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LIST OF ABBREVIATIONS

Abbreviation	Definition
AUC	area under the concentration versus time curve
ADME	Absorption, Distribution, Metabolism, Excretion
AUC _(0-t)	area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed
AUC _{last}	area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed
bDNA	branched DNA
C _{max}	maximum plasma concentration
CMV	cytomegalovirus
CoV	coronavirus
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
DMG	myristoyl diglyceride
DP	drug product
EIC	Extracted ion current
gB	glycoprotein B
gH	glycoprotein H
gL	glycoprotein L
GLP	Good Laboratory Practice
IM	intramuscular(ly)
LC	liquid chromatography
LLOQ	lower limit of quantitation
LNP	lipid nanoparticle
Luc	luciferase
mRNA	messenger RNA
MTX	matrix ratios (tissue-to-serum)
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NHP	Non-human primate
NPI	nascent peptide imaging
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000

Abbreviation	Definition
PG	propylene glycol
PK	pharmacokinetic
PD	pharmacodynamics
S	spike
S-2P	spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
SARS-CoV-2	severe acute respiratory disease coronavirus 2
SM-102	a custom-manufactured ionizable lipid
T _{1/2}	half-life
Tris	tris(hydroxymethyl)aminomethane
T _{max}	time to peak (maximum) plasma concentration

2.6.4.1 BRIEF SUMMARY

ModernaTX, Inc. (Sponsor) has developed mRNA-1273 and mRNA-1273 variant vaccines, which are lipid nanoparticle (LNP)-encapsulated messenger RNA (mRNA)-based vaccines against the severe acute respiratory disease coronavirus (CoV) 2 (SARS-CoV-2). mRNA-1273 contains a single mRNA that encodes the full-length wild-type spike (S) protein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilize the S protein into the prefusion conformation. The substitutions in mRNA-1273 variant vaccines are described in Modules 2.6.1 and 2.4. The mRNA is combined in a mixture of 4 lipids common to the Sponsor's mRNA vaccine platform: SM-102, cholesterol, DSPC, and PEG2000-DMG.

Nonclinical PK evaluations included in vivo distribution, persistence, and clearance of mRNAs, SM-102 lipid, and/or expressed proteins using other mRNA-LNP DPs formulated in the same 4 lipids as mRNA-1273. When not formulated in LNPs, unprotected mRNA is degraded within minutes in biologic fluids and is unlikely to persist in tissues; therefore, the biodistribution of mRNA-based vaccines formulated in LNPs is predicted to be driven by the LNP characteristics and route of administration and not the encapsulated mRNA. Consequently, mRNAs are expected to distribute similarly when encapsulated in LNPs of the same composition and administered via the same route of administration. As described below, data derived using DPs with mRNA formulated in the same lipid composition provide evidence to substantiate this claim.

Results from 3 biodistribution studies using mRNA DPs comprised of the same 4 lipids (ie, SM-102, cholesterol, DSPC, and PEG2000-DMG) and generally similar lipid-to-mRNA ratios were used to characterize the kinetics and tissue distribution of mRNA-1273 (see

). These studies evaluated biodistribution following IM administration of:

(1) a DP comprised of reporter mRNA (NPI-Luc mRNA) encapsulated in SM-102/PEG2000-DMG-containing LNPs, (2) mRNA-1273, and (3) mRNA-1647. The reporter mRNA (NPI-Luc) encodes an intracellularly-expressed NPI-Luc protein, mRNA-1273 encodes the full-length SARS-CoV-2 protein, and mRNA-1647 is a mRNA-based CMV vaccine that contains 6 distinct mRNA sequences (1 that encodes the full-length CMV gB and 5 that encode the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex) formulated at a target mass ratio of 1:1:1:1:1:1. The in vivo distribution, persistence, and clearance of the mRNAs, SM-102 lipid, and/or expressed proteins from these IM-administered DPs are considered to be representative of mRNA-1273 given that the LNP composition is the same.

Collectively, data derived from the 3 biodistribution studies provide evidence that LNP composition and route of administration drive tissue distribution. Specifically, the general similarities in exposure tissue rank order and kinetics across DPs demonstrate that mRNA cargo and presence or absence of an immune response do not alter tissue distribution of mRNA-LNP DPs. Furthermore, tissue distribution is similar following one or two administration(s) of mRNA-1273, demonstrating that there is low to no risk of accumulation and no differences in distribution of DP components with repeat dosing.

The PK of NPI-Luc mRNA and SM-102 lipid and the PD of expressed NPI-Luc protein were evaluated following a single IM injection of 100 µg NPI-Luc mRNA encapsulated in SM-102/PEG2000-DMG-containing LNPs in male and female Sprague Dawley rats

(1). The systemic exposure of NPI-Luc mRNA appeared to be

independent of sex for serum and tissues. Tissues with the highest NPI-Luc mRNA and SM-102 exposures were generally similar for both analytes with highest exposures in the injection site, axillary lymph nodes, and spleen; and all of these had exposures greater than serum or plasma. Effective $T_{1/2}$ of systemic mRNA and SM-102 was 2.95 (serum) and 8.44 (plasma) hours. Across both analytes, the effective $T_{1/2}$ in tissues with higher exposures than systemic (based on AUC in serum or plasma) ranged from 2.98 to 60.5 hours. Likely due to the limitations of the analytical method, the expressed NPI-Luc was only quantifiable in 5 of 769 female samples, including 3 in the liver and 2 in the injection site (concentrations ranged from 211 to 339 ng/g).

