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## List of Abbreviations

Abbreviation	Definition
A2M	$\alpha$ 2-macroglobulin
A/G	albumin to globulin
AGP	$\alpha$ 1-acid glycoprotein
APTT	activated partial thromboplastin time
BLA	Biologics License Application
CoV	coronavirus
CMV	cytomegalovirus
DTPA	diethylenetriamine pentaacetic acid
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
ELISA	enzyme-linked immunosorbent assay
GLP	Good Laboratory Practice
F	fusion
g	glycoprotein
hMPV	human metapneumovirus
ICH	International Council for Harmonisation
IE	immature erythrocytes
IFN	interferon
IgG	immunoglobulin G
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
MIE	micronucleated immature erythrocytes
MIP-1- $\alpha$	macrophage inflammatory protein 1 alpha
mRNA	messenger RNA
MTD	maximum tolerated dose
NCE	normochromatic erythrocytes
NaCl	sodium chloride
NPI	nascent peptide imaging

Abbreviation	Definition
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PCE	polychromatic erythrocytes
PEG2000-DMG	1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol polyethylene 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 with 2 proline substitutions within the heptad repeat 1 domain
SARS-CoV-2	2019 novel coronavirus
SM-102	heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6(undecyloxy)hexyl)amino)octanoate
TNF- $\alpha$	tumor necrosis factor alpha
Tris	trometamol
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 SARS-CoV-2. mRNA-1273 contains a single mRNA that encodes for the full-length SARS-CoV-2 spike (S) glycoprotein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilize the prefusion conformation. The mRNA-1273 is combined in a mixture of 4 ionizable, structural, helper, and polyethylene glycol lipids common to the Sponsor's mRNA vaccine platform: heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate (SM-102); cholesterol; 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); and 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol polyethylene glycol (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-based vaccines that encode for 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 (mRNA-1706, mRNA-1653, mRNA-1893, 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 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 9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks is similar and consistent despite the different mRNA constructs encoding for 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 expressed antigens of the mRNA vaccine, and 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.

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

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

This brief summary provides an overview of the mRNA-1273 nonclinical toxicology program. 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. The mRNA-1273 clinical presentations are summarized in [Module 2.4](#).

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

[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
<b>Repeat-Dose Toxicity</b>					
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706 <sup>a</sup>	Rat, Sprague Dawley	IM; 0, 13, 65, 129 µg/dose (Days 1, 15, 29)	Yes	5002045
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706 <sup>a</sup>	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 <sup>b</sup>	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 <sup>c</sup>	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 <sup>d</sup>	Rat, Sprague Dawley	IM; 0, 8.9, 27, 89 µg/dose (Days 1, 15, 29, 43)	Yes	5002034
6-week (4 doses) repeat-dose study with 2-week recovery	mRNA-1443 <sup>e</sup>	Rat, Sprague Dawley	IM; 0, 9.6, 29, 96 µg/dose (Days 1, 15, 29, 43)	Yes	5002158
<b>In Vitro Genotoxicity</b>					
Bacterial reverse mutation test	SM-102	<i>Salmonella typhimurium</i> , <i>Escherichia coli</i>	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 faction	Yes	9601567
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 faction	Yes	9601568
<b>In Vivo Genotoxicity</b>					
In vivo mammalian erythrocyte micronucleus test	mRNA-1706 <sup>a</sup>	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 <sup>f</sup>	Yes	9800399
In vivo mammalian erythrocyte micronucleus test	NPI luciferase mRNA <sup>g</sup>	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.1250 12NGLCH.BTL
<b>Other Toxicology</b>					
5-week (2 doses) repeat-dose immunogenicity study with safety endpoints	mRNA-1273 <sup>h</sup>	Rat, Sprague Dawley	IM; 0, 30, 60, 100 µg/dose (Days 1 and 22)	No	2308-123

Abbreviations: CMV = cytomegalovirus; DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine;

DTPA = diethylenetriamine pentaacetic acid; F = female; g = glycoprotein; IM = intramuscular;

IV = intravenous; GLP, Good Laboratory Practice; h = hour; hMPV = human metapneumovirus;

g = glycoprotein; M = male; min = minute; NaCl = sodium chloride; NPI = nascent peptide imaging;

PG = propylene glycol; PIV3 = parainfluenza virus type 3; pp65 = phosphoprotein 65; prME = pre-membrane and envelope; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain;

SM-102 = heptadecan-9-yl 8((2hydroxyethyl)(6oxo6-(undecyloxy) hexyl)amino)octanoate; Tris = trometamol.

