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2.6.7.1 TOXICOLOGY: OVERVIEW

Type of Study	Species and Strain	Method of Adm.	Test Article	Duration of Dosing	Doses or Concentration	GLP Compliance	Testing Facility	Report Number	Location in eCTD
Repeat-dose toxicity	Rat, Sprague Dawley	IM	mRNA-1706[a]	3 doses over 1 month (every 2 weeks)	0, 13, 65, and 129 µg/dose[b]	Yes	CRL Sherbrooke, QC, Canada	5002045	4.2.3.2
		IM	mRNA-1706[a]	3 doses over 1 month (every 2 weeks)	0, 10, 50, and 100 µg/dose	Yes	CRL Sherbrooke, QC, Canada	5002231	4.2.3.2
		IM	mRNA-1653[c]	3 doses over 1 month (every 2 weeks)	0, 10, 50, and 150 µg/dose	Yes	CRL Sherbrooke, QC, Canada	5002033	4.2.3.2
		IM	mRNA-1893[d]	3 doses over 1 month (every 2 weeks)	0, 10, 30, and 96 µg/dose	Yes	CRL Sherbrooke, QC, Canada	5002400	4.2.3.2
		IM	mRNA-1647[e]	4 doses over 6 weeks (every 2 weeks)	0, 8.9, 27, and 89 µg/dose[f]	Yes	CRL Sherbrooke, QC, Canada	5002034	4.2.3.2
		IM	mRNA-1443[g]	4 doses over 6 weeks (every 2 weeks)	0, 9.6, 29, and 96 µg/dose[h]	Yes	CRL Sherbrooke, QC, Canada	5002158	4.2.3.2

Type of Study	Species and Strain	Method of Adm.	Test Article	Duration of Dosing	Doses or Concentration	GLP Compliance	Testing Facility	Report Number	Location in eCTD
Genotoxicity	*Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 *Escherichia coli* strain WP2 *uvr*A	In vitro	SM-102	Incubation 67 h and 29 min	0, 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000 µg/plate SM-102	Yes	CRL (b) (6)	9601567	4.2.3.3.1
		In vitro	PEG2000-DMG (b) (4)	Incubation 67 h and 57 min	0, 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000 µg/plate PEG2000-DMG	Yes	CRL (b) (6)	9601035	4.2.3.3.1
	Human peripheral blood lymphocytes	In vitro	SM-102	Incubation 4 and 24 h with or without supplemented rat liver fraction	0, 3.25, 5.68, 9.95, 17.4, 30.5, 53.3, 93.3, 163, 286, 500 µg/mL SM-102	Yes	CRL (b) (6)	9601568	4.2.3.3.1
		In vitro	PEG2000-DMG (b) (4)	Incubation 4 and 24 h with or without supplemented rat liver fraction	0, 3.25, 5.68, 9.95, 17.4, 30.5, 53.3, 93.3, 163, 286, 500 µg/mL PEG2000-DMG	Yes	CRL (b) (6)	9601036	4.2.3.3.1
	Rat, Sprague Dawley	IV (bolus)	mRNA-1706[a]	Single dose evaluated at 24 h and 48 h post dose	0, 0.6/6.2 (F), 1.3/13.5, 2.6/27.0, 5.2/54.1 (M) mg/kg mRNA-1706/ SM-102[j, k]	Yes	CRL (b) (6)	9800399	4.2.3.3.2
		IV (bolus)	NPI luciferase mRNA[l]	Single dose evaluated at 24 h and 48 h post-dose	0.32/6.0, 1.07/20, 3.21/60 mg/kg NPI luciferase mRNA/SM-102	No	BioReliance Corporation Rockville, MD, USA	AF87FU.125012NGLPICH.BTL	4.2.3.3.2

Type of Study	Species and Strain	Method of Adm.	Test Article	Duration of Dosing	Doses or Concentration	GLP Compliance	Testing Facility	Report Number	Location in eCTD
Reproductive and developmental toxicity	Rat, Sprague Dawley	IM	mRNA-1273[m]	4 doses over 6 weeks (every 2 weeks)	0 and 100 µg/dose	Yes	CRL Horsham, PA, USA	20248897	4.2.3.5.3
Other toxicity studies	Rat, Sprague Dawley	IM	mRNA-1273[n]	2 doses over 3 weeks	0, 30, 60, and 100 µg/dose	No	CRL Mattawan, MI, USA	2308-123	4.2.3.7.7

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

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

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

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

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

[e] mRNA-1647 contains 6 mRNAs 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.

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

[g] mRNA-1443 contains a single mRNA sequence that encodes 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.

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

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

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

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

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

[m] mRNA-1273 contains a single mRNA sequence that encodes 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, 17.5 mM sodium acetate, pH 7.5.

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

2.6.7.4 TOXICOLOGY: DRUG PRODUCTS

Test Article	Lot/Batch No.	Purity	Specified Impurities			Report Number	Type of Study
Proposed specification:			Pre-main peak area	Post-main peak area	mRNA-adduct species		
mRNA-1706[a]	MTDP16064	(b) (4)	(b) (4)		NE	5002045	Repeat-dose toxicity
	MTDP17036				NE	5002231	Repeat-dose toxicity
	MTDP16064				NE	9800399	Genotoxicity
mRNA-1653[a]	MTDP17038				NE	5002033	Repeat-dose toxicity
mRNA-1893[a]	MTDP18195				(b) (4)	5002400	Repeat-dose toxicity
mRNA-1647[a]	MTDP17015		NE	NE	NE	5002034	Repeat-dose toxicity
mRNA-1443[a]	MTDS17017		NE	NE	NE	5002158	Repeat-dose toxicity
SM-102	RL-100-211-1		NE	NE	NE	9601567	Genotoxicity
	RL-100-211-1		NE	NE	NE	9601568	Genotoxicity
PEG2000-DMG (b) (4)	G14904		NE	NE	NE	9601035	Genotoxicity
	G14904		NE	NE	NE	9601036	Genotoxicity
NPI luciferase mRNA[c]	MTDS18021		NE	NE	NE	AF87FU.125012NGLPICH.BTL	Genotoxicity
mRNA-1273	DH-03026		(b) (4)			20248897	Reproductive and developmental toxicity
mRNA-1273	8520100101					2308-123	Other toxicity

Abbreviations: mRNA = messenger RNA; NE = not evaluated; NPI = nascent peptide imaging.

[a] mRNA-based vaccine formulated in SM-102 lipid nanoparticles, as described in Section 2.6.7.1.

(b) (4)

[c] NPI luciferase mRNA formulated in SM-102 lipid nanoparticles, as described in Section 2.6.7.1.

2.6.7.7A REPEAT-DOSE TOXICITY: PIVOTAL STUDIES

Study Title: Zika: A 1-month (3 doses) intramuscular injection toxicity study of mRNA-1706 in Sprague Dawley rats with a 2-week recovery period

Test Article: mRNA-1706[a]

Species/Strain: Rat/Crl:CD(SD) Sprague Dawley

Duration of Dosing: 1 month (3 doses; Days 1, 15, and 29)

Formulation: 20 mM Tris, 8% sucrose, pH 7.4

Initial Age: 8 weeks

Duration of Postdose: 2 weeks

Report Number: 5002045

Date of First Dose: 20 Oct 2016 (males) and 21 Oct 2016 (females)

Route of Administration: Intramuscular injection

Location in eCTD: 4.2.3.2

Dose Volume: 200 µL/dose

GLP Compliance: Yes

Control Article: Phosphate-buffered saline, pH 7.2

Special Features: IFN-α[b], IL-1β, IL-6, TNF-α, IP-10, MIP-1α, MCP-1, and anti-ZIKV lysate antibody titers

Dose (µg/dose)[c]	**0 (control)**		**13**		**65**		**129**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals								
Main study	10	10	10	10	10	10	10	10
Recovery period	5	5	0	0	0	0	5	5
Died or sacrificed moribund	0	0	0	0	0	0	0	0
Mean anti-ZIKV lysate antibody titers (units/mL)[d]								
Main study animals								
Day 1	1.48	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Day 30	1.76	1.47	3.94	13.88	10.55	124.9	80.48	316.8
Recovery period animals								
Day 1	1.40	1.40	NE	NE	NE	NE	1.40	1.77
Day 43	1.40	1.40	NE	NE	NE	NE	1330	3574
Noteworthy findings – main study								
Clinical observations								
Warm to touch	0	0	0	0	0	0	0	15[e]

Dose (µg/dose)[c]	0 (control)		13		65		129	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mean body weight	–	–	–	–	–	–	–	–
Mean body weight gains (g)								
Days −1 to 7	62.9	24.6	–	–	–	–	43.0$^{\#\#\#}$	15.8**
Days 7 to 14	57.2	20.3	–	–	–	–	65.1**	26.3
Days 14 to 21	48.7	19.9	–	–	–	–	32.9***	16.2
Days 21 to 28	48.9	13.8	–	–	–	–	56.7*	17.9
Days −1 to 28	217.6	78.7	–	–	–	–	197.6	76.3
Mean food consumption (g or %)[f]								
Days 1 to 8	31.01	21.72	–	–	–	–	−12.23	−7.64
Days 8 to 15	32.30	23.35	–	–	–	–	4.95	−2.17
Days 15 to 22	32.97	23.59	–	–	–	–	−8.51	−6.44
Days 22 to 29	33.59	24.48	–	–	–	–	5.81	−0.65
Ophthalmic examinations	–	–	–	–	–	–	–	–
Hematology (Day 30; mean or mean fold change)[g]								
White blood cells (10^3/µL)	7.999	7.061	1.6***	–	2.3***	–	1.8***	–
Neutrophils (10^3/µL)	0.794	0.802	8.2	5.5$^{\#}$	14.1$^{\#\#\#}$	7.2$^{\#\#\#}$	11.7$^{\#\#\#}$	7.0$^{\#\#\#}$
Lymphocytes (10^3/µL)	6.906	5.959	–	0.82	–	0.47***	–	0.39***
Eosinophils (10^3/µL)	0.070	0.058	2.1	4.0$^{\#\#\#}$	2.7$^{\#\#}$	3.1$^{\#}$	2.1	3.6$^{\#\#}$
Large unstained cells (10^3/µL)	0.057	0.058	6.0$^{\#\#\#}$	–	4.1$^{\#\#\#}$	–	2.4	–
Reticulocytes (10^9/L)	257.59	194.90	0.77***	–	0.68***	–	0.69***	–
Platelets (10^3/µL)	1117.7	1145.8	0.92	0.97	0.92	0.87*	0.85	0.76***
Coagulation (Day 30; mean or mean fold change)[g]								
Fibrinogen (mg/dL, %)	300.8	236.3	2.2***	2.0***	2.5***	2.2***	2.4***	1.9***
Clinical chemistry (Day 30; mean or mean fold change)[g]								
Globulin (g/dL)	1.77	1.77	1.3***	–	1.3***	–	1.3***	–
Albumin/globulin ratio (ratio)	2.21	2.61	0.70$^{\#\#\#}$	–	0.71$^{\#\#\#}$	–	0.71$^{\#\#\#}$	–

Dose (µg/dose)[c]	0 (control)		13		65		129	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Cytokines (recovery period animals only; mean, pg/mL)								
IL-1β	–	–	NE	NE	NE	NE	–	–
IL-6	–	–	NE	NE	NE	NE	–	–
IP-10								
Day 1, 6 h post dose	184.084	98.670	NE	NE	NE	NE	773.962[§§§]	1099.716[^]
Day 15, 6 h post dose	106.720	70.428	NE	NE	NE	NE	678.824[^]	991.920[^]
Day 29, 6 h post dose	106.406	64.514	NE	NE	NE	NE	868.406[§§§]	956.216[§§§]
MCP-1								
Day 1, 6 h post dose	364.498	257.378	NE	NE	NE	NE	824.392[§§]	938.538[§§]
Day 15, 6 h post dose	454.146	484.518	NE	NE	NE	NE	707.672[§§]	1053.934[^]
Day 29, 6 h post dose	306.984	446.772	NE	NE	NE	NE	700.854[§§]	1125.104[§]
MIP-1α								
Day 1, 6 h post dose	11.700[h]	11.700[h]	NE	NE	NE	NE	–	31.488
Day 15, 6 h post dose	11.700[h]	11.700[h]	NE	NE	NE	NE	–	30.314[^]
Day 29, 6 h post dose	11.700[h]	11.700[h]	NE	NE	NE	NE	–	41.260[^^]
TNF-α								
Day 1, 6 h post dose	2.930[h]	2.930[h]	NE	NE	NE	NE	4.688	–
Day 15, 6 h post dose	2.930[h]	7.234	NE	NE	NE	NE	6.146[^]	–
Day 29, 6 h post dose	2.930[h]	7.136	NE	NE	NE	NE	5.168	–
Gross pathology (Day 30)								
Site, injection (number examined)	10	10	10	10	10	10	10	10
Abnormal consistency, firm	0	0	10	10	10	10	10	10
Swelling	0	0	4	6	7	8	7	9
Lymph node, inguinal (number examined)	10	10	10	10	10	10	10	10
Enlargement	0	0	2	1	5	1	6	4
Lymph node, popliteal (number examined)	10	10	10	10	10	10	10	10
Enlargement	0	0	3	4	8	5	7	4

Dose (µg/dose)[c]	**0 (control)**		**13**		**65**		**129**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Lymph node[i] (number examined)	0	0	1	2	7	4	4	6
Enlargement	0	0	1	2	7	4	4	6
Organ weight (Day 30)	–	–	–	–	–	–	–	–
Histopathology (Day 30; number of animals affected)[j]								
Liver (number examined)	10	10	10	10	10	10	10	10
Vacuolation	(1)	(7)	(3)	(8)	(4)	(8)	(5)	(5)
Minimal	1	7	3	8	4	8	5	5
Spleen (number examined)	10	10	10	10	10	10	10	10
Decreased cellularity; lymphoid, periarteriolar lymphoid sheath	(0)	(0)	(4)	(5)	(7)	(9)	(10)	(10)
Minimal	0	0	4	5	7	9	7	5
Mild	0	0	0	0	0	0	3	5
Injection site (number examined)	10	10	10	10	10	10	10	10
Inflammation	(0)	(0)	(10)	(10)	(10)	(10)	(10)	(10)
Minimal	0	0	0	1	0	0	0	0
Mild	0	0	0	6	0	2	0	4
Moderate	0	0	10	3	10	8	10	6
Lymph node, inguinal (number examined)	10	10	10	10	10	10	10	10
Infiltration, mixed cell	(0)	(0)	(0)	(0)	(0)	(1)	(2)	(3)
Minimal	0	0	0	0	0	0	1	0
Mild	0	0	0	0	0	1	1	3
Lymph node, popliteal (number examined)	10	10	10	10	10	10	10	10
Infiltration, mixed cell	(0)	(0)	(6)	(9)	(7)	(10)	(8)	(9)
Minimal	0	0	0	7	2	4	6	2
Mild	0	0	6	2	5	6	2	7
Noteworthy findings – recovery period								
Clinical observations	–	–	–	–	–	–	–	–

Dose (µg/dose)[c]	0 (control)		13		65		129	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mean body weight gains (g)								
Days 28 to 42	50.0	31.4	NE	NE	NE	NE	58.0	30.6
Mean food consumption	–	–	NE	NE	NE	NE	–	–
Hematology (Day 43)	–	–	NE	NE	NE	NE	–	–
Coagulation (Day 43)	–	–	NE	NE	NE	NE	–	–
Clinical chemistry (Day 43)	–	–	NE	NE	NE	NE	–	–
Cytokines (Day 43; recovery period animals only)	–	–	NE	NE	NE	NE	–	–
Gross pathology (Day 43)								
Lymph node, inguinal (number examined)	5	5	NE	NE	NE	NE	5	5
Enlargement	0	0	NE	NE	NE	NE	2	0
Lymph node, popliteal (number examined)	5	5	NE	NE	NE	NE	5	5
Enlargement	0	0	NE	NE	NE	NE	2	0
Histopathology (Day 43)	–	–	NE	NE	NE	NE	–	–

Abbreviations: – = no mRNA-1706-related effect; eCTD = electronic common technical document; F, female; GLP = Good Laboratory Practice; IFN = interferon; IL = interleukin; IP-10 = interferon gamma-induced protein 10; M = male; MCP-1 = monocyte chemoattractant protein 1; MIP-1α = macrophage inflammatory protein 1 alpha; mRNA = messenger RNA; NE = not evaluated; SoA = summary of analysis; TNF = tumor necrosis factor; ZIKV = Zika virus.

Notes: Statistical significance is based on actual data (not on the percent differences); * = $p \leq .05$, ** = $p \leq .01$, *** = $p \leq .001$ (mean value significantly different from control mean value using a Dunnett test); # = $p \leq .05$, ## = $p \leq .01$, ### = $p \leq .001$ (mean value significantly different from control mean value using a Dunn test); § = $p \leq .05$, §§ = $p \leq .01$, §§§ = $p \leq .001$ (mean value significantly different from control mean value using a *t* test); ^ = $p \leq .05$, ^^ = $p \leq .01$ (mean value significantly different from control mean value using a Wilcoxon test).

[a] mRNA-based vaccine formulated in SM-102 lipid nanoparticles, as described in Section 2.6.7.1.