To evaluate the distribution after a single or two dose administrations of mRNA-1273, the PK of mRNA from mRNA-1273 and SM-102 lipid and the PD of expressed SARS-CoV-2 S protein were evaluated following IM injections of 78 µg/dose mRNA-1273 on Day 1 and Day 28 in male and female Sprague Dawley rats ([Report 20456513 Amendment 1](#)). There were no differences in exposure for mRNA from mRNA-1273, SM-102 lipid, or SARS-CoV-2 S protein expression when comparing exposures after single or two dose administrations in any plasma or tissue matrix. Across matrices and analytes, exposures were generally sex dependent, where female exposures were often higher than males. Since the dose level administered was not adjusted for body weight (fixed dose) and male body weights were higher than female body weights in this study, the dose administered per body weight (g) was higher for females than males, which likely contributed to the observation of higher exposure in females. Compared with serum/plasma, mRNA from mRNA-1273 and SM-102 lipid exposures were generally higher in lymph nodes (inguinal/popliteal and axillary), the injection site, and spleen. Effective $T_{1/2}$ of systemic mRNA and SM-102 ranged from 1.77 to 3.89 (serum) and 4.83 to 7.02 hours (plasma). Across both analytes, the effective $T_{1/2}$ in tissues with higher exposures than systemic (based on AUC) ranged from 2.49 to 64.4 hours. Expressed SARS-CoV-2 protein was also generally higher in tissues than in serum, where lymph nodes (inguinal/popliteal), axillary lymph nodes, injection site, spleen and/or liver had highest exposures. Exposures of expressed SARS-CoV-2 protein in brain, heart, and lung had E_{max} values that were below the limit of quantification or had unreportable AUEC values due to limited number of samples with quantifiable values. All animals were positive for anti-SARS-CoV-2 S protein antibodies at 336 hours after each dose with increasing titer values after the second dose that were generally associated with lower exposure to SARS-CoV-2 S protein in all matrices, demonstrating a robust immunological response to the vaccine.

The results of a biodistribution study of mRNA-1647 also support the development of mRNA-1273 and mRNA-1273 variant vaccines. mRNA-1647 is a novel mRNA-based cytomegalovirus vaccine that contains 6 distinct mRNA sequences (1 that encodes the full-length cytomegalovirus glycoprotein B [gB], and 5 that encode the pentameric glycoprotein H [gH]/glycoprotein L [gL]/UL128/UL130/UL131A glycoprotein complex) combined at a target mass ratio of 1:1:1:1:1:1 in the Sponsor's standard proprietary SM-102-containing LNPs.

The biodistribution of mRNA-1647 was evaluated in a non-Good Laboratory Practice (GLP), single-dose, intramuscular (IM) injection study in Sprague Dawley rats. The objectives of this study were to determine the tissue distribution and pharmacokinetic (PK) characteristics of mRNA-1647 following IM administration. The biodistribution of mRNA-based vaccines in LNPs is predicted to be driven by the LNP characteristics. Therefore, mRNAs that are within an LNP of the same composition (eg, mRNA-1273 and mRNA-1647) are expected to distribute similarly.

Concentrations for all 6 mRNA-1647 constructs, gB, gH, gL, UL128, UL130, and UL131A, were detectable in plasma and tissues in a 1:1:1:1:1:1 ratio. After a single IM dose in male rats, the time after dosing at which the maximum concentration was observed in plasma (T_{\max}) was 2 hours for all constructs and was followed by a rapid elimination phase, with a half-life ($T_{1/2}$) estimated to range from 2.7 to 3.8 hours. The maximum plasma concentration (C_{\max}) ranged from 1.60 to 2.30 ng/mL, and the area under the concentration versus time curve (AUC) from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed ($AUC_{[0-t]}$) ranged from 22.7 to 25.5 ng \times h/mL.

Concentrations for all 6 mRNA-1647 constructs were detected at levels above the lower limit of quantitation (LLOQ) in most tissues analyzed, except for the kidney, where all levels were below the LLOQ. For highly exposed tissues (injection site [muscle], lymph nodes [proximal and distal], and spleen), the C_{\max} was observed between 2- and 24-hours post-dose. The $T_{1/2}$ was calculated using the average tissue $T_{1/2}$ values for all 6 constructs. The results were 14.9 hours for injection site (muscle), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

As observed with other IM-delivered vaccines, the highest mRNA concentrations were observed at the injection site followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0 .

The persistence of mRNA in tissues was evaluated in Sprague Dawley rats and indicated that for the majority of tissues, administered mRNA was not detectable after 1-3 days, the only exceptions were at the site of injection (muscle) and within the draining lymph nodes and spleen where the mRNA had a calculated half-life of 14.9-63.0 hrs.

Across the 3 biodistribution studies, the highest concentrations of SM-102 and mRNA were observed at the injection site, spleen, and lymph nodes in a manner similar to other IM delivered vaccines (Gómez-Mantilla et al 2016). The highest tissue-to-serum/plasma ratios based on C_{\max} of mRNA and SM-102 lipid were observed in the injection site followed by the lymph nodes and spleen. The highest tissue-to-plasma ratios based on AUC were observed in the injection site followed lymph nodes for SM-102 lipid. The mRNA tissue-to-serum ratios based on AUC in the spleen were higher in the studies with NPI-Luc mRNA and mRNA-1273 compared to the study with mRNA-1647, which is likely attributed to the wider range of collection times in the studies with NPI-Luc mRNA and mRNA-1273 (0.16 to 168 or 336 hours) compared to study with mRNA-1647 (2 to 120 hours). Results from these rat studies are corroborated by a published report by Hassett et al (2023), where NHPs were administered a single IM dose of a mRNA-LNP DP formulated in the same 4 lipids, and a select number of tissues (lymph nodes, spleen, liver, and injection site muscle) were evaluated for mRNA concentrations. Although the collection period in that publication was limited (ranging from 8 to 168 hours postdose), results were generally consistent with those observed in the rat studies. Specifically, mRNA in plasma and spleen were detected over 168 hours, and the spleen had the highest exposures, most likely from infiltrating cells. In that report, mRNA was not detected in the injection site, lymph nodes, and liver beyond 24 hours. Overall, the data derived from the rat studies confirms similarities in tissue distribution and kinetics, consistent with distribution via the lymphatic system and indicates cross-species similarities to NHPs.

