-type and SARS-CoV-2 BA.4/5 S2P titer levels, sponsor provided (Study no. 20462697).

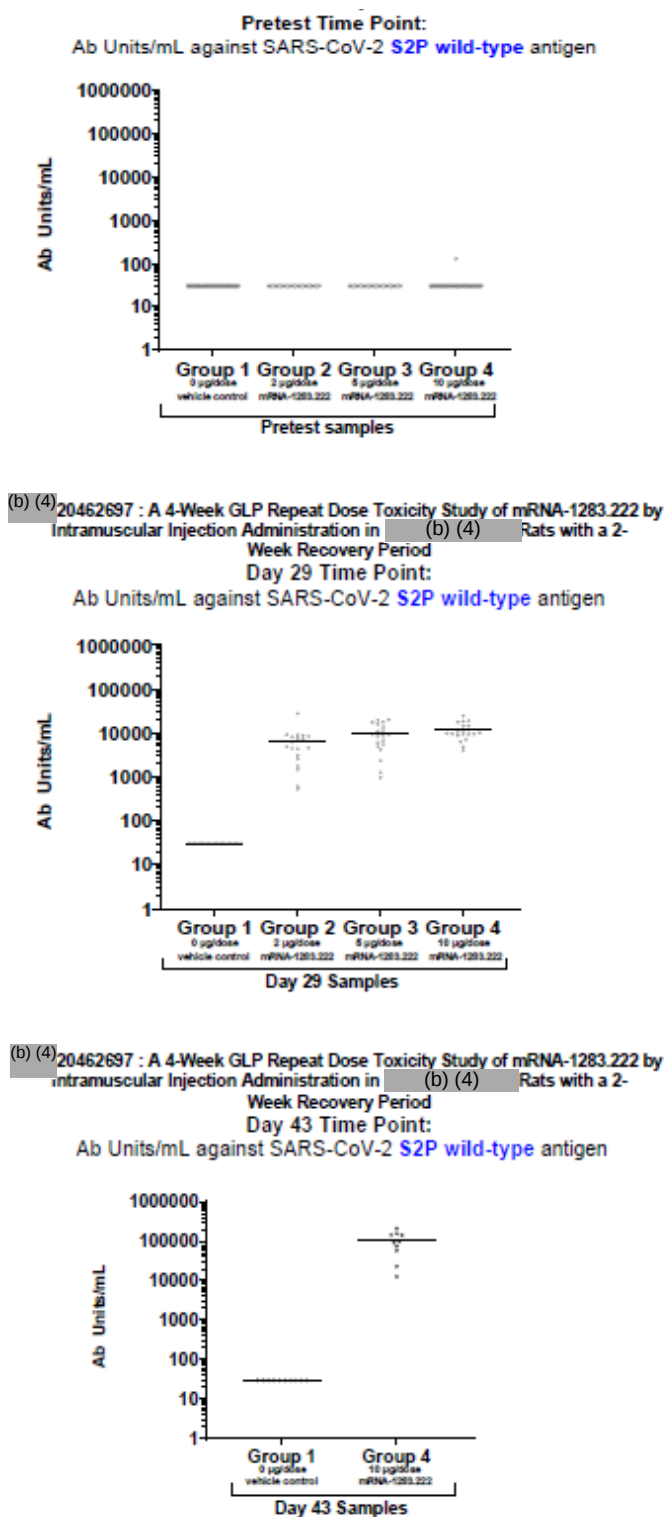


Figure 5: IgG antibody titers against the S2P wild-type antigen. Antibody Units/mL (AU/mL). Circles represent the AU/mL mean values; horizontal lines represent mean for each group, sponsor provided. (Study no. 20462697).

Test article related effects are listed in the table below:

Test article related effects
↑ Eosinophils ↑ Neutrophils ↑ Fibrinogen Injection site findings Neutrophil infiltrate in right iliac lymph node Immune responses

Table 148: Test article related effects (Study no. 20462697).

Assessment:

No treatment-related mortality, clinical signs, body weight, food consumption, ophthalmology, or macroscopic findings were reported.

Eosinophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. They are granulocytes that develop during haematopoiesis in the bone marrow before migrating into blood.

Neutrophils are key components in the system of defense against infection. An individual with absence or scarcity of neutrophils (neutropenia) is vulnerable to infection. The increase in neutrophils might be related to the immune responses initiated by the test article treatment.

Fibrinogen changes were indication of an acute phase response/inflammation. The increase in fibrinogen levels might be related to treatment and is considered as an expected (inflammatory) response following treatment with immunogenic substances.

Mixed cell inflammation and fibrosis were reported at the injection sites. The inflammation ranged from minimal to moderate in severity. Mixed cell inflammation was characterized by an infiltrate of mononuclear cells (predominantly macrophages) with varying numbers of neutrophils accompanied by fluid accumulation, generally limited to tissue spaces in fascial planes and subcutis. Cellular infiltrate sporadically extended slightly around (perimysium) and into muscle bundles (endomysium), with occasional minimal degeneration of muscle fibers. Fibrosis was characterized by pale loose material in the loose connective tissue of the subcutaneous area.

Neutrophil infiltrates were reported in the right iliac lymph node (draining lymph node of injection site 2) in groups 3 and 4. This finding occurred in rats with mixed cell inflammation in the injection site and was considered part of or secondary to the inflammatory process in the injection site.

Immune responses were reported in all test article-treated animals.

Based on the overall findings in this study, it can be concluded that in (b) (4) rats, test article [mRNA-1283.222]-administered by IM injection caused increases in hematology parameters (eosinophils, neutrophil, and fibrinogen), injection site inflammation, and immune responses.

Study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results. However, clinical pathology sample processing error were reported. This made clinical chemistry parameters (albumin, globulin, total protein, sodium, potassium chloride, and glucose concentrations and albumin to globulin ratio) not reliable to be evaluated due to the inadequate sample mixing prior to sample analysis.

Conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues reported in this study.

In Vivo Tox Studies

Study number 1: (b) (4) **Vaccine**

(b) (4)

10 pages have been determined to be not releasable: (b)(4)

(b) (4)

(b) (4)

In Vitro Tox Studies

Study number 1: (b) (4) Vaccine:

(b) (4)

One page has been determined to be not releasable: (b)(4)

(b) (4)

Study number 2:

(b) (4)

(b) (4)

14 pages have been determined to be not releasable: (b)(4)

(b) (4)

(b) (4)

Overall conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues reported in this BLA.

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