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Abbreviation	Definition
2AA	2-aminoanthracene
2NF	2-nitrofluorene
9AC	9-aminoacridine hemihydrate
A2M	α2-macroglobulin
A/G	albumin to globulin
AGP	α1-acid glycoprotein
APTT	activated partial thromboplastin time
BaP	benzo[a]pyrene
CBPI	cytokinesis-block proliferation index
CoV	coronavirus
CMV	cytomegalovirus
CP	cyclophosphamide
DMSO	dimethyl sulfoxide
DTPA	diethylenetriamine pentaacetic acid
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
ELISA	enzyme-linked immunosorbent assay
GLP	Good Laboratory Practice
gB	glycoprotein B
gH	glycoprotein H
gL	glycoprotein L
hMPV	human metapneumovirus
ICH	International Council for Harmonisation
IE	immature erythrocytes
IFN	interferon
Ig	immunoglobulin
IL	interleukin
IM	intramuscular
IP-10	interferon gamma-induced protein 10
IV	intravenous
LNP	lipid nanoparticle
MCP-1	monocyte chemoattractant protein 1
ME	mature erythrocytes

Abbreviation	Definition
MIE	micronucleated immature erythrocytes
MIP-1-α	macrophage inflammatory protein 1 alpha
MMC	mitomycin C
mRNA	messenger RNA
MTD	maximum tolerated dose
NaAz	sodium azide
NaCl	sodium chloride
NPI	nascent peptide imaging
NQO	4 nitroquinoline-N-oxide (NQO)
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PCE	polychromatic erythrocyte
PEG	polyethylene glycol
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PG	propylene glycol
PIV3	parainfluenza virus type 3
prME	pre-membrane and envelope
pp65	phosphoprotein 65
RDW	red blood cell distribution width
S	spike
S-2P	spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
S9 mix	Supplemented rat liver fraction
SARS-CoV-2	2019 novel coronavirus
SM-102	heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate
TNF	tumor necrosis factor
Tris	tris(hydroxymethyl)aminomethane
WBC	white blood cell

2.6.6.1 BRIEF SUMMARY

ModernaTX, Inc. (Sponsor) has used its messenger RNA (mRNA)-based rapid-response proprietary vaccine platform to develop mRNA-1273, a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine against the 2019 novel coronavirus (CoV; SARS-CoV-2). mRNA-1273 contains a single mRNA that encodes the full-length SARS-CoV-2 spike (S) protein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilize the S protein into the prefusion conformation. The mRNA is combined in a mixture of 4 lipids common to the Sponsor's mRNA vaccine platform: SM-102, cholesterol, DSPC, and PEG2000-DMG. The nonclinical toxicity studies to support the use of mRNA-1273 formulated in SM-102–containing LNPs were conducted in accordance with best scientific principles. Definitive studies were conducted according to Good Laboratory Practice (GLP) and include the appropriate documentation. The dates of study conduct, and location of the raw data are noted in the final reports provided in Module 4.

The safety and tolerability of mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102–containing LNPs have been evaluated in multiple GLP-compliant repeat-dose toxicity studies in Sprague Dawley rats (2 Zika virus vaccines: mRNA-1706 and mRNA-1893; 1 human metapneumovirus [hMPV] and parainfluenza virus type 3 [PIV3] vaccine: mRNA-1653; and 2 cytomegalovirus [CMV] vaccines: mRNA-1647 and mRNA-1443). The Sprague Dawley rat was selected as the animal model for the toxicity studies because it is an accepted rodent species for nonclinical toxicology testing by regulatory agencies and is a relevant species to assess the toxicity and immunogenicity of mRNA vaccines, as evidenced by immunogenic response.

The aggregate rat repeat-dose toxicity profile observed in these GLP studies at intramuscular (IM) doses ranging from 8.9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks is similar and consistent despite the different mRNA constructs that encode different antigens. Therefore, the Sponsor proposes that the toxicity associated with mRNA vaccines formulated in LNPs is driven primarily by the LNP composition and, to a lesser extent, the biologic activity of the antigen(s) encoded by the mRNA. Thus, the aggregate GLP repeat-dose rat data is considered to be representative of mRNA vaccines formulated in the same SM-102–containing LNPs and supports the development of mRNA-1273.

SM-102, the novel lipid used in mRNA-1273, and the commercially available PEG2000-DMG (b) (4) were evaluated in genotoxicity studies as individual agents using a standard International Council for Harmonisation (ICH) S2 (R1) approach (ICH 2011), including GLP-compliant bacterial reverse mutation (Ames) tests in *Salmonella*

typhimurium and *Escherichia coli* and GLP-compliant in vitro micronucleus tests in human peripheral blood lymphocytes. In addition, SM-102 was evaluated for in vivo genotoxicity risk in a GLP-compliant in vivo rat micronucleus test using an mRNA-based vaccine formulated in SM-102 LNPs (mRNA-1706) and in a non-GLP–compliant in vivo rat micronucleus test using a reporter mRNA (nascent peptide imaging [NPI] luciferase mRNA) formulated in SM-102 LNPs.

The Sponsor completed a GLP-compliant combined developmental and perinatal/postnatal reproductive toxicity study to assess the potential effects of mRNA-1273 on fertility and pre- and postnatal development in pregnant and lactating female Sprague Dawley rats. The Sponsor also completed a repeat-dose non-GLP study in Sprague Dawley rats to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses.

The pivotal toxicology studies were conducted with mRNA vaccines developed with the Sponsor's mRNA-based platform using SM-102–containing LNPs and support IM administration in humans. Rats were administered doses up to the anticipated maximum tolerated dose of 150 µg/dose, where clinical observations including vocalization (100 µg/dose) were accompanied by body weight loss and decrease in food consumption. The number of doses selected for the individual GLP studies was 1 more than the intended number of doses proposed for the individual clinical studies. The number of doses ranged from 3 to 4, and doses were administered every 2 weeks, or as determined based on the frequency of the anticipated clinical dosing regimen. The mRNA-1273 clinical presentations are summarized in Module 2.4.

All toxicology studies supportive of mRNA-1273 are listed in the Section 2.6.7 Toxicology Tabulated Summary, including information on the GLP compliance status and testing facility.

Table 1 summarizes the nonclinical toxicity program for mRNA-1273.

Table 1: Summary of Nonclinical Toxicology Program for mRNA-1273

Study Type	Test Article	Species, Strain	Method of Administration; Dose	GLP	Report Number
Repeat-Dose Toxicity					
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706[a]	Rat, Sprague Dawley	IM; 0, 13, 65, 129 µg/dose[b] (Days 1, 15, 29)	Yes	5002045
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706[a]	Rat, Sprague Dawley	IM; 0, 10, 50, 100 µg/dose (Days 1, 15, 29)	Yes	5002231
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1653[c]	Rat, Sprague Dawley	IM; 0, 10, 50, 150 µg/dose (Days 1, 15, 29)	Yes	5002033
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1893[d]	Rat, Sprague Dawley	IM; 0, 10, 30, 96 µg/dose (Days 1, 15, 29)	Yes	5002400
6-week (4 doses) repeat-dose study with 2-week recovery	mRNA-1647[e]	Rat, Sprague Dawley	IM; 0, 8.9, 27, 89 µg/dose[f] (Days 1, 15, 29, 43)	Yes	5002034
6-week (4 doses) repeat-dose study with 2-week recovery	mRNA-1443[g]	Rat, Sprague Dawley	IM; 0, 9.6, 29, 96 µg/dose[h] (Days 1, 15, 29, 43)	Yes	5002158
In Vitro Genotoxicity					
Bacterial reverse mutation test	SM-102	*Salmonella typhimurium, Escherichia coli*	Incubation for 67 h 29 min with 0, 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000 µg/plate SM-102 with or without supplemented rat liver fraction	Yes	9601567
	PEG2000-DMG (b) (4)	*Salmonella typhimurium, Escherichia coli*	Incubation for 67 h 57 min with 0, 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000 µg/plate PEG2000-DMG with or without supplemented rat liver fraction	Yes	9601035
Mammalian cell micronucleus test	SM-102	Human peripheral blood lymphocytes	Incubation for 4 and 24 h with 0, 163, 286, 500 µg/mL SM-102 with or without supplemented rat liver fraction	Yes	9601568
	PEG2000-(b) (4)	Human peripheral blood lymphocytes	Incubation for 4 and/or 24 h with 0, 53.3, 93.3, 163, 286, 500 µg/mL PEG2000-DMG with or without supplemented rat liver fraction	Yes	9601036
In Vivo Genotoxicity					
In vivo mammalian erythrocyte micronucleus test	mRNA-1706[a]	Rat, Sprague Dawley	Single IV; 0, 0.6/6.2 (F), 1.3/13.5, 2.6/27.0, 5.2/54.1 (M) mg/kg mRNA-1706/SM-102[j, k]	Yes	9800399

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

Abbreviations: CMV = cytomegalovirus; CoV = coronavirus; F = female; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; h = hour; hMPV = human metapneumovirus; IM = intramuscular; IV = intravenous; M = male; min = minute; mRNA = messenger RNA; NPI = nascent peptide imaging; PIV3 = parainfluenza virus type 3; pp65 = phosphoprotein 65; prME = pre-membrane and envelope; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus; SoA = summary of analysis.

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

[b] The original dose levels selected were 0, 10, 50, and 100 µg/dose, respectively (SoA issued on 11 October 2016). The calculated dose levels were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064 (SoA issued on 03 May 2017). The change in the reported mRNA content for mRNA-1706 was (b) (4)

[c] mRNA-1653 contains 2 distinct mRNA sequences that encode the full-length membrane-bound fusion proteins of hMPV and PIV3. The 2 mRNAs are combined at a target mass ratio of 1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 7% PG, 1 mM DTPA, pH 7.4.

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

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

[f] The original dose levels selected were 0, 10, 30, and 100 µg/dose, respectively (SoA issued on 16 March 2017). The calculated dose levels were revised based on the updated concentration reported for mRNA-1647 Lot No. MTDP17015 (SoA issued on 31 May 2017). The change in the reported mRNA content for mRNA-1647 was (b) (4)

[g] mRNA-1443 contains a single mRNA sequence that encodes a phosphorylation mutant of the CMV pp65 protein (ie, deletion of amino acids 435-438) combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

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

[i] Multiple test articles (b) (4) and MC3) were assessed in this study. Only data relevant to the development of mRNA-1273 are discussed in this dossier.

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

[k] The original dose levels selected were 0, 1.0, 2.0, 4.0, 0.5, 1.0, and 2.0 mg/kg mRNA-1706, respectively (SoA issued on 11 October 2016). The calculated dose levels were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064 (SoA issued on 03 May 2017). The change in the reported mRNA content for mRNA-1706 was (b) (4)

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

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

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

2.6.6.2 SINGLE-DOSE TOXICITY

No single-dose toxicity studies have been performed with mRNA-1273.

2.6.6.3 REPEAT-DOSE TOXICITY

The pivotal Biologics License Application-enabling toxicology studies were conducted with SM-102–containing LNPs.

2.6.6.3.1 A 1-Month (3 Doses) Intramuscular Injection Toxicity Study of mRNA-1706 in Sprague Dawley Rats With a 2-Week Recovery Period

The objectives were to examine the potential toxicity of mRNA-1706 when given by IM injection for 1 month (3 doses) to Sprague Dawley rats on Days 1, 15, and 29 and to evaluate the potential reversibility of any findings after a 2-week recovery period (Report 5002045; GLP-compliant). mRNA-1706 contains a single mRNA sequence that encodes the pre-membrane and envelope (prME) structural proteins of Zika virus combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 20 mM Tris, 8% sucrose, pH 7.4.