[b] IFN-α results were considered invalid because samples were analyzed with a rat IFN-α antibody enzyme-linked immunosorbent assay kit that detects anti-IFN-α antibodies instead of the cytokine IFN-α.

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

[d] This study phase was not within the scope of regulations governing the conduct of nonclinical laboratory studies and was not intended to comply with such regulations. However, this non-GLP study phase was conducted in accordance with the standard operating procedures of Integrated Biotherapeutics, Inc.

[e] Day 2 only.

[f] For controls, group means are presented. For treated groups, percent differences from controls are presented.

[g] For controls, group means are presented. For treated groups, mean fold changes from controls are presented.

[h] Value given is the lower limit of quantification; sample values were below the lower limit of quantification.

[i] The tissue lymph node included iliac and mediastinal lymph nodes at collection. Here, only iliac lymph nodes are presented. Tissues presented are considered as gross lesions.

[j] Numbers in parentheses indicate the number of animals with findings.

2.6.7.7B REPEAT-DOSE TOXICITY: PIVOTAL STUDIES

Study Title: A 1-month (3 doses) intramuscular injection vaccine study of mRNA-1706 in Sprague Dawley rats with a 2-week recovery period

Test Article: mRNA-1706[a]

Species/Strain: Rat/Crl:CD(SD) Sprague Dawley

Duration of Dosing: 1 month (3 doses; Days 1, 15, and 29)

Formulation: 20 mM Tris, 8% sucrose, pH 7.4

Initial Age: 13 weeks

Duration of Postdose: 2 weeks

Report Number: 5002231

Date of First Dose: 03 May 2017 (males) and 04 May 2017 (females)

Route of Administration: Intramuscular injection

Location in eCTD: 4.2.3.2

Dose volume: 200 µL/dose

GLP Compliance: Yes

Control Article: Phosphate-buffered saline, pH 7.2

Special Features: IL-1β, IL-6, TNF-α, IP-10, MIP-1α, MCP-1, and anti-ZIKV lysate antibody titers

Dose (µg/dose)	**0 (control)**		**10**		**50**		**100**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals								
Main study	10	10	10	10	10	10	10	10
Recovery period	5	5	0	0	0	0	5	5
Died or sacrificed moribund	0	0	0	0	0	0	0	0
Mean anti-ZIKV lysate antibody titers (antibody unit/mL)[b]								
Main study animals								
Day 1	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Day 30	1.2	1.2	52.83	165.11	365.95	1028.26	538.73	1387.4
Recovery period animals								
Day 1	1.2[c]		NE	NE	NE	NE	1.2	1.2
Day 43	1.2[c]		NE	NE	NE	NE	2707.2	5099.2
Noteworthy findings – main study								
Clinical observations								
Hunched posture	0	0	0	0	0	0	1	5
Limited usage	0	0	0	0	0	1	1	10
Swollen soft	7	0	10	10	10	10	6	6
Swollen firm	0	0	10	7	10	10	15	15

Dose (µg/dose)	0 (control)		10		50		100	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Warm to touch	0	0	0	0	0	6	6	14
Skin, red	5	3	4	4	9	8	13	14
Backbone prominent	0	0	0	0	0	0	0	3
Dehydrated suspected	0	0	0	0	0	0	0	6
Thin	0	0	0	0	0	0	0	3
Mean body weights (g or %)[d]	–	–	–	–	–	–	–	–
Mean body weight gains (g)								
Days −1 to 7	19.9	3.3	11.4**	0.6	0.3***	−2.6	−11.5***	−0.7
Days 7 to 14	18.0	7.9	21.8	10.6	29.8###	7.9	29.1###	9.8
Days 14 to 21	22.1	7.2	16.5*	3.6	4.4***	2.5	0.3***	-1.3**
Days 21 to 28	16.8	4.2	17.8	4.7	29.4***	11.9**	28.5***	9.9*
Days -1 to 28	76.8	22.6	67.5	19.5	63.9*	19.7	46.4***	17.7
Mean food consumption	–	–	–	–	–	–	–	–
Ophthalmic examinations	–	–	–	–	–	–	–	–
Mean body temperature (°C)[e]								
Day 1, pre-dose	37.36	38.58	–	–	36.97	37.88#	37.09	38.06
Day 1, 6 h post dose	37.50	37.83	–	–	38.30***	38.56	38.73***	39.15###
Day 2, 24 h post-Day 1 dose	36.52	37.03	–	–	37.54##	38.54***	37.77###	39.30***
Day 3, 48 h post-Day 1 dose	NR	NR	NR	NR	NR	NR	NR	37.85
Day 29, pre-dose	36.28	38.19	–	–	36.40	37.85	36.81	38.04
Day 29, 6 h post dose	37.06	37.01	–	–	38.54***	38.16#	38.66***	38.69###
Day 30, 24 h post-Day 29 dose	36.65	37.39	–	–	38.70###	38.97***	38.86###	39.21***
Day 31, 48 h post-Day 29 dose	NR	NR	NR	NR	NR	NR	NR	37.40
Hematology (Day 30; mean or mean fold change)[f]								
White blood cell count (10^3/µL)	7.084	4.257	2.1***	2.5***	2.3***	1.8**	1.8***	0.90
Neutrophil count (10^3/µL)	0.991	0.715	10.0#	9.7###	13.0###	7.9###	11.3###	4.0
Monocyte count (10^3/µL)	0.149	0.084	2.0**	1.8*	2.1**	1.3	1.5	0.85
Lymphocyte count (10^3/µL)	5.814	3.373	0.78	0.97	0.49##	0.48#	0.26###	0.24###
Eosinophil count (10^3/µL)	0.084	0.049	3.0***	6.5###	2.3*	3.8##	1.1	1.6
Large unstained cell	0.041	0.035	4.7###	3.0**	5.1###	2.4*	3.0	1.3
Platelet (10^3/µL)	1168.0	1123.4	–	1.01	–	0.96	–	0.80**
Reticulocytes (10^9/L)	235.0.1	170.35	0.87	0.90	0.80**	0.98	0.72***	0.82

Dose (µg/dose)	0 (control)		10		50		100	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Coagulation (Day 30; mean or mean fold change)[f]								
Activated partial thromboplastin time (seconds)	15.07	14.94	1.2***	1.2***	1.2***	1.4***	1.3***	1.4***
Fibrinogen (mg/dL)	280.2	188.1	2.3##	2.4##	2.5###	2.6###	2.3###	2.4###
Clinical chemistry (Day 30; mean or mean fold change)[f]								
Albumin (g/dL)	3.8	4.48	0.9***	0.9##	0.9***	0.9	0.9***	0.9***
Globulin (g/dL)	2.37	2.13	1.2**	1.1*	1.2***	1.1	1.2**	1.0
Albumin/globulin ratio	1.63	2.14	0.8##	0.8###	0.8##	0.8#	0.8##	0.8#
Glucose (mg/dL)	203.9	188.1	0.8**	0.9	0.8***	0.8***	0.7***	0.7***
Cytokines (mean, pg/mL)								
IL-1β	–	–	–	–	–	–	–	–
IL-6	–	–	–	–	–	–	–	–
TNF-α	–	–	–	–	–	–	–	–
IP-10								
Day 1, 6 h post dose	58.762	70.984	NE	NE	NE	NE	852.920^	1401.728^
Day 15, 6 h post dose	62.900	62.736	NE	NE	NE	NE	591.852^	862.310^
Day 29, 6 h post dose	75.906	68.774	NE	NE	NE	NE	811.432§§§	1283.416^
MIP-1α								
Day 1, 6 h post dose	11.720[g]	11.720[g]	NE	NE	NE	NE	20.682	67.320^^
Day 15, 6 h post dose	11.720[g]	11.720[g]	NE	NE	NE	NE	14.536	21.990
Day 29, 6 h post dose	11.720[g]	11.720[g]	NE	NE	NE	NE	14.684	37.930^
MCP-1								
Day 1, 6 h post dose	292.366	178.856	NE	NE	NE	NE	500.112§	671.020§§
Day 15, 6 h post dose	203.094	172.824	NE	NE	NE	NE	411.536§§	500.678§§
Day 29, 6 h post dose	321.152	140.630[g]	NE	NE	NE	NE	473.198	666.018^^
Gross pathology (Day 30)								
Site, injection (number examined)	10	10	10	10	10	10	10	10
Abnormal consistency; firm	0	0	8	4	9	9	9	9
Swelling	0	0	1	5	3	6	2	7
Material accumulation; clot	0	0	0	0	2	0	0	0

Dose (µg/dose)	0 (control)		10		50		100	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Lymph node (number examined)[h]	10	10	10	10	10	10	10	10
Enlargement	0	0	0	1	0	3	4	4
Lymph node, inguinal (number examined)	10	10	10	10	10	10	10	10
Enlargement	0	0	0	0	1	0	0	1
Lymph node, popliteal (number examined)	10	10	10	10	10	10	10	10
Enlargement	1	0	3	3	0	0	1	0
Organ weights (Day 30; g or %)[d]								
Gland, adrenal (number weighed)	10	10	10	10	10	10	10	10
Absolute value	NR	NR	7	4	2	13*	14	21**
% of body weight	NR	NR	15*	6	5	11	27***	23**
% of brain weight	NR	NR	7	3	-2	13	11	24***
Histopathology (Day 30; number of animals affected)[i]								
Bone marrow (number examined)	10	10	10	10	10	10	10	10
Increased cellularity; myeloid	(0)	(0)	(5)	(3)	(6)	(5)	(7)	(8)
Minimal	0	0	5	3	6	5	7	8
Gland, adrenal (number examined)	10	10	10	10	10	10	10	10
Hypertrophy; cortical	(0)	(0)	(0)	(0)	(5)	(4)	(10)	(9)
Minimal	0	0	0	0	5	4	10	9
Liver (number examined)	10	10	10	10	10	10	10	10
Hypertrophy; Kupffer cell	(0)	(0)	(2)	(2)	(1)	(2)	(2)	(5)
Minimal	0	0	2	2	1	2	2	4
Mild	0	0	0	0	0	0	0	1
Degeneration/necrosis; centrilobular	(0)	(0)	(1)	(0)	(0)	(1)	(0)	(3)
Minimal	0	0	1	0	0	1	0	2
Mild	0	0	0	0	0	0	0	1
Lymph node; inguinal (number examined)	10	10	9	10	8	10	10	10
Infiltration, mixed cell	(0)	(0)	(0)	(0)	(1)	(1)	(1)	(5)
Minimal	0	0	0	0	1	1	1	4
Mild	0	0	0	0	0	0	0	1

Dose (µg/dose)	0 (control)		10		50		100	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Lymph node; popliteal (number examined)	10	10	9	10	9	10	10	10
Infiltration, mixed cell	(0)	(0)	(0)	(3)	(1)	(5)	(3)	(5)
Minimal	0	0	0	3	1	5	2	4
Mild	0	0	0	0	0	0	1	1
Lymph node (number examined)[j]	0	0	0	1	0	3	4	4
Infiltration, mixed cell	NR	NR	NR	(1)	NR	(3)	(4)	(4)
Minimal	NR	NR	NR	1	NR	1	1	0
Mild	NR	NR	NR	0	NR	2	2	3
Moderate	NR	NR	NR	0	NR	0	1	1
Lymph node; mesenteric (number examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Decreased cellularity; lymphoid, paracortex	0	0	3	2	4	4	4	8
Minimal	0	0	3	2	4	4	4	8
Site, injection (number examined)	10	10	10	10	10	10	10	10
Inflammation	(0)	(2)	(10)	(10)	(10)	(10)	(9)	(10)
Minimal	0	2	0	1	0	0	0	0
Mild	0	0	5	3	1	1	2	0
Moderate	0	0	5	6	6	3	4	2
Marked	0	0	0	0	3	6	3	8
Hemorrhage	(1)	(0)	(1)	(1)	(5)	(0)	(3)	(0)
Minimal	1	0	1	1	0	0	3	0
Mild	0	0	0	0	3	0	0	0
Moderate	0	0	0	0	2	0	0	0
Spleen (number examined)	10	10	10	10	10	10	10	10
Decreased cellularity; lymphoid, periarteriolar lymphoid sheath	(0)	(0)	(9)	(7)	(8)	(8)	(10)	(10)
Minimal	0	0	7	6	4	5	8	5
Mild	0	0	2	1	4	3	2	5
Single cell necrosis; lymphoid	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(6)
Minimal	0	0	0	0	0	0	1	6

Dose (µg/dose)	0 (control)		10		50		100	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Thymus (number examined)	10	10	10	10	10	10	10	10
Single cell necrosis; lymphoid	(0)	(0)	(0)	(0)	(3)	(0)	(3)	(8)
Minimal	0	0	0	0	3	0	3	6
Mild	0	0	0	0	0	0	0	2
Decreased cellularity; lymphoid	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
Minimal	0	0	0	0	0	0	1	0
Noteworthy findings – recovery period								
Clinical observations	–	–	NE	NE	NE	NE	–	–
Mean body weight gains (g)								
Days 28 to 35	16.2	-0.4	NE	NE	NE	NE	1.4§§	-3.4
Days 35 to 42	19.8	13.4	NE	NE	NE	NE	31.2§§§	12.8
Days 28 to 42	36.0	13.0	NE	NE	NE	NE	32.6	9.4
Hematology (Day 43; mean or mean fold change)[f]								
White blood cell count (10^3/µL)	7.672	4.772	NE	NE	NE	NE	1.1	1.1
Neutrophil count (10^3/µL)	1.184	0.754	NE	NE	NE	NE	1.7	1.6
Monocyte count (10^3/µL)	0.162	0.130	NE	NE	NE	NE	1.5	1.2
Lymphocyte count (10^3/µL)	6.160	3.786	NE	NE	NE	NE	1.0	1.1
Eosinophil count (10^3/µL)	0.086	0.050	NE	NE	NE	NE	0.86	0.96
Large unstained cell counts (10^3/µL)	0.064	0.054	NE	NE	NE	NE	1.5	1.2
Platelet (10^3/µL)	1221.6	1111.0	NE	NE	NE	NE	–	1.1
Reticulocyte count (10^9/µL)	224.28	170.62	NE	NE	NE	NE	1.2§	1.2
Coagulation (Day 43; mean or mean fold change)[f]								
Activated partial thromboplastin time (seconds)	14.14	15.34	NE	NE	NE	NE	1.0	1.0
Fibrinogen (mg/dL)	273.8	14.82	NE	NE	NE	NE	0.9§	1.0
Clinical chemistry (Day 43; mean or mean fold change)[f]								
Albumin (g/dL)	3.72	4.50	NE	NE	NE	NE	1.0	1.0
Globulin (g/dL)	2.40	2.00	NE	NE	NE	NE	0.9	1.0
Albumin/globulin ratio	1.56	2.26	NE	NE	NE	NE	1.1	1.0

Dose (µg/dose)	**0 (control)**		**10**		**50**		**100**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Glucose (mg/dL)	219.0	173.0	NE	NE	NE	NE	1.0	1.3[§]
Cytokines (Day 43)	–	–	NE	NE	NE	NE	–	–
Gross pathology (Day 43)	–	–	NE	NE	NE	NE	–	–
Organ weights (Day 43)	–	–	NE	NE	NE	NE	–	–
Histopathology (Day 43; number of animals affected)[i]								
Site injection (number examined)	5	5	NE	NE	NE	NE	5	5
Infiltration, mononuclear cell	(0)	(5)	NE	NE	NE	NE	(1)	(2)
Minimal	0	5	NE	NE	NE	NE	1	2

Abbreviations: – = no mRNA-1706-related effect; eCTD = electronic common technical document; F = female; GLP = Good Laboratory Practice; IL = interleukin; IP-10 = interferon gamma-induced protein 10; M = male; MCP-1, monocyte chemoattractant protein 1; MIP-1α = macrophage inflammatory protein 1 alpha; mRNA = messenger RNA; NE = not evaluated; NR = not recorded; TNF = tumor necrosis factor; ZIKV = Zika virus.

Notes: Statistical significance is based on actual data (not on the fold changes or percent differences); [*] = $p \leq .05$, [**] = $p \leq .01$, [***] = $p \leq .001$ (mean value significantly different from control mean value using a Dunnett test); [#] = $p \leq .05$, [##] = $p \leq .01$, [###] = $p \leq .001$ (mean value significantly different from control mean value using a Dunn test); [§] = $p \leq .05$, [§§] = $p \leq .01$, [§§§] = $p \leq .001$ (mean value significantly different from control mean value using a *t* test); [^] = $p \leq .05$, [^^] = $p \leq .01$ (mean value significantly different from control mean value using a Wilcoxon test).

[a] mRNA-based vaccine formulated in SM-102 lipid nanoparticles, as described in Section 2.6.7.1.

[b] This study phase was not within the scope of regulations governing the conduct of nonclinical laboratory studies and was not intended to comply with such regulations. However, this non-GLP study phase was conducted in accordance with the standard operating procedures of Integrated Biotherapeutics, Inc.

[c] Value represents the mean antibody titers of males and females.

[d] For controls, group means are presented. For treated groups, percent differences from controls are presented.

[e] Body temperatures were recorded on Day 1 and Day 29 at pre-dose, and 6- and 24-hours post dose. Additional body temperature measurements were recorded on Day 3, at 48 hours post-Day 1 dose for all females (Day 1) and 2 females a (Day 29) in the 100 µg/dose group.