- <sup>a</sup> 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.
- <sup>b</sup> 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.
- <sup>c</sup> 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.
- <sup>d</sup> mRNA-1647 contains 6 mRNAs which 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.
- <sup>e</sup> mRNA-1443 contains a single mRNA sequence that encodes for 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.
- <sup>f</sup> 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)
- <sup>g</sup> 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.
- <sup>h</sup> 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 (BLA)-enabling toxicology studies were conducted with formulations of SM-102-containing LNPs used in the clinic.



#### **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 trometamol (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, and clinical chemistry), and cytokine analysis (interleukin [IL]-1β, IL-6, tumor necrosis factor alpha [TNF-α], interferon gamma-induced protein 10 [IP-10], macrophage inflammatory protein 1 alpha [MIP-1-α], monocyte chemoattractant protein 1 [MCP-1], interferon alpha [IFN-α]). Concentrations of IFN-α were not reported due to technical issues with the assay. Immunoglobulin G (IgG) 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 <sup>a</sup> (µg/dose)	Dose Volume (µL/dose)	Dose Concentration <sup>a</sup> (mg/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	10/13	200	0.05/0.07	10	10	NE	NE
3	mRNA-1706	50/65	200	0.25/0.33	10	10	NE	NE
4	mRNA-1706	100/129	200	0.5/0.65	10	10	5	5

Abbreviations: F = female; M = male; NE = not evaluated; PBS = phosphate-buffered saline; SoA = Summary of Analysis.

<sup>a</sup> Value based on the SoA issued on 11 October 2016 /Value based on the SoA issued on 03 May 2017 (in bold). The original doses calculated for Groups 2, 3, and 4 were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064. The change in the reported mRNA content for the mRNA-1706 was 29%, and this percentage difference in reported RNA content was used to revise the calculated doses.

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-1 $\beta$  and IL-6.

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

mRNA-1706-related clinical observations were noted at  $\geq 13$  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  $\geq 13$  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  $\geq 13$  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, and clinical chemistry) changes were observed at  $\geq 13$  µg/dose on Day 30 and included increases in neutrophil, eosinophil (males and females  $\geq 13$  µg/dose), and large unstained cell counts with

concomitant increases in white blood cell (WBC) counts (males  $\geq 13$   $\mu\text{g}/\text{dose}$ ); decreases in lymphocyte counts (females  $\geq 13$   $\mu\text{g}/\text{dose}$ ), reticulocyte counts (males  $\geq 13$   $\mu\text{g}/\text{dose}$ ), and platelet counts (males 129  $\mu\text{g}/\text{dose}$ , females  $\geq 65$   $\mu\text{g}/\text{dose}$ ); increases in globulin and decreases in albumin with concomitant decreases in albumin to globulin (A/G) ratio (males  $\geq 13$   $\mu\text{g}/\text{dose}$ ).

mRNA-1706-related changes in cytokines were observed at 129  $\mu\text{g}/\text{dose}$  (the only treatment group analyzed for cytokine levels) and included statistically significant increases in IP-10, MCP-1, MIP-1 $\alpha$  (females only), and TNF- $\alpha$  (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  $\geq 13$   $\mu\text{g}/\text{dose}$  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  $\geq 13$   $\mu\text{g}/\text{dose}$  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  $\mu\text{g}/\text{dose}$ . mRNA-1706-related enlargement of the lymph nodes (inguinal and popliteal) noted at the terminal euthanasia was still observed in 129  $\mu\text{g}/\text{dose}$  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  $\mu\text{g}/\text{dose}$ . At  $\geq 13$   $\mu\text{g}/\text{dose}$ , 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-1β, 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 (mg/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	0.05	10	10	NE	NE
3	mRNA-1706	50	200	0.25	10	10	NE	NE
4	mRNA-1706	100	200	0.5	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-1 $\beta$ , TNF- $\alpha$ , and IL-6.

All mRNA-1706-treated animals, except for one 10  $\mu$ g/dose male animal, showed detectable antibody titers to the Zika virus after two doses (Day 30), but the administration of the third 100  $\mu$ g/dose boosted the antibody titers further by Day 43.

mRNA-1706-related clinical observations were noted at  $\geq 10$   $\mu$ g/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  $\mu$ g/dose; and skin redness at the injection site with higher incidence throughout the dosing period at  $\geq 50$   $\mu$ g/dose; and prominent backbone, thinness, hunched posture, and suspected dehydration in individual females at 100  $\mu$ g/dose.

mRNA-1706-related, lower mean body weight gains were observed after each dose at  $\geq 10$   $\mu$ g/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  $\geq 50$   $\mu$ g/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, and clinical chemistry) changes were observed at  $\geq 10$   $\mu$ g/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  $\geq 50$   $\mu$ g/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  $\mu$ g/dose and included significantly increased IP-10 and increased MIP-1 $\alpha$  and MCP-1.