No ADME studies have been performed with mRNA-1273 or mRNA-1273 variant vaccines, however, the metabolism and elimination of the novel amino-lipid component in mRNA-1273, SM-102, have been examined in vivo (Study QV-0236-DA-RE) and in vitro (Study NCS-BA-2022-010). Overall, the primary circulating species after IV dosing Sprague-Dawley rats **CCI** (Study QV-0236-DA-RE) was intact SM-102 (>95% of extracted ion current (EIC) with principally ester hydrolysis and β -oxidative metabolites cleared via both the renal and hepatic routes of elimination. These ester hydrolysis and β -oxidation products account for the majority of all the detected SM-102 metabolites in both the urine (>99% of EIC) and bile (~70% of EIC) with the balance comprising intact SM-102. Low molecular weight, hydrophilic metabolites were detected in relatively higher amounts (~10-fold) in urine compared to larger, more hydrophobic metabolites in plasma and bile. The extensive metabolism of SM-102; the oxidative nature of the metabolites; and the multiple, ubiquitous, high capacity systems by which they are formed combined with the rapid overall clearance of SM-102 (within 24 h) and the elimination of SM-102 and its metabolites via kidney (metabolites only) and liver (intact SM-102 and metabolites) to <3.0% of the maximum level indicate that the SM-102 is unlikely to accumulate upon repeat IM dosing or present an issue for elimination in patients with hepatic or renal insufficiency.

The list of completed nonclinical studies are presented in [Table 1](#)

Table 1: Nonclinical Studies Supporting Development of mRNA-1273

Study Type	Test Article	Study System	Method of Administration, Dose ^b	GLP	Report Number
Single-dose tissue distribution study	mRNA-1647 ^a	Rat, Sprague-Dawley	Single IM injection, dose of 100 μ g on Day 1	No	5002121 Amendment 2
A non-GLP biodistribution study of NPI-Luc mRNA in SM-102/PEG2000-DMG following single intramuscular injection in Sprague Dawley rats	NPI-Luc mRNA in SM-102/PEG2000-DMG-containing LNPs	Rat, Sprague-Dawley	Single IM injection, dose of 100 μ g on Day 1	No	2308-582 Amendment 1
A single or repeat dose biodistribution study of mRNA-1273 by intramuscular administration in	mRNA-1273	Rat, Sprague-Dawley	One or 2 IM injections, dose of 78 μ g on Day 1 or Days 1 and 28, respectively	No	20456513 Amendment 1

Study Type	Test Article	Study System	Method of Administration, Dose ^b	GLP	Report Number
Sprague Dawley rats					
Identification and profiling of metabolites of SM-102 in Rat, Monkey and Human Hepatocytes	NPI-Luciferase mRNA in SM-102	Rat, monkey and human hepatocytes	In vitro incubation	No	NCS-BA-2022-010
Metabolite Profile and Identification of SM-102 in Rat Plasma, Urine and Bile Following IV Infusion of SM-102 containing Lipid Nanoparticles to Male Sprague Dawley Rats	NPI-Luciferase mRNA in SM-102	Rat, Sprague-Dawley, M	IV dose of 0.7 mg/kg	No	QV-0236-DA-RE

Abbreviations: CMV = cytomegalovirus; gB = glycoprotein B; gH = glycoprotein; gL = glycoprotein L; GLP = Good Laboratory Practice; IM = intramuscular; mRNA = messenger RNA.

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

^b Dose refers to the total dose of mRNA encapsulated in the administered LNP.

2.6.4.2 METHODS OF ANALYSIS

The methods used to quantify mRNA, SM-102, and/or expressed proteins in the non-GLP PK studies supporting the development of mRNA-1283 are summarized in [Table 2](#). Details on the analytical methods can be found in the study reports.

Additionally, identification of SM-102 lipid metabolites was performed in vitro in Sprague Dawley rat, cynomolgus monkey, and human hepatocytes ([Report NCS-BA-2022-010](#)) and in vivo in Sprague Dawley rat urine, bile, and plasma ([E](#)) using HRMS methods. Details of the methods can be found in the study reports.

Table 2: Analytical Methods Used in Non-GLP PK Studies

Analyte	Analytical Method	Species	Matrix	Assay Rigor	LLOQ	Report Number
NPI-Luc mRNA	bDNA singleplex	Rats	Serum, tissues	Qualified	0.125 ng/mL	
SM-102	LC-MS/MS	Rats	Plasma, tissues	Qualified	0.500 ng/mL	20456513 Amendment 1
			Plasma, bile, urine	Fit for purpose	0.2 ng/mL	-
NPI-Luc protein	ECL	Rats	Tissue	Qualified	200 ng/g	
mRNA from mRNA-1273	RT-qPCR	Rats	Serum	Qualified	0.850 pg/mL	
			Tissues	Qualified	0.850 pg/g	
SARS-CoV-2 S protein	Ligand-binding assay	Rats	Serum	Qualified	4.57 ng/mL	1
			Tissues	Qualified	91.4 ng/g	
Anti-SARS-CoV-2 S protein IgG antibody	ELISA	Rats	Serum	Qualified	0.059 AU/mL	1
mRNAs from mRNA 1647 (gB, gH, gL, UL128, UL130, and UL131A)	bDNA multiplex	Rats	Plasma, tissues	Fit for purpose	0.01 ng/mL (gH, gL, UL128, and UL131A) ^a 0.05 ng/mL (gB and UL130) ^a	2

Abbreviations: bDNA = branched DNA; ECL = electrochemiluminescence; LC-MS/MS = liquid chromatography with tandem mass spectrometry; LLOQ = lower limit of quantification; Luc = luciferase; mRNA = messenger

RNA; NPI = nascent peptide imaging; RT-qPCR = reverse transcriptase-quantitative polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Note: For the calculation of tissue-to-plasma/serum ratios, where tissue concentration is ng/g and plasma/serum concentration is ng/mL, the assumption is made that 1 g of tissue is equivalent to 1 mL of plasma/serum.