[f] For controls, group means are presented. For treated groups, mean fold changes from controls are presented.

[g] Value given is the lower limit of quantification; sample values were below the lower limit of quantification.

[h] Iliac left as documented in individual animals.

[i] Numbers in parentheses indicate the number of animals with findings.

[j] Iliac lymph nodes were only examined if a gross abnormality was observed.

2.6.7.7C REPEAT-DOSE TOXICITY: PIVOTAL STUDIES

Study Title: A 1-month (3 doses) study of mRNA-1653 by intramuscular injection in Sprague Dawley rat with a 2-week recovery period

Test Article: mRNA-1653[a]

Species/Strain: Rat/Crl:CD(SD) Sprague Dawley

Duration of Dosing: 1 month (3 doses; Days 1, 15, and 29)

Formulation: 93 mM Tris, 7% PG, 1 mM DTPA, pH 7.4

Initial Age: 8 weeks

Duration of Postdose: 2 weeks

Report Number: 5002033

Date of First Dose: 19 Apr 2017

Route of Administration: Intramuscular injection

Location in eCTD: 4.2.3.2

Dose Volume: 200 µL/dose

GLP Compliance: Yes

Control Article: Phosphate-buffered saline, pH 7.2

Special Features: IL-1β, IL-6, IP-10, MIP-1α, MCP-1, and neutralizing antibodies against hMPV/A2 and PIV3

Dose (µg/dose)	**0 (control)**		**10**		**50**		**150**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals								
Main study	10	10	10	10	10	10	10	10
Recovery period	5	5	0	0	0	0	5	5
Died or sacrificed moribund	0	0	0	0	0	0	0	0
Mean neutralizing antibody titers (geometric mean Log2 NT)[b]								
Anti-hMPV/A2 neutralizing antibodies								
Day 0	4.321928095		4.321928095		4.321928095		4.321928095	
Day 30	4.32192809		9.34510442		9.03195791		9.20840797	
Day 43	4.32192809		NE		NE		10.3469815	
Anti-PIV3 neutralizing antibodies								
Day 0	4.463114795		4.442326308		4.385988211		4.645495461	
Day 30	6.14455777		9.97667311		9.01389519		9.26123399	
Day 43	6.2867719		NE		NE		10.09877	

Dose (µg/dose)	0 (control)		10		50		150	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Noteworthy findings – main study								
Clinical observations (number of animals affected)								
Swollen soft	0	0	0	0	0	3	0	3
Swollen firm	0	0	10	10	10	10	15	15
Skin, red	0	3	0	3	0	2	5	8
Mean body weights	–	–	–	–	–	–	–	–
Mean body weight gains (g)								
Days -1 to 7	65.00	30.60	59.80	–	57.60	20.70$^{\#\#}$	47.53$^{\#\#\#}$	18.67$^{\#\#\#}$
Days 7 to 14	56.20	13.80	54.90	–	61.80	16.30	58.07	12.47
Days 14 to 21	54.33	15.73	49.70	–	47.20	13.60	33.73***	11.40
Days 21 to 28	38.13	12.60	35.70	–	44.40	16.00	43.00	14.60
Days -1 to 28	213.67	72.73	200.10	–	211.00	66.60	182.33$^{\#}$	57.13$^{\#\#\#}$
Mean food consumption (g/animal/day or %)[c]								
Days -7 to 1	30.93	21.63	-0.62	-1.97	-0.53	-3.78	-1.42	3.14
Days 1 to 8	33.55	23.08	-4.28	-2.08	-5.36	-7.06	-12.94	-9.07
Days 8 to 15	34.37	25.52	-1.33	-8.97	3.59	-13.44	-0.35	-9.59
Days 15 to 22	35.72	22.99	-6.47	0.03	-3.47	-5.93	-11.33	-8.52
Days 22 to 29	36.81	23.63	-2.51	0.28	2.25	-5.39	-4.26	-0.90
Ophthalmic examinations	–	–	–	–	–	–	–	–
Mean body temperature (°C)[d]								
Day 1, pre-dose	36.94	37.13	36.72	37.41	36.86	37.38	36.90	37.36
Day 1, 6 h post dose	37.67	37.89	38.57**	38.00	39.12***	38.95***	39.13***	39.38***
Day 2, 24 h post-Day 1 dose	36.81	37.79	37.32*	38.05	38.29***	38.40*	38.56***	39.11***
Day 3, 48 h post-Day 1 dose	NR	NR	NR	NR	–	NR	–	–
Day 29, pre-dose	36.62	38.43	36.62	38.85	36.79	38.60	36.68	38.56
Day 29, 6 h post dose	37.96	38.42	38.22	38.10	38.13	38.80	38.88***	39.25$^{\#\#}$
Day 30, 24 h post-Day 29 dose	36.26	38.37	36.89*	38.60	36.97**	38.53	37.90***	38.93*

Dose (µg/dose)	0 (control)		10		50		150	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Hematology (Day 30; mean or mean fold change)[e]								
White blood cell count (10^3/µL)	8.500	6.188	1.4	1.6**	1.9***	1.7***	2.0***	1.6**
Neutrophil count (10^3/µL)	0.865	0.487	5.7***	7.8	11.2***	13.0###	12.6***	14.0###
Lymphocyte count (10^3/µL)	7.245	5.393	0.8	–	0.7*	0.7*	0.7*	0.6**
Eosinophil count (10^3/µL)	0.074	0.071	2.8#	3.2##	3.4	4.6###	4.3###	3.9###
Large unstained cell count (10^3/µL)	0.087	0.083	3.0***	1.7	2.4**	1.3	1.7	1.2
Platelet count (10^3/µL)	1087.3	1189.2	–	0.9	–	0.9*	–	0.7***
Reticulocyte count (10^9/µL)	258.03	181.32	0.8***	–	0.7***	–	0.7***	–
Coagulation (Day 30; mean or mean fold change)[e]								
Activated partial thromboplastin time (seconds)	16.21	15.29	–	1.1	1.1***	1.1**	1.2***	1.3***
Fibrinogen (mg/dL)	302.8	280.4	2.0***	1.6***	2.1***	2.1***	2.3***	2.2***
Clinical chemistry (Day 30; mean or mean fold change)[e]								
Albumin (g/dL)	3.83	4.31	0.9***	0.9*	0.9***	0.9*	0.9***	0.9***
Globulin (g/dL)	1.96	1.96	1.3***	1.2**	1.4***	1.2***	1.4***	1.3**
Albumin/globulin ratio	1.98	2.22	0.7#	0.8***	0.6###	0.7***	0.6###	0.7***
Cytokines (mean, pg/mL)								
IL-1β	–	–	NE	NE	NE	NE	–	–
IL-6	–	–	NE	NE	NE	NE	–	–
TNF-α	–	–	NE	NE	NE	NE	–	–
IP-10								
Day 1, 6 h post dose	100.940	65.580	NE	NE	NE	NE	1336.402§§§	1447.742^
Day 15, 6 h post dose	87.850	71.542	NE	NE	NE	NE	1042.548^	1353.880^
Day 29, 6 h post dose	86.848	50.424	NE	NE	NE	NE	1053.640§§§	962.920§§§
MCP-1								
Day 1, 6 h post dose	319.814	179.780	NE	NE	NE	NE	1060.686§§	586.194§§§
Day 15, 6 h post dose	299.612	176.052	NE	NE	NE	NE	897.894§§	969.196^^
Day 29, 6 h post dose	310.128	182.138	NE	NE	NE	NE	940.052§	417.398§§

Dose (µg/dose)	**0 (control)**		**10**		**50**		**150**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
MIP-1α								
Day 1, 6 h post dose	11.720[f]	11.720[f]	NE	NE	NE	NE	29.100	32.154
Day 15, 6 h post dose	11.720[f]	11.720[f]	NE	NE	NE	NE	22.136	26.284
Day 29, 6 h post dose	11.720[f]	11.720[f]	NE	NE	NE	NE	–	–
Gross pathology (Day 30)								
Injection site (number examined)	10	10	10	10	10	10	10	10
Abnormal consistency; firm	0	0	8	6	10	10	9	10
Swelling	0	0	4	1	6	6	10	9
Thick	0	0	0	0	0	1	3	1
Popliteal lymph node (number examined)	10	10	10	10	10	10	10	10
Enlargement	0	0	3	0	4	1	1	3
Lymph node, inguinal (number examined)	10	10	10	10	10	10	10	10
Enlargement	0	0	1	0	1	0	3	3
Lymph node, iliac (number examined)	0	0	1	0	1	2	3	4
Enlargement	0	0	1	0	1	2	3	4
Organ weights (Day 30; g or %)[c]								
Liver (number weighed)	10	10	10	10	10	10	10	10
Absolute value	15.0571	8.3382	–	0.47	–	-1.69	–	2.72
% of body weight	2.93967	2.86516	–	1.71	–	2.40	–	9.34**
% of brain weight	688.91370	419.70333	–	-2.08	–	-0.97	–	2.68
Spleen (number weighed)	10	10	10	10	10	10	10	10
Absolute value	0.9533	0.5710	3.65	24.86**	15.17	31.14***	14.79	21.37**
% of body weight	0.18797	0.19685	10.27	26.40**	21.80*	37.21***	27.58**	28.66**
% of brain weight	43.63288	28.75383	1.96	21.44*	15.35	32.18***	15.47	21.17*

Dose (µg/dose)	0 (control)		10		50		150	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Histopathology (Day 30; number of animals affected)[g]								
Injection site (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell	(1)	(3)	(10)	(9)	(10)	(10)	(10)	(10)
Minimal	1	2	1	1	–	–	–	–
Mild	–	1	1	4	1	–	–	–
Moderate	–	–	8	4	5	6	5	5
Marked	–	–	–	–	4	4	5	5
Liver (number examined)	10	10	10	10	10	10	10	10
Vacuolation, hepatocellular	(1)	(2)	(1)	(1)	(2)	(2)	(7)	(9)
Minimal	1	2	1	1	2	2	3	5
Mild	–	–	–	–	–	–	4	4
Bone marrow (number examined)	10	10	10	10	10	10	10	10
Increased hematopoiesis myeloid	(0)	(0)	(10)	(10)	(10)	(10)	(10)	(10)
Minimal	–	–	10	10	7	8	3	5
Mild	–	–	–	–	3	2	7	5
Popliteal lymph node (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell	(0)	(0)	(9)	(10)	(8)	(9)	(10)	(9)
Minimal	–	–	1	3	3	1	2	4
Mild	–	–	8	7	5	6	7	3
Moderate	–	–	–	–	–	2	1	2
Inguinal lymph node (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell	(0)	(0)	(2)	(0)	(1)	(0)	(4)	(6)
Minimal	–	–	2	–	1	–	3	2
Mild	–	–	–	–	–	–	1	4
Iliac lymph node (number examined)	0	0	1	0	1	2	3	4
Inflammation, mixed cell	(0)	(0)	(1)	(0)	(1)	(2)	(2)	(4)
Minimal	–	–	1	–	1	1	1	–

Dose (µg/dose)	0 (control)		10		50		150	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mild	–	–	–	–	–	1	1	4
Sciatic nerve (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell	(0)	(0)	(9)	(10)	(10)	(10)	(10)	(7)
Minimal	–	–	8	9	9	9	9	6
Mild	–	–	1	1	1	1	1	1
Spleen (number examined)	10	10	10	10	10	10	10	10
Decreased cellularity; periarteriolar lymphoid sheath	(0)	(0)	(2)	(1)	(4)	(2)	(7)	(7)
Minimal	–	–	2	1	4	2	3	6
Mild	–	–	–	–	–	–	4	1
Increased macrophages; periarteriolar lymphoid sheath	(0)	(0)	(0)	(0)	(3)	(2)	(5)	(7)
Minimal	–	–	–	–	3	2	5	7
Noteworthy findings – recovery period								
Clinical observations								
Swollen soft	0	0	NE	NE	NE	NE	0	0
Swollen firm	1	0	NE	NE	NE	NE	15	15
Skin, red	0	3	NE	NE	NE	NE	5	8
Mean body weight gains (g)								
Days 28 to 35	37.20	8.80	NE	NE	NE	NE	21.40[§§]	7.20
Days 35 to 42	23.80	12.40	NE	NE	NE	NE	36.00[§]	14.60
Days 28 to 42	61.00	21.20	NE	NE	NE	NE	57.40	21.80
Mean food consumption (g/animal/day or %)[c]								
Days 29 to 36	33.16	22.66	NE	NE	NE	NE	-7.60	3.18
Days 36 to 42	32.32	22.42	NE	NE	NE	NE	5.75	8.03

Dose (µg/dose)	0 (control)		10		50		150	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Hematology (Day 43; mean or mean fold change)[e]								
White blood cell count (10^3/µL)	8.374	7.124	NE	NE	NE	NE	1.0	1.0
Neutrophil count (10^3/µL)	1.112	1.506	NE	NE	NE	NE	1.1	1.2
Lymphocyte count (10^3/µL)	6.916	5.302	NE	NE	NE	NE	1.0	0.9
Eosinophil count (10^3/µL)	0.104	0.066	NE	NE	NE	NE	1.0	1.2
Large unstained cell count (10^3/µL)	0.040	0.054	NE	NE	NE	NE	1.1	0.8
Platelet count (10^3/µL)	1184.8	1204.2	NE	NE	NE	NE	–	1.0
Reticulocyte count (10^9/µL)	218.66	159.92	NE	NE	NE	NE	1.3[§]	–
Coagulation (Day 43; mean or mean fold change)[e]								
Activated partial thromboplastin time (seconds)	15.74	15.40	NE	NE	NE	NE	1.0	1.1
Fibrinogen (mg/dL)	323.2	234.0	NE	NE	NE	NE	0.9	1.1
Clinical chemistry (Day 43; mean or mean fold change)[e]								
Albumin (g/dL)	3.82	4.40	NE	NE	NE	NE	1.0	0.9
Globulin (g/dL)	2.18	1.84	NE	NE	NE	NE	1.0	1.2[§§]
Albumin/globulin ratio	1.76	2.42	NE	NE	NE	NE	0.9	0.9[§]
Cytokines (Day 43)	–	–	NE	NE	NE	NE	–	–
Gross pathology (Day 43)								
Popliteal lymph node (number examined)	5	5	NE	NE	NE	NE	5	5
Enlargement	0	0	NE	NE	NE	NE	0	2
Organ weights (Day 43; g or %)[c]								
Liver (number weighed)	5	5	NE	NE	NE	NE	5	5
Absolute value	14.4982	8.0992	NE	NE	NE	NE	–	8.62
% of body weight	2.78476	2.63975	NE	NE	NE	NE	–	13.45[§]
% of brain weight	659.15779	409.67189	NE	NE	NE	NE	–	6.93

Dose (µg/dose)	0 (control)		10		50		150	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Histopathology (Day 43; number of animals affected)[g]								
Injection site (number examined)	5	5	NE	NE	NE	NE	5	5
Inflammation, mononuclear cell	(0)	(0)	NE	NE	NE	NE	(5)	(5)
Minimal	–	–	NE	NE	NE	NE	4	5
Mild	–	–	NE	NE	NE	NE	1	–
Popliteal lymph node (number examined)	5	5	NE	NE	NE	NE	5	5
Inflammation, mixed cell	(0)	(0)	NE	NE	NE	NE	(3)	(5)
Minimal	–	–	NE	NE	NE	NE	3	5
Sciatic nerve (number examined)	5	5	NE	NE	NE	NE	5	5
Infiltration, mononuclear cell	(0)	(0)	NE	NE	NE	NE	(5)	(4)
Minimal	–	–	NE	NE	NE	NE	5	4

Abbreviations: – = no mRNA-1653-related effect; eCTD = electronic common technical document; F = female; GLP = Good Laboratory Practice; hMPV/A2 = human metapneumovirus subgroup A2; IL = interleukin; IP-10 = interferon gamma-induced protein 10; M = male; MCP-1 = monocyte chemoattractant protein 1; MIP-1α = macrophage inflammatory protein 1 alpha; mRNA = messenger RNA; NE = not evaluated; NT = neutralizing antibody titer; PIV3 = human parainfluenza virus type 3; TNF = tumor necrosis factor.

Notes: Statistical significance is based on actual data (not on the fold changes or percent differences); $^{*} = p \leq .05$, $^{**} = p \leq .01$, $^{***} = p \leq .001$ (mean value significantly different from control mean value using a Dunnett test); $^{\#} = p \leq .05$, $^{\#\#} = p \leq .01$, $^{\#\#\#} = p \leq .001$ (mean value significantly different from control mean value using a Dunn test); $^{\S} = p \leq .05$, $^{\S\S} = p \leq .01$, $^{\S\S\S} = p \leq .001$ (mean value significantly different from control mean value using a *t* test); $^{\wedge} = p \leq .05$, $^{\wedge\wedge} = p \leq .01$ (mean value significantly different from control mean value using a Wilcoxon test).

[a] mRNA-based vaccine formulated in SM-102 lipid nanoparticles, as described in Section 2.6.7.1.

[b] Values represent the geometric mean Log2-transformed neutralizing antibody titers of males and females in each group.

[c] For controls, group means are presented. For treated groups, percent differences from controls are presented.