At the terminal necropsy (Day 30), mRNA-1706-related increases in adrenal weights were observed at 100  $\mu$ g/dose in males and at  $\geq 50$   $\mu$ g/dose in females and correlated with cortical hypertrophy.

mRNA-1706-related macroscopic changes were observed at  $\geq 10$   $\mu\text{g}/\text{dose}$  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, and popliteal), bone marrow, adrenal glands, liver, mesenteric lymph node, thymus, and/or spleen.

At the injection site, minimal to marked inflammation was observed in  $\geq 10$   $\mu\text{g}/\text{dose}$  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  $\mu\text{g}/\text{dose}$  males.

In the lymph nodes (iliac, inguinal, and/or popliteal), minimal to moderate mixed cell infiltration was observed in  $\geq 50$   $\mu\text{g}/\text{dose}$  males and  $\geq 10$   $\mu\text{g}/\text{dose}$  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  $\geq 10$   $\mu\text{g}/\text{dose}$ .

In the adrenal glands, dose-dependent minimal cortical hypertrophy was present at  $\geq 50$   $\mu\text{g}/\text{dose}$  and correlated with the increased adrenal weights.

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

Microscopic findings in the spleen and mesenteric lymph nodes in  $\geq 10$   $\mu\text{g}/\text{dose}$  and in the thymus in  $\geq 50$   $\mu\text{g}/\text{dose}$  males and 100  $\mu\text{g}/\text{dose}$  females were consistent with minimal to mild decreased lymphoid cellularity and/or single cell necrosis of lymphocyte. 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 dendritic 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 (F) proteins of human metapneumovirus (hMPV) and parainfluenza virus type 3 (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, 1mM 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-1β, IL-6, TNF-α, IP-10,

MIP-1- $\alpha$ , 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 ( $\mu\text{g}/\text{dose}$ )	Dose Volume ( $\mu\text{L}$ )	Dose Concentration ( $\mu\text{g}/\text{mL}$ )	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 test article-related effects on mortality or ophthalmology findings. No mRNA-1653-related cytokine changes were observed for IL-1 $\beta$ , IL-6, and TNF- $\alpha$ .

All mRNA-1653-treated groups showed detectable antibody titers to hMPV/A2 and PIV/3 after two doses (Day 30), but the administration of the third 150  $\mu\text{g}/\text{dose}$  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  $\geq 10$   $\mu\text{g}/\text{dose}$  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  $\mu\text{g}/\text{dose}$ ; swelling (soft or firm) and localized skin redness at 150  $\mu\text{g}/\text{dose}$  after the second dose and occasionally present after the third dose.

mRNA-1653-related, dose-dependent lower mean body weight gains were noted in  $\geq 10$   $\mu\text{g}/\text{dose}$  males and  $\geq 50$   $\mu\text{g}/\text{dose}$  females; these changes were only cumulative at 150  $\mu\text{g}/\text{dose}$  and were associated with a slightly reduced food consumption at that dose.



mRNA-1653-related, minimal increases in body temperature were observed at  $\geq 10$   $\mu\text{g}/\text{dose}$  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  $\geq 10$   $\mu\text{g}/\text{dose}$  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  $\geq 10$   $\mu\text{g}/\text{dose}$ , females  $\geq 50$   $\mu\text{g}/\text{dose}$ ), reticulocyte counts (males  $\geq 10$   $\mu\text{g}/\text{dose}$ ), and platelet counts (females  $\geq 10$   $\mu\text{g}/\text{dose}$ ); 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  $\mu\text{g}/\text{dose}$  and included increases in IP-10, MCP-1, and MIP-1 $\alpha$ .

At the terminal necropsy (Day 30), mRNA-1653-related significant increases were observed in liver weights (150  $\mu\text{g}/\text{dose}$  females) and spleen weights ( $\geq 50$   $\mu\text{g}/\text{dose}$  males,  $\geq 10$   $\mu\text{g}/\text{dose}$  females). These weight changes had no microscopic correlation.

mRNA-1653-related macroscopic changes were observed at  $\geq 10$   $\mu\text{g}/\text{dose}$  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, and 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 intramuscular connective tissue and subcutis. Edema, necrotic debris, hemorrhage, and/or rare degenerated myofibers were also present. Minimal to mild mixed cell inflammation was also noted in the sciatic nerve (also present in surrounding connective tissue) of animals at  $\geq 10$   $\mu\text{g}/\text{dose}$ . The sciatic nerve inflammatory changes were regarded as secondary to the injection site inflammation.

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  $\geq 10$   $\mu\text{g}/\text{dose}$  and correlated macroscopically with enlargement. Minimal to mild mixed cell inflammation was also noted in the sciatic nerve of animals at  $\geq 10$   $\mu\text{g}/\text{dose}$  in

connective tissue surrounding the nerve. 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 in all groups including the control group 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  $\geq 10$  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  $\geq 10$  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  $\geq 10$  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% propylene glycol, 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,  $\alpha$ 1-acid glycoprotein [AGP], and  $\alpha$ 2-macroglobulin [A2M]), and cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$ , IP-10, MIP-1 $\alpha$ , 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 (mg/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	0.05	10	10	NE	NE
3	mRNA-1893	30	200	0.15	10	10	NE	NE
4	mRNA-1893	96	200	0.48	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- $\alpha$ .