Methods:

Sprague Dawley rats were administered 3 doses of 13, 65, or 129 µg/dose mRNA-1706 or control (phosphate-buffered saline [PBS]) via IM injection into the lateral compartment of the thigh alternating legs on Days 1, 15, and 29 (Table 2). Animals were monitored for clinical observations, body weight, food consumption, ophthalmology examinations, clinical pathology parameters (hematology, coagulation, clinical chemistry), and cytokine analysis (interleukin [IL]-1ß, IL-6, tumor necrosis factor [TNF]-α, interferon gamma-induced protein 10 [IP-10], macrophage inflammatory protein 1 alpha [MIP-1-α], monocyte chemoattractant protein 1 [MCP-1], interferon [IFN]-α). Concentrations of IFN-α were not reported due to technical issues with the assay. Immunoglobulin (Ig) G antibody titers were analyzed using an enzyme-linked immunosorbent assay (ELISA). Main study animals were euthanized the day after completion of dose administration (Day 30), and recovery animals were euthanized after a 14-day recovery period (Day 43). Gross necropsy findings, organ weights, and histopathologic examinations were performed at termination. Study findings were expressed as pair-wise comparisons made with the control group.

Table 2: Experimental Design for 1-Month (3 Doses) Repeat-Dose Toxicity Study of mRNA-1706 in Sprague Dawley Rats

Group Number	Test Material	Dose Level[a] (µg/dose)	Dose Volume (µL/dose)	Dose Concentration (µg/mL)	Number of Animals			
					Main Study		Recovery Study	
					M	F	M	F
1	Control (PBS)	0	200	0	10	10	5	5
2	mRNA-1706	13	200	70	10	10	NE	NE
3	mRNA-1706	65	200	330	10	10	NE	NE
4	mRNA-1706	129	200	650	10	10	5	5

Abbreviations: F = female; M = male; mRNA = messenger RNA; NE = not evaluated; PBS = phosphate-buffered saline; SoA = summary of analysis.

[a] The original dose levels selected were 0, 10, 50, and 100 µg/dose, respectively (SoA issued on 11 October 2016). The calculated dose levels were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064 (SoA issued on 03 May 2017). The change in the reported mRNA content for mRNA-1706 was (b) (4)

Source: Report 5002045.

Results:

There were no mRNA-1706–related effects on mortality, ophthalmology findings, or organ weights. No mRNA-1706–related cytokine changes were observed for IL-1ß and IL-6 levels.

All mRNA-1706–treated groups showed detectable antibody titers to the Zika virus after 2 doses (Day 30), and administration of the third 129 µg/dose boosted the antibody titers further by Day 43.

mRNA-1706–related clinical observations were noted at ≥ 13 µg/dose and included warm to the touch in 129 µg/dose females (Day 2); and dose-dependent edema and, less frequently, erythema at the injection site after the first dose at ≥ 13 µg/dose. The apex of severity was generally 24 hours post-dose and was decreased at 72 hours post-dose. Injection site observations consisted of slight to moderate edema and slight to mild erythema with occasional severe edema noted at 129 µg/dose. Increased severity of the edema was observed in 129 µg/dose females from Day 2 through Day 4 and correlated with warm to the touch observed clinically on Day 2; and at ≥ 13 µg/dose after the third dose (Day 29).

mRNA-1706–related, minimal decreases in body weight and food consumption were observed at 129 µg/dose. Higher body weight gains were noted for both sexes while food consumption returned to control values during between doses 1 and 2 and doses 2 and 3.

mRNA-1706–related, minimal to moderate clinical pathology (hematology, coagulation, clinical chemistry) changes were observed at ≥ 13 µg/dose on Day 30 and included increases in neutrophil, eosinophil (males and females ≥ 13 µg/dose), and large unstained cell counts with concomitant increases in white blood cell (WBC) counts (males ≥ 13 µg/dose); decreases in lymphocyte counts (females ≥ 13 µg/dose), reticulocyte counts (males ≥ 13 µg/dose), and platelet counts (males 129 µg/dose, females ≥ 65 µg/dose); and increases in globulin and decreases in albumin with concomitant decreases in albumin to globulin (A/G) ratio (males ≥ 13 µg/dose).

mRNA-1706–related changes in cytokines were observed at 129 µg/dose (the only treatment group analyzed for cytokine levels) and included statistically significant increases in IP-10, MCP-1, MIP-1-α (females only), and TNF-α (males only). The highest cytokine levels were generally reached on Day 29 and correlated with the severity of edema and/or erythema noted at the injection site.

At the terminal necropsy (Day 30), mRNA-1706–related macroscopic changes were observed at ≥ 13 µg/dose and were limited to the injection site (firm consistency, swelling) and to the inguinal, popliteal, and iliac lymph nodes (enlargement).

mRNA-1706–related microscopic changes were observed at ≥ 13 µg/dose and included minimal to moderate inflammation at the injection site; minimal to mild mixed cell infiltration in and around the popliteal and inguinal lymph nodes; dose-dependent minimal to mild depletion of lymphocytes in the periarteriolar sheath in the spleen; and dose-dependent increases in the incidence of minimal hepatocytic vacuolation with no macroscopic changes.

At the end of the recovery period (Day 43), all changes were partially or fully recovered at 129 µg/dose. mRNA-1706–related enlargement of the lymph nodes (inguinal and popliteal) noted at the terminal euthanasia was still observed in 129 µg/dose males, but at a lower incidence and without microscopic correlation.

Conclusions:

Administration of mRNA-1706 by IM injection for 1 month (3 doses) was clinically well tolerated in rats up to 129 µg/dose. At ≥ 13 µg/dose, dose-dependent changes in clinical signs, clinical pathology parameters, and cytokine levels were consistent with an inflammatory response at the injection site. Dose-dependent target organ effects were observed at the injection site, tissues surrounding lymph nodes regional to the injection site, spleen, and liver of mRNA-1706–treated animals. At the end of the recovery period, all changes were partially or fully recovered.

2.6.6.3.2 A 1-Month (3 Doses) Intramuscular Injection Toxicity Study of mRNA-1706 in Sprague Dawley Rats With a 2-Week Recovery Period

The objectives were to examine the potential toxicity of mRNA-1706 when given by IM injection for 1 month (3 doses) to Sprague Dawley rats on Days 1, 15, and 29 and to evaluate the potential reversibility of any findings over a 2-week recovery period (Report 5002231; GLP-compliant). mRNA-1706 contains a single mRNA sequence that encodes the prME structural proteins of Zika virus combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 20 mM Tris, 8% sucrose, pH 7.4.

Methods:

Sprague Dawley rats were administered 3 doses of 10, 50, or 100 µg/dose mRNA-1706 or control (PBS) via IM injection into the lateral compartment of the thigh alternating legs on Days 1, 15, and 29 (Table 3). Animals were monitored for clinical observations, body weight, food consumption, ophthalmic examinations, body temperature, clinical pathology parameters

(hematology, coagulation, clinical chemistry), and cytokine analysis (IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α, MCP-1). IgG antibody titers were analyzed using an ELISA. Main study animals were euthanized the day after completion of dose administration (Day 30), and recovery animals were euthanized after a 14-day recovery period (Day 43). Gross necropsy findings, organ weights, and histopathologic examinations were performed at termination. Study findings were expressed as pair-wise comparisons made with the control group, when appropriate.

Table 3: Experimental Design for 1-Month (3 Doses) Repeat-Dose Toxicity Study of mRNA-1706 in Sprague Dawley Rats

Group Number	Test Material	Dose Level (µg/dose)	Dose Volume (µL/dose)	Dose Concentration (µg/mL)	Number of Animals			
					Main Study		Recovery Study	
					M	F	M	F
1	Control (PBS)	0	200	0	10	10	5	5
2	mRNA-1706	10	200	50	10	10	NE	NE
3	mRNA-1706	50	200	250	10	10	NE	NE
4	mRNA-1706	100	200	500	10	10	5	5

Abbreviations: F = female; M = male; NE = not evaluated; PBS = phosphate-buffered saline.
Source: Report 5002231.

Results:

There were no mRNA-1706–related effects on mortality, food consumption, or ophthalmology findings. No mRNA-1706–related cytokine changes were observed for IL-1β, TNF-α, and IL-6 levels.

All mRNA-1706–treated animals, except for one 10 µg/dose male animal, showed detectable antibody titers to the Zika virus after 2 doses (Day 30), and administration of the third 100 µg/dose boosted the antibody titers further by Day 43.

mRNA-1706–related clinical observations were noted at ≥ 10 µg/dose and included a general dose-dependent increase in incidence and/or severity of injection site swelling after dose administration; extension of the injection site swelling to hindlimb and inguinal areas at 100 µg/dose; skin redness at the injection site with higher incidence throughout the dosing period at ≥ 50 µg/dose; and prominent backbone, thinness, hunched posture, and suspected dehydration in some 100 µg/dose females.

mRNA-1706–related, lower mean body weight gains was observed after each dose at ≥ 10 µg/dose and sometimes reached statistical significance. From Days –1 to 28, the changes were 0.60-fold (males) and 0.78-fold (females) of control means.

mRNA-1706–related, significant, minimal increases in mean body temperature were observed at ≥ 50 µg/dose at 6 and/or 24 hours post-dose on Days 1 and 29 compared with the control group and pre-dose measurements.

mRNA-1706–related, minimal to moderate clinical pathology (hematology, coagulation, clinical chemistry) changes were observed at ≥ 10 µg/dose on Day 30 and included increases in neutrophil, monocyte, eosinophil, and large unstained cell counts with concomitant increases in WBC counts; decreases in lymphocyte counts and platelet counts (females ≥ 50 µg/dose); increases in activated partial thromboplastin time (APTT) and fibrinogen; dose-dependent decreases in glucose concentration; and increases in globulin and decreases in albumin with concomitant decreases in A/G ratio.

mRNA-1706–related changes in cytokines were observed at 100 µg/dose and included significantly increased IP-10 and increased MIP-1-α and MCP-1.

At the terminal necropsy (Day 30), mRNA-1706–related increases in adrenal weights were observed in 100 µg/dose males and ≥ 50 µg/dose females and correlated with cortical hypertrophy.

mRNA-1706–related macroscopic changes were observed at ≥ 10 µg/dose and were limited to the injection site (firm consistency, swelling, occasional presence of a clot) and/or hemorrhage; and to the inguinal, popliteal, and iliac lymph nodes (enlargement).

mRNA-1706–related microscopic changes were observed at the injection site, draining lymph nodes of the injection site (iliac, inguinal, popliteal), bone marrow, adrenal glands, liver, mesenteric lymph node, thymus, and/or spleen.

At the injection site, minimal to marked inflammation was observed in ≥ 10 µg/dose males and females and 2 control females. It was characterized locally by extensive infiltration of mixed inflammatory cells, mainly granulocytes, with associated edema and fibrin exudates and correlated macroscopically with firmness of the thigh (abnormal consistency; firm). It was accompanied by mild to moderate hemorrhage (correlating macroscopically with material accumulation; clot) in some 50 µg/dose males.

In the lymph nodes (iliac, inguinal, and/or popliteal), minimal to moderate mixed cell infiltration was observed in ≥ 50 µg/dose males and ≥ 10 µg/dose females. The inflammatory infiltrate was composed of clusters of degenerated granulocytes in the lymph node sinuses or in the adjacent adipose/connective tissue.

In the bone marrow, dose-dependent minimal increases in myeloid cellularity were observed at ≥ 10 µg/dose.

In the adrenal glands, dose-dependent minimal cortical hypertrophy was present at ≥ 50 µg/dose and correlated with the increased adrenal weights.

In the liver, minimal to mild hypertrophy of the Kupffer cells was observed in low incidence at ≥ 10 µg/dose with no clear dose relationship. The hypertrophied Kupffer cells sometimes contained finely granular brownish pigment. In 1 male at 10 µg/dose, 1 female at 50 µg/dose, and 3 females at 100 µg/dose, there was minimal to mild degeneration in the centrilobular region characterized by presence of mixed inflammatory cells in the sinusoid with single cell necrosis or degeneration of hepatocytes.

Microscopic findings in the spleen and mesenteric lymph nodes at ≥ 10 µg/dose and in the thymus in ≥ 50 µg/dose males and 100 µg/dose females were consistent with minimal to mild decreased lymphoid cellularity and/or single cell necrosis of lymphocytes. The decreased lymphoid cellularity was present in the periarteriolar sheath of the spleen, in the cortex of the thymus, and in the paracortex of the mesenteric lymph node. In these areas, the lymphoid cells were less densely populated with prominent dendric cells. Single cell necrosis of lymphocytes in the spleen and decreased lymphoid cellularity in the thymus were observed at 100 µg/dose.