[d] Body temperatures were recorded on Day 1 and Day 29 at pre-dose, and 6- and 24-hours post dose. Additional body temperature measurements were recorded on Day 3, 48 hours post-Day 1 dose for the 50 and 100 µg/dose males and 100 µg/dose females.

[e] For controls, group means are presented. For treated groups, mean fold changes from controls are presented.

[f] Value given is the lower limit of quantification; sample values were below the lower limit of quantification.

[g] Numbers in parentheses indicate the number of animals with findings.

2.6.7.7D REPEAT-DOSE TOXICITY: PIVOTAL STUDIES

Study Title: A 1-month (3 doses) intramuscular injection toxicity study of mRNA-1893 in Sprague Dawley rats followed by a 2-week recovery period

Test Article: mRNA-1893[a]

Species/Strain: Rat/Crl:CD(SD) Sprague Dawley

Duration of Dosing: 1 month (3 doses; Days 1, 15, and 29)

Formulation: 100 mM Tris, 7% PG, 1 mM DTPA, pH 7.5

Initial Age: 7 weeks

Duration of Postdose: 2 weeks

Report Number: 5002400

Date of First Dose: 19 Nov 2018 (main study males and recovery study males and females) and 20 Nov 2018 (main study females)

Route of Administration: Intramuscular injection

Location in eCTD: 4.2.3.2

Dose Volume: 200 µL/dose

GLP Compliance: Yes

Control Article: Phosphate-buffered saline, pH 7.2

Special Features: α1-acid glycoprotein, α2-macroglobulin, IL-1β, IL-6, IP-10, MIP-1α, MCP-1, TNF-α, anti-ZIKV neutralizing antibody titers

Dose (µg/dose)	**0 (control)**		**10**		**30**		**96**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals								
Main study	10	10	10	10	10	10	10	10
Recovery period	5	5	0	0	0	0	5	5
Died or sacrificed moribund	0	0	0	0	0	0	0	0
Mean anti-ZIKV neutralizing antibody titers (range MN_{50} titers)								
Day 0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Day 30 (main study animals)	<LOD	<LOD	138-11,891	321-4391	62-2159	422-4545	488-3270	872-4953
Day 43 (recovery period animals)	<LOD	<LOD	NE	NE	NE	NE	3809-13,805	6190-14,249
Noteworthy findings – main study								
Clinical observations (number of animals affected)								
Vocalization	0	0	0	0	0	0	6	2
Limited usage	0	0	0	0	0	0	0	1
Skin thickening	0	0	0	0	0	0	14	10

Dose (µg/dose)	**0 (control)**		**10**		**30**		**96**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Skin, discolored	0	0	1	3	0	4	5	14
Skin, scab	3	3	4	2	5	6	3	4
Swollen	0	0	1	10	7	10	15	15
Abnormal gait	0	0	0	0	0	0	1	2
Mean body weight	–	–	–	–	–	–	–	–
Mean body weight gains	–	–	–	–	–	–	–	–
Mean food consumption	–	–	–	–	–	–	–	–
Ophthalmic examinations	–	–	–	–	–	–	–	–
Hematology (Day 30; mean or mean fold change)[b]								
White blood cell count (10^3/µL)	7.276	6.404	1.12	1.27	1.77$^{\#\#}$	1.75$^{\#\#}$	2.21$^{\#\#}$	1.34
Neutrophil count (10^3/µL)	0.917	0.868	2.54	3.34$^{\#\#}$	7.15**	7.53$^{\#\#}$	11.61**	6.34$^{\#\#}$
Lymphocyte count (10^3/µL)	6.142	5.302	–	–	–	–	0.80	0.51$^{\#\#}$
Monocyte count (10^3/µL)	0.097	0.098	1.46	1.32	1.95$^{\#}$	1.65$^{\#}$	2.40$^{\#\#}$	–
Eosinophil count (10^3/µL)	0.055	0.069	2.05*	2.73$^{\#\#}$	3.04**	3.09$^{\#\#}$	3.13**	2.53$^{\#\#}$
Red blood cell distribution width (%)	12.26	10.87	–	–	1.09$^{\#\#}$	1.09$^{\#\#}$	1.16$^{\#\#}$	1.16$^{\#\#}$
Reticulocyte count (10^9/µL)	263.81	204.79	–	–	–	–	0.77$^{\#}$	–
Coagulation (Day 30; mean or mean fold change)[b]								
Activated partial thromboplastin time (seconds)	15.79	15.32	–	1.08$^{\#}$	1.10*	1.20$^{\#\#}$	1.28**	1.28$^{\#\#}$
Fibrinogen (mg/dL)	310.7	219.7	1.75$^{\#\#}$	1.65	2.36$^{\#\#}$	2.81**	2.81$^{\#\#}$	3.24**
Clinical chemistry (Day 30; mean or mean fold change)[b]								
Glucose (mg/dL)	201.9	192.2	–	–	0.98	0.86$^{\#}$	–	0.76$^{\#\#}$
Albumin (g/dL)	3.77	4.67	–	–	0.91$^{\#\#}$	0.90$^{\#}$	0.90$^{\#\#}$	0.89$^{\#\#}$
Globulin (g/dL)	1.65	1.53	1.14$^{\#}$	1.11	1.33$^{\#\#}$	1.36$^{\#\#}$	1.51$^{\#\#}$	1.44$^{\#\#}$
Albumin/globulin ratio	2.30	3.11	0.83$^{\#\#}$	0.84	0.68$^{\#\#}$	0.65**	0.60$^{\#\#}$	0.61**
Blood markers (Day 30; mean, ng/mL)								
α1-acid glycoprotein	33,308.095	25,825.490	–	–	384,683.154**	30,2753.384**	690,352.583**	649,982.890**
α2-macroglobulin	15,123.563	8803.271	–	–	280,453.324**	70,097.595**	1,222,406.563**	496,666.713**

Dose (µg/dose)	0 (control)		10		30		96	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Cytokines (mean, pg/mL)[c]								
TNF-α	–	–	–	–	–	–	–	–
IL-1β								
Day 1, 2 h post dose	46.880[d]	61.000	–	77.254	–	–	96.958	–
Day 1, 6 h post dose	46.880[d]	46.880[d]	–	–	–	–	–	145.560
Day 15, 2 h post dose	46.880[d]	63.956	–	–	–	–	–	–
Day 15, 6 h post dose	46.880[d]	62.352	–	–	133.586	104.794	90.420	317.806
Day 29, 2 h post dose	46.880[d]	76.056	–	–	–	–	–	–
Day 29, 6 h post dose	59.630	46.880[d]	–	–	67.080	168.982	181.542	158.660
IL-6								
Day 1, 2 h post dose	351.560[d]	351.560[d]	–	981.652	–	–	–	–
Day 1, 6 h post dose	351.560[d]	754.376	–	–	–	–	–	–
Day 15, 2 h post dose	443.044	351.560[d]	–	–	–	1792.092	–	–
Day 15, 6 h post dose	351.560[d]	1224.226	–	–	–	–	462.366	–
Day 29, 2 h post dose	351.560[d]	421.914	–	–	–	1033.880	–	–
Day 29, 6 h post dose	351.560[d]	351.560[d]	–	–	485.434	–	–	–
IP-10								
Day 1, 2 h post dose	219.712	174.448	–	203.092	261.868	–	227.458	–
Day 1, 6 h post dose	207.056	146.446	–	252.414	382.598[#]	384.030[*]	481.888[##]	678.100[**]
Day 15, 2 h post dose	216.828	200.000	–	320.048	–	–	283.052	–
Day 15, 6 h post dose	254.364	203.444	–	260.030	403.934	547.292[*]	657.816[*]	752.920[**]
Day 29, 2 h post dose	251.398	209.086	–	242.568	251.738	–	–	–
Day 29, 6 h post dose	250.220	129.876	–	–	368.698	573.080[*]	656.202[#]	1133.914[**]
MCP-1								
Day 1, 2 h post dose	703.150	703.150	–	–	–	–	–	–
Day 1, 6 h post dose	703.150	703.150	859.250	–	–	–	1058.836	851.774
Day 15, 2 h post dose	1014.942	703.150	880.202	861.458	884.732	–	–	–
Day 15, 6 h post dose	703.150	703.150	–	–	–	–	–	1222.286
Day 29, 2 h post dose	864.292	703.150	–	–	879.228	–	–	–
Day 29, 6 h post dose	703.150	703.150	–	–	1086.050	–	877.354	–

Dose (µg/dose)	**0 (control)**		**10**		**30**		**96**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
MIP-1α								
Day 1, 2 h post dose	11.720[d]	11.720[d]	–	–	–	–	39.888	–
Day 1, 6 h post dose	11.720[d]	11.720[d]	–	–	–	–	–	–
Day 15, 2 h post dose	14.736	11.720[d]	–	–	–	–	17.794	–
Day 15, 6 h post dose	11.720[d]	11.720[d]	–	–	–	–	–	–
Day 29, 2 h post dose	11.720[d]	11.720[d]	–	–	–	–	–	–
Day 29, 6 h post dose	11.720[d]	11.720[d]	–	–	–	–	–	–
Gross pathology (Day 30)								
Site, injection (number examined)	10	10	10	10	10	10	10	10
Abnormal consistency; firm	0	0	1	2	6	1	9	7
Swelling	0	0	4	4	5	5	7	7
Thick	0	0	0	1	3	10	8	10
Focus, dark	0	0	0	0	0	1	0	0
Lymph node, iliac (number examined)	10	10	10	10	10	10	10	10
Enlargement	0	0	0	1	0	1	3	6
Lymph node, inguinal (number examined)	10	10	10	10	10	10	10	10
Enlargement	0	0	0	0	0	0	1	2
Lymph node, popliteal (number examined)	10	10	10	10	10	10	10	10
Enlargement	0	0	1	0	0	1	0	2
Organ weights (Day 30; g or %)[c]								
Spleen								
Absolute value	0.7680	0.5604	–0.7552	–0.6424	6.3021	7.5482	14.0625	10.4925
% of body weight	0.19581	0.22661	–1.88364	2.34641	7.62303	13.71379[#]	14.68763[##]	15.82386[#]
% of brain weight	38.60257	29.28032	–1.92392	2.14876	4.06712	11.45926	12.83219	14.05730

Dose (µg/dose)	0 (control)		10		30		96	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Histopathology (Day 30; number of animals affected)[e]								
Site, injection (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell; dermal	(0)	(0)	(1)	(2)	(0)	(0)	(2)	(2)
Minimal	0	0	1	2	0	0	1	2
Mild	0	0	0	0	0	0	1	0
Inflammation, mixed cell; subcutis/perimuscular	(1)	(5)	(10)	(10)	(10)	(10)	(10)	(10)
Minimal	1	5	2	1	0	1	0	0
Mild	0	0	4	8	7	7	2	3
Moderate	0	0	4	1	3	2	8	7
Inflammation, mixed cell; muscular	(0)	(0)	(10)	(10)	(10)	(10)	(10)	(10)
Minimal	0	0	4	7	5	5	1	2
Mild	0	0	6	3	5	5	9	8
Hyperplasia; epidermal	(0)	(0)	(0)	(2)	(0)	(2)	(3)	(7)
Minimal	0	0	0	2	0	2	3	7
Lymph node, iliac (number examined)	10	10	10	10	10	10	10	10
Increased cellularity; lymphoid	(0)	(3)	(1)	(5)	(4)	(9)	(9)	(6)
Minimal	0	2	1	5	2	1	2	3
Mild	0	1	0	0	1	8	6	3
Moderate	0	0	0	0	1	0	0	0
Marked	0	0	0	0	0	0	1	0
Inflammation, mixed cell; perinodal	(0)	(0)	(0)	(1)	(0)	(6)	(1)	(8)
Minimal	0	0	0	1	0	6	1	7
Mild	0	0	0	0	0	0	0	1
Inflammation, neutrophilic	(0)	(0)	(0)	(0)	(0)	(5)	(0)	(7)
Minimal	0	0	0	0	0	4	0	3
Mild	0	0	0	0	0	1	0	3
Moderate	0	0	0	0	0	0	0	1

Dose (µg/dose)	0 (control)		10		30		96	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Lymph node, inguinal (number examined)	10	10	10	10	10	10	10	9
Increased cellularity; lymphoid	(0)	(0)	(0)	(0)	(2)	(0)	(9)	(2)
Minimal	0	0	0	0	2	0	3	2
Mild	0	0	0	0	0	0	6	0
Inflammation, neutrophilic	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(2)
Minimal	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Inflammation, mixed cell; perinodal	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(2)
Minimal	0	0	0	0	1	0	0	1
Moderate	0	0	0	0	0	0	0	1
Lymph node, popliteal (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell; perinodal	(0)	(0)	(9)	(10)	(10)	(10)	(9)	(10)
Minimal	0	0	6	7	6	7	6	4
Mild	0	0	3	3	4	3	3	6
Increased cellularity; lymphoid	(0)	(0)	(0)	(3)	(1)	(6)	(1)	(0)
Minimal	0	0	0	3	1	6	1	0
Inflammation, neutrophilic	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(2)
Minimal	0	0	0	0	0	1	0	2
Nerve, sciatic (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell; perineurial	(1)	(1)	(10)	(10)	(10)	(10)	(9)	(10)
Minimal	1	1	1	5	2	2	4	2
Mild	0	0	4	1	1	3	3	6
Moderate	0	0	5	4	7	5	2	2

Dose (µg/dose)	0 (control)		10		30		96	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Spleen (number examined)	10	10	10	10	10	10	10	10
Extramedullary hematopoiesis; increased	(0)	(0)	(0)	(0)	(1)	(0)	(3)	(0)
Minimal	0	0	0	0	1	0	3	0
Infiltration, neutrophilic; red pulp	(0)	(0)	(0)	(7)	(10)	(10)	(10)	(10)
Minimal	0	0	0	7	8	8	3	5
Mild	0	0	0	0	2	2	7	5
Decreased cellularity; periarteriolar lymphoid sheath	(0)	(0)	(0)	(0)	(0)	(0)	(4)	(2)
Minimal	0	0	0	0	0	0	3	2
Mild	0	0	0	0	0	0	1	0
Increased cellularity, red pulp	(0)	(0)	(0)	(0)	(0)	(1)	(4)	(7)
Minimal	0	0	0	0	0	1	2	6
Mild	0	0	0	0	0	0	2	1
Bone marrow (number examined)	10	10	10	10	10	10	10	10
Increased cellularity; myeloid	(0)	(0)	(0)	(1)	(2)	(1)	(8)	(10)
Minimal	0	0	0	1	2	1	8	10
Liver (number examined)	10	10	10	10	10	10	10	10
Hypertrophy; Kupffer cell	(0)	(0)	(0)	(2)	(2)	(6)	(4)	(7)
Minimal	0	0	0	2	2	4	4	6
Mild	0	0	0	0	0	2	0	1
Vacuolation; hepatocellular, periportal to midzonal	(0)	(1)	(1)	(1)	(1)	(5)	(7)	(9)
Minimal	0	1	1	1	1	3	5	8
Mild	0	0	0	0	0	2	2	1
Gland, seminal vesicle (number examined)	10	NA	10	NA	10	NA	10	NA
Single cell necrosis; increased	(0)	NA	(0)	NA	(0)	NA	(4)	NA
Minimal	0	NA	0	NA	0	NA	4	NA

Dose (µg/dose)	0 (control)		10		30		96	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Noteworthy findings – recovery period								
Clinical observations								
Swollen	0	0	NE	NE	NE	NE	0	3
Hematology (Day 43; mean or mean fold change)[b]								
White blood cell count (10^3/µL)	7.184	5.124	NE	NE	NE	NE	1.37	1.56*
Neutrophil count (10^3/µL)	1.068	0.780	NE	NE	NE	NE	1.77[#]	2.11[#]
Lymphocyte count (10^3/µL)	5.852	4.146	NE	NE	NE	NE	-	-
Monocyte count (10^3/µL)	0.122	0.098	NE	NE	NE	NE	1.80[#]	1.76[#]
Eosinophil count (10^3/µL)	0.066	0.034	NE	NE	NE	NE	1.52	2.82*
Red blood cell distribution width (%)	12.74	11.38	NE	NE	NE	NE	1.13[#]	1.18[##]
Reticulocyte count (10^9/µL)	223.86	203.90	NE	NE	NE	NE	–	–
Coagulation (Day 43)	–	–	NE	NE	NE	NE	–	–
Clinical chemistry (Day 43; mean or mean fold change)[b]								
Glucose (mg/dL)	216.0	184.2	NE	NE	NE	NE	–	0.80
Blood markers (Day 43; mean, ng/mL)								
α1-acid glycoprotein	34,976.510	22,092.828	NE	NE	NE	NE	31,990.776	20,828.976
α2-macroglobulin	8305.714	7422.756	NE	NE	NE	NE	23,133.352[#]	12,457.842
Cytokines (Day 43)	–	–	NE	NE	NE	NE	–	–
Gross pathology (Day 43)								
Lymph node, iliac (No. examined)	5	5	NE	NE	NE	NE	5	5
Enlargement	0	0	NE	NE	NE	NE	1	0
Organ weight (Day 43)	–	–	NE	NE	NE	NE	–	–