All mRNA-1893-treated groups showed detectable antibody titers to the Zika virus after two doses (Day 30), but the administration of the third 96  $\mu$ g/dose boosted the antibody titers further by Day 43.

mRNA-1893-related, dose-dependent clinical observations were noted at  $\geq 10$   $\mu$ g/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; 2 females and 1 male animal at 96  $\mu$ g/dose with an abnormal gait; and 1 female animal at 96  $\mu$ g/dose with limited usage of the left hindlimb.

mRNA-1893-related, minimal dose-dependent increases in mean body temperature (from 37.47 to 39.13°C) were observed in  $\geq 10$   $\mu$ g/dose males at generally 6 hours post-dose on Days 1 and 29 and generally returned to pre-dose values at 24-hour post-dose (Days 2 and 30), except for a few animals at 10 and 30  $\mu$ g/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  $\geq 10$   $\mu$ g/dose on Day 30 and included increases in neutrophil, monocyte (except females 96  $\mu$ g/dose), and eosinophil counts with concomitant increases in WBC counts; increases in red blood cell distribution width (RDW) ( $\geq 30$   $\mu$ g/dose) and decreases in reticulocyte counts (males 96  $\mu$ g/dose); decreases in lymphocytes (96  $\mu$ g/dose); increases in APTT (males  $\geq 30$   $\mu$ g/dose, females  $\geq 10$   $\mu$ g/dose) and fibrinogen; increases in globulin and/or decreases in albumin ( $\geq 30$   $\mu$ g/dose) with concomitant decreases in A/G ratio; and decreases in glucose (males 30  $\mu$ g/dose, females  $\geq 30$   $\mu$ g/dose). Dose-dependent increases in AGP and A2M were also noted at  $\geq 10$   $\mu$ g/dose on Day 30.

mRNA-1893-related changes in cytokines were observed at  $\geq 10$   $\mu$ g/dose and included increases in IL-1 $\beta$ , IL-6, MCP-1, IP-10, and MIP-1 $\alpha$ .

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

mRNA-1893-related macroscopic changes were observed at  $\geq 10$   $\mu\text{g}/\text{dose}$  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, and 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  $\geq 10$   $\mu\text{g}/\text{dose}$  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  $\mu\text{g}/\text{dose}$  males and  $\geq 10$   $\mu\text{g}/\text{dose}$  females, with higher incidence at 96  $\mu\text{g}/\text{dose}$ . 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  $\geq 10$   $\mu\text{g}/\text{dose}$ . 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 males and/or females at  $\geq 10$   $\mu\text{g}/\text{dose}$  compared with the control group and minimal to moderate focal/multifocal neutrophilic inflammation with necrosis in females at  $\geq 30$   $\mu\text{g}/\text{dose}$ . These lymph node changes were regarded as 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  $\mu\text{g}/\text{dose}$  males and  $\geq 30$   $\mu\text{g}/\text{dose}$  females. In addition, minimal to mild hypertrophy of Kupffer cells was observed in  $\geq 30$   $\mu\text{g}/\text{dose}$  males and  $\geq 10$   $\mu\text{g}/\text{dose}$  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  $\mu\text{g}/\text{dose}$ , minimal to mild increased cellularity of macrophages in red pulp in 96  $\mu\text{g}/\text{dose}$  males and  $\geq 30$   $\mu\text{g}/\text{dose}$  females, minimal to mild neutrophilic infiltration in the red

pulp in  $\geq 30$   $\mu\text{g}/\text{dose}$  males  $\geq 10$   $\mu\text{g}/\text{dose}$  females, and minimal increased extramedullary hematopoiesis in  $\geq 30$   $\mu\text{g}/\text{dose}$  males.

In the bone marrow, there was minimal increased cellularity of myeloid lineage in  $\geq 30$   $\mu\text{g}/\text{dose}$  males and  $\geq 10$   $\mu\text{g}/\text{dose}$  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 a minimal increased epithelial single cell necrosis in 96  $\mu\text{g}/\text{dose}$  males.

At the end of the 2-week recovery period (Day 43), all changes were partially or fully recovered at 96  $\mu\text{g}/\text{dose}$ . 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 a decreased incidence and/or severity indicating partial recovery.

### **Conclusions:**

Administration of mRNA-1893 by intramuscular injection for 1 month (over 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  $\mu\text{g}/\text{dose}$ . At  $\geq 10$   $\mu\text{g}/\text{dose}$ , 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 which encode the full-length cytomegalovirus (CMV) glycoprotein B (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.