At the end of the 2-week recovery period (Day 43), all changes were partially or fully recovered at 100 µg/dose. At the injection site, minimal mononuclear infiltration was observed in 100 µg/dose males and females and in 1 control female. This microscopic finding was interpreted to result from the healing process from previous inflammation at the injection site.

Conclusions:

Administration of mRNA-1706 by IM injection for 1 month (3 doses) was clinically well tolerated in rats up to 100 µg/dose. At ≥ 10 µg/dose, generally dose-dependent changes in clinical signs, clinical pathology parameters, and cytokines were consistent with an inflammatory response at the injection site. Dose-dependent target organ effects were observed at the injection site, bone marrow, tissues surrounding lymph nodes regional to the injection site, adrenal gland, liver, spleen, and thymus of mRNA-1706–treated animals. At the end of the recovery period, all changes were partially or fully recovered.

2.6.6.3.3 A 1-Month (3 Doses) Intramuscular Injection Toxicity Study of mRNA-1653 in Sprague Dawley Rats With a 2-Week Recovery Period

The objectives were to examine the potential toxicity of mRNA-1653 when given by IM injection for 1 month (3 doses) to Sprague Dawley rats on Days 1, 15, and 29 and to evaluate the potential reversibility of any findings over a 2-week recovery period (Report 5002033; GLP-compliant). mRNA-1653 contains 2 distinct mRNA sequences that encode the full-length membrane-bound fusion proteins of hMPV and PIV3. The 2 mRNAs are combined at a target mass ratio of 1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 7% PG, 1 mM DTPA, pH 7.4.

Methods:

Sprague Dawley rats were administered 3 doses of 10, 50, or 150 µg/dose mRNA-1653 or control (PBS) via IM injection into the lateral compartment of the thigh alternating legs on Days 1, 15, and 29 (Table 4). Animals were monitored for clinical observations, body weight, food consumption, ophthalmic examinations, body temperature, clinical pathology parameters (hematology, coagulation, clinical chemistry), and cytokine analysis (IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α, MCP-1). IgG antibody titers were analyzed using a neutralizing antibody assay. Main study animals were euthanized the day after completion of dose administration (Day 30), and recovery animals were euthanized after a 14-day recovery period (Day 43). Gross necropsy findings, organ weights, and histopathologic examinations were performed at termination. Study findings were expressed as pair-wise comparisons made with the control group, when appropriate.

Table 4: Experimental Design for 1-Month (3 Doses) Repeat-Dose Toxicity Study of mRNA-1653 in Sprague Dawley Rats

Group Number	Treatment	Dose Level (µg/dose)	Dose Volume (µL)	Dose Concentration (µg/mL)	Number of Animals			
					Main Study		Recovery Study	
					M	F	M	F
1	Control (PBS)	0	200	0	10	10	5	5
2	mRNA-1653	10	200	50	10	10	NE	NE
3	mRNA-1653	50	200	250	10	10	NE	NE
4	mRNA-1653	150	200	750	10	10	5	5

Abbreviations: F = female; M = male; NE = not evaluated; PBS = phosphate-buffered saline.
Source: Report 5002033.

Results:

There were no mRNA-1653–related effects on mortality or ophthalmology findings. No mRNA-1653–related cytokine changes were observed for IL-1ß, IL-6, and TNF-α levels.

All mRNA-1653–treated groups showed detectable antibody titers to hMPV/A2 and PIV3 after 2 doses (Day 30), and administration of the third 150 µg/dose boosted the antibody titers further by Day 43.

mRNA-1653–related clinical observations related to dose-dependent local inflammation at the injection site were noted at ≥ 10 µg/dose and included slight to severe edema peaking 24 hours post-dose (Days 2, 16, and 30) and generally decreasing by 72 hours post-dose (Days 4, 18, and 32 [recovery animals only]); sporadic, slight to severe (on rare occasions) erythema after each dose administration that was considered mRNA-1653–related at 150 µg/dose; and swelling (soft or firm) and localized skin redness at 150 µg/dose after the second dose and occasionally present after the third dose.

mRNA-1653–related, dose-dependent lower mean body weight gains were noted in ≥ 10 µg/dose males and ≥ 50 µg/dose females; these changes sometimes reached statistical significance and were associated with slightly reduced food consumption at 150 µg/dose.

mRNA-1653–related, minimal increases in body temperature were observed at ≥ 10 µg/dose at 6 and/or 24 hours post-dose after the first and third doses (Days 1 and 29) and were generally statistically significant.

mRNA-1653–related, minimal to marked clinical pathology (hematology, coagulation, clinical chemistry) changes were observed at ≥ 10 µg/dose on Day 30 and included increases in neutrophil, eosinophil, and large unstained cell counts with concomitant increases in WBC counts; decreases in lymphocyte counts (males ≥ 10 µg/dose, females ≥ 50 µg/dose), reticulocyte counts (males ≥ 10 µg/dose), and platelet counts (females ≥ 10 µg/dose); increases in APTT and fibrinogen; and increases in globulin and decreases in albumin with concomitant decreases in A/G ratio.

mRNA-1653–related changes in cytokines were observed at 150 µg/dose and included increases in IP-10, MCP-1, and MIP-1-α.

At the terminal necropsy (Day 30), mRNA-1653–related significant increases were observed in liver weights (150 µg/dose females) and spleen weights (≥ 50 µg/dose males, ≥ 10 µg/dose females). These weight changes had no microscopic correlation.

mRNA-1653–related macroscopic changes were observed at ≥ 10 µg/dose and were limited to the injection site (firm consistency, swelling, thick); and popliteal and iliac lymph nodes (enlargement).

mRNA-1653–related microscopic changes were observed at the injection site, draining lymph nodes of the injection site (iliac, inguinal, popliteal), bone marrow, liver, and spleen.

At the injection site, dose-dependent minimal to marked mixed cell inflammation was observed in all groups including the control group and correlated macroscopically with firm abnormal consistency, swelling, and/or thick. It was characterized by an infiltration of mostly neutrophils but also macrophages and lymphocytes in the IM connective tissue and subcutis. Edema, necrotic debris, hemorrhage, and/or rare degenerated myofibers were also present.

In the lymph nodes (iliac, inguinal, and/or popliteal), minimal to moderate perinodal mixed cell inflammation with generally increased incidence and severity in the popliteal lymph node was observed at ≥ 10 µg/dose and correlated macroscopically with enlargement. Minimal to mild mixed cell inflammation was also noted in the sciatic nerve and connective tissue surrounding the nerve of animals at ≥ 10 µg/dose. The lymph nodes and sciatic nerve inflammatory changes were regarded as secondary to the injection site inflammation.

In the liver, minimal to mild hepatocellular vacuolation were observed in all groups including the control group and consisted of the presence of intracytoplasmic microvesicles with enlarged hepatocytes. The increased incidence and severity observed at 150 µg/dose was considered mRNA-1653–related.

In the bone marrow, minimal to mild increased hematopoiesis of the myeloid lineage was observed at ≥ 10 µg/dose and was likely a reactive response to the inflammation observed at the injection site.

In the spleen, minimal to mild decreased cellularity of the periarteriolar lymphoid sheath was observed at ≥ 10 µg/dose and was often associated with an increase in macrophages.

At the end of the 2-week recovery period (Day 43), all changes partially or fully recovered at 150 µg/dose. mRNA-1653–related microscopic changes at the recovery necropsy included mixed cell inflammation around the popliteal lymph node and mononuclear cell infiltration around the sciatic nerve and at the injection site. These remaining findings generally occurred with a decreased incidence and/or severity indicating partial recovery.

Conclusions:

Administration of mRNA-1653 by IM injection for 1 month (3 doses) was clinically well tolerated in rats up to 150 µg/dose. At ≥ 10 µg/dose, generally dose-dependent changes in clinical signs, clinical pathology parameters, and cytokines were consistent with an inflammatory response at the injection site. Dose-dependent target organ effects were observed at the injection site, bone marrow, tissues surrounding lymph nodes regional to the injection site, the connective tissue surrounding the sciatic nerve, the spleen, and the liver of mRNA-1653–treated animals. At the end of the 2-week recovery period, all changes were fully recovered with exception of the injection site, popliteal lymph node, and the connective tissue surrounding the sciatic nerve which were considered to be partially recovered.

2.6.6.3.4 A 1-Month (3 Doses) Intramuscular Injection Toxicity Study of mRNA-1893 in Sprague Dawley Rats With a 2-Week Recovery Period

The objectives were to examine the potential toxicity of mRNA-1893 when given by IM injection for 1 month (3 doses) to Sprague Dawley rats on Days 1, 15, and 29 and to evaluate the potential reversibility of any findings after a 2-week recovery period (Report 5002400; GLP-compliant). mRNA-1893 contains a single mRNA sequence that encodes the prME structural proteins of Zika virus in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 100 mM Tris, 7% PG, 1 mM DTPA, pH 7.5.

Methods:

Sprague Dawley rats were administered 3 doses of 10, 30, or 96 µg/dose mRNA-1893 or control (PBS) via IM injection into the lateral compartment of the thigh alternating legs on Days 1, 15, and 29 (Table 5). Animals were monitored for clinical observations, body weight, food consumption, ophthalmic examinations, body temperature, clinical pathology parameters (hematology, coagulation, clinical chemistry, α1-acid glycoprotein [AGP], α2-macroglobulin [A2M]), and cytokines (IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α, MCP-1). IgG antibody titers were analyzed using a neutralizing antibody assay. Main study animals were euthanized the day after completion of dose administration (Day 30), and recovery animals were euthanized after a 14-day recovery (Day 43). Gross necropsy findings, organ weights, and histopathologic

examinations were performed at termination. Study findings were expressed as pair-wise comparisons made with the control group.

Table 5: Experimental Design for 1-Month (3 Doses) Repeat-Dose Toxicity Study of mRNA-1893 in Sprague Dawley Rats

Group Number	Test Material	Dose Level (µg/dose)	Dose Volume (µL/dose)	Dose Concentration (µg/mL)	Number of Animals			
					Main Study		Recovery Study	
					M	F	M	F
1	Control (PBS)	0	200	0	10	10	5	5
2	mRNA-1893	10	200	50	10	10	NE	NE
3	mRNA-1893	30	200	150	10	10	NE	NE
4	mRNA-1893	96	200	480	10	10	5	5

Abbreviations: F = female; M = male; NE = not evaluated; PBS = phosphate-buffered saline.
Source: Report 5002400.

Results:

There were no mRNA-1893–related effects on mortality, body weight, food consumption, or ophthalmology findings. No mRNA-1893–related cytokine changes were observed for TNF-α levels.

All mRNA-1893–treated groups showed detectable antibody titers to the Zika virus after 2 doses (Day 30), and administration of the third 96 µg/dose boosted the antibody titers further by Day 43.

mRNA-1893–related, dose-dependent clinical observations were noted at ≥ 10 µg/dose and included inflammatory changes at the injection site characterized by firmness, swelling, localized skin redness, and/or scabbed; increased vocalization from individual male and female animals; abnormal gait in 2 females and 1 male animal at 96 µg/dose; and limited usage of the left hindlimb in 1 female animal at 96 µg/dose.

mRNA-1893–related, minimal dose-dependent increases in mean body temperature (from 37.47 to 39.13°C) were observed in ≥ 10 µg/dose males at generally 6 hours post-dose on Days 1 and 29. Body temperatures generally returned to pre-dose values at 24-hour post-dose (Days 2 and 30), except for a few animals at 10 and 30 µg/dose, where body temperatures returned to baseline at 48 hours post-dose (Day 3).

mRNA-1893–related, minimal to moderate clinical pathology (hematology, coagulation, clinical chemistry, AGP, A2M) changes were observed at ≥ 10 µg/dose on Day 30 and included increases

in neutrophil, monocyte (except females 96 µg/dose), and eosinophil counts with concomitant increases in WBC counts; increases in red blood cell distribution width (RDW) (≥ 30 µg/dose) and decreases in reticulocyte counts (males 96 µg/dose); decreases in lymphocytes (96 µg/dose); increases in APTT (males ≥ 30 µg/dose, females ≥ 10 µg/dose) and fibrinogen; increases in globulin and/or decreases in albumin (≥ 30 µg/dose) with concomitant decreases in A/G ratio; and decreases in glucose (males 30 µg/dose, females ≥ 30 µg/dose). Dose-dependent increases in AGP and A2M were also noted at ≥ 10 µg/dose on Day 30.

mRNA-1893–related changes in cytokines were observed at ≥ 10 µg/dose and included increases in IL-1β, IL-6, MCP-1, IP-10, and MIP-1-α.