^a. The LLOQ values used for pharmacokinetic reporting reflect the standard range in neat plasma, while the calibration range in Section 2.6.5.2 reflects the range after minimum required dilution (100-fold).

The following is a summary of the analytical methods used in Study 5002121 Amendment 2, which was included in the original mRNA-1273 dossier.

The procedure followed during the course of the biodistribution study, along with the assay acceptance criteria, was detailed in a bioanalytical protocol. The LLOQs for plasma and tissues was set at 0.05 ng/mL for the gB and UL130 constructs and 0.01 ng/mL for the gH, gL, UL128, and UL131A constructs. Samples were analyzed in duplicate. Details on how biological samples were collected and processed are provided in the study report (Report 5002121 Amendment 2 Section 4.12).

Samples were analyzed for all 6 mRNA constructs (gB, gH, gL, UL128, UL130, and UL131A) present in mRNA-1647. To quantify these multiple constructs in mRNA-1647, a multiplex branched DNA (bDNA) assay was used. This assay is a hybridization-based method that combines multi-analyte profiling beads and bDNA signal amplification to enable the detection and quantitation of multiple RNA targets simultaneously. After preparation, a sample is combined with an array of fluorescent microspheres (capture beads) and probe sets specific for each RNA molecule of interest and allowed to incubate overnight. The capture beads are used as a support to capture RNA molecules, and the probe sets are used to quantify multiple target-specific RNA molecules within a single sample. Signal amplification is mediated by DNA amplification molecules that hybridize to one of the synthetic probes within each RNA-specific probe set. The capture beads are hybridized with pre-amplifier, amplifier, and label probe solutions. The label probes bind to streptavidin-conjugated R-phycoerythrin, and the resulting fluorescence signal associated with individual capture beads is read on a Luminex® flow cytometer. The signal is reported as the median fluorescence intensity and is proportional to the number of target RNA molecules present in the sample.

Samples for the metabolite identification studies were generated either in vitro from hepatocytes (Study NCS-BA-2022-010) or from in vivo samples following a 0.7 mg/kg intravenous dose of SM-102 containing lipid nanoparticles to Sprague-Dawley rats (Study QV-0236-DA-RE) and the treatment of samples are detailed in the study reports. Similar analytical methods were applied to the analysis of the samples from both studies: a reverse phase (C18) LC separation method in tandem with accurate mass and data-dependent acquisition method was used for acquiring the data from the samples mentioned above. Agilent 1290 UPLC coupled with Agilent 6550 QTOF Accurate Mass with reference mass correction enabled. Positive ion spectra were acquired in profile mode. The ESI source and MS and MS/MS parameters were optimized by using SM102 standard. In addition, data independent targeted MS/MS experiments were performed for identification of low abundant metabolites. SM-102 was also quantified from the in vivo samples: A reverse phase (C8) LC separation method was coupled to a QQQ MS (Sciex QTRAP 6500+) for quantitation of SM102 in the samples. SM102 reference standards ranging from (0.2-500 ng/mL) were spiked into the matrices (Plasma, Bile and Urine) to generate a calibration curve for the method. Sample concentrations were calculated using linear regression of the standard curve based on the EIC areas of the unknown samples.

2.6.4.3 ABSORPTION

No absorption studies with mRNA-1273 have been performed.

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Data up to date as of 12 June 2025

2.6.4.4 DISTRIBUTION

2.6.4.4.1. A Non-GLP Biodistribution Study of NPI-Luc mRNA in SM-102/PEG2000-DMG Following Single Intramuscular Injection in Sprague Dawley Rats (Report 2308-582 Amendment 1)

The objectives of this study were to evaluate the PK and biodistribution of NPI-Luc mRNA and SM-102 lipid and the PD and biodistribution of expressed NPI-Luc protein when given by a single IM injection (100 µg/dose) to rats ().

The test article in this study was NPI-Luc mRNA encapsulated in SM-102/PEG2000-DMG-containing LNPs that encodes for NPI-Luc protein.

Methods:

Male and female Sprague Dawley rats were administered a single IM injection of 100 µg of NPI-Luc mRNA encapsulated in SM-102/PEG2000-DMG-containing LNPs in a dose volume of 205 µL (dose concentration of 0.486 mg/mL) on Day 1 (Table 3). Subgroups of 6 rats (3/sex) were subsequently sacrificed predose (0) and 0.167, 1, 4, 10, 24, 72, 120, 168 hours after IM dosing. Serum and tissues were collected and analyzed for NPI-Luc mRNA using a qualified bDNA multiplex method, SM-102 lipid using qualified LC-MS/MS, and NPI-Luc protein using qualified ECL assay (Section 2.6.4.2).