##### **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-1β, 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 <sup>a</sup> (µg/dose)	Dose Volume (µL/dose)	Dose Concentration <sup>a</sup> (mg/mL)	Number of Animals			
					Main Study		Recovery Study	
					Males	Females	Males	Females
1	PBS (control)	0	200	0	10	10	5	5
2	mRNA-1647	10/ <b>8.9</b>	200	0.05/ <b>0.045</b>	10	10	NE	NE
3	mRNA-1647	30/ <b>27</b>	200	0.15/ <b>0.14</b>	10	10	NE	NE
4	mRNA-1647	100/ <b>89</b>	200	0.5/ <b>0.45</b>	10	10	5	5

Abbreviations: NE = not evaluated; PBS = phosphate-buffered saline; SoA = Summary of Analysis.

<sup>a</sup> Value based on SoA issued on 16 March 2017/Value based on SoA issued on 31 May 2017 (in bold). The original doses calculated for Groups 2, 3, and 4 were revised based on the updated concentration reported for mRNA-1647 Lot No. MTDP17015. The change in the reported mRNA content for the mRNA-1647 was -11%, and this percentage difference in reported RNA content was used to revise the calculated doses.

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-1 $\beta$ , IL-6, MIP-1 $\alpha$ , and TNF- $\alpha$  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  $\geq 8.9$  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 were observed after each dose in  $\geq 8.9$  µg/dose males and 89 µg/dose females and sometimes reached statistical significance. From Day -1 to 42, the changes were cumulative in males only and were 0.86-fold mean controls.

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  $\geq 8.9$  µg/dose on Day 44 and included increases in neutrophil, eosinophil, and large unstained cell counts with concomitant increases in WBC counts (males  $\geq 27$  µg/dose, females  $\geq 8.9$  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  $\geq 8.9$  µg/dose; were statistically significant in 89 µg/dose males and  $\geq 27$  µg/dose females; and were not correlated with macroscopic or microscopic findings.

mRNA-1647-related macroscopic changes were observed at  $\geq 8.9$  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  $\geq 8.9$  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  $\geq 8.9$   $\mu\text{g}/\text{dose}$ . 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  $\geq 8.9$   $\mu\text{g}/\text{dose}$ . This finding was considered to be an extension of the inflammatory reaction at the intramuscular injection sites to this region.

In the bone marrow, minimal increased myeloid hematopoiesis was noted at  $\geq 27$   $\mu\text{g}/\text{dose}$ . This finding was characterized by increased numbers of myeloid precursors in the marrow and was secondary or compensatory inflammatory reaction noted at the intramuscular injection sites.

In the spleen, a dose-dependent minimal to mild decreased cellularity of the periarteriolar lymphoid sheath was noted at  $\geq 8.9$   $\mu\text{g}/\text{dose}$ .

At the end of the 2-week recovery period (Day 57), all changes were partially or fully recovered at 89  $\mu\text{g}/\text{dose}$ . The concentrations of A2M levels were still slightly higher than controls. 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 intramuscular injection for 6 weeks (4 doses) was clinically well tolerated in rats up to 89  $\mu\text{g}/\text{dose}$ . At  $\geq 8.9$   $\mu\text{g}/\text{dose}$ , 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 ([Study 5002158](#); GLP-compliant). mRNA-1443 contains a single mRNA sequence that encodes for 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-1β, 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 1-Month (3 Doses) Repeat-Dose Toxicity Study of mRNA-1443 in Sprague Dawley Rats**

Group Number	Treatment	Dose Level <sup>a</sup> (µg/dose)	Dose Volume (µL/dose)	Dose Concentration <sup>a</sup> (mg/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	10/ <b>9.6</b>	200	0.05/ <b>0.048</b>	10	10	NE	NE
3	mRNA-1443	30/ <b>29</b>	200	0.15/ <b>0.145</b>	10	10	NE	NE
4	mRNA-1443	100/ <b>96</b>	200	0.5/ <b>0.48</b>	10	10	5	5

Abbreviations: F = female; M = male; NE = not evaluated; PBS = phosphate-buffered saline; SoA = Summary of Analysis.

<sup>a</sup> Value based on the SoA issued on 16 March 2017/value based on the SoA issued on 30 May 2017 (in bold). The original doses calculated for Groups 2, 3, and 4 were revised based on the updated concentration reported for mRNA-1443 Lot No. MTDP17017. The change in the reported mRNA content for the mRNA-1443 was 4%, and this percentage difference in reported RNA content was used to revise the calculated doses.