Dose (µg/dose)	**0 (control)**		**10**		**30**		**96**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Histopathology (Day 43; number of animals affected)[e]								
Site, injection (number examined)	5	5	NE	NE	NE	NE	5	5
Inflammation, mixed cell; subcutis/perimuscular	(0)	(0)	NE	NE	NE	NE	(5)	(5)
Minimal	0	0	NE	NE	NE	NE	5	5
Inflammation, mixed cell; muscular	(0)	(0)	NE	NE	NE	NE	(0)	(1)
Minimal	0	0	NE	NE	NE	NE	0	1
Infiltration, mononuclear cell; muscular	(1)	(3)	NE	NE	NE	NE	(4)	(4)
Minimal	1	3	NE	NE	NE	NE	4	3
Mild	0	0	NE	NE	NE	NE	0	1
Hyperplasia; epidermal	(0)	(0)	NE	NE	NE	NE	(1)	(0)
Minimal	0	0	NE	NE	NE	NE	1	0
Lymph node, iliac (number examined)	5	5	NE	NE	NE	NE	5	5
Increased cellularity; lymphoid	(0)	(0)	NE	NE	NE	NE	(3)	(2)
Minimal	0	0	NE	NE	NE	NE	3	1
Mild	0	0	NE	NE	NE	NE	0	1
Inflammation, mixed cell; perinodal	(0)	(0)	NE	NE	NE	NE	(1)	(2)
Minimal	0	0	NE	NE	NE	NE	1	2
Lymph node, inguinal (number examined)	5	5	NE	NE	NE	NE	5	5
Inflammation, mixed cell; perinodal	(0)	(0)	NE	NE	NE	NE	(0)	(1)
Minimal	0	0	NE	NE	NE	NE	0	1

Dose (µg/dose)	0 (control)		10		30		96	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Lymph node, popliteal (number examined)	5	5	NE	NE	NE	NE	5	5
Inflammation, mixed cell; perinodal	(0)	(0)	NE	NE	NE	NE	(2)	(4)
Minimal	0	0	NE	NE	NE	NE	2	4
Increased cellularity; lymphoid	(0)	(0)	NE	NE	NE	NE	(1)	(0)
Minimal	0	0	NE	NE	NE	NE	1	0
Nerve, sciatic (number examined)	5	5	NE	NE	NE	NE	5	5
Inflammation, mixed cell; perineurial	(0)	(0)	NE	NE	NE	NE	(5)	(5)
Minimal	0	0	NE	NE	NE	NE	5	5
Liver (number examined)	5	5	NE	NE	NE	NE	5	5
Vacuolation; hepatocellular, periportal to midzonal	(0)	(0)	NE	NE	NE	NE	(1)	(3)
Minimal	0	0	NE	NE	NE	NE	1	3

Abbreviations: – = no mRNA-1893-related effect; eCTD = electronic common technical document; F = female; GLP = Good Laboratory Practice; IL = interleukin; IP-10 = interferon gamma-inducible protein 10; LOD = limit of detection; M = male; MCP-1 = monocyte chemoattractant protein 1; MIP-1α = macrophage inflammatory protein 1 alpha; MN50 = 50% neutralization; mRNA = messenger RNA; NA = not applicable; NE = not evaluated; TNF = tumor necrosis factor; ZIKV = Zika virus.

Notes: Statistical significance is based on actual data (not on the fold changes or percent differences); $^{*}p \leq .05$, $^{**}p \leq .01$ (significantly different from controls; Kruskal-Wallis test followed by pairwise comparisons using a Dunn test); $^{\#}p \leq .05$, $^{\#\#}p \leq .01$ (significantly different from controls; one-way analysis of variance followed by pairwise comparison using a Dunnett test).

[a] mRNA-based vaccine formulated in SM-102 lipid nanoparticles, as described in Section 2.6.7.1.

[b] For controls, group means are presented. For treated groups, mean fold changes from controls are presented.

[c] For controls, group means are presented. For treated groups, percent differences from controls are presented.

[d] Value given is the lower limit of quantification; sample values were below the lower limit of quantification.

[e] Numbers in parentheses indicate the number of animals with findings.

2.6.7.7E REPEAT-DOSE TOXICITY: PIVOTAL STUDIES

Study Title: A 6-week (4 doses) intramuscular injection toxicity study of mRNA-1647 in Sprague Dawley rats followed by a 2-week recovery period

Test Article: mRNA-1647[a]

Species/Strain: Rat/Crl:CD(SD) Sprague Dawley

Duration of Dosing: 6 weeks (4 doses; Days 1, 15, 29, and 43)

Formulation: 100 mM Tris, 60 mM NaCl 7% (w/v PG)

Initial Age: 8 weeks

Duration of Postdose: 2 weeks

Report Number: 5002034

Date of First Dose: 22 Mar 2017 (males) and 23 Mar 2017 (females)

Route of Administration: Intramuscular injection

Location in eCTD: 4.2.3.2

Dose volume: 200 µL/dose

GLP Compliance: Yes

Control Article: Phosphate-buffered saline, pH 7.2

Special Features: IL-1β, IL-6, IP-10, MIP-1α, MCP-1, and TNF-α, Anti-CMV gH pentamer complex and anti-CMV gB protein antibody titers, and IFN-γ-producing T cells

Dose (µg/dose)[b]	**0 (control)**		**8.9**		**27**		**89**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals								
Main study	10	10	10	10	10	10	10	10
Recovery period	5	5	0	0	0	0	5	5
Died or sacrificed moribund	1[c]	0	0	0	0	0	0	0
Mean antibody titers (antibody units/mL)[d]								
Anti-CMV gH pentamer complex antibodies								
Main Study animals								
Day 1	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Day 43	2.8	2.8	953	1721	685	4229	2973	7987
Recovery Study animals								
Day 1	2.8	2.8	NE	NE	NE	NE	2.8	2.8
Day 57	2.8	2.8	NE	NE	NE	NE	5806	12,594

Dose (µg/dose)[b]	**0 (control)**		**8.9**		**27**		**89**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Anti-CMV gB protein antibodies								
Main Study animals								
Day 1	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Day 43	1.5	1.5	1785	2347	1902	3377	2179	4460
Recovery Study animals								
Day 1	1.5	1.5	NE	NE	NE	NE	1.5	1.5
Day 57	1.5	1.5	NE	NE	NE	NE	2970	6652
Pentamer-specific T cells (Day 44; range % IFN-γ+ cells)[e]								
CD4+ T cells	0.00-0.46	0.00-1.13	0.00-1.20	0.00-1.39	0.00-0.46	0.00-2.16	0.00-6.99	0.00-3.16
CD8+ T cells	0.00-0.32	0.00-0.33	0.00-2.73	0.00-3.91	0.00-0.74	0.00-2.70	0.00-8.96	0.00-4.35
Glycoprotein B-specific T cells (Day 44; range % IFN-γ+ cells)[e]								
CD4+ T cells	0.00-0.57	0.00-0.79	0.00-0.79	0.00-1.30	0.00-0.91	0.00-2.15	0.00-3.17	0.00-3.00
CD8+ T cells	0.00-0.12	0.00-0.69	0.00-0.26	0.00-0.00	0.00-0.64	0.004.17	0.00-4.52	0.00-4.76
Noteworthy findings – main study								
Clinical observations (number of animals affected)								
Swollen Soft	0	0	10	10	10	10	12	13
Swollen firm	0	0	0	0	0	0	3	10
Skin, red	2	2	2	0	1	2	6	15
Mean body weight	–	–	–	–	–	–	–	–
Mean body weight gains (g)								
Days −1 to 7	66.9	23.2	56.9**	–	52.7***	–	50.0***	17.6
Days 7 to 14	68.0	21.3	60.8	–	62.1	–	70.3	27.9
Days 14 to 21	53.3	16.6	44.9**	–	43.5**	–	36.6***	11.7
Days 21 to 28	42.9	16.3	38.6	–	36.7	–	47.2	17.2
Days 28 to 35	36.2	13.2	29.1*	–	30.6	–	26.9***	9.9
Days 35 to 42	30.7	12.1	24.8	–	29.2	–	36.4	13.7
Mean food consumption	–	–	–	–	–	–	–	–

Dose (µg/dose)[b]	0 (control)		8.9		27		89	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Ophthalmic examinations	–	–	–	–	–	–	–	–
Mean body temperature (°C)[g]								
Day 1, pre-dose	36.24	37.32	–	–	–	–	36.61	37.64
Day 1, 6 h post dose	35.26	36.25	–	–	–	–	38.05***	37.55***
Day 2, 24 h post-Day 1 dose	37.45	37.62	–	–	–	–	37.47	38.56***
Day 3, 48 h post-Day 1 dose	NR	NR	–	–	–	–	NR	–
Day 43, pre-dose	36.23	38.01	–	–	–	–	36.81*	37.77
Day 43, 6 h post dose	36.79	37.04	–	–	–	–	37.53**	38.59***
Day 44, 24 h post-Day 43 dose	36.91	37.39	–	–	–	–	37.29	38.12**
Hematology (Day 44; mean or mean fold change)[h]								
White blood cells (10^3/µL)	10.570	6.541	–	1.2	1.3	1.4#	1.8###	1.8###
Neutrophils (10^3/µL)	1.595	0.724	1.8	4.6	4.4***	6.2###	7.2***	8.9###
Lymphocytes (10^3/µL)	8.402	5.498	0.84	0.73	0.74	0.73	0.77	0.83
Eosinophils (10^3/µL)	0.108	0.069	2.6##	4.0##	2.8##	3.9##	3.8###	6.5###
Large unstained cells (10^3/µL)	0.138	0.087	2.2**	1.9	2.2**	2.1	1.8	2.0
Platelet count (10^3/µL)	1078.6	1060.4	–	–	–	–	–	0.82**
Coagulation (Day 44; mean or mean fold change)[h]								
Activated partial thromboplastin time (seconds)	15.50	14.97	1.1	1.2***	1.1***	1.2**	1.2***	1.2***
Fibrinogen (mg/dL)	302.6	252.0	1.7***	1.7***	1.9***	1.9***	2.1***	2.1***
Clinical chemistry (Day 44; mean or mean fold change)[h]								
Albumin (g/dL)	3.64	4.54	0.9***	0.9***	0.9***	0.9**	0.9***	0.9***
Globulin (g/dL)	2.13	1.97	1.2***	1.2***	1.2***	1.2***	1.3***	1.2***
Albumin/globulin ratio	1.72	2.32	0.8***	0.8***	0.8***	0.8***	0.7***	0.7***
Blood markers (Day 44; mean, µg/mL)								
α1-acid glycoprotein	94.060	72.220	257.432***	235.634***	390.988***	339.454***	551.569***	505.421***
α2-macroglobulin	23.506	42.357	115.194	55.994	293.504###	123.631##	382.531###	186.357##

Dose (µg/dose)[b]	0 (control)		8.9		27		89	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Cytokines (mean, pg/mL)								
IL-1β	–	–	–	–	–	–	–	–
IL-6	–	–	–	–	–	–	–	–
MIP-1α	–	–	–	–	–	–	–	–
TNF-α	–	–	–	–	–	–	–	–
IP-10								
Day 1, 6 h post dose	119.183	101.380	NE	NE	NE	NE	1215.712[§§]	1484.720[^]
Day 15, 6 h post dose	81.600	78.686	NE	NE	NE	NE	882.356[^]	1254.524[§§§]
Day 29, 6 h post dose	108.196	75.830	NE	NE	NE	NE	993.816[§§]	1374.798[^]
Day 43, 6 h post dose	114.608	105.752	NE	NE	NE	NE	667.464[§§§]	947.402[^]
MCP-1								
Day 1, 6 h post dose	385.368	128.680	NE	NE	NE	NE	–	525.168[§]
Day 15, 6 h post dose	407.454	158.156	NE	NE	NE	NE	–	1032.490[§]
Day 29, 6 h post dose	387.200	174.606	NE	NE	NE	NE	–	1032.040[§§]
Day 43, 6 h post dose	352.102	169.772	NE	NE	NE	NE	–	–
Gross pathology (Day 44)								
Site, injection (number examined)	10	10	10	10	10	10	10	10
Abnormal consistency, firm	0	0	1	3	5	5	9	7
Swelling	0	0	5	5	6	5	9	7
Focus, dark	0	0	0	1	0	1	0	3
Lymph node, inguinal (number examined)	10	10	10	10	10	10	10	10
Enlargement	1	0	1	0	0	0	5	1
Lymph node, popliteal (number examined)	10	10	10	10	10	10	10	10
Enlargement	0	0	3	8	7	6	7	7

Dose (µg/dose)[b]	0 (control)		8.9		27		89	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Organ weights (Day 44; g or %)[f]								
Spleen (number weighed)	10	10	10	10	10	10	10	10
Absolute weight	0.9560	0.6045	12.9079	17.8660	13.2218	25.1613*	26.2552**	31.2490**
% of body weight	0.17474	0.19011	21.26283*	19.57208	24.05873*	26.46140**	34.07596***	33.57762***
% of brain weight	43.31597	30.40901	12.65112	14.12509	14.99651	21.61765*	27.20966**	27.60228**
Histopathology (Day 44)[i]								
Site, injection (number examined)	10	10	10	10	10	10	10	10
Inflammation: mixed cell, subcutaneous	(0)	(0)	(10)	(10)	(9)	(10)	(10)	(10)
Minimal	–	0	1	1	0	1	0	1
Mild	–	0	6	7	3	8	4	2
Moderate	–	0	3	2	6	1	6	7
Edema; subcutaneous	(0)	(0)	(5)	(6)	(8)	(8)	(9)	(10)
Minimal	–	–	2	2	0	2	2	0
Mild	–	–	3	3	2	4	3	3
Moderate	–	–	0	1	6	2	4	7
Degeneration, myofiber	(5)	(5)	(7)	(5)	(9)	(8)	(6)	(6)
Minimal	5	4	7	4	8	6	5	6
Mild	0	1	0	1	1	2	1	0
Lymph node, popliteal (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell	(0)	(0)	(2)	(10)	(10)	(10)	(9)	(10)
Minimal	–	–	0	4	2	2	1	0
Mild	–	–	2	6	4	7	3	5
Moderate	–	–	0	0	4	1	4	5
Marked	–	–	0	0	0	0	1	0
Lymph node, inguinal (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell	(0)	(0)	(0)	(0)	(1)	(0)	(3)	(0)
Minimal	–	–	–	–	1	–	1	–
Mild	–	–	–	–	0	–	2	–

Dose (µg/dose)[b]	**0 (control)**		**8.9**		**27**		**89**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Sciatic nerve (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed, perineurial	(0)	(0)	(10)	(10)	(10)	(10)	(10)	(10)
Minimal	–	–	0	4	2	3	1	1
Mild	–	–	2	2	4	4	1	1
Moderate	–	–	3	4	4	3	8	7
Marked	–	–	5	0	0	0	0	1
Bone marrow (number examined)	10	10	10	10	10	10	10	10
Increased hematopoiesis, myeloid	(0)	(0)	(0)	(0)	(4)	(2)	(9)	(9)
Minimal	–	–	–	–	4	2	9	9
Spleen (number examined)	10	10	10	10	10	10	10	10
Decreased cellularity, periarteriolar lymphoid sheath	(0)	(0)	(5)	(7)	(9)	(10)	(10)	(10)
Minimal	–	–	1	5	5	6	3	1
Mild	–	–	4	2	4	4	7	9
Noteworthy findings – recovery period								
Clinical observations	–	–	–	–	–	–	–	–
Body weight gains	–	–	–	–	–	–	–	–
Hematology (Day 57)	–	–	–	–	–	–	–	–
Coagulation (Day 57)	–	–	–	–	–	–	–	–
Clinical chemistry (Day 57)	–	–	–	–	–	–	–	–
Blood markers (Day 57; mean, µg/mL)								
α1-acid glycoprotein	–	–	NE	NE	NE	NE	–	–
α2-macroglobulin	20.773	35.152	NE	NE	NE	NE	37.996[§]	54.802
Cytokines (Day 57)	–	–	NE	NE	NE	NE	–	–
Gross pathology (Day 57)	–	–	–	–	–	–	–	–

Dose (µg/dose)[b]	0 (control)		8.9		27		89	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Organ weights (Day 57; g or %)[f]								
Spleen (number weighed)	4[c]	5	NE	NE	NE	NE	5	5
Absolute weight	0.9270	0.5948	NE	NE	NE	NE	18.8997	9.6503
% of body weight	0.15520	0.17949	NE	NE	NE	NE	21.49843[§]	11.74440
% of brain weight	40.34433	28.59109	NE	NE	NE	NE	19.85703	11.57676
Histopathology (Day 57)[i]								
Site, injection (number examined)	4	5	NE	NE	NE	NE	5	5
Infiltration, mononuclear cell, myofiber	(0)	(0)	NE	NE	NE	NE	(2)	(5)
Minimal	–	–	NE	NE	NE	NE	1	4
Mild	–	–	NE	NE	NE	NE	1	1
Nerve, sciatic (number examined)	4	5	NE	NE	NE	NE	5	5
Inflammation, mixed cell; perineurial	(0)	(0)	NE	NE	NE	NE	(0)	(2)
Minimal	–	–	NE	NE	NE	NE	–	2
Bone marrow (number examined)	4	5	NE	NE	NE	NE	5	5
Increased hematopoiesis; myeloid	(0)	(0)	NE	NE	NE	NE	(2)	(1)
Minimal	–	–	NE	NE	NE	NE	2	1
Spleen (number examined)	4	5	NE	NE	NE	NE	5	5
Decreased cellularity; periarteriolar lymphoid sheath	(0)	(0)	NE	NE	NE	NE	(2)	(0)
Minimal	–	–	NE	NE	NE	NE	2	–

Abbreviations: – = no mRNA-1647-related effect; CMV = cytomegalovirus; eCTD = electronic common technical document; F = female; GLP = Good Laboratory Practice; IFN = interferon; IL = interleukin; IP-10 = interferon gamma-inducible protein 10; M = male; MCP-1 = monocyte chemoattractant protein 1; MIP-1α = macrophage inflammatory protein 1 alpha; NE = not evaluated; NR = not recorded; SoA = summary of analysis; TNF = tumor necrosis factor.