Table 3: A Single Dose IM NPI-Luc mRNA in Sprague Dawley Rats

Group Numbers	Test Article ^a (Method of Administration)	Species/ Strain	Number of Animals	NPI-Luc mRNA			Sample Collection Timepoints (h)
				Dose Level (µg)	Dose Volume ^b (µL)	Dose Concentration (mg/mL)	
1	NA	Rats/	3/sex	NA	NA	NA	0 (predose)
2	NPI-Luc mRNA (single IM injection)	Sprague Dawley	27/sex ^c	100 ^d	205	0.486 ^e	0.167, 1 ^f , 4, 10, 24, 72, 120, 168

Abbreviations: h = hour(s); IM = intramuscular; Luc = luciferase; mRNA = messenger RNA; NA = not applicable; NPI = nascent peptide imaging.

- a. The test article in this study was NPI-Luc mRNA CCI
- b. Fixed dose volume per animal.
- c. 3 rats/sex/timepoint.
- d. Each dose contained 100 µg of mRNA and 0.84 mg of the SM-102 lipid, unless otherwise noted.
- e. The SM-102 lipid dose concentration was 4.1 mg/mL.
- f. N = 12 animals were sacrificed at 1 hour postdose; of these, data for 6 rats was retained but not reported.

Source: Report 2308-582 Amendment 1.

Results:

NPI-Luc mRNA in SM-102/PEG2000-DMG-containing LNPs was clinically tolerated with no clinical observations at the 100 µg/dose tested in this study.

NPI-Luc mRNA

Following a single IM administration of NPI-Luc mRNA **CCI**, systemic exposure of NPI-Luc mRNA appeared to be independent of sex for serum and tissues (axillary lymph node, bone marrow, heart, inguinal lymph node, injection site, left kidney, liver, lung, popliteal lymph node, and spleen). Peak concentrations (T_{max}) were reached between 0.167 (first collection postdose) and 24 hours postdose in tissues with exposures above that of serum (by AUC_{last}) (Table 4). Only 1 sample at 10 hours was quantifiable above the LLOQ in the popliteal lymph node and all samples collected were below the LLOQ for brain, eye, jejunum, pancreas, stomach, testes, thymus, and uterus (Table 4). The Effective $T_{1/2}$ of NPI-Luc mRNA in serum was 2.95 hours and in tissues greater than serum (by AUC_{last}) were injection site, axillary lymph nodes and spleen with average values of 4.88, 24.1 and 45.8 hours, respectively (Table 4). Tissue-to-serum ratios based on C_{max} for NPI-Luc mRNA were ranked from the highest level observed to the lowest level observed and were injection site, spleen, axillary lymph node, inguinal lymph node, liver, lung (female), heart, popliteal lymph node (female), bone marrow, and left kidney (female). Tissue-to-serum ratios based on AUC_{last} for NPI-Luc mRNA were ranked from the highest level observed to the lowest level observed and were spleen, injection site, axillary lymph node, liver, lung (female) and bone marrow.

Table 4: Summary of NPI-Luc mRNA PK Parameters in Serum and Tissues Following a Single IM Dose of 100 µg of NPI-Luc mRNA in Sprague Dawley Rats (Male and Female Combined)

Matrix	T_{max} (h)	Effective $T_{1/2}$ (h)	C_{max} MTX	AUC_{last} MTX
Serum	1	2.95	NA	NA
Axillary lymph node	24	24.1	84.6	686
Bone marrow	10	7.59	0.461	1.32
Brain	NC	NC	NC	NC
Eye	NC	NC	NC	NC
Heart	1	NC	2.54	NC
Inguinal lymph node	120	NC	72.2	NC
Injection site	0.167	4.88	4220	2250
Jejunum	NC	NC	NC	NC
Kidney (left) ^a	1	NA	0.848	NC
Liver	4	2.98	25.2	31.3
Lung ^a	1	3.07	3.18	4.59
Pancreas	NC	NC	NC	NC

Matrix	T _{max} (h)	Effective T _{1/2} (h)	C _{max} MTX	AUC _{last} MTX
Popliteal lymph node	NC	NC	NC	NC
Spleen	24	45.8	277	4800
Stomach	NC	NC	NC	NC
Testis (left)	NC	NC	NC	NC
Thymus	NC	NC	NC	NC
Uterus	NC	NC	NC	NC

Abbreviations: AUC_{last} = area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed; C_{max} = maximum observed concentration; effective T_{1/2} = half-life determination using mean residence time; Luc = luciferase; MTX = matrix ratios (tissue-to-serum); NC = not calculable (insufficient data points above the lower limit of quantification); NPI = nascent peptide imaging; T_{max} = time after dosing at which the maximum concentration was observed. Note: For NPI-Luc mRNA, right kidney, right testis, and ovaries tissue samples were not analyzed due to small tissue sample size.

^a. Results reported for females only. NPI-Luc mRNA concentrations in males were all below the LLOQ (0.125 ng/mL).

Source:

SM-102 Lipid

Following a single IM administration of NPI-Luc mRNA encapsulated in SM-102/PEG2000-DMG-containing LNPs, systemic exposure of SM-102 lipid appeared to be independent of sex for plasma and tissues (axillary lymph node, bone marrow femur, brain, heart, inguinal lymph node, injection site, jejunum, left kidney, liver, lung, ovaries, pancreas, popliteal lymph node, right kidney, spleen, stomach, thymus, and uterus). Peak concentrations (T_{max}) were reached between 0.167 (first collection postdose) and 10 hours postdose in tissues with exposures above that of plasma (by AUC_{last}) (Table 5). All samples collected were below the LLOQ for eye, testes, female thymus, and male pancreas. The Effective T_{1/2} of SM-102 in plasma was 8.44 hours and in tissues greater than or equal to plasma (by AUC_{last}) were injection site, axillary, inguinal and popliteal lymph nodes and spleen with average values of 14.4, 60.5, 37.5, 18.9 and 22.8 hours, respectively (Table 5). Tissue-to-plasma ratios based on C_{max} for the SM-102 lipid were ranked from the highest level observed to the lowest level observed following dosing on Day 1 and were popliteal lymph nodes, injection site, inguinal lymph node, spleen, axillary lymph node, liver, bone marrow, lung, ovaries (female), jejunum, right kidney, heart, left kidney, uterus (female), pancreas (female), stomach, thymus (male), and brain. Tissue-to-plasma ratios based on AUC_{last} for SM-102 lipid were ranked from highest level observed to the lowest level observed following dosing on Day 1 and were injection site, inguinal lymph node, popliteal lymph node, axillary lymph node, spleen, liver, bone marrow, ovaries (female), lung, jejunum, right kidney, and left kidney.