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-1 $\beta$ , IL-6, MIP-1 $\alpha$ , and TNF- $\alpha$  levels.

mRNA-1443 elicited minimal and variable CD4 and CD8 T cell responses to phosphoprotein 65 (pp65) in all groups.

mRNA-1443-related, dose-dependent clinical observations at the injection site were noted at  $\geq 9.6$  µg/dose and included very slight to moderate edema, with occasional severe edema noted at  $\geq 29$  µg/dose; and very slight erythema in 96 µg/dose males and very slight to mild erythema in  $\geq 29$  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  $\geq 9.6$  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  $\geq 9.6$  µg/dose on Day 44 and included increases in neutrophil, eosinophil, and/or large unstained cell (males only  $\geq 9.6$  µg/dose) counts with concomitant increases in WBC counts (males  $\geq 29$  µg/dose, females  $\geq 9.6$  µg/dose); decreases in lymphocyte (males only  $\geq 29$  µg/dose), reticulocyte (males only  $\geq 9.6$  µg/dose), and platelet (females only  $\geq 9.6$  µg/dose) counts; increases in APTT (males  $\geq 29$  µg/dose, females 96 µg/dose) and fibrinogen; decreases in albumin (males 96 µg/dose, females  $\geq 29$  µg/dose) and

increases in globulin (males and females  $\geq 29$   $\mu\text{g}/\text{dose}$ ) with concomitant decreases in A/G ratio (males and females  $\geq 29$   $\mu\text{g}/\text{dose}$ ). Dose-dependent increases in AGP and A2M were also noted on Day 44 and were statistically significant at  $\geq 29$   $\mu\text{g}/\text{dose}$ .

mRNA-1443-related changes in cytokines were observed at 96  $\mu\text{g}/\text{dose}$  and included IP-10 and MCP-1.

At the terminal necropsy (Day 44), mRNA-1443-related increases in spleen weights were observed in  $\geq 9.6$   $\mu\text{g}/\text{dose}$  males and 96  $\mu\text{g}/\text{dose}$  females and were not correlated with macroscopic or microscopic findings.

mRNA-1443-related macroscopic changes were observed at  $\geq 9.6$   $\mu\text{g}/\text{dose}$  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  $\geq 9.6$   $\mu\text{g}/\text{dose}$  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  $\geq 9.6$   $\mu\text{g}/\text{dose}$ . 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  $\geq 9.6$   $\mu\text{g}/\text{dose}$ . 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  $\geq 9.6$   $\mu\text{g}/\text{dose}$  compared with the control group.

In the sciatic nerve, dose-dependent, minimal to marked mixed cellular inflammation of the perineurial tissue was observed in  $\geq 9.6$   $\mu\text{g}/\text{dose}$  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 breach 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  $\geq 9.6$   $\mu\text{g}/\text{dose}$  males and  $\geq 29$   $\mu\text{g}/\text{dose}$ . 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 a minimally decreased cellularity of the periarteriolar lymphoid sheath was noted at  $\geq 9.6$   $\mu\text{g}/\text{dose}$ . 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  $\geq 9.6$   $\mu\text{g}/\text{dose}$ , 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  $\mu\text{g}/\text{dose}$ . 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  $\mu\text{g}/\text{dose}$  and control groups. These remaining findings generally occurred with a decreased incidence and/or severity indicating partial recovery.

### **Conclusions:**

Administration of mRNA-1443 by intramuscular 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  $\geq 9.6$  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**

The objective was to determine the potential genotoxicity of SM-102 using the bacterial reverse mutation test (Ames test) in *Salmonella typhimurium* (*S. typhimurium*) and *Escherichia coli* (*E. coli*) ([Study 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 uvrA, were treated with SM-102 in the absence (0S9) and presence (+S9) of a supplemented rat liver fraction (S9 mix) at 37°C for 67 hours and 29 minutes. SM-102 was tested over a wide range of dose levels from 19.0 µg/mL to 50000 µg/mL (the standard limit dose for this assay) ([Table 8](#)). The negative control was ethanol. The positive controls included 2-nitrofluorene, sodium azide, 9-Aminoacridine hemihydrate, and 4 nitroquinoline-N-oxide in the absence of S9 mix; and 2 aminoanthracene and benzo[a]pyrene 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 in *S. typhimurium* and *E. coli***

Dose Number	Final Concentration (µL/plate)	Number of Strains	Number of Cultures	
			67 h 29 min (0S9)	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 <sup>a</sup>	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 <sup>b</sup>	2	NE	3
benzo[a]pyrene (positive control)	5	3	NE	3

Abbreviations: 0S9 = without S9 mix activation; +S9 = with S9 mix activation; *E. coli* = *Escherichia coli*; h = hour; min = minute; NE = not evaluated; SM-102 = heptadecan-9-yl 8((2hydroxyethyl)(6oxo6-(undecyloxy) hexyl)amino)octanoate; *S. typhimurium* = *Salmonella typhimurium*.