At the terminal necropsy (Day 30), mRNA-1893–related increases in spleen weights were observed in 96 µg/dose males and ≥ 30 µg/dose females and had no macroscopic or microscopic correlates.

mRNA-1893–related macroscopic changes were observed at ≥ 10 µg/dose and were limited to the injection site (firm consistency, swelling, thick, dark focus); and to the inguinal, popliteal, and iliac lymph nodes (enlargement).

mRNA-1893–related microscopic changes were observed at the injection site, sciatic nerve, draining lymph nodes of injection site (iliac, inguinal, popliteal lymph nodes), liver, spleen, bone marrow, and seminal vesicle (males).

At the injection site, minimal to moderate mixed cell inflammation was observed in all groups including the control group with exacerbation of inflammation noted at ≥ 10 µg/dose based on the distribution and increased incidence. In mRNA-1893–treated groups, the mixed cell inflammation was accompanied by edema, rarely hemorrhage and formation of microabscesses, and was found in the dermis, subcutaneous, and perimuscular tissue and skeletal muscle. Minimal epidermal hyperplasia was also noted in 96 µg/dose males and ≥ 10 µg/dose females, with higher incidence at 96 µg/dose. There was also evidence of an extension of mixed cell inflammation from the injection site into the surrounding connective tissue affecting mainly sciatic nerve and iliac, inguinal and/or popliteal lymph nodes at ≥ 10 µg/dose. The popliteal lymph nodes were the most frequently affected lymph nodes followed by the iliac lymph nodes. Of note, the mixed cell inflammation was sometimes extending into other tissues adjacent to the injection site (perifemoral tissue, inguinal skin/mammary gland, and quadriceps femoris muscle).

In the lymph nodes (iliac, inguinal, and/or popliteal), there was a higher incidence and/or severity of increased lymphoid cellularity in ≥ 10 µg/dose males and/or females compared with

the control group and minimal to moderate focal/multifocal neutrophilic inflammation with necrosis in ≥ 30 µg/dose females. These lymph node changes were regarded as a secondary or reactive response to the injection site inflammation and correlated with the enlargement described grossly.

In the liver, there was minimal to mild microvesicular periportal to midzonal hepatocellular vacuolation in 96 µg/dose males and ≥ 30 µg/dose females. In addition, minimal to mild hypertrophy of Kupffer cells was observed in ≥ 30 µg/dose males and ≥ 10 µg/dose females. The Kupffer cells were enlarged with a prominent nucleus and abundant vacuolated and/or granular cytoplasm.

In the spleen, there was minimal to mild decreased cellularity of the periarteriolar lymphoid sheath at 96 µg/dose; minimal to mild increased cellularity of macrophages in red pulp in 96 µg/dose males and ≥ 30 µg/dose females; minimal to mild neutrophilic infiltration in the red pulp in ≥ 30 µg/dose males and ≥ 10 µg/dose females; and minimal increased extramedullary hematopoiesis in ≥ 30 µg/dose males.

In the bone marrow, there was minimal increased cellularity of myeloid lineage in ≥ 30 µg/dose males and ≥ 10 µg/dose females. This change as well as the increased extramedullary hematopoiesis seen in the spleen was considered to be a secondary response to the inflammation observed at the injection site.

In the seminal vesicle, there was minimally increased epithelial single cell necrosis in 96 µg/dose males.

At the end of the 2-week recovery period (Day 43), all changes were partially or fully recovered at 96 µg/dose. mRNA-1893–related minimal to moderate increases in neutrophils, monocytes, eosinophils, RDW, and A2M; and minimal decreases in glucose (females only) were still noted. mRNA-1893–related enlarged iliac lymph node was observed in 1 male. mRNA-1893–related microscopic changes were observed in males and/or females and included mixed cell inflammation without edema or mononuclear cell infiltration and epidermal hyperplasia at the injection site; perineurial mixed cell inflammation in the surrounding connective tissue of sciatic nerve; perinodal mixed cell inflammation and increased lymphoid cellularity in the lymph nodes (iliac, inguinal and/or popliteal); and periportal to midzonal hepatocellular vacuolation in the liver. These remaining findings occurred with decreased incidence and/or severity indicating partial recovery.

Conclusions:

Administration of mRNA-1893 by IM injection for 1 month (3 doses) was clinically well tolerated (no mortality, changes in food consumption or deleterious changes in body weight, hematology, coagulation, or clinical chemistry parameters) in rats up to 96 µg/dose. At ≥ 10 µg/dose, dose-dependent clinical signs (swelling, firmness, redness, scabs) at the injection site, clinical pathology parameters, and cytokines levels along with minimal to mild increase in body temperatures were consistent with an inflammatory reaction. Dose-dependent target organ effects were limited to the injection site, perineural tissue of the sciatic nerve, iliac, inguinal, and popliteal lymph nodes, liver, spleen, seminal vesicle, and bone marrow of animals given mRNA-1893. In general, changes were partially or fully recovered at the end of the 2-week recovery period.

2.6.6.3.5 A 6-Week (4 Doses) Intramuscular Injection Toxicity Study of mRNA-1647 in Sprague Dawley Rats With a 2-Week Recovery Period

The objectives were to examine the potential toxicity of mRNA-1647 when given by IM injection for 6 weeks (4 doses) to Sprague Dawley rats on Days 1, 15, 29, and 43 and to evaluate the potential reversibility of any findings over a 2-week recovery period (Report 5002034; GLP-compliant). mRNA-1647 contains 6 mRNAs that encode the full-length CMV glycoprotein B (gB) and the pentameric glycoprotein H (gH)/glycoprotein L (gL)/UL128/UL130/UL131A glycoprotein complex. The 6 mRNAs are combined at a target mass ratio of 1:1:1:1:1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

Methods:

Sprague Dawley rats were administered 4 doses of 8.9, 27, or 89 µg/dose mRNA-1647 or control (PBS) via IM injection into the lateral compartment of the thigh alternating legs on Days 1, 15, 29, and 43 (Table 6). Animals were monitored for clinical observations, body weight, food consumption, ophthalmic examinations, body temperature, clinical pathology parameters (hematology, coagulation, clinical chemistry, AGP, A2M), cytokine analysis (IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α, MCP-1), and peripheral blood mononuclear cell (PBMC) analysis. IgG antibody titers were analyzed using an ELISA. Main study animals were euthanized the day after completion of dose administration (Day 44), and recovery animals were euthanized after a 14-day recovery (Day 57). Gross necropsy findings, organ weights, and histopathologic

examinations were performed at termination. Study findings were expressed as pair-wise comparisons made with the control group unless otherwise noted, when appropriate.

Table 6: Experimental Design for 6-Week (4 Doses) Repeat-Dose Toxicity Study of mRNA-1647 in Sprague Dawley Rats

Group Number	Treatment	Dose Level[a] (µg/dose)	Dose Volume (µL/dose)	Dose Concentration[a] (µg/mL)	Number of Animals			
					Main Study		Recovery Study	
					M	F	M	F
1	PBS (control)	0	200	0	10	10	5	5
2	mRNA-1647	8.9	200	45	10	10	NE	NE
3	mRNA-1647	27	200	140	10	10	NE	NE
4	mRNA-1647	89	200	450	10	10	5	5

Abbreviations: F = female; M = males; mRNA = messenger RNA; NE = not evaluated; PBS = phosphate-buffered saline; SoA = summary of analysis.

[a] The original dose levels selected were 0, 10, 30, and 100 µg/dose, respectively (SoA issued on 16 March 2017). The calculated dose levels were revised based on the updated concentration reported for mRNA-1647 Lot No. MTDP17015 (SoA issued on 31 May 2017). The change in the reported mRNA content for mRNA-1647 was (b) (4)

Source: Report 5002034.

Results:

There were no mRNA-1647–related effects on mortality, food consumption, or ophthalmology findings. No mRNA-1647–related cytokine changes were observed in IL-1β, IL-6, MIP-1-α, and TNF-α levels.

All mRNA-1647–treated groups showed detectable antibody titers to the CMV gB protein and gH pentamer complex after 3 doses (Day 43). The antibody titer levels at 89 µg/dose were similar between Days 43 and 57.

mRNA-1647 elicited both CD4+ and CD8+ T cell responses to the CMV gB protein and gH pentamer complex. T cell responses during PBMC analysis were significantly variable within each test group with data trending towards higher T cells responses at higher doses of mRNA-1647.

One male in the control group was found dead on Day 43. The pathological evaluation revealed gross abnormal findings in the adrenal gland, kidneys, thymus, and lungs which were incidental and did not explain the cause of death.

mRNA-1647–related clinical observations were noted at ≥ 8.9 µg/dose at the injection site and included dose-dependent soft swelling after the fourth dose (Day 44); severe, firm swelling at 89 µg/dose after the third dose (Day 31) and fourth dose (Day 44) in 1 male only; dose-dependent slight to severe edema with the apex of severity noted 24 hours post-dose (Days 2, 14, 28, 44) and generally decreased 72 hours post-dose; and sporadic, slight to moderately severe (rare) erythema following the third and fourth doses, which was only mRNA-1647–related at 89 µg/dose.

mRNA-1647–related, lower mean body weight gains was observed after each dose in ≥ 8.9 µg/dose males and 89 µg/dose females and sometimes reached statistical significance. From Day –1 to 42, the changes were 0.86-fold (males) of control means.

mRNA-1647–related, significant increases in mean body temperature were observed at 89 µg/dose at 6 and/or 24 hours post-dose on Days 1 and 43 compared with the control group and pre-dose measurements.

mRNA-1647–related, minimal to moderate clinical pathology (hematology, coagulation, clinical chemistry, AGP, A2M) changes were observed at ≥ 8.9 µg/dose on Day 44 and included increases in neutrophil, eosinophil, and large unstained cell counts with concomitant increases in WBC counts (males ≥ 27 µg/dose, females ≥ 8.9 µg/dose); decreases in lymphocyte counts and platelet counts (females 89 µg/dose); increases in APTT and fibrinogen; increases in globulin and decreases in albumin with concomitant decreases in A/G ratio. Dose-dependent increases in AGP and A2M were also noted on Day 44.

mRNA-1647–related changes in cytokines were observed at 89 µg/dose and included significantly increased IP-10 and increased MCP-1.

At the terminal necropsy (Day 44), mRNA-1647–related increases in spleen weights were observed at ≥ 8.9 µg/dose; were statistically significant in 89 µg/dose males and ≥ 27 µg/dose females; and were not correlated with macroscopic or microscopic findings.

mRNA-1647–related macroscopic changes were observed at ≥ 8.9 µg/dose and were limited to the injection site (firm consistency, swelling, dark focus) and/or edema; and to the inguinal and/or popliteal lymph nodes (enlargement).

mRNA-1647–related microscopic changes were observed at the injection site, draining lymph nodes of the injection site (popliteal and/or inguinal), sciatic nerve, bone marrow, and spleen.

At the injection site, dose-dependent minimal to moderate mixed cell inflammation involving the subcutaneous tissues, skeletal muscle, and to a lesser extent the dermis, as well as associated minimal to moderate subcutaneous edema and minimal to mild myofiber degeneration was observed ≥ 8.9 μg/dose. The inflammatory reaction, which increased in severity with increasing dose, often extended along and expanded endomysial and perimysial tissue layers, encircling individual muscle fibers and/or bundles. This reaction was characterized by varying numbers of intact and degenerating neutrophils, mononuclear cells, and macrophages (mixed cell inflammation); accumulations of protein-rich fluid (edema); and varying degrees of myofiber degeneration.

In the lymph nodes (popliteal and/or inguinal), an increased incidence and/or severity of minimal to marked mixed cell inflammation were noted at ≥ 8.9 μg/dose. The inflammation often involved the adventitia surrounding the lymph nodes, and most commonly involved the popliteal lymph nodes.