Table 5: Summary of SM-102 Lipid PK Parameters in Serum and Tissues Following a Single IM Dose of 100 µg of NPI-Luc mRNA (0.84 mg of SM-102 Lipid) in Sprague Dawley Rats (Male and Female Combined)

Matrix	T _{max} (h)	Effective T _{1/2} (h)	C _{max} MTX	AUC _{last} MTX
Plasma	0.167	8.44	NA	NA
Axillary lymph node	4	60.5	25.7	195
Bone marrow (femur)	1	7.94	10.1	8.44
Brain ^a	1	NC	0.265	NC
Eye	NC	NC	NC	NC
Heart ^b	1	NC	1.22	NC
Inguinal lymph node	10	37.5	478	1190
Injection site	24	14.4	588	2170
Jejunum	4	10.9	2.03	2.33
Kidney (left)	4	1.73	0.425	0.115
Kidney (right)	4	1.85	0.847	0.208
Liver	4	6.33	25.5	31.0
Lung	1	4.80	2.74	2.84
Ovaries (females)	10	27.8	2.72	5.51
Pancreas ^b	10	NC	0.246	NC
Popliteal lymph node	1	18.9	969	1010
Spleen	10	22.8	33.5	99.6
Stomach	1	NC	0.210	NC
Testis (right) (males)	NC	NC	NC	NC
Thymus ^a	0.167	NC	0.163	NC
Uterus (females)	0.167	NC	0.331	NC

Abbreviations: AUC_{last} = area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed; C_{max} = maximum observed concentration; effective T_{1/2} = half-life determination using mean residence time; Luc = luciferase; MTX = matrix ratios (tissue-to-serum); NA = not applicable; NC = not calculable (insufficient data points above the lower limit of quantification); NPI = nascent peptide imaging; T_{max} = time after dosing at which the maximum concentration was observed.

a. Results reported for males only. SM-102 concentrations in females were all below the LLOQ (0.5 ng/mL).

b. Results reported for females only. SM-102 concentrations in males were all below the LLOQ (0.5 ng/mL).

Source:

Expressed NPI-Luc Proteins

Out of 769 samples, only 5 tissue samples had values for expressed NPI-Luc protein that were quantifiable: 3 samples in liver and 2 samples in the injection site. All other samples were below the LLOQ and no PD parameters could be estimated.

Conclusions:

In conclusion, NPI-Luc mRNA encapsulated in SM-102/PEG2000-DMG was clinically tolerated after a single IM dose in Sprague Dawley rats with no clinical observations at the 100 µg/dose level tested in this study. Across matrices and analytes, exposures were generally independent of sex. Compared with serum/plasma, NPI-Luc mRNA and SM-102 lipid exposures were generally higher in lymph nodes (inguinal, popliteal and/or axillary), the injection site, and spleen, whereas exposures were lower in bone marrow, brain, heart, jejunum, liver, lung, ovary, kidney, stomach, thymus, and uterus. The majority of samples were below the LLOQ for NPI-Luc protein in tissues.

2.6.4.4.2. A Single or Repeat Dose Biodistribution Study of mRNA-1273 by Intramuscular Administration in Sprague Dawley Rats (Report 20456513 Amendment 1)

The objectives of this study were to determine the PK and biodistribution of mRNA from mRNA-1273 and SM-102 lipid and PD and biodistribution of SARS-CoV-2 S protein following IM injections on Day 1 and Day 28 to Sprague Dawley rats ([Report 20456513 Amendment 1](#)). Additionally, the immunogenic response was assessed as determined by serum antibodies levels.

The test article in this study was mRNA-1273, an LNP-encapsulated mRNA-based vaccine against SARS-CoV-2 S protein.

Methods:

Male and female Sprague Dawley rats were administered an IM injection of 78 µg of mRNA-1273 on Day 1 for groups 1-10 (38 male and 38 female rats) and on Day 1 and Day 28 for groups 11-19 (38 male and 38 female rats) ([Table 6](#)). Rats administered a single IM dose of mRNA-1273 on Day 1 (8 rats [4/sex]) were sacrificed predose (0), 0.16, 1, 4, 10, 24, 72, 120, 168, and 336 hours after Dose 1. Rats administered 2 doses of mRNA-1273 on Day 1 and 28 (8 rats [4/sex]) were sacrificed predose (0), 0.16, 1, 4, 10, 24, 72, 120, 168, and 336 hours after Dose 2. Serum and tissues were collected and analyzed for mRNA from mRNA-1273 using a qualified RT-qPCR method, SM-102 lipid concentration was quantified using LC-MS/MS, and SARS-CoV-2 S protein expression was determined using a ligand-binding assay ([Section 2.6.4.2](#)). IgG antibodies against SARS-CoV-2 S protein after a single and repeat administration of mRNA-1273 were evaluated predose Day 1 (0 hours), 336 hours after the Dose-1, predose Day 28 (0 hours), and 336 hours after Dose 2.