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

<sup>b</sup> Final concentrations of 5 and 20 µg/plate were incubated with *S. typhimurium* TA1535 strain and *E. coli* WP2 uvrA 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  $\geq 1581$  µg/plate in the absence of S9 mix and at concentrations  $\geq 500$  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.

**2.6.6.4.2 In Vitro Mammalian Cell System**

The objective was to determine the potential genotoxicity of SM-102 using an in vitro mammalian cell micronucleus test in human peripheral blood lymphocytes ([Study 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 (0S9) and presence (+S9) 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 9](#)). The negative control was ethanol. The positive controls were mitomycin C and nocodazole in the absence of S9 mix, and cyclophosphamide in the presence of S9 mix. All concentrations of SM-102, as well as positive and negative controls, were evaluated in duplicate.

**Table 9: Experimental Design for Mammalian Cell Micronucleus Test in Human Peripheral Blood Lymphocytes**

Dose Number	Final Concentration (µg/mL) <sup>a</sup>	Number of Cultures		
		4 h (0S9)	4 h (+S9)	24 h (0S9)
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: 0S9 = without S9 mix activation; +S9 = with S9 mix activation; h = hour; NE = not evaluated;  
SM-102 = heptadecan-9-yl 8((2hydroxyethyl)(6oxo6-(undecyloxy) hexyl)amino)octanoate.

<sup>a</sup> 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 and 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 absence of S9 mix

and at 500 µg/mL in the presence of S9 mix; and at 24-hours post-treatment at  $\geq 286$  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.

## **2.6.6.4.3 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 ([Study 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  $\geq 3.9$  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 5.2, 2.6, or 1.3 mg/kg and females were administered 2.6, 1.3, or 0.6 mg/kg as an IV injection via the tail vein. The main phase study design is outlined in [Table 10](#). 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, temporarily 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 10: Experimental Design for Mammalian Erythrocyte Micronucleus Test of mRNA-1706 in Rats**

Group Number	Test Material	Dose Level <sup>a</sup> (mg/kg [mRNA/SM-102])	Dose Volume (mL/kg)	Dose Conc. <sup>a</sup> (mg/mL)	Sampling Time (h)	Number of Animals			
						Main Study		Bioanalysis Study <sup>b</sup>	
						M	F	M	F
1	Negative Control	0	5	0	24	5	5	3	3
					48	5	5	NE	NE
2	mRNA-1706	1.0/ <b>1.3</b> /13.5	5	0.2/ <b>0.26</b> / <b>2.7</b>	24	5	NE	3	NE
3	mRNA-1706	2.0/ <b>2.6</b> /27.0	5	0.4/ <b>0.52</b> / <b>5.4</b>	24	5	NE	3	NE
4	mRNA-1706	4.0/ <b>5.2</b> /54.1	5	0.8/ <b>1.03</b> / <b>10.82</b>	24	5	NE	3	NE
					48	5	NE	NE	NE
9	mRNA-1706	0.5/ <b>0.6</b> /6.2	5	0.1/ <b>0.129</b> / <b>1.24</b>	24	NE	5	NE	3
10	mRNA-1706	1.1/ <b>1.3</b> /13.5	5	0.2/ <b>0.26</b> / <b>2.7</b>	24	NE	5	NE	3
11	mRNA-1706	2.0/ <b>2.6</b> /27.0	5	0.4/ <b>0.52</b> / <b>5.4</b>	24	NE	5	NE	3
					48	NE	5	NE	NE
5	Positive Control	c							

Conc = concentration; F = Females; M = Males; NE = not evaluated; SoA = Summary of Analysis.

<sup>a</sup> Value based on the SoA issued on 11 October 2016 /Value based on the SoA issued on 03 May 2017 (in bold) /SM-102 concentration. The original doses calculated for Groups 2, 3, and 4 were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064. The change in the reported mRNA content for the mRNA-1706 was 29%, and this percentage difference in reported RNA content was used to revise the calculated doses.

<sup>b</sup> Bioanalysis animals were used for quantification of exposure in plasma only.

<sup>c</sup> Positive scoring control slides (taken from three 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  $\leq 2.6/27.0$  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, or redness of the tail skin were observed. In general, females also had decreased weight gain, which increased with dose, 24 hours post-dose, but recovered by 48 hours.

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 over negative control, a non-dose dependent (no change at the mid dose), 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 the 48-hour sampling time, 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 of this non-GLP-compliant study 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 ([Study AF87FU.125012NGLPICH.BTL](#)). NPI luciferase mRNA is combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 25 mM Tris/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 11). Animals were monitored for clinical observations and body temperature measurements before and after dosing. Two and 6 hours after dosing, 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 after dosing using a branched DNA assay; however, the mRNA quantification was not reported due to technical issues with the assay. Cytokine concentrations for MIP-1 $\alpha$ , MCP-1, IL-6, IL-1 $\beta$ , TNF $\alpha$ , and IP-10 were quantified in plasma samples from 6 hours after dosing using a Luminex assay. Twenty-four and 48 hours after dosing, 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 [NCEs]) 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 after treatment. Study findings were expressed as pair-wise comparisons made with the control group.