In the perineurial tissue surrounding the sciatic nerve, minimal to marked mixed cell inflammation was frequently observed at ≥ 8.9 μg/dose. This finding was considered to be an extension of the inflammatory reaction at the IM injection sites to this region.

In the bone marrow, minimal increased myeloid hematopoiesis was noted at ≥ 27 μg/dose. This finding was characterized by increased numbers of myeloid precursors in the marrow and was secondary or compensatory inflammatory reaction noted at the IM injection sites.

In the spleen, a dose-dependent minimal to mild decreased cellularity of the periarteriolar lymphoid sheath was noted at ≥ 8.9 μg/dose.

At the end of the 2-week recovery period (Day 57), all changes were partially or fully recovered at 89 μg/dose. The concentrations of A2M levels were still slightly higher than the control group. In the spleen, a slight increase in absolute and/or relative organ weights were noted and were not correlated with macroscopic or microscopic findings. Microscopic findings at the recovery necropsy included a shift to mononuclear cell infiltration rather than mixed cell inflammation at the injection site; residual inflammatory reaction characterized by minimal to mild mononuclear cell infiltration involving the subcutaneous tissues and skeletal muscle at the injection site; minimal mixed cell inflammation in the perineurial tissue surrounding the sciatic nerve; minimal increased myeloid hematopoiesis in the bone marrow; and minimal decreased cellularity of the periarteriolar lymphoid sheath in the spleen. In general, these remaining findings occurred with a decreased incidence and/or severity indicating partial recovery.

Conclusions:

Administration of mRNA-1647 by IM injection for 6 weeks (4 doses) was clinically well tolerated in rats up to 89 µg/dose. At ≥ 8.9 µg/dose, generally dose-dependent changes in clinical signs at the injection site, clinical pathology parameters, cytokines, and acute protein levels were consistent with an inflammatory reaction. Dose-related target organ effects were limited to the injection site, bone marrow, inguinal and popliteal lymph nodes, connective tissue surrounding the sciatic nerve, and spleen of animals given mRNA-1647. At the end of the 2-week recovery period, all changes were generally partially or fully recovered.

2.6.6.3.6 A 6-Week (4 Doses) Intramuscular Injection Toxicity Study of mRNA-1443 in Sprague Dawley Rats With a 2-Week Recovery Period

The objectives were to examine the potential toxicity of mRNA-1443 when given by IM injection for 6 weeks (4 doses) to Sprague Dawley rats on Days 1, 15, 29, and 43 and to evaluate the potential reversibility of any findings over a 2-week recovery period (Report 5002158; GLP-compliant). mRNA-1443 contains a single mRNA sequence that encodes a phosphorylation mutant of the CMV phosphoprotein 65 (pp65) protein (ie, deletion of amino acids 435-438) combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

Methods:

Sprague Dawley rats were administered 4 doses of 9.6, 29, or 96 µg/dose mRNA-1443 or control (PBS) via IM injection into the lateral compartment of the thigh alternating legs on Days 1, 15, 29, and 43 (Table 7). Animals were monitored for clinical observations, body weight, food consumption, ophthalmic examinations, body temperature, clinical pathology parameters (hematology, coagulation, clinical chemistry, AGP, A2M), cytokines analysis (IL-1ß, IL-6, TNF-α, IP-10, MIP-1-α, MCP-1), and PBMC analysis. Blood samples were collected to assess IgG antibody titers but not analyzed due to technical issues with the assay. Main study animals were euthanized the day after completion of dose administration (Day 44), and recovery animals were euthanized after a 14-day recovery (Day 57). Gross necropsy findings, organ weights, and histopathologic examinations were performed at termination. Study findings were expressed as pair-wise comparisons made with the control group.

Table 7: Experimental Design for 6-Week (4 Doses) Repeat-Dose Toxicity Study of mRNA-1443 in Sprague Dawley Rats

Group Number	Treatment	Dose Level[a] (µg/dose)	Dose Volume (µL/dose)	Dose Concentration[a] (µg/mL)	Number of Animals			
					Main Study		Recovery Study	
					M	F	M	F
1	Control (PBS)	0	200	0	10	10	5	5
2	mRNA-1443	9.6	200	48	10	10	NE	NE
3	mRNA-1443	29	200	145	10	10	NE	NE
4	mRNA-1443	96	200	480	10	10	5	5

Abbreviations: F = female; M = male; mRNA = messenger RNA; NE = not evaluated; PBS = phosphate-buffered saline; SoA = summary of analysis.

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

Source: Report 5002158.

Results:

There were no mRNA-1443–related effects on mortality, clinical observation, body weight, food consumption, or ophthalmology findings. No mRNA-1443–related cytokines changes were observed in IL-1β, IL-6, MIP-1-α, and TNF-α levels.

mRNA-1443 elicited minimal and variable CD4+ and CD8+ T cell responses to pp65 in all groups.

mRNA-1443–related, dose-dependent clinical observations at the injection site were noted at ≥ 9.6 µg/dose and included very slight to moderate edema, with occasional severe edema noted at ≥ 29 µg/dose; very slight erythema in 96 µg/dose males; and very slight to mild erythema in ≥ 29 µg/dose females. The apex of severity was generally noted 24 hours post-dose and decreased 72 hours post-dose and were generally noted with a higher extent in female rats.

In general, minimal, dose-dependent mRNA-1443–related increases in mean body temperature were observed at ≥ 9.6 µg/dose from 6 hours to 24 hours post-dose.

mRNA-1443–related, minimal to moderate clinical pathology (hematology, coagulation, clinical chemistry, AGP, A2M) changes were observed at ≥ 9.6 µg/dose on Day 44 and included increases in neutrophil, eosinophil, and/or large unstained cell (males ≥ 9.6 µg/dose) counts with concomitant increases in WBC counts (males ≥ 29 µg/dose, females ≥ 9.6 µg/dose); decreases in lymphocyte (males ≥ 29 µg/dose), reticulocyte (males ≥ 9.6 µg/dose), and platelet

(females ≥ 9.6 µg/dose) counts; increases in APTT (males ≥ 29 µg/dose, females 96 µg/dose) and fibrinogen; decreases in albumin (males 96 µg/dose, females ≥ 29 µg/dose) and increases in globulin (males and females ≥ 29 µg/dose) with concomitant decreases in A/G ratio (males and females ≥ 29 µg/dose). Dose-dependent increases in AGP and A2M were also noted on Day 44 and were statistically significant at ≥ 29 µg/dose.

mRNA-1443–related changes in cytokines were observed at 96 µg/dose and included IP-10 and MCP-1.

At the terminal necropsy (Day 44), mRNA-1443–related increases in spleen weights were observed in ≥ 9.6 µg/dose males and 96 µg/dose females and were not correlated with macroscopic or microscopic findings.

mRNA-1443–related macroscopic changes were observed at ≥ 9.6 µg/dose and were limited to the injection site (firm consistency, swelling, dark focus, pale focus); and to the inguinal and popliteal lymph nodes (enlargement).

mRNA-1443–related microscopic changes were observed at ≥ 9.6 µg/dose at the injection site, draining lymph nodes of the injection site (inguinal and popliteal), sciatic nerve, bone marrow, liver, and spleen.

At the injection site, dose-dependent, minimal to marked mixed cellular inflammation accompanied by edema was noted at ≥ 9.6 µg/dose. This change was characterized by a stereotypic acute inflammatory milieu comprising increased clear space expanding the interstitium (ie, edema) accompanied by numerous neutrophils variably admixed with foamy macrophages, lymphocytes, and rare plasma cells and hemosiderophages, as well as variable quantities of extravasated erythrocytes (ie, hemorrhage).

In the lymph nodes (inguinal and/or popliteal), minimal to moderate mixed cellular inflammation was observed at ≥ 9.6 µg/dose. These changes were reflected by perinodal interstitial mixed cell inflammation variably accompanied by small amounts of edema. Inflammation did not extend into the lymph node capsule. The inguinal lymph node site generally had less incidence and severity of peripheral inflammation compared with the popliteal site. Intrinsic lymph node changes included lymphoid hyperplasia and medullary plasmacytosis; these changes were orderly and of a character and magnitude that would be expected of reactive lymph nodes that are a non-specific and appropriate secondary consequence of injection site inflammation. These non-specific reactive changes were observed in relevant locoregional injection site lymph nodes

(ie, inguinal, popliteal) at a higher rate and slightly higher magnitude at ≥ 9.6 µg/dose compared with the control group.

In the sciatic nerve, dose-dependent, minimal to marked mixed cellular inflammation of the perineurial tissue was observed in ≥ 9.6 µg/dose males and females and control group females and was variably accompanied by edema. Of note, the sciatic nerve contained no changes within the nerve fibers, inflammation did not broach the epineurium, and inflammation/edema was generally of a lesser severity than the injection site proper.

In the bone marrow, increased incidence of minimal to mild increased myeloid hematopoiesis (ie, myeloid hyperplasia) with a distinctive appearance was noted in ≥ 9.6 µg/dose males and ≥ 29 µg/dose females. Incidence in males and females generally trends upward with increasing dose, suggesting a dose-dependent effect. Microscopically, this change was consistently characterized by multifocal aggregates of precursor cells predominantly composed of early myeloid lineage and typically found adjacent to the cortex and/or trabeculae.

In the spleen, slightly increased incidence of minimally decreased cellularity of the periarteriolar lymphoid sheath was noted at ≥ 9.6 µg/dose. Microscopically, this change was characterized by subtle attrition of periarteriolar lymphocytes and variably accompanied by a slight increase in tingible body macrophages.

In the liver, microvascular hepatocellular vacuolation without nuclear displacement throughout periportal to midzonal regions was noted in all groups including the control group. However, this change demonstrated a dose-dependent increase in incidence and magnitude at ≥ 9.6 µg/dose, consistent with mRNA-1443–related exacerbation of a background lesion.

At the end of the 2-week recovery period (Day 57), all changes were partially or fully recovered at 96 µg/dose. Changes in clinical pathology parameters included increases in lymphocytes, eosinophils, and WBC counts in males; and decreases in neutrophils, eosinophils, and platelets in females. Microscopic findings at the recovery necropsy included interstitial to perivascular inflammatory population at the injection site that was minimal and comprised a mixture of lymphocytes and macrophages with rare plasma cells and was consistent with a healing process; minimal to mild infiltration of mononuclear cells in the sciatic nerve; minimal mononuclear cell infiltration was occasionally present within the interstitium and/or perivascularly in the inguinal and/or popliteal lymph nodes; intrinsic lymph node changes including lymphoid hyperplasia and medullary plasmacytosis; and microvascular hepatocellular vacuolation throughout the periportal to midzonal regions in the liver of 96 µg/dose and control groups. These remaining findings generally occurred with a decreased incidence and/or severity indicating partial recovery.

Conclusions:

Administration of mRNA-1443 by IM injection for 6 weeks (4 doses) was clinically well tolerated (no mortality, changes in body weight or food consumption, or deleterious changes in hematology, coagulation, or clinical chemistry parameters) in rats up to 96 µg/dose. At ≥ 9.6 µg/dose, dose-dependent changes clinical signs (edema/erythema) at the injection site, clinical pathology parameters, and cytokines/protein levels along with slight increase in body temperature were consistent with a systemic inflammatory response. Dose-dependent target organ effects were limited to the injection site, the tissues surrounding the sciatic nerve, the popliteal and inguinal lymph nodes, the spleen, the bone marrow, and the liver of animals given mRNA-1443. At the end of the recovery period, generally, all changes were partially or fully recovered.

2.6.6.4 GENOTOXICITY

2.6.6.4.1 In Vitro Nonmammalian Cell System

2.6.6.4.1.1 SM-102 Bacterial Reverse Mutation Test in *Salmonella typhimurium* and *Escherichia coli*

The objective was to determine the potential genotoxicity of SM-102 using the bacterial reverse mutation test (Ames test) in *S. typhimurium* and *E. coli* (Report 9601567; GLP-compliant). SM-102 was prepared as a stock solution (50 mg/mL) in ethanol, and all lower level formulations were made by serial dilution.