Notes: Statistical significance is based on actual data (not on the fold changes or percent differences); $^{*} = p \leq .05$, $^{**} = p \leq .01$, $^{***} = p \leq .001$ (mean value significantly different from control mean value using a Dunnett test); $^{\#} = p \leq .05$, $^{\#\#} = p \leq .01$, $^{\#\#\#} = p \leq .001$ (mean value significantly different from control mean value using a Dunn test); $^{\S} = p \leq .05$, $^{\S\S} = p \leq .01$, $^{\S\S\S} = p \leq .001$ (mean value significantly different from control mean value using a *t* test); $^{\wedge} = p \leq .05$, $^{\wedge\wedge} = p \leq .01$, $^{\wedge\wedge\wedge} = p \leq .001$ (mean value significantly different from control mean value using a Wilcoxon test).

[a] mRNA-based vaccine formulated in SM-102 lipid nanoparticles, as described in Section 2.6.7.1.

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

[c] One control male was found dead on Day 43. This death was considered not mRNA-1647 related.

[d] This study phase was not within the scope of regulations governing the conduct of nonclinical laboratory studies and was not intended to comply with such regulations. However, this non-GLP study phase was conducted in accordance with the standard operating procedures of Integrated Biotherapeutics, Inc.

[e] For purpose of range calculation, value < .0.00 following substraction of unstimulated control values were set to 0.00 for reporting.

[f] For controls, group means are presented. For treated groups, percent differences from controls are presented.

[g] Body temperatures were recorded on Day 1 and 43 at pre-dose, and 6- and 24-hours post dose. Additional body temperature measurements were recorded on Day 3, 48 hours post-Day 1 dose for the 100 µg/dose females.

[h] For controls, group means are presented. For treated groups, mean fold changes from controls are presented.

[i] Numbers in parentheses indicate the number of animals with findings.

2.6.7.7F REPEAT-DOSE TOXICITY: PIVOTAL STUDIES

Study Title: A 6-week (4 doses) intramuscular injection toxicity study of mRNA-1443 in Sprague Dawley rats followed by a 2-week recovery period

Test Article: mRNRA-1443[a]

Species/Strain: Rat/Crl:CD(SD) Sprague Dawley

Duration of Dosing: 6 weeks (4 doses; Days 1, 15, 29, and 43)

Formulation: 93 mM Tris, 60 mM NaCl, 7% PG

Initial Age: 7 weeks

Duration of Postdose: 2 weeks

Report Number: 5002158

Date of First Dose: 20 Mar 2017 (males) and 21 Mar 2017 (females)

Route of Administration: Intramuscular injection

Location in eCTD: 4.2.3.2

Dose volume: 200 µL/dose

GLP Compliance: Yes

Control Article: Phosphate-buffered saline, pH 7.2

Special Features: α1-acid glycoprotein, α2-macroglobulin, IL-1β, IL-6, IP-10, MIP-1α, MCP-1, TNF-α, IFN-γ-producing T cells

Dose (µg/dose)[b]	**0 (control)**		**9.6**		**29**		**96**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals								
Main study	10	10	10	10	10	10	10	10
Recovery period	5	5	0	0	0	0	5	5
Died or sacrificed moribund	0	0	0	0	0	0	0	0
pp65-specific T cells (range % IFN-γ+ cells)[c]								
CD4+ T cells	0.00-0.18	0.00-0.48	0.00-0.27	0.00-0.62	0.00-0.22	0.00-0.48	0.00-0.10	0.00-0.68
CD8+ T cells	0.00-0.00	0.00-0.18	0.00-1.07	0.00-0.22	0.00-0.57	0.00-0.17	0.00-0.41	0.00-0.55
Noteworthy findings – main study								
Clinical observations	–	–	–	–	–	–	–	–
Mean body weights	–	–	–	–	–	–	–	–
Mean body weight gains	–	–	–	–	–	–	–	–
Mean food consumption	–	–	–	–	–	–	–	–
Ophthalmic examinations	–	–	–	–	–	–	–	–

Dose (µg/dose)[b]	0 (control)		9.6		29		96	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mean body temperature (°C)								
Day 1, pre-dose	36.76	36.85	36.95	36.75	37.27	37.29	36.91	37.07
Day 1, 6 h post dose	36.09	37.00	36.63*	37.23	37.41***	37.48*	37.74***	37.77***
Day 2, 24 h post-Day 1dose	36.85	37.12	36.79	36.80	36.74	36.88	37.46***	37.35
Day 43, pre-dose	37.11	38.13	37.65	38.17	38.38***	38.41	37.49	38.55
Day 43, 6 h post dose	37.06	37.51	36.95	37.46	37.28	37.97	38.15***	38.71***
Day 44, 24 h post-Day 43 dose	36.83	36.81	36.77	36.81	37.22	36.79	37.85***	37.27
Hematology (Day 44; mean or mean fold change)[d]								
White blood cell count (10^3/µL)	9.447	5.567	–	1.3	1.3	1.4	2.0***	2.0***
Neutrophil count (10^3/µL)	1.274	0.926	1.6	1.7	3.3$^{\#\#}$	3.6***	9.0$^{\#\#\#}$	6.8***
Lymphocyte count (10^3/µL)	7.657	4.359	–	–	0.91	–	0.87	–
Eosinophil count (10^3/µL)	0.085	0.068	1.8	2.4$^{\#}$	2.0$^{\#}$	3.3$^{\#\#\#}$	2.8$^{\#\#\#}$	4.1$^{\#\#\#}$
Large unstained cell count (10^3/µL)	0.129	0.070	1.8	–	1.6	–	2.7***	–
Reticulocyte count (10^9/µL)	238.18	202.09	0.90	–	0.85*	–	0.82**	–
Platelet count (10^3/µL)	1120.6	1155.8	–	0.84*	–	0.93	–	0.77***
Coagulation (Day 44; mean or mean fold change)[d]								
Activated partial thromboplastin time (seconds)	15.92	16.04	–	–	1.1***	–	1.2***	1.1***
Fibrinogen (mg/dL)	281.9	214.3	1.6***	1.5	2.0***	2.1$^{\#\#\#}$	2.5***	2.4$^{\#\#\#}$
Clinical chemistry (Day 44; mean or mean fold change)[d]								
Albumin (g/dL)	3.81	4.61	–	–	–	0.89**	0.89***	0.87**
Globulin (g/dL)	2.16	1.91	–	–	1.2***	1.2***	1.3***	1.3***
Albumin/globulin ratio	1.77	2.45	–	–	0.78***	0.72$^{\#\#\#}$	0.70***	0.67$^{\#\#\#}$
Blood markers (Day 44; mean, µg/mL)								
α1-acid glycoprotein	68.251	39.036	–	–	294.610$^{\#\#\#}$	251.813$^{\#\#\#}$	467.607$^{\#\#\#}$	449.381$^{\#\#\#}$
α2-macroglobulin	19.900	23.679	–	–	158.635$^{\#\#}$	52.681	1180.908$^{\#\#\#}$	261.184$^{\#\#\#}$

Dose (µg/dose)[b]	0 (control)		9.6		29		96	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Cytokines (mean, pg/mL)								
IL-1β	–	–	NE	NE	NE	NE	–	–
IL-6	–	–	NE	NE	NE	NE	–	–
MIP-1α	–	–	NE	NE	NE	NE	–	–
TNF-α	–	–	NE	NE	NE	NE	–	–
IP-10								
Day 1, 6 h post dose	201.838	108.658	NE	NE	NE	NE	170.262	122.778
Day 15, 6 h post dose	146.606	99.730	NE	NE	NE	NE	222.820	196.752
Day 29, 6 h post dose	10.802	84.002	NE	NE	NE	NE	308.492^	522.986^
Day 43, 6 h post dose	167.726	82.884	NE	NE	NE	NE	484.112§	566.746
MCP-1								
Day 1, 6 h post dose	303.614	70.500	NE	NE	NE	NE	–	519.054§§
Day 15, 6 h post dose	295.866	236.046	NE	NE	NE	NE	–	440.528^
Day 29, 6 h post dose	439.954	326.604	NE	NE	NE	NE	–	544.322§
Day 43, 6 h post dose	280.644	158.990	NE	NE	NE	NE	–	439.100^
Gross pathology (Day 44)								
Injection site (number examined)	10	10	10	10	10	10	10	10
Abnormal consistency; firm	0	0	2	2	9	10	10	10
Swelling	0	0	1	0	4	1	7	2
Focus; dark	0	0	0	0	3	1	1	1
Focus; pale	0	0	0	0	0	0	1	0
Lymph node, inguinal (number examined)	10	10	10	10	10	10	10	10
Enlargement	0	0	2	0	2	0	2	1
Lymph node, popliteal (number examined)	10	10	10	10	10	10	10	10
Enlargement	0	0	3	1	3	2	5	4
Organ weights (Day 44; g or %)[e]								
Spleen (number weighed)	10	10	10	10	10	10	10	10
Absolute value	0.9193	0.5984	16.5343#	14.0207	15.7402	13.9037	22.8217#	20.0535
% of body weight	0.17228	0.20695	21.13059*	9.34063	20.11467*	13.78629	31.40503***	20.28219##
% of brain weight	41.70881	29.12029	14.89285	15.32076	18.35570#	13.87448	24.47485##	20.57586**

Dose (µg/dose)[b]	0 (control)		9.6		29		96	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Histopathology (Day 44; number of animals affected)[f]								
Site, injection (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell	(3)	(1)	(10)	(10)	(10)	(10)	(10)	(10)
Minimal	3	0	1	2	0	0	0	0
Mild	0	1	3	7	0	0	0	0
Moderate	0	0	6	1	4	7	0	0
Marked	0	0	0	0	6	3	10	10
Liver (number examined)	10	10	10	10	10	10	10	10
Vacuolation, microvesicular, periportal to midzonal	(1)	(5)	(6)	(8)	(9)	(8)	(9)	(9)
Minimal	1	5	6	5	6	5	4	5
Mild	0	0	0	3	3	2	4	3
Moderate	0	0	0	0	0	1	1	1
Spleen (number examined)	10	10	10	10	10	10	10	10
Decreased cellularity, lymphoid, periarteriolar sheath	(0)	(0)	(2)	(1)	(1)	(1)	(1)	(4)
Minimal	0	0	2	1	1	1	1	4
Inguinal lymph node (number examined)	10	10	10	9	10	9	10	10
Inflammation, mixed cell; perinodal	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
Mild	0	0	0	0	0	0	1	0
Plasmacytosis	(0)	(0)	(1)	(0)	(0)	(0)	(7)	(3)
Minimal	0	0	1	0	0	0	5	3
Mild	0	0	0	0	0	0	2	0
Hyperplasia; lymphoid	(1)	(0)	(6)	(5)	(4)	(7)	(5)	(4)
Minimal	1	0	2	3	2	2	3	3
Mild	0	0	3	1	1	5	2	1
Moderate	0	0	1	1	1	0	0	0

Dose (µg/dose)[b]	**0 (control)**		**9.6**		**29**		**96**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Popliteal lymph node (number examined)	9	10	10	10	10	10	10	10
Inflammation, mixed cell; perinodal	(0)	(0)	(9)	(9)	(10)	(9)	(6)	(7)
Minimal	0	0	3	5	6	3	1	2
Mild	0	0	6	4	2	5	4	4
Moderate	0	0	0	0	2	1	1	1
Plasmocytosis	(1)	(1)	(0)	(1)	(0)	(1)	(3)	(5)
Minimal	1	1	0	1	0	1	3	2
Mild	0	0	0	0	0	0	0	3
Hyperplasia; lymphoid	(0)	(0)	(9)	(8)	(8)	(9)	(5)	(8)
Minimal	0	0	6	3	5	6	3	5
Mild	0	0	3	5	3	3	2	3
Sciatic nerve (number examined)	10	10	10	10	10	10	10	10
Inflammation, mixed cell; perineurial	(0)	(5)	(10)	(10)	(10)	(9)	(10)	(10)
Minimal	0	5	1	0	4	4	0	2
Mild	0	0	5	6	3	3	8	7
Moderate	0	0	3	3	3	2	2	1
Marked	0	0	1	1	0	0	0	0
Bone marrow (number examined)	10	10	10	10	10	10	10	10
Increased hematopoiesis: myeloid	(0)	(1)	(2)	(0)	(2)	(4)	(5)	(4)
Minimal	0	1	2	0	2	4	4	4
Mild	0	0	0	0	0	0	1	0

Dose (µg/dose)[b]	0 (control)		9.6		29		96	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Noteworthy findings – recovery period								
Hematology (Day 57; mean or mean fold change)[d]								
White blood cell count (10^3/µL)	9.192	4.554	NE	NE	NE	NE	1.3	–
Neutrophil count (10^3/µL)	1.204	0.776	NE	NE	NE	NE	–	0.74
Lymphocyte count (10^3/µL)	7.520	3.488	NE	NE	NE	NE	1.4	–
Eosinophil count (10^3/µL)	0.072	0.094	NE	NE	NE	NE	1.4	0.69
Large unstained cell count (10^3/µL)	0.178	0.066	NE	NE	NE	NE	–	–
Reticulocyte count (10^9/µL)	242.88	165.32	NE	NE	NE	NE	–	–
Platelet count (10^3/µL)	1003.8	1080.2	NE	NE	NE	NE	–	0.93
Coagulation (Day 57)	–	–	NE	NE	NE	NE	–	–
Clinical chemistry (Day 57)	–	–	NE	NE	NE	NE	–	–
Blood markers (Day 57)	–	–	NE	NE	NE	NE	–	–
Cytokines (Day 57)	–	–	NE	NE	NE	NE	–	–
Gross pathology (Day 57)	–	–	NE	NE	NE	NE	–	–
Organ weights (Day 57)	–	–	NE	NE	NE	NE	–	–
Histopathology (Day 57; number of animals affected)[f]								
Injection site (number examined)	5	5	NE	NE	NE	NE	5	5
Inflammation, mononuclear cell	(1)	(0)	NE	NE	NE	NE	(4)	(4)
Minimal	1	0	NE	NE	NE	NE	4	4
Liver (number examined)	5	5	NE	NE	NE	NE	5	5
Vacuolation, microvesicular, periportal to midzonal	(0)	(2)	NE	NE	NE	NE	(1)	(4)
Minimal	0	2	NE	NE	NE	NE	0	1
Mild	0	0	NE	NE	NE	NE	1	3
Inguinal lymph node	5	5	NE	NE	NE	NE	5	5
Plasmacytosis	(1)	(0)	NE	NE	NE	NE	(2)	(0)
Minimal	1	0	NE	NE	NE	NE	2	0
Hyperplasia; lymphoid	(2)	(1)	NE	NE	NE	NE	(3)	(1)
Minimal	2	1	NE	NE	NE	NE	2	1
Mild	0	0	NE	NE	NE	NE	1	0

Dose (µg/dose)[b]	0 (control)		9.6		29		96	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Popliteal lymph node (number examined)	5	5	NE	NE	NE	NE	5	5
Infiltration, mononuclear cell; perinodal	(0)	(0)	NE	NE	NE	NE	(1)	(1)
Minimal	0	0	NE	NE	NE	NE	1	1
Plasmacytosis	(2)	(0)	NE	NE	NE	NE	(3)	(2)
Minimal	2	0	NE	NE	NE	NE	2	0
Mild	0	0	NE	NE	NE	NE	1	2
Hyperplasia; lymphoid	(1)	(0)	NE	NE	NE	NE	(3)	(5)
Minimal	0	0	NE	NE	NE	NE	3	5
Mild	1	0	NE	NE	NE	NE	0	0
Sciatic nerve (number examined)	5	5	NE	NE	NE	NE	5	5
Infiltration, mononuclear cell; perineurial	(0)	(1)	NE	NE	NE	NE	(5)	(4)
Minimal	0	1	NE	NE	NE	NE	5	2
Mild	0	0	NE	NE	NE	NE	0	2

Abbreviations: – = no mRNA-1443-related effect; eCTD = electronic common technical document; F = female; GLP = Good Laboratory Practice; IFN = interferon; IL = interleukin; IP-10 = interferon gamma-induced protein 10; M = male; MCP-1 = monocyte chemoattractant protein 1; MIP-1α = macrophage inflammatory protein 1 alpha; mRNA = messenger RNA; NE = not evaluated; SoA = summary of analysis; TNF = tumor necrosis factor.