**Table 11: Experimental Design for Mammalian Erythrocyte Micronucleus Test of SM-102 in Rats**

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

Abbreviation: DTPA = diethylenetriamine pentaacetic acid; F = female; M = male; NPI = nascent peptide imaging; SM-102 = heptadecan-9-yl 8((2hydroxyethyl)(6oxo6-(undecyloxy) hexyl)amino)octanoate; Tris = trometamol.

<sup>a</sup> Based upon individual body weight measurements.

<sup>b</sup> Time point ranged from 24 to 27 hours and 45 to 48 hours, respectively.

<sup>c</sup> Control is 25 mM Tris/sucrose 1 mM DTPA pH 7.5.

<sup>d</sup> Test article is NPI luciferase mRNA in SM-102-containing lipid nanoparticles.

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 ( $\geq 1^{\circ}\text{C}$  increase for at least 4.5 hours).

Test article-related increases in IL-6, MCP-1, MIP-1 $\alpha$ , 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 $\alpha$ , 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**

Reproductive and developmental toxicity studies have not been conducted with mRNA-1273.

## **2.6.6.7 STUDIES IN JUVINILE 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 of this non-GLP-compliant study 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 ([Study 2308-123](#)).

##### **Methods:**

Sprague Dawley rats were administered 2 doses of 30, 60, or 100 µg/dose mRNA-1273 or control (Tris/sucrose buffer) via IM bolus injection into alternating quadriceps (hind leg, thigh) on Days 1 and 22 ([Table 12](#)). Animals were monitored for clinical observations, body weight, and clinical pathology parameters (hematology, coagulation, and 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 12: Experimental Design for Toxicity Study of mRNA-1273 in Sprague Dawley Rats**

Group Number	Treatment	Dose Level (µg/dose)	Dose Volume (µL/dose)	Dose Concentration (µg/mL)	Number of Animals	
					M	F
1	Control (Tris/sucrose buffer)	0	0.2	0	5	5
2	mRNA-1273	30	0.2	150	5	5
3	mRNA-1273	60	0.2	300	5	5
4	mRNA-1273	100	0.2	500	5	5

Abbreviations: F = female; M = male; Tris = trometamol.

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 two doses (Day 35); full immunogenicity results are presented in [Section 2.6.2 Pharmacology Written Summary](#).

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

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

All clinical observations fully recovered by Day 35.

### **Conclusions:**

Administration of mRNA-1273 by intramuscular bolus injection on Days 1 and 22 to Sprague Dawley rats was well tolerated up to 100  $\mu\text{g}/\text{dose}$ .

### **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, are driven primarily by the LNP composition

and, to a lesser extent, the biologic activity of the expressed antigens of the vaccine. 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 vaccines, together with a non-GLP rat repeat-dose immunogenicity rat study with safety endpoints and genotoxicity assessments of the SM-102 lipid, supports the 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 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 observed at  $\geq 9$  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  $\geq 9$  µg/dose. Hematology changes included increases in white blood cells, 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  $\geq 9$  µg/dose at 6 hours post-dose including interferon (IFN)  $\gamma$ , IP-10, MCP-1, and MIP-1 $\alpha$ . Cytokine changes were generally reversing by the end of the 2-week recovery period.

Post-mortem test article-related and generally dose-dependent changes in organ weights and macroscopic and microscopic findings were observed at  $\geq 9$  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 periarticular 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 to characterize the immunogenic response and potential toxicity of mRNA-1273 at IM doses levels of 30, 60, and 100 ug/dose on Days 1 and 22 was conducted. A strong immunogenic response against SARS-CoV-2 S-2P was observed on Day 35, with measured IgG titers above  $10^6$  at all dose levels. mRNA-1273 had no effect on body weights and limited, transient clinical signs starting at 30  $\mu$ g/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  $\mu$ g/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 in the SM-102-containing LNPs.

Genotoxicity assessments of the SM-102 lipid concluded the lipid is not genotoxic in the bacterial mutagenicity and the human peripheral blood lymphocytes chromosome aberration assays. Two intravenous in vivo micronucleus assays were conducted with mRNA-based drug products formulated in the SM-102-containing LNPs. Results from [Report AF87FU.125012NGLPICH.BTL](#) were negative, 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 intramuscular administration, limited duration of exposure, and negative in vitro results.

#### **2.6.6.11 TABLES AND FIGURES**

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

#### **2.6.6.12 REFERENCES**

International Council 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>.

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