Methods:

Four strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537) and an *E. coli* strain (WP2 *uvr*A) were treated with SM-102 in the absence and presence of a supplemented rat liver fraction (S9 mix) at 37ºC for 67 hours and 29 minutes. SM-102 was tested over a wide range of dose levels from 19.0 µg/mL to 50,000 µg/mL (the standard limit dose for this assay) (Table 8). The negative control was ethanol. The positive controls included 2-nitrofluorene (2NF), sodium azide (NaAz), 9-aminoacridine hemihydrate (9AC), and 4-nitroquinoline-N-oxide (NQO) in the absence of S9 mix; and 2-aminoanthracene (2AA) and benzo[a]pyrene (BaP) in the presence of S9 mix. All concentrations of SM-102, as well as positive and negative controls, were evaluated in triplicate.

Table 8: Experimental Design for Bacterial Reverse Mutation Test of SM-102 in *Salmonella typhimurium* and *Escherichia coli*

Dose Number	Final Concentration (µg/plate)	Number of Strains	Number of Cultures	
			67 h 29 min (–S9)	67 h 29 min (+S9)
Ethanol (negative control)	–	5	3	3
1/SM-102	1.58	5	3	3
2/SM-102	5.0	5	3	3
3/SM-102	15.8	5	3	3
4/SM-102	50	5	3	3
5/SM-102	158	5	3	3
6/SM-102	500	5	3	3
7/SM-102	1581	5	3	3
8/SM-102	5000[a]	5	3	3
2-nitrofluorene (positive control)	1	1	3	NE
Sodium azide (positive control)	0.5	2	3	NE
9-Aminoacridine hemihydrate (positive control)	50	1	3	NE
4-nitroquinoline-N-oxide (positive control)	0.5	1	3	NE
2-aminoanthracene (positive control)	5 or 20[b]	2	NE	3
Benzo[a]pyrene (positive control)	5	3	NE	3

Abbreviations: – = concentration not measured; –S9 = without S9 mix activation; +S9 = with S9 mix activation; *E. coli* = *Escherichia coli;* h = hour; min = minute; NE = not evaluated; *S. typhimurium* = *Salmonella typhimurium*.

[a] SM-102 was tested at levels up to 5000 µg/plate, which is the standard limit dose recommended by regulatory guidelines.

[b] Final concentrations of 5 and 20 µg/plate were incubated with *S. typhimurium* TA1535 strain and *E. coli* WP2 *uvr*A strain, respectively.

Source: Report 9601567.

After the incubation period, visual counts were performed on the plates; an inverted microscope was also employed to facilitate observations. Plates were evaluated for the quality of the background lawn and the number of revertant colonies using an automated colony counter. The mean number of revertant colonies for all treatment groups was compared with those obtained for the concurrent negative control level.

Results:

Incomplete, or absent, background lawns of non-revertant bacteria, or substantial reductions in revertant colony counts, were not obtained after exposure to SM-102, indicating that SM-102 was non-toxic to the bacteria at the levels tested. Precipitation was observed at concentrations ≥ 1581 µg/plate in the absence of S9 mix and at concentrations ≥ 500 µg/plate in the presence of S9 mix.

No substantial increases in revertant colony numbers were obtained with any of the tester strains, after exposure to SM-102 at any dose level, in either the presence or absence of S9 mix.

Conclusions:

SM-102 did not show any evidence of genotoxic activity in this in vitro mutagenicity assay when tested in accordance with ICH guidelines.

2.6.6.4.1.2 PEG2000-DMG (b) (4) Bacterial Reverse Mutation Test in *Salmonella typhimurium* and *Escherichia coli*

The objective was to determine the potential genotoxicity of the commercially available PEG2000-DMG (b) (4) using the bacterial reverse mutation test (Ames test) in *S. typhimurium* and *E. coli* (Report 9601035; GLP-compliant). PEG2000-DMG was prepared as a stock solution (50 mg/mL) in dimethyl sulfoxide (DMSO), and all lower level formulations were made by serial dilution.

Methods:

Four strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537) and an *E. coli* strain (WP2 *uvr*A) were treated with PEG2000-DMG in the absence and presence of a supplemented rat liver fraction (S9 mix) at 37ºC for 67 hours and 57 minutes. PEG2000-DMG was tested over a wide range of dose levels from 158 µg/mL to 50,000 µg/mL (the standard limit dose for this assay) (Table 9). The negative control was DMSO (vehicle). The positive controls included 2NF, NaAz, 9AC, and NQO in the absence of S9 mix; and 2AA and BaP in the presence of S9 mix. All concentrations of PEG2000-DMG, as well as positive and negative controls, were evaluated in triplicate.

Table 9: Experimental Design for Bacterial Reverse Mutation Test of PEG200-DMG in *Salmonella typhimurium* and *Escherichia coli*

Dose Number	Final Concentration (µg/plate)	Number of Strains	Number of Cultures	
			67 h 57 min (–S9)	67 h 57 min (+S9)
DMSO (negative control)	–	5	3	3
1/PEG2000-DMG	1.58	5	3	3
2/PEG2000-DMG	5.0	5	3	3
3/PEG2000-DMG	15.8	5	3	3
4/PEG2000-DMG	50	5	3	3
5/PEG2000-DMG	158	5	3	3
6/PEG2000-DMG	500	5	3	3
7/PEG2000-DMG	1581	5	3	3
8/PEG2000-DMG	5000[a]	5	3	3
2-nitrofluorene (positive control)	1	1	3	NE
Sodium azide (positive control)	0.5	2	3	NE
9-aminoacridine hemihydrate (positive control)	50	1	3	NE
4 nitroquinoline-N-oxide (positive control)	0.5	1	3	NE
2-aminoanthracene (positive control)	5 or 20[b]	2	NE	3
Benzo[a]pyrene (positive control)	5	3	NE	3

Abbreviations: – = concentration not measured; –S9 = without S9 mix activation; +S9 = with S9 mix activation; DMSO = dimethyl sulfoxide; *E. coli* = *Escherichia coli;* h = hour; min = minute; NE = not evaluated; *S. typhimurium* = *Salmonella typhimurium*.

[a] PEG2000-DMG was tested at levels up to 5000 µg/plate, which is the standard limit dose recommended by regulatory guidelines.

[b] Final concentrations of 5 and 20 µg/plate were incubated with *S. typhimurium* TA1535 strain and *E. coli* WP2 *uvr*A strain, respectively.

Source: Report 9601035.

After the incubation period, visual counts were performed on the plates; an inverted microscope was also employed to facilitate observations. Plates were evaluated for the quality of the background lawn and the number of revertant colonies using an automated colony counter. The mean number of revertant colonies for all treatment groups was compared with those obtained for the concurrent negative control level.

Results:

(b) (4)

(b) (4)

No substantial increases in the revertant colony counts were obtained with any strains, after exposure to PEG2000-DMG in the presence or absence of S9 mix.

Conclusions:

PEG2000-DMG did not show any evidence of genotoxic activity in this in vitro mutagenicity assay when tested in accordance with ICH guidelines.

2.6.6.4.2 In Vitro Mammalian Cell System

2.6.6.4.2.1 SM-102 In Vitro Mammalian Cell Micronucleus Test in Human Peripheral Blood Lymphocytes

The objective was to determine the potential genotoxicity of SM-102 using an in vitro mammalian cell micronucleus test in human peripheral blood lymphocytes (Report 9601568; GLP-compliant). SM-102 was prepared as a stock solution (50 mg/mL) in ethanol, and all lower level formulations were made by serial dilution.

Methods:

Human peripheral blood lymphocytes were treated with SM-102 in the absence and presence of a supplemented rat liver fraction for 4 hours and 24 hours. SM-102 was tested over a wide range of dose levels (3.25 to 500 µg/mL) using all treatment regimens (4-hour treatment period in the absence and presence of S9 mix and 24-hour treatment period in the absence of S9 mix) (Table 10). The negative control was ethanol. The positive controls were mitomycin C (MMC) and nocodazole in the absence of S9 mix, and cyclophosphamide (CP) in the presence of S9 mix. All concentrations of SM-102, as well as positive and negative controls, were evaluated in duplicate.

Table 10: Experimental Design for Mammalian Cell Micronucleus Test of SM-102 in Human Peripheral Blood Lymphocytes

Dose Number	Final Concentration (µg/mL)[a]	Number of Cultures		
		4 h (–S9)	4 h (+S9)	24 h (–S9)
Ethanol (negative control)	–	2	2	2
1/SM-102	3.25	2	2	2
2/SM-102	5.68	2	2	2
3/SM-102	9.95	2	2	2
4/SM-102	17.4	2	2	2
5/SM-102	30.5	2	2	2
6/SM-102	53.3	2	2	2
7/SM-102	93.3	2	2	2
8/SM-102	163	2	2	2
9/SM-102	286	2	2	2
10/SM-102	500	2	2	2
Nocodazole (positive control)	0.25	2	NE	NE
	0.30	2	NE	NE
Cyclophosphamide (positive control)	10	NE	2	NE
	15	NE	2	NE
Mitomycin C (positive control)	0.10	NE	NE	2
	0.20	NE	NE	2

Abbreviations: – = concentration not measured; –S9 = without S9 mix activation; +S9 = with S9 mix activation; h = hour; NE = not evaluated.

[a] Theoretical concentrations, actual concentrations may differ slightly due to the limitations of the instruments used.

Source: Report 9601568.

Cell cultures were harvested, fixed, placed on slides, air-dried, stained with acridine orange, and examined visually for toxicity. A total of 2000 binucleate cells per experimental point (1000 per culture) were examined for the presence of micronuclei, which are indicative of chromosomal damage. The cytokinesis-block proliferation index (CBPI) was determined by examination of at least 500 cells (if available) per culture. The results for all treatment groups were compared with those obtained for the concurrent negative control level.

Results:

Cultures treated with SM-102 at levels up to 500 µg/mL did not show any statistically significant increases in the incidence of micronucleated binucleate cells. Precipitation was observed at the end of treatment at 500 µg/mL in the absence of S9 mix at 4- and 24-hours post-treatment. Cloudy media was observed at 4-hours post-treatment at ≥ 93.3 µg/mL in the in the absence of

S9 mix and at 500 µg/mL in the presence of S9 mix; and at 24-hours post-treatment at ≥ 286 µg/mL. No cytotoxicity was observed in the assay.

Conclusions:

SM-102 did not show any evidence of genotoxic activity in this in vitro test for induction of micronuclei in human peripheral blood lymphocytes when tested in accordance with ICH regulatory guidelines.

2.6.6.4.2.2 PEG2000-DMG (b) (4) In Vitro Mammalian Cell Micronucleus Test in Human Peripheral Blood Lymphocytes

The objective was to determine the potential genotoxicity of the commercially available PEG2000-DMG (b) (4)) using an in vitro mammalian cell micronucleus test in human peripheral blood lymphocytes (Report 9601036; GLP-compliant). PEG2000-DMG was prepared as a stock solution (50 mg/mL) in DMSO, and all lower level formulations were made by serial dilution.

Methods:

Human peripheral blood lymphocytes were treated with PEG2000-DMG in the absence and presence of a supplemented rat liver fraction (S9 mix) for 4 hours and 24 hours. PEG2000-DMG was tested over a wide range of dose levels (3.25 to 500 µg/mL) using all treatment regimens (4-hour treatment period in the absence and presence of S9 mix and 24-hour treatment period in the absence of S9 mix) (Table 11). The negative control was DMSO (vehicle). The positive controls were MMC and colcemid in the absence of S9 mix, and CP in the presence of S9 mix. All concentrations of PEG2000-DMG, as well as positive and negative controls, were evaluated in duplicate.

Table 11: Experimental Design for Mammalian Cell Micronucleus Test of PEG2000-DMG in Human Peripheral Blood Lymphocytes

Dose Number	Final Concentration (µg/mL)	Number of Cultures		
		4 h (–S9)	4 h (+S9)	24 h (–S9)
DMSO (negative control)	–	2	2	2
1/PEG2000-DMG	3.25	2	2	2
2/PEG2000-DMG	5.68	2	2	2
3/PEG2000-DMG	9.95	2	2	2
4/PEG2000-DMG	17.4	2	2	2
5/PEG2000-DMG	30.5	2	2	2
6/PEG2000-DMG[a]	53.3	2	2	2
7/PEG2000-DMG[a]	93.3	2	2	2
8/PEG2000-DMG[a]	163	2	2	2
9/PEG2000-DMG	286	2	2	2
10/PEG2000-DMG	500	2	2	2
Mitomycin C (positive control)	0.30	2	NE	NE
	0.45	2	NE	NE
	0.60	2	NE	NE
Cyclophosphamide (positive control)	5.0	NE	2	NE
	10	NE	2	NE
	15	NE	2	NE
Colcemid (positive control)	0.035	NE	NE	2
	0.050	NE	NE	2
	0.070	NE	NE	2

Abbreviations: – = concentration not measured; –S9 = without S9 mix activation; +S9 = with S9 mix activation; DMSO = dimethyl sulfoxide; h = hours; NE = not evaluated.