Table 6: mRNA-1273 Study Design in Sprague Dawley Rats

Group Numbers	Test Article (Method of Administration)	Species / Strain	Number of Animals	Dose Level (µg)	Dose Volume (µL)	Dose Concentration (µg/mL)	Dosing Schedule	Sample Collection Timepoints (h)
1-10	mRNA-1273 (IM injection)	Rats/ Sprague Dawley	38/sex ^a	78 ^b	200 ^c	390	Day 1	0 (predose) After Dose 1: 0.16, 1, 4, 10, 24, 72, 120, 168, and 336
11-19			38/sex ^a				Day 1 and Day 28	0 (predose) After Dose 2: 0.16, 1, 4, 10, 24, 72, 120, 168, and 336

Abbreviations: h = hour; IM = intramuscular; mRNA = messenger RNA

a. 3 rats/sex/timepoint.

b. Each dose contains 78 µg of mRNA-1273 and 0.98 mg of SM-102 lipid (dose concentration of 4.9 mg/mL), unless otherwise specified.

c. Fixed dose volume per animal.

Source:

Results:

mRNA-1273 was clinically tolerated after a single or two dose administration(s) of 78 µg/dose with minimal mRNA-1273-related clinical observations (including fur erect, hindlimb swelling and decreased activity) likely related to the IM injection that were observed within 24 hours postdose and resolved within a few days of the respective dose.

mRNA from mRNA-1273

Following a single IM administration of 78 µg/dose of mRNA-1273, systemic exposure to mRNA from mRNA-1273 generally appeared to be sex dependent with females having a higher exposure for serum and tissues than males (Table 8); therefore data below are discussed in terms of males and females separately. Exposure in brain, heart, and lung was lower than exposure in serum; exposure in liver was generally similar to exposure in serum and lymph nodes (inguinal/popliteal and axillary); and exposures in the injection site and spleen were generally greater than exposure in serum for males and females. Exposures and kinetics of mRNA from mRNA-1273 were generally similar between Day 1 and Day 28.

In female animals postdose Day 1, the peak concentration of mRNA from mRNA-1273 (T_{max}) was reached at 4 hours postdose in serum (Table 8). Peak concentrations (T_{max}) were reached between 0.16 and 72 hours postdose in tissues with exposures above that of serum (by AUC_{tlast}). The effective $T_{1/2}$ of mRNA from mRNA-1273 in serum was 2.90 hours. In tissues with exposures greater than serum by AUC_{tlast} (ie, spleen, injection site, inguinal/popliteal lymph nodes, axillary lymph nodes, and liver), effective $T_{1/2}$ values were 37.7, 14.8, 62.3, 45.5, and 8.68 hours, respectively (Table 8).

In female animals postdose Day 28, the peak concentrations of mRNA from mRNA-1273 (T_{max}) was reached at 1 hour postdose in serum (Table 8). Peak concentrations (T_{max}) were reached between 1 and 24 hours postdose in tissues with exposures above that of serum (by AUC_{tlast}).

The effective $T_{1/2}$ of mRNA from mRNA-1273 in serum was 3.89 hours. In tissues with exposures greater than serum by AUC_{tlast} (ie, spleen, axillary lymph nodes, inguinal/popliteal lymph nodes, injection site, and liver), effective $T_{1/2}$ values were 51.4, 41.5, 52.2, 9.36, and 7.48 hours, respectively (Table 8).

In male animals postdose Day 1, the peak concentrations of mRNA from mRNA-1273 (T_{max}) was reached at 1 hour postdose in serum (Table 8). Peak concentrations (T_{max}) were reached between 0.183 and 24 hours postdose in tissues with exposures above that of serum (by AUC_{tlast}). The effective $T_{1/2}$ of mRNA from mRNA-1273 in serum was 1.88 hours. In tissues with exposures greater than serum by AUC_{tlast} (ie, spleen, inguinal/popliteal lymph nodes, injection site, axillary lymph nodes, and liver), effective $T_{1/2}$ values were 62.1, 23.3, 15.5, 50.4, and 5.26 hours, respectively (Table 8).

In male animals postdose Day 28, the peak concentrations of mRNA from mRNA-1273 (T_{max}) was reached at 0.133 hours postdose (Table 8). Peak concentrations (T_{max}) were reached between 0.133 and 10 hours postdose in tissues with exposures above that of serum (by AUC_{tlast}). The effective $T_{1/2}$ of mRNA from mRNA-1273 in serum was 1.77 hours. In tissues with exposures greater than serum by AUC_{tlast} (ie, spleen, injection site, inguinal/popliteal lymph nodes, axillary lymph nodes, and liver), effective $T_{1/2}$ values were 64.4, 10.8, 40.6, 33.4, and 5.61 hours, respectively (Table 8).

Tissue-to-serum ratios based on C_{max} and AUC_{tlast} for mRNA-1273 were ranked from the highest level observed to the lowest level observed on Days 1 and 28 and are presented in Table 7.

Table 7: mRNA from mRNA-1273 Tissue-to-Serum Ratios Ranked From High to Low Based on C_{max} and AUC_{tlast}

mRNA from mRNA-1273:	Day	MTX Rank Order	
		F	M
C_{max} MTX	1	spleen > injection site > inguinal/popliteal lymph nodes > axillary lymph nodes > lung > liver > heart > brain	inguinal/popliteal lymph nodes > spleen > lung > injection site > axillary lymph nodes > liver > heart > brain
	28	spleen > injection site > axillary lymph nodes > inguinal/popliteal lymph nodes > liver > heart > lung > brain	injection site > spleen > axillary lymph nodes > inguinal/popliteal lymph nodes > liver > lung > heart > brain
AUC_{tlast} MTX	1	spleen > injection site > inguinal/popliteal lymph nodes > axillary lymph nodes > liver > lung > heart > brain	spleen > inguinal/popliteal lymph nodes > injection site > axillary lymph nodes > lung > liver > heart > brain (AUC not reportable)
	28	spleen > axillary lymph nodes > inguinal/popliteal lymph nodes > injection site > liver > heart > lung > brain (AUC not reportable)	spleen > injection site > inguinal/popliteal lymph nodes > axillary lymph nodes > liver > lung > heart > brain (AUC not reportable)

Abbreviations: AUC_{tlast} = area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed; C_{max} = maximum observed concentration; mRNA = messenger RNA; MTX = matrix ratios (tissue-to-serum).