Notes: Statistical significance is based on actual data (not on the fold changes or percent differences); $^{*} = p \leq .05$, $^{**} = p \leq .01$, $^{***} = p \leq .001$ (mean value significantly different from control mean value using a Dunnett test); $^{\#} = p \leq .05$, $^{\#\#} = p \leq .01$, $^{\#\#\#} = p \leq .001$ (mean value significantly different from control mean value using a Dunn test); $^{\S} = p \leq .05$, $^{\S\S} = p \leq .01$, $^{\S\S\S} = p \leq .001$ (mean value significantly different from control mean value using a *t* test); $^{\wedge} = p \leq .05$, $^{\wedge\wedge} = p \leq .01$ (mean value significantly different from control mean value using a Wilcoxon test).

[a] mRNA-based vaccine formulated in SM-102 lipid nanoparticles, as described in Section 2.6.7.1.

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

[c] For purpose of range calculation, values < 0.00 following substraction of unstimulated control values were set to 0.00 for reporting.

[d] For controls, group means are presented. For treated groups, mean fold changes from controls are presented.

[e] For controls, group means are presented. For treated groups, percent differences from controls are presented.

[f] Numbers in parentheses indicate the number of animals with findings.

2.6.7.8A GENOTOXICITY IN VITRO

Study Title: Bacterial reverse mutation test in *Salmonella Typhimurium* and *Escherichia Coli*
Test for Induction of: Reverse mutation in bacterial cells
Strains:
S typhimurium TA1535 *his*G46 *rfa ΔuvrB*
S typhimurium TA1537 *his*C3076 *rfa ΔuvrB*
S typhimurium TA98 *his*D3052 *rfa ΔuvrB* pKM101
S typhimurium TA100 *his*G46 *rfa ΔuvrB* pKM101
E coli WP2 *trp uvrA*

Number of Independent Assays: Single assay
No. of Replicate Cultures:
–S9 Fraction: 3/concentration
+S9 Fraction: 3/concentration
Positive Controls: NaAz, 9AC, 2NF, NQO, 2AA, and BaP
Negative Control: Ethanol

Test Article: SM-102
Report Number: 9601567
Location in eCTD: 4.2.3.3.1

Metabolizing System: Rat liver S9 fraction (S9)
Vehicle for Test Article: Ethanol

No. of Cells Analyzed/Culture: ≥ 1000×10^6 bacteria/mL
Vehicle for Positive Controls:
Sterile water for NaAz
DMSO for 9AC, 2NF, NQO, 2AA, BaP

GLP Compliance: Yes

Treatment: A 0.5 mL aliquot of S9 mix (+S9) or phosphate buffer 0.2 M pH 7.4 (–S9) was combined with 0.1 mL bacterial culture in a sterile container. A 0.1 mL aliquot of the test/reference item was added followed by 2 mL of molten top agar supplemented with 0.05 mM biotin, minimal (0.05 mM) histidine and minimal (0.05 mM) tryptophan. The solution was mixed and overlaid onto a minimal glucose plate (1.5% agar, Vogel-Bonner medium E, 2% glucose). After the overlay solidified, the plates were inverted and placed in an incubator set to maintain 37°C for 67 hours and 29 minutes.

Date of Treatment:
13 Sep 2016

Cytotoxic Effects: None observed
Genotoxic Effects: None observed

(b) (4)

2.6.7.8B GENOTOXICITY IN VITRO

Study Title: (b) (4) PEG 2K-DMG) and MC3 bacterial reverse mutation test in *Salmonella Typhimurium* and *Escherichia Coli*[a]

Test Article: PEG2000-DMG (b) (4)

Test for Induction of: Reverse mutation in bacterial cells

Number of Independent Assays: Single assay

Report Number: 9601035

Strains:
S typhimurium TA1535 *his*G46 *rfa ΔuvrB*
S typhimurium TA1537 *his*C3076 *rfa ΔuvrB*
S typhimurium TA98 *his*D3052 *rfa ΔuvrB* pKM101
S typhimurium TA100 *his*G46 *rfa ΔuvrB* pKM101
E coli WP2 *trp uvrA*

No. of Replicate Cultures:
–S9 Fraction: 3/concentration
+S9 Fraction: 3/concentration

Location in eCTD: 4.2.3.3.1

Positive Controls: NaAz, 9AC, 2NF, NQO, 2AA, and BaP
Negative Control: DMSO

Metabolizing System: Rat liver S9 fraction (S9)

No. of Cells Analyzed/Culture: ≥ 1000 × 10^6 bacteria/mL

Vehicle for Test Article: DMSO

Vehicle for Positive Controls:
Purified sterile water for NaAz
DMSO for 9AC, 2NF, NQO, 2AA, BaP

GLP Compliance: Yes

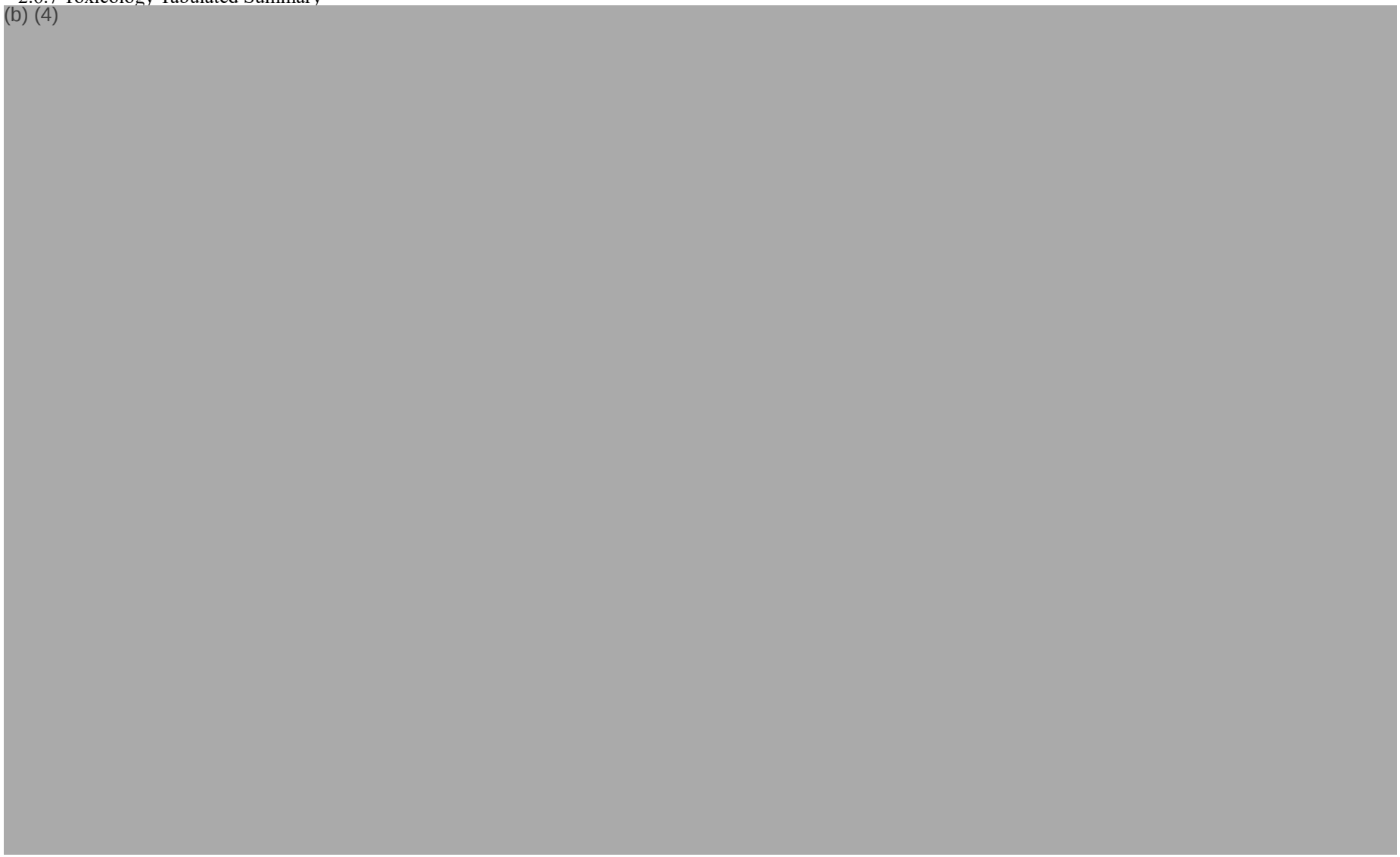
Treatment: A 0.5 mL aliquot of S9 mix (+S9) or phosphate buffer 0.2 M pH 7.4 (–S9) was combined with 0.1 mL bacterial culture in a sterile container. A 0.1 mL aliquot of PEG2000-DMG, negative control, or positive control was added followed immediately by 2 mL of molten top agar supplemented with 0.05 mM biotin, minimal (0.05 mM) histidine, and minimal (0.05 mM) tryptophan. The solution was mixed and overlaid onto a minimal glucose plate (1.5% agar, Vogel-Bonner medium E, 2% glucose). After the overlay solidified, the plates were inverted and placed in an incubator set to maintain 37°C for 67 hours and 57 minutes.

Date of Treatment:
10 Feb 2015

Cytotoxic Effects: None observed

Genotoxic Effects: None observed

(b) (4)



2.6.7.8C GENOTOXICITY IN VITRO

Study Title: In vitro mammalian cell micronucleus test in human peripheral blood lymphocytes

Test Article: SM-102

Test for Induction of: Micronuclei in human peripheral blood lymphocytes

Number of Independent Assays: Single assay

Report Number: 9601568

Strains: Human peripheral blood lymphocytes

No. of Replicate Cultures: 2 cultures per condition

Location in eCTD: 4.2.3.3.1

Positive Controls: MMC, NOC, and CP

Negative Controls: EtOH

Metabolizing System: Rat liver S9 fraction (S9)

No. of Cells Analyzed/Culture: 2000 binucleate cells

Vehicle for Test Article: Ethanol

Vehicle for Positive Controls: Not applicable

GLP Compliance: Yes

Treatment: Treatments were performed approximately 48 hours (44 to 48 hours) after culture initiation. Appropriate dilutions of test item and positive control formulations were prepared to reach the final concentrations indicated in the experimental design. Cultures tested in the absence of S9 mix were treated as indicated in the experimental design then returned to the incubator for 4 or 24 hours as appropriate. For cultures tested in the presence of S9 mix, 1 mL of S9 mix was added immediately prior to treatment, and then the cultures were returned to the incubator for 4 hours. A standard dose volume of 10 µL test item, negative control, or positive control per mL culture was used throughout. The test item 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 a 24-hour treatment period in the absence of S9 mix) so that analyzable cells would be available for at least 3 dose levels for each regimen.

Date of Treatment: 15 Sep 2016

Cytotoxic Effects: None observed

Genotoxic Effects: None observed

Metabolic Activation	Test Article	Concentration or Dose Level (µg/mL)	4-Hour Treatment			24-Hour Treatment		
			CBPI (mean)	MBC (%)	Cytotoxicity (%)[b]	CBPI (mean)	MBC (%)	Cytotoxicity (%)[b]
Absence of S9 (–S9)	EtOH	–	1.8	0.2	0.0	1.7	0.4	0.0
	SM-102	163	1.9	0.5	-8.0	1.7	0.2	-8.0
		286	1.9	0.6	-8.0	1.7	0.2	-2.0
		500[a]	1.8	0.1	0.0	1.7	0.3	1.0
	NOC	0.25	1.5	4.4**	43.0	–	–	–
	MMC	0.10	–	–	–	1.6	2.0**	3.0
Presence of S9 (+S9)	EtOH	–	1.8	0.4	0.0	–	–	–
	SM-102	163	1.8	0.5	-3.0	–	–	–
		286	1.8	0.6	-1.0	–	–	–
		500	1.8	0.3	1.0	–	–	–
	CP	10	1.4	2.2**	46.0	–	–	–

Abbreviations: – = not applicable/determined; –S9 = absence of S9 mix; +S9 = presence of S9 mix; CBPI = cytokinesis-block proliferation index; CP = cyclophosphamide; eCTD = electronic common technical document; EtOH = ethanol 100%; GLP = Good Laboratory Practice; MBC = micronucleated binucleate cells; MMC = mitomycin C; NOC = nocodazole.

Note: Fisher's exact test with single-sided probabilities, ** = p value ≤ .01.

[a] Precipitate visible in the culture medium at the end of treatment.

[b] Relative to vehicle control.

2.6.7.8D GENOTOXICITY IN VITRO

Study Title: (b) (4) (PEG 2K-DMG) and MC3 in vitro mammalian cell micronucleus test in human peripheral blood lymphocytes[a]

Test Article: PEG2000-DMG (b) (4) (b) (4)

Test for Induction of: Micronuclei in human peripheral blood lymphocytes

Number of Independent Assays: Single assay

Report Number: 9601036

Strains: Human peripheral blood lymphocytes

No. of Replicate Cultures: 2 cultures per condition

Positive Controls: MMC, CP, and colcemid

Negative Controls: DMSO

Location in eCTD: 4.2.3.3.1

Metabolizing System: Rat liver S9 fraction (S9)

No. of Cells Analyzed/Culture: 2000 binucleated cells

Vehicle for Test Article: DMSO

Vehicle for Positive Controls: Sterile purified water for MMC and colcemid. Sterile water for irrigation, USP for CP.

GLP Compliance: Yes

Treatment: Treatments were performed approximately 48 hours (44 to 48 hours) after culture initiation. Appropriate dilutions of test item and positive control solutions were prepared to reach the final concentrations indicated in the experimental design. Cultures tested in the absence of S9 mix were treated as indicated in the experimental design then returned to the incubator for 4 or 24 hours as appropriate. For cultures tested in the presence of S9 mix, 0.2 mL of S9 mix per mL of culture was added immediately prior to treatment, and then the cultures were returned to the incubator for 4 hours. A standard dose volume of 10 µL PEG2000-DMG, negative control, or positive control per mL culture was used throughout. The test item 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 a 24-hour treatment period in the absence of S9 mix) so that analyzable cells would be available for at least 3 dose levels for each regimen. No cytotoxicity was observed in either of the 4-hour regimen in the absence or presence of S9 mix, therefore the top 3 dose levels (163, 286, and 500 µg/mL) were chosen for micronucleus assessment. In the 24-hour treatment regimen, cytotoxicity of approximately 60.8% was observed at a concentration of 163 µg/mL; this concentration and the next 2 lower dose levels (93.3 and 53.3 µg/mL) were chosen for micronucleus assessment.

Date of Treatment: 11 Feb 2015/25 Feb 2015

Cytotoxic Effects: Cytotoxicity was observed in the 24-hour treatment regimen at ≥ 163 µg/mL

Genotoxic Effects: None observed

Metabolic Activation	Test Article	Concentration or Dose Level (µg/mL)	4-Hour Treatment			24-Hour Treatment		
			CBPI (mean)	MBC (%)	Cytotoxicity (%)[b]	CBPI (mean)	MBC (%)	Cytotoxicity (%)[b]
Absence of S9 (–S9)	DMSO	–	2.0	0.5	0.0	1.7	0.20	0.0
	PEG 2K-DMG[c]	53.3	–	–	–	1.6	0.40	9.3
		93.3	–	–	–	1.4	0.20	45.1
		163	2.0	0.50	0.6	1.3	0.60	60.8
		286	1.9	0.55	5.8	–	–	–
		500	2.0	0.85	-3.7	–	–	–
	MMC	0.45	1.4	12.50*	56.5	–	–	–
	Colcemid	0.035	–	–	–	1.9	3.55*	-28.0
Presence of S9 (+S9)	DMSO	–	1.8	0.20	0.0	–	–	–
	PEG 2K-DMG	163	2.0	0.35	-20.4	–	–	–
		286	1.9	0.50	-9.8	–	–	–
		500	1.9	0.55	-8.5	–	–	–
	CP	10	1.6	3.65*	26.2	–	–	–

Abbreviations: – = not applicable/determined; –S9 = absence of S9 mix; +S9 = presence of S9 mix; CBPI = cytokinesis-block proliferation index; CP = cyclophosphamide; DMSO = dimethyl sulfoxide; eCTD = electronic common technical document; GLP = Good Laboratory Practice; MBC = micronucleated binucleate cells; MMC = mitomycin C.

Note: * = Substantial increase compared to concurrent vehicle control.

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

[b] Relative to vehicle control.

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

2.6.7.9A GENOTOXICITY IN VIVO

Study Title: Zika mRNA: mammalian erythrocyte micronucleus test in rat

Test Article: mRNA-1706[a]

Test for Induction of: bone marrow micronuclei

Treatment Schedule: In the main phase of the study, male and female rats (5 to 8 animals/sex/group) were given a single intravenous injection in the following dose-range: 0.6 to 2.6 mg/kg for female rats and 1.3 to 5.2 mg/kg for male rats.

Report Number: 9800399

Species/Strain: Rat/Sprague Dawley (male and female)

Sampling Time: 24- or 48-hours post dose bone marrow collection

Location in eCTD: 4.2.3.3.2

Age: 49 to 56 days

Method of Administration: Intravenous injection (slow bolus 1 to 2 minutes)

Cells Evaluated: immature erythrocytes (IE) in rat bone marrow

Vehicle/Formulation: Test Article was diluted with control (PBS) to achieve target concentrations.