[a] Cytotoxicity of approximately 60.8% was observed in the 24-hour treatment regimen at a concentration of 163 µg/mL; this concentration and the next 2 lower dose levels, 93.3 and 53.3 µg/mL, were chosen for micronucleus assessment for the 24-hour treatment regimen.

Source: Report 9601036.

Cell cultures were harvested, fixed, placed on slides, air-dried, stained with acridine orange, and examined visually for toxicity. A total of 2000 binucleate cells per experimental point (1000 per culture) were examined for the presence of micronuclei, which are indicative of chromosomal damage. The CBPI was determined by examination of at least 500 cells per culture. The results for all treatment groups were compared with those obtained for the concurrent negative control group.

Results:

The standard positive controls caused substantial increases in the incidence of micronucleated binuclear cells, confirming the sensitivity of the system and the effectiveness of the S9 mix.

Cultures treated with PEG2000-DMGs at concentrations up to 500 µg/mL did not show substantial increases in the incidence of micronucleated binucleated cells. No precipitation was observed at any dose level of PEG2000-DMG; however, cytotoxicity was observed in the 24-hour treatment regimen at concentrations ≥ 163 µg/mL.

Conclusions:

PEG2000-DMG did not induce micronuclei in human peripheral blood lymphocytes, with or without an exogenous metabolic activation system, when tested in accordance with ICH regulatory guidelines.

2.6.6.4.3 In Vivo Mammalian System

2.6.6.4.3.1 Mammalian Erythrocyte Micronucleus Test in Rat

The objective was to determine the potential genotoxicity of mRNA-1706 when administered to rats as a single intravenous (IV) bolus injection, using a bone marrow micronucleus test (Report 9800399; GLP-compliant). mRNA-1706 contains a single mRNA sequence that encodes the prME structural proteins of Zika virus combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 20 mM Tris, 8% sucrose, pH 7.4.

Methods:

A dose-range–finding experiment was initially conducted to determine the maximum tolerated dose (MTD). Sprague Dawley rats (3/sex/group) were administered a single dose of 2.6, 3.9, or 5.2 mg/kg for females and 2.6, 5.2, or 10.3 mg/kg for males as an IV injection via the tail vein. Doses ≥ 3.9 mg/kg in the female rat resulted in body weight loss; therefore, the female MTD was determined to be 2.6 mg/kg. In males, 10.3 mg/kg resulted in mortality (2 out of 3 animals) and no clinical signs at 5.2 mg/kg; therefore, the male MTD was determined to be 5.2 mg/kg.

During the main test, male Sprague Dawley rats were administered a single dose of 1.3, 2.6, or 5.2 mg/kg and females were administered 0.6, 1.3, or 2.6 mg/kg as an IV injection via the tail vein (Table 12). In addition, 3 animals/sex/group were administered a single IV injection for the quantification of exposure in plasma. Animals were monitored for clinical observations and body weight. Twenty-four and 48 hours post-dose, bone marrow smears (from both femurs) were collected, fixed, stained with acridine orange, and examined under code using fluorescence microscopy. A total of 4000 immature erythrocytes (IE) per animal were examined for the presence of micronuclei indicative of chromosome damage. In addition, the proportion of IE in

the total population (IE and mature erythrocytes [ME]) was assessed for each animal as a measure of potential bone marrow toxicity. Positive scoring control slides (taken from 3 male rats previously dosed with the positive control cyclophosphamide at 20 mg/kg) were added for evaluation. The data for the concurrent negative control (group mean % IE/[IE + ME] and micronucleated immature erythrocytes [MIE]) were within the ranges determined from laboratory historical data.

Table 12: Experimental Design for Mammalian Erythrocyte Micronucleus Test of SM-102 in Rats

Group Number	Test Material	mRNA/SM-102 Dose Level[a, b] (mg/kg)	mRNA/SM-102 Dose Concentration[b] (mg/mL)	Dose Volume (mL/kg)	Sampling Time (h)	Number of Animals			
						Main Study		Bioanalysis Study[c]	
						M	F	M	F
1	Negative Control	0	0	5	24	5	5	3	3
					48	5	5	NE	NE
2	mRNA-1706	1.3/13.5	0.26/2.70	5	24	5	NE	3	NE
3	mRNA-1706	2.6/27.0	0.52/5.41	5	24	5	NE	3	NE
4	mRNA-1706	5.2/54.1	1.03/10.71	5	24	5	NE	3	NE
					48	5	NE	NE	NE
9	mRNA-1706	0.6/6.2	0.129/1.34	5	24	NE	5	NE	3
10	mRNA-1706	1.3/13.5	0.26/2.70	5	24	NE	5	NE	3
11	mRNA-1706	2.6/27.0	0.52/5.41	5	24	NE	5	NE	3
					48	NE	5	NE	NE
5	Positive Control	[d]							

F = Females; LNP = lipid nanoparticle; M = Males; mRNA = messenger RNA; NE = not evaluated; SoA = summary of analysis.

[a] The original dose levels selected were 0, 1.0, 2.0, 4.0, 0.5, 1.0, and 2.0 mg/kg mRNA-1706, respectively (SoA issued on 11 October 2016). The calculated dose levels were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064 (SoA issued on 03 May 2017). The change in the reported mRNA content for mRNA-1706 was (b) (4)

[b] Test article is mRNA-1706 in SM-102-containing LNPs.

[c] Bioanalysis animals were used for quantification of exposure in plasma only.

[d] Positive scoring control slides (taken from 3 male rats previously dosed with positive control cyclophosphamide at 20 mg/kg) were added to the slides for evaluation.

Source: Report 9800399.

Results:

During the main test, there was no mortality. At doses ≤ 2.6/27.0 mg/kg mRNA-1706/SM-102, no mRNA-1706–related clinical signs were observed. In the 5.2/54.1 mg/kg mRNA-1706/SM-102 males, decreased body weight gain, yellow staining of the fur at the urogenital and abdominal

areas, and redness of the tail skin were observed. In general, females also had dose-dependent decreased weight gain at 24 hours post-dose that recovered by 48 hours post-dose.

Animals treated with mRNA-1706 did not show any significant decreases in the proportion of IE at the 24-hour and 48-hour sampling times, indicating that there was minimal bone marrow toxicity.

Male animals treated with mRNA-1706 showed 2-fold increase over negative control, a non-dose-dependent (no change at the mid dose), and statistically significant increases in the incidence of MIE at the 24-hour sampling time. The increased incidences observed were outside the mean distribution of the historical negative control data, and a positive trend test was obtained. Additionally, a 3-fold increase in the number of MIE was observed in males at 5.2/54.1 mg/kg mRNA-1706/SM-102 at 48-hours post-dose, meeting the criteria for a positive response. mRNA-1706 was therefore considered positive for the induction of chromosome damage in male rat IE.

Female animals treated with mRNA-1706 showed statistically significant increases in the incidence of MIE, at the 48-hour sampling time only at 2.6/27.0 mg/kg mRNA-1706/SM-102, however the increased incidences were within the historical control range. No statistically significant increases in the incidence of MIE were obtained at the 24-hour sampling time.

Conclusions:

Although statistically significant increases in MIE were observed in male rats at both 24 and 48 hours and in females at 48 hours only, there was no clear dose response after IV administration of mRNA-1706, and the increases were generally weak and associated with minimal bone marrow toxicity. These observations are unlikely to indicate a risk to humans after IM administration due to minimal systemic exposure.

2.6.6.4.3.2 Mammalian Erythrocyte Micronucleus Test in Rat

The objectives were to evaluate the test article, NPI luciferase mRNA in SM-102–containing LNPs, for in vivo clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte (PCE) cells in Sprague Dawley rat bone marrow (Report AF87FU.125012NGLPICH.BTL; non-GLP–compliant). The NPI luciferase mRNA is combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 25 mM Tris, 123 g/L sucrose, 1 mM DTPA, pH 7.5.

Methods:

Sprague Dawley rats (13/sex/group) were administered a single dose of 0.32/6.0, 1.07/20, or 3.21/60 mg/kg NPI luciferase mRNA/SM-102, respectively, or control (vehicle; 25 mM Tris/sucrose, 1 mM DTPA, pH 7.5) as an IV injection via the tail vein at a volume of 5 mL/kg (Table 13). Animals were monitored for clinical observations and body temperature measurements before and after dosing. Two and 6 hours post-dose, blood samples were collected from 3 animals/sex/group, plasma was harvested from the blood, and then animals were euthanized without necropsy after the 6-hour blood collection. mRNA was quantified in plasma samples from 2 hours post-dose using a branched DNA assay; however, the mRNA quantification was not reported due to technical issues with the assay. Cytokine concentrations for MIP-1-α, MCP-1, IL-6, IL-1β, TNF-α, and IP-10 were quantified in plasma samples from 6 hours post-dose using a Luminex assay. Twenty-four and 48 hours post-dose, 5 animals/sex/group were euthanized, bone marrow was collected, and then processed for the micronucleus assay. The bone marrow suspension for each animal was spread onto a glass slide and stained with acridine orange for microscopic evaluation. 4000 PCEs/animal were scored for the presence of micronuclei, whenever possible. In addition, at least 500 total erythrocytes (PCEs + normochromatic erythrocytes) were scored per animal to determine the proportion of PCEs as an index of bone marrow cytotoxicity. To verify scoring, positive control slides were generated from male rats treated once with cyclophosphamide monohydrate at 40 mg/kg, and the bone marrow harvested 24 hours post-dose. Study findings were expressed as pair-wise comparisons made with the control group.

Table 13: Experimental Design for Mammalian Erythrocyte Micronucleus Test of SM-102 in Rats

Treatment	mRNA/SM-102[a] Dose Level (mg/kg)	mRNA/SM-102[a] Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals					
				2- and 6-hour blood collection		24-hour bone marrow collection		48-hour bone marrow collection	
				M	F	M	F	M	F
Control (vehicle)[b]	0/0	0/0	5	3	3	5	5	5	5
Test article[d]	0.32/6.0	0.064/1.2	5	3	3	5	5	5	5
Test article[d]	1.07/20	0.22/4	5	3	3	5	5	5	5
Test article[d]	3.21/60	0.64/12	5	3	3	5	5	5	5

Abbreviation: F = female; LNP = lipid nanoparticle; M = male; NPI = nascent peptide imaging.

[a] Test article is NPI luciferase mRNA in SM-102-containing LNPs.

[b] Control is 25 mM Tris/sucrose 1 mM DTPA pH 7.5.

Source: Report AF87FU.125012NGLPICH.BTL.

Results:

There were no test article-related effects on mortality or clinical observations.

Test article-related increases in body temperature were observed at 3.21/60 mg/kg NPI luciferase mRNA/SM-102 from 1-2 hours post-dose to 8 hours post-dose and met the protocol-specified parameters for hyperthermia (≥ 1°C increase for at least 4.5 hours).

Test article-related increases in IL-6, MCP-1, MIP-1-α, and/or IP-10 were observed at 6 hours post-dose in one or both sexes at 1.07/20 mg/kg NPI luciferase mRNA/SM-102 and in both sexes at 3.21/60 mg/kg NPI luciferase mRNA/SM-102. Fold increases observed for IL-6, MCP-1, MIP-1-α, and IP-10 were up to 3.68-, 4.66-, 2.62-, and 30.47-fold the levels in controls, respectively.

There was no significant increase in the incidence of micronuclei in the test article-treated animals at either time point after dose administration (24 or 48 hours).

A slight, but statistically significant, decrease in %PCEs was observed in the 0.32/6.0 mg/kg NPI luciferase mRNA/SM-102 group males at the 48-hour time point.

Conclusions:

NPI luciferase mRNA in SM-102–containing LNPs was determined to be negative (non-clastogenic) after a single dose of 0.32/6.0, 1.07/20, or 3.21/60 mg/kg NPI luciferase mRNA/SM-102 in Sprague Dawley rats.

2.6.6.5 CARCINOGENICITY

Carcinogenicity studies have not been conducted with mRNA-1273.

2.6.6.6 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

2.6.6.6.1 A GLP Intramuscular Combined Developmental and Perinatal/Postnatal Reproductive Toxicity Study of mRNA-1273 in Rats

The objective of this study was to assess the potential effects of mRNA-1273 on fertility and pre- and postnatal development in pregnant and lactating female Sprague Dawley rats (Report 20248897; GLP-compliant).

Methods:

Sprague Dawley rats were administered 4 doses of 100 µg/dose mRNA-1273 or control (20 mM Tris, 87 mg/mL sucrose, 17.5 mM sodium acetate, pH 7.5) on Study Days 1 and 15 (28 and 14 days prior to mating, respectively) and Gestation Days 1 and 13, via IM injection into alternating quadriceps (hindlimbs). In each dose group, female rats were divided into either Caesarean-sectioning phase cohort (Cohort 1) or natural delivery phase cohort (Cohort 2, Table 14).

In Cohort 1, F_0 generation rats (dams) were euthanized on Gestation Day 21 for Caesarean-sectioning, gross pathology, organ weights (gravid uterus, placentae), and ovarian and uterine contents examinations; and F_1 generation rats (pups) were euthanized for gross pathology and fetal examinations (external abnormalities, visceral examination, skeletal examination, fetal ossification site averages).

In Cohort 2, F_0 rats were allowed to deliver their litters naturally, and litters were reduced to 8 pups each (when possible). F_0 rats were monitored for clinical observations, body weight, food consumption, estrous cycling, and mating and fertility. F_1 rats were monitored for clinical

observations and body weight. Sera samples from F_0 and F_1 rats were analyzed on Study Days 1, 15, Gestational Days 1, 13, 21 and Lactation Day 21 for antibody responses to SARS-CoV2 S-2P protein using optimized indirect ELISA. The serum antibody analyses were not performed with GLP compliance. In Cohort 2, F_0 rats were euthanized after a 21-day postpartum period for gross pathology and F_1 rats were euthanized for gross pathology. Study findings were expressed as pair-wise comparisons made with the control group, where appropriate.

Table 14: Experimental Design for Reproductive Toxicity Study of mRNA-1273 in Sprague Dawley Rats

Group Number	Test Material	Dose Level (µg/dose)	Dose Volume (µL/dose)	Dose Concentration (mg/mL)	Number of Females	
					Cohort 1 (Caesarean-Sectioning Phase)	Cohort 2 (Natural Delivery Phase)
1	Control (Vehicle)[a]	0	200	0	22	22
2	mRNA-1273	100	200	0.5	22	22

[a] Control is 20 mM Tris, 87 mg/mL sucrose, 17.5 mM sodium acetate, pH 7.5.
Source: Report 20248897.

Results:

In the F_0 generation, there were no mRNA-1273–related effects or changes on mortality, body weight, body weight gain, food consumption, macroscopic observations, estrous cycling during pre-cohabitation, mating and fertility, ovarian/uterine examination findings, or natural delivery or litter observation parameters. In the F_1 generation, there were no mRNA-1273–related effects on mortality, body weight, clinical observations, macroscopic observations, gross pathology, external or visceral malformations or variations, skeletal malformations, or mean number of ossification sites per fetus per litter.

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

In the F_0 generation, mRNA-1273–related clinical observations included transient thin fur cover, swollen hindlimbs, and limited usage of the hindlimb during the premating, gestation, and/or lactation phases of the study, with the most observations noted following dose administration on

Gestation Day 13. These mRNA-1273–related observations were not considered adverse as these effects did not significantly impair the animal's mobility, access to food, or ability to thrive. Only thin fur cover was still present during the lactation phase and was resolved by Lactation Day 18.

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

Conclusions:

Maternal administration of mRNA-1273 on Study Days 1 and 15 (28 and 14 days prior to mating, respectively) and Gestation Days 1 and 13 did not have any adverse effects on F_0 and F_1 generations. The mRNA-1273-related, non-adverse effects were limited to an increase in the number of fetuses with common skeletal variations of 1 or more rib nodules and 1 or more wavy ribs with no effect on the viability and growth on the F_1 generation. Robust IgG titers were observed in the rats following 4 immunizations of mRNA-1273 vaccine. Peak titer was reached on Gestation Day 13 and plateaued at the time of parturition Gestation Day 21 and stayed constant through Lactation Day 21. Strong maternal-to-fetal and maternal-to-pup transfer of antibodies was observed with mRNA-1273.

2.6.6.7 STUDIES IN JUVENILE ANIMALS

Studies in juvenile animals have not been conducted with mRNA-1273.

2.6.6.8 LOCAL TOLERANCE

No local tolerance studies have been performed with mRNA-1273. Injection sites were examined for signs of erythema and edema in the GLP-compliant toxicity studies.

2.6.6.9 OTHER TOXICITY STUDIES

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

The objective was to examine the immunogenicity and potential toxicity of mRNA-1273 when given by IM bolus injection to Sprague Dawley rats on Days 1 and 22 (Report 2308-123; non-GLP–compliant).

Methods:

Sprague Dawley rats were administered 2 doses of 30, 60, or 100 µg/dose mRNA-1273 or control (vehicle; 20 mM Tris, 87 mg/mL sucrose, 10.7 mM sodium acetate, pH 7.5) via IM bolus injection into alternating quadriceps (hind leg, thigh) on Days 1 and 22 (Table 15). Animals were monitored for clinical observations, body weight, and clinical pathology parameters (hematology, coagulation, clinical chemistry). IgG antibody titers were analyzed using an ELISA. All animals were euthanized without necropsy following collection for antibodies on Day 35. Study findings were expressed as pair-wise comparisons made with the control group, where appropriate.

Table 15: Experimental Design for 5-Week (2 Doses) Repeat-Dose Immunogenicity and Toxicity Study of mRNA-1273 in Sprague Dawley Rats

Group Number	Test Material	Dose Level (µg/dose)	Dose Volume (µL/dose)	Dose Concentration (µg/mL)	Number of Animals	
					M	F
1	Control (vehicle)[a]	0	200	0	5	5
2	mRNA-1273	30	200	150	5	5
3	mRNA-1273	60	200	300	5	5
4	mRNA-1273	100	200	500	5	5

Abbreviations: F = female; M = male.

[a] Control is 20 mM Tris, 87 mg/mL sucrose, 10.7 mM sodium acetate, pH 7.5.

Source: Report 2308-123.

Results:

There were no mRNA-1273–related effects on mortality or body weight.

All mRNA-1273–treated groups showed detectable antibody titers against SARS-CoV-2 S-2P after 2 doses (Day 35) with measured IgG antibody titers above 10^6 at all dose levels; full immunogenicity results are presented in Section 2.6.2 Pharmacology Written Summary.

mRNA-1273–related clinical observations were noted at ≥ 30 µg/dose and included transient, dose-dependent edema with or without hindlimb impairment at 24 hours post-dose (Days 2 and 23) that resolved by 5 days post-dose (Days 6 and 27).

mRNA-1273–related hematology changes at ≥ 30 µg/dose on Day 23 were consistent with inflammation and included increases in neutrophil (range: 5.86-fold to 10.81-fold of control means) and eosinophil (range: 2.60-fold to 4.67-fold of control means) counts and decreases in mean albumin (range: 0.90-fold to 0.85-fold of control means) and A/G ratio (range: 0.86-fold to 0.75-fold of control means) at all dose levels, with increased mean globulin (range: 1.12-fold to 1.15-fold of control means) in ≥ 60 µg/dose males. Other test article-related changes observed at 30, 60, and/or 100 µg/dose included decreases in mean reticulocyte (range: 0.80-fold to 0.65-fold of control means), lymphocyte (range: 0.74-fold to 0.47-fold of control means), and/or monocyte (range: 0.58-fold to 0.52-fold of control means) counts. The decreases in reticulocyte counts were associated with mild decreases in red blood cell mass (erythrocytes, hemoglobin, and/or hematocrit) in ≥ 30 µg/dose males (hemoglobin range: 0.93-fold to 0.91-fold of control means), and increases in RDW (range: 1.05-fold to 1.10-fold of control means) at all doses.

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

All clinical observations fully recovered by Day 35.

Conclusions:

Administration of mRNA-1273 by IM bolus injection on Days 1 and 22 to Sprague Dawley rats was well tolerated up to 100 µg/dose.

2.6.6.10 DISCUSSION AND CONCLUSIONS

The toxicological profile associated with mRNA-based vaccines formulated in the SM-102–containing LNPs, including mRNA-1273, is driven primarily by the LNP composition and, to a lesser extent, the biologic activity of the antigen(s) encoded by the mRNA. The toxicological profile of mRNA-based vaccines formulated in the SM-102–containing LNPs has been extensively characterized across various programs. The aggregate toxicology profile

observed across 6 GLP rat repeat-dose toxicology studies for 5 different SM-102–containing LNPs vaccine programs, together with a non-GLP rat repeat-dose immunogenicity rat study with safety endpoints and genotoxicity assessments of the SM-102 lipid supports development of mRNA-1273.

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

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

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

Additionally, a non-GLP study in rats was conducted to characterize the immunogenic response and potential toxicity of mRNA-1273 at IM doses levels of 30, 60, and 100 µg/dose on Days 1 and 22. A strong immunogenic response against SARS-CoV-2 S-2P was observed on Day 35, with measured IgG antibody titers above 10^6 at all dose levels. mRNA-1273 had no effect on

body weights and limited, transient clinical signs starting at 30 µg/dose consisting of transient dose-dependent injection site edema with or without hindlimb impairment. Clinical pathology findings consisted, in part, of changes associated with inflammation starting at 30 µg/dose. In general, the changes observed are consistent with the results from the previous GLP rat toxicity studies conducted with other mRNA-based vaccines formulated with SM-102–containing LNPs.

Genotoxicity assessments of the SM-102 lipid concluded that the lipid is not genotoxic in the bacterial mutagenicity and human peripheral blood lymphocytes chromosome aberration assays. Two intravenous in vivo micronucleus assays were conducted with mRNA-based vaccines formulated in the SM-102–containing LNPs. Results from Report AF87FU.125012NGLPICH.BTL were negative up to 3.21/60 mg/kg NPI luciferase mRNA/SM-102, while results from Report 9800399 were positive at 2.6/27.0 mg/kg mRNA-1706/SM-102 in females and at 5.2/54.1 mg/kg mRNA-1706/SM-102 in males, indicating that there was minimal bone marrow toxicity. The equivocal results are likely driven by micronuclei formation secondary to elevated body temperature induced by LNP-driven systemic inflammation at high systemic (intravenous) doses. Overall, the genotoxic risk to humans is considered to be low due to minimal systemic exposure following IM administration, limited duration of exposure, and negative in vitro results. Genotoxicity assessments of the PEG2000-DMG lipid concluded that the lipid is not genotoxic in the bacterial mutagenicity and human peripheral blood lymphocytes chromosome aberration assays.

A GLP study was conducted to assess the potential effects of mRNA-1273 on fertility and pre- and postnatal development in the pregnant and lactating female Sprague Dawley rats. Administration of a 100 µg dose of mRNA-1273 did not result in any adverse effects on the F_0 and F_1 generations; a small increase in the common non-adverse malformation of wavy ribs/nodules was observed in mRNA-1273-treated animals at this dose. Strong maternal-to-fetal and maternal-to-pup transfer of SARS-CoV-2 S-2P antibodies was observed with mRNA-1273.

2.6.6.11 TABLES AND FIGURES

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

2.6.6.12 REFERENCES

Carney EW, Kimmel CA. Interpretation of skeletal variations for human risk assessment: delayed ossification and wavy ribs. Birth Defects Res B Dev Reprod Toxicol. 2007;80(6):473-96.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use: S2(R1). 09 November 2011 [cited 2019 Apr 16] [29 screens]. Available from: https://database.ich.org/sites/default/files/S2%28R1%29%20Guideline.pdf.