GLP Compliance: Yes

No. of Cells Analyzed/Animals: 4000 cells (IE)

Date of Dosing:

Dose-range finding: 17 Oct 2016

Main phase: 08 Nov 2016

Special Features: 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, 3.9, and 5.2 mg/kg for females, and 2.6, 5.2, and 10.3 mg/kg for males)

Toxic/Cytotoxic Effects: In the dose-range finding phase of the study, doses ≥ 3.9 mg/kg in females resulted in body weight loss. In males, a dose of 10.3 mg/kg resulted in mortality (2 out of 3 animals) but there were no clinical signs at 5.2 mg/kg. The MTD was determined to be 2.6 mg/kg for females and 5.2 mg/kg for males.

During the main phase males dosed at 5.2 mg/kg, decreased weight gain, yellow staining of the fur at the urogenital and abdominal areas or redness of the tail skin were observed. Females also suffered a depression in weight gain which increased with dose, 24 hours post dose, but recovered by 48 hours.

Genotoxic Effects: Statistically significant increases in MIE were observed in males at 24 and 48 hours and in females at 48 hours only with no clear dose response after IV administration of mRNA-1706; the increases in MIE were generally weak and associated with minimal bone marrow toxicity.

Evidence of Exposure: Exposure was verified by measurement of plasma Zika mRNA concentrations at 15 minutes after mRNA-1706 administration in a separate bioanalysis study. Zika mRNA was quantifiable for all mRNA-1706 dose groups.

Treatment	mRNA/SM-102 Dose (mg/kg)[b]	Sex	Time (h)	Number of Animals	%IE/(IE+ME)	%MIE	%MME	Incidence MIE (Mean ± SD)
Negative Control	0	M	24	5	54.6	0.155	0.00	6.2 ± 2.3
		F	24	5	43.9	0.135	0.00	5.4 ± 4.7
mRNA-1706[a]	0.6/6.2	F	24	5	51.4	0.200	0.00	8.0 ± 3.7
	1.3/13.5	M	24	5	55.8	0.315	0.00	12.6 ± 5.1***
		F	24	5	44.6	0.205	0.00	8.2 ± 3.7
	2.6/27.0	M	24	5	53.8	0.170	0.00	6.8 ± 3.6
		F	24	5	45.0	0.135	0.00	5.4 ± 2.1
	5.2/54.1	M	24	5	40.6	0.295	0.00	11.8 ± 4.9#/**
CP[c]	0	M	24	3	43.3	2.142	0.00	85.7 ± 9.7***
Negative Control	0	M	48	5	53.6	0.140	0.00	5.6 ± 3.9
		F	48	5	46.2	0.135	0.00	5.4 ± 1.9
mRNA-1706[a]	2.6/27.0	F	48	5	35.5	0.210	0.00	8.4 ± 1.5*
	5.2/54.1	M	48	5	36.6	0.480	0.06	19.2 ± 7.6***

Abbreviations: CP = cyclophosphamide; eCTD = electronic common technical document; GLP = Good Laboratory Practice; F = female; IE = immature erythrocytes; M = male; ME = mature erythrocytes; MIE = micronucleated immature erythrocytes; MME = micronucleated mature erythrocytes; mRNA = messenger RNA; MTD = maximum tolerated dose; PBS = phosphate-buffered saline; SD = standard deviation; SoA = summary of analysis.

Note: Results from main phase of the study reported. Fisher's exact test (* = p value < .05, ** = p value < .01, *** = p value < .001) or Cochran-Armitage's test (# = p value < .05).

[a] mRNA-based vaccine formulated in SM-102 lipid nanoparticles, as described in Section 2.6.7.1.

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

[c] Cyclophosphamide, 20 mg/kg was used as positive control.

2.6.7.9B GENOTOXICITY IN VIVO

Study Title: NPI luciferase mRNA in SM-102-containing lipid nanoparticles: in vivo mammalian bone marrow erythrocyte micronucleus assay in the rat

Test Article: NPI luciferase mRNA in SM-102-containing lipid nanoparticles

Test for Induction of: bone marrow micronuclei

Treatment Schedule: 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) as an IV injection.

Report Number: AF87FU.125012NGLPICH.BTL

Species/Strain: Rat/Sprague Dawley (male and female)

Sampling Time: 24- or 48-hours post dose bone marrow collection

Location in eCTD: 4.2.3.3.2

Age: 6 weeks

Method of Administration: Intravenous injection (slow push over 1.5 to 2.5 minutes)

Cells Evaluated: PCEs in rat bone marrow

Vehicle/Formulation: Test article was diluted with control (vehicle; 25 mM Tris/sucrose 1 mM DTPA pH 7.5) to achieve target concentrations.

GLP Compliance: No

No. of Cells Analyzed/Animal: 4000 cells (PCE)

Date of Dosing: 09 Dec 2019

Special Features[a]: Cytokine concentrations for MIP-1α, MCP-1, IL-6, IL-1β, TNF-α, and IP-10 were quantified in plasma samples from 6 hours post dose using a Luminex® assay.

Toxic/Cytotoxic Effects[b]: There were no test article-related effects on mortality or clinical observations. Test article-related increases in body temperature at 3.21/60 mg/kg (mRNA/SM-102, respectively) were observed from 1 to 2 hours postdose to 8 hours postdose). Test article-related cytokine changes were also observed.

Genotoxic Effects: There was no significant increase in the incidence of micronuclei in the test article-treated animals at either time point (24 or 48 hours). No clastogenic effects were observed.

Evidence of Exposure: Increase in cytokines following dose administration.

Treatment	mRNA/SM-102 Dose (mg/kg)	Sex	Time (h)	Number of Animals	%PCE (Mean ± SD)	Toxicity (%)	%MnPCE (Mean ± SD)	Number of MnPCE/PCE Scored
Control (vehicle)	0/0	M	24	5	58.3 ± 6.2	ND	0.10 ± 0.04	19/20000
		F	24	5	66.7 ± 5.4	ND	0.12 ± 0.04	23/20000
NPI luciferase mRNA in SM-102-containing LNPs	0.32/6.0	M	24	5	66.2 ± 4.8*	14	0.11 ± 0.02	22/20000
		F	24	5	68.7 ± 7.2	3	0.10 ± 0.04	20/20000
	1.07/20	M	24	5	61.7 ± 4.6	6	0.10 ± 0.04	20/20000
		F	24	5	64.1 ± 5.7	-4	0.10 ± 0.03	19/20000
	3.21/60	M	24	5	66.3 ± 3.1*	14	0.09 ± 0.03	18/20000
		F	24	5	61.0 ± 7.2	-9	0.11 ± 0.02	21/20000
CP	40[a]	M	24	5	27.7 ± 4.3**	-53	3.70 ± 0.47**	740/20000
Control (vehicle)	0/0	M	48	5	70.0 ± 4.4	ND	0.08 ± 0.02	15/20000
		F	48	5	61.8 ± 11.3	ND	0.10 ± 0.04	20/20000
NPI luciferase mRNA in SM-102-containing LNPs	0.32/6.0	M	48	5	57.9 ± 9.2**	-17	0.09 ± 0.02	17/20000
		F	48	5	62.1 ± 7.6	1	0.12 ± 0.03	23/20000
	1.07/20	M	48	5	63.8 ± 3.2	-9	0.09 ± 0.04	17/20000
		F	48	5	64.4 ± 2.5	4	0.13 ± 0.03	25/20000
	3.21/60	M	48	5	67.7 ± 4.7	-3	0.08 ± 0.03	15/20000
		F	48	5	66.9 ± 9.0	8	0.11 ± 0.05	21/20000

Abbreviations: ANOVA = analysis of variance; CP = cyclophosphamide; eCTD = electronic common technical document; F = female; GLM = generalized linear model; GLP = Good Laboratory Practice; IV = intravenous; IL = interleukin; IP-10 = interferon gamma-induced protein 10; LNP = lipid nanoparticle; M = male; MCP-1 = monocyte chemoattractant protein 1; MIP-1α = macrophage inflammatory protein 1 alpha; MnPCE = micronucleated polychromatic erythrocyte; mRNA = messenger RNA; ND = not determined; NPI = nascent peptide imaging; PCE = polychromatic erythrocyte; SD = standard deviation; TNF = tumor necrosis factor.

Notes: * = $p < .05$, ** = $p < .01$, One-way ANOVA with a post hoc Dunnett test or *t* test; 24 h MnPCE male GLM $p = 0.80$, R-square = 6.04%; 24 h MnPCE female GLM $p = 0.791$, R-square = 6.13%.

[a] mRNA was quantified in plasma samples from 2 hours post dose using a branched DNA assay; however, the mRNA quantification was not reported due to technical issues with the assay.

[b] Test article-related increases in IL-6, MCP-1, MIP-1α, and/or IP-10 were observed at 6 hours following dose administration 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; levels of IL-6, MCP-1, MIP-1α, and IP-10 were up to 3.68-, 4.66-, 2.62-, and 30.47-fold the levels in controls, respectively.

2.6.7.11 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Study Title: A GLP intramuscular combined developmental and perinatal/postnatal reproductive toxicity study of mRNA-1273 in rats

Design similar to ICH 4.1.2: Yes

Species/Strain: Rat/Sprague Dawley

Initial Age: Approximately 74 days

Date of First Dose: 30 Jun 2020

Day of Mating: GD 0

Litters Culled/Not Culled: Culled to 8/litter (if possible)

Special Features: None

Duration of Dosing: F_0 (dams): 6 weeks (4 doses, SD 1, SD 15 [28 and 14 days prior to mating, respectively], GD 1, and GD 13)

Route of Administration: Intramuscular injection

Vehicle/Formulation: 20 mM Tris, 87 mg/mL sucrose, 17.5 mM sodium acetate, pH 7.5

Test Article: mRNA-1273

Report Number: 20248897

Location in eCTD: 4.2.3.5.3

GLP Compliance: Yes (except for the antibody titer analysis, which was non-GLP)

Dose (µg/dose)	0 (control)	100
Cohort 1 (Cesaearan-sectioning phase)		
Number of F_0 (dams)	22	22
Number of litters evaluated	21	22
Number of F_1 (fetuses) examined	278	308
Cohort 2 (Natural Delivery phase)		
Number of F_0 dams	22	22
Number of litters evaluated	20	15
Number of F_1 (pups) (number of F1 pups examined)	274 (166)	205 (123)
Died, stillborn, or sacrificed moribund		
F_0 (dams)	1	0
F_1 (pups)	18	5
Mean S-2P antibody titers (antibody unit/mL)		
F_0 dams		
SD 1	< 30	< 30
SD 15	< 30	44,362
GD 1	< 30	220,596
GD 13	< 30	442,138
GD 21	< 30	149,443
LD 21	< 30	117,903
F_1 (fetuses/pups)		
GD 21	< 30	15,315

Dose (µg/dose)	0 (control)	100
LD 21	< 30	167,478
Noteworthy findings – F_0 (dams)		
Clinical observations (number of animals affected)		
Premating		
Fur, thin cover	1	9
Swollen hindlimb	0	5
Gestation		
Limited usage, hindlimb	0	20
Fur, thin cover	0	16
Swollen hindlimb	0	39
Lactation		
Fur, thin cover	1	4
Mean body weight	–	–
Mean body weight gains	–	–
Mean food consumption	–	–
Estrous cycling	–	–
Mating and fertility	–	–
Ovarian and uterine observations and litter observations	–	–
Organ weights (gravid uterus and placentae)	–	–
Gross pathology	–	–
Noteworthy findings – F_1 (fetuses/pups)		
Fetal examinations	–	–
External abnormalities	–	–
Visceral examination	–	–
Skeletal examination		
Malformations	–	–
Variations		
1 or more nodules of the ribs		
Fetuses N (%)	0 (0.00)	5 (3.27)*
Litters N (%)	0 (0.0)	4 (18.2)
1 or more wavy ribs		
Fetuses N (%)	0 (0.00)	6 (4.03)*
Litters N (%)	0 (0.0)	4 (18.2)
Fetal ossification site averages	–	–

Dose (µg/dose)	0 (control)	100
Natural delivery or litter observations	–	–
Clinical observations	–	–
Mean body weights	–	–
Gross pathology	–	–

Abbreviations: – = no mRNA-1273-related effect; eCTD = electronic common technical document; f_0 = dams; f_1 = fetuses/pups; GD = Gestation Day; GLP = Good Laboratory Practice; ICH = International Council for Harmonisation; LD = Lactation Day; SD = Study Day.

Note: * = $p < .05$, Kruskal-Wallis and Dunn.

2.6.7.17 OTHER TOXICITY STUDIES

Study Title: A non-GLP repeat-dose immunogenicity and toxicity study of mRNA-1273 by intramuscular injection in Sprague Dawley rats

Test Article: mRNA-1273

Species/Strain: Rat/CD® [Crl:CD® (SD)] Sprague Dawley

Duration of Dosing: 22 days (2 doses; Days 1 and 22)

Formulation: 20 mM Tris, 87 mg/mL sucrose, and 10.7 mM sodium acetate, pH 7.5

Initial Age: Approximately 7 weeks

Duration of Postdose: 13 days after the last immunization

Report Number: 2308-123

Date of First Dose: 13 Feb 2020

Route of Administration: Intramuscular injection

Location in eCTD: 4.2.3.7.7

Dose Volume: 0.2 mL/dose

GLP Compliance: No

Control Article: 20 mM Tris, 8.7% (w/v) sucrose, and 10.7 mM sodium acetate

Special Features: none

Dose (µg/dose)	**0 (control)**		**30**		**60**		**100**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals	5	5	5	5	5	5	5	5
Died or sacrificed moribund	0	0	0	0	0	0	0	0
Mean anti-SARS-Cov-2 S-2P antibody titers (Day 35)[a]	LOQ	LOQ	2,486,970.54	4,492,100.43	3,571,545.26	3,221,503.68	2,361,125.79	4,949,493.90
Noteworthy Findings								
Clinical observations (number of animals affected)								
External appearance								
Limb function impairment	0	0	1	0	5	4	5	5
Edema	0	0	5	5	5	5	5	5
Body weight and body weight gains	–	–	–	–	–	–	–	–

Dose (µg/dose)	0 (control)		30		60		100	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Hematology (mean or mean fold change)[b]								
Hemoglobin (g/dL)	17.42	16.44	0.92**	–	0.91**	–	0.93**	–
Erythrocytes (10^6 cells/µL)	8.600	8.618	0.94	–	0.94	–	0.96	–
Hematocrit (%)	53.46	49.48	0.93**	–	0.91**	–	0.93**	–
Reticulocytes (10^3 cells/µL)	223.48	179.28	0.78*	0.79	0.77*	0.80	0.77*	0.65**
Neutrophils (10^3 cells/µL)	0.946	1.178	9.79**	7.67**	10.81**	6.58**	8.26**	5.86**
Lymphocytes (10^3 cells/µL)	7.978	6.004	0.65**	–	0.58**	0.74	0.47**	0.61
Monocytes (10^3 cells/µL)	0.220	0.124	–	–	0.58*	–	0.52*	–
Eosinophils (10^3 cells/µL)	0.040	0.054	3.30##	4.67##	4.00##	3.26##	2.60##	3.48##
RDW (%)	12.52	11.28	1.06	1.05	1.08**	1.07*	1.10**	1.07*
Clinical chemistry (mean or mean fold change)[b]								
Creatinine (mg/dL)	0.28	0.40	1.36**	1.32*	–	1.26	1.43**	1.37*
Albumin (g/dL)	3.53	3.86	0.90**	0.90*	0.87**	0.88*	0.88**	0.85**
Globulin (g/dL)	3.12	3.47	–	–	1.12	–	1.15*	–
Albumin/Globulin ratio	1.13	1.12	0.83**	0.87	0.78**	0.87	0.75**	0.86
Triglycerides (mg/dL)	40.0	35.5	1.88*	–	2.30**	2.02	1.66	–
Cholesterol (mg/dL)	55.6	76.9	1.62**	–	1.57**	–	1.58**	–
Glucose (mg/dL)	69.0	84.5	–	–	–	–	1.26§§	–

Abbreviations: – = no mRNA-1273-related effect; CoV = coronavirus; eCTD = electronic common technical document; F = female; GLP = Good Laboratory Practice; LOQ = limit of quantitation; M = male; RDW = red cell distribution width; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus.

Notes: Statistical significance is based on actual data (not on the fold differences); * = $p \leq .05$, ** = $p \leq .01$ (mean value significantly different from control mean value; one-way analysis of variance followed by pairwise comparison using a Dunnett test); # = $p \leq .05$, ## = $p \leq .01$ (log transformed mean value significantly different from control mean value; one-way analysis of variance followed by pairwise comparison using a Dunnett test); §§ = $p \leq .01$ (mean value significantly different from control mean value; Kruskal-Wallis test followed by pairwise comparison using a Dunn test).

[a] mRNA-1273 antibody titer levels were determined on Day 1 (before administration of the first mRNA-1273 dose) and on Day 35 using a serum enzyme-linked immunosorbent assay with an LOQ ≤ 100.00. Titer levels on Day 1 were not detectable and therefore are not presented.

[b] On Day 23, 24 hours after administration of the last mRNA-1273 dose. For the control group, calculated group means are provided for comparison purposes. For treated groups, mean fold changes over the control group means are provided.