Source:

Table 8: Summary of mRNA PK Parameters in Serum and Tissues Following IM Administration of 78 µg of mRNA-1273 on Day 1 and Day 28 in Male and Female Sprague Dawley Rats

Matrix	Day	T _{max} (h)		Effective T _{1/2} (h)		C _{max} MTX		AUC _{tlast} MTX	
		F	M	F	M	F	M	F	M
Serum	1	4	1	2.90	1.88	NA	NA	NA	NA
	28	1	0.133	3.89	1.77	NA	NA	NA	NA
Liver	1	4	1	8.68	5.26	39.0	14.5	69.3	47.2
	28	1	1	7.48	5.61	39.8	13.4	67.7	90.7
Brain	1	4	1	1.68	NC	0.183	0.151	0.0773	NC
	28	1	1	NC	NC	0.0647	0.129	NC	NC
Heart	1	4	0.183	2.84	2.40	8.51	6.79	8.14	5.97
	28	1	4	2.49	2.51	21.2	2.78	18.0	7.73
Lung	1	0.16	0.183	5.66	0.905	51.1	342	16.6	62.6
	28	1	4	7.95	7.28	8.72	4.58	15.0	24.4
Lymph nodes (inguinal/popliteal)	1	72	1	62.3	23.3	124	1570	1550	5760
	28	24	10	52.2	40.6	113	382	1540	8920
Lymph node (axillary)	1	10	4	45.5	50.4	107	151	918	1710
	28	24	10	41.5	33.4	180	576	1560	4260
Injection site	1	24	24	14.8	15.5	435	259	2210	2410
	28	4	0.133	9.36	10.8	436	1830	514	9470
Spleen	1	10	10	37.7	62.1	728	679	4600	12000
	28	10	4	51.4	64.4	1130	908	6120	30900

Abbreviations: AUC_{tlast} = area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed; C_{max} = maximum observed concentration; effective T_{1/2} = half-life determination using mean residence time; MTX = matrix ratios (tissue-to-serum); NA = not applicable; NC = not calculable (insufficient data points above the lower limit of quantification); T_{max} = time after dosing at which the maximum concentration was observed.

Source: [Report 20456513 Amendment 1](#).

SM-102 Lipid

Following a single IM administration of 78 µg/dose of mRNA-1273 (containing 0.98 mg/dose of SM-102 lipid), systemic exposure to SM-102 generally appeared to be sex dependent with females having higher exposure for plasma and tissues than males ([Table 10](#)). Exposure in brain, heart, liver, and lung was generally lower than exposure in plasma, while exposure in lymph nodes (inguinal/popliteal and axillary), the injection site, and spleen was generally greater than exposure in plasma for males and females. Exposures and kinetics of SM-102 lipid were generally similar between Day 1 and Day 28.

In female rats postdose Day 1, the peak concentration of SM-102 (T_{max}) was reached at 4 hours postdose in plasma. Peak concentrations of SM-102 (T_{max}) were between 4 and 24 hours postdose in tissues with exposures above that of plasma (by AUC_{tlast}). The effective $T_{1/2}$ of SM-102 in plasma was 4.83 hours. In tissues greater than plasma by AUC_{tlast} (ie, injection site, axillary lymph nodes, pooled inguinal/popliteal lymph nodes, and spleen), effective $T_{1/2}$ values were 16.2, 34.2, 43.7, and 24.4 hours, respectively (Table 10).

In female rats postdose Day 28, the peak concentration of SM-102 (T_{max}) was reached at 1 hour postdose in plasma. Peak concentrations of SM-102 (T_{max}) were between 4 and 24 hours postdose in tissues with exposures above that of plasma (by AUC_{tlast}). The effective $T_{1/2}$ of SM-102 in plasma was 4.88 hours. In tissues greater than plasma by AUC_{tlast} (ie, injection site, axillary lymph nodes, pooled inguinal/popliteal lymph nodes, and spleen) effective $T_{1/2}$ values were 15, 23.4, 32.2, and 15.6 hours, respectively (Table 10).

In male rats postdose Day 1, the peak concentration of SM-102 (T_{max}) was reached at 1 hour postdose in plasma. Peak concentrations of SM-102 (T_{max}) were between 4 and 24 hours postdose in tissues with exposures above that of plasma (by AUC_{tlast}). The effective $T_{1/2}$ of SM-102 in plasma was 7.02 hours. In tissues greater than plasma by AUC_{tlast} (ie, injection site, axillary lymph nodes, pooled inguinal/popliteal lymph nodes, and spleen), effective $T_{1/2}$ values were 15.5, 11.7, 27.1, and 30.0 hours, respectively (Table 10).

In male rats postdose Day 28, the peak concentration of SM-102 (T_{max}) was reached at 0.133 hours postdose in plasma. Peak concentrations of SM-102 (T_{max}) were between 4 and 24 hours postdose in tissues with exposures above that of plasma (by AUC_{tlast}). The effective $T_{1/2}$ of SM-102 in plasma was 6.34 hours. In tissues greater than plasma by AUC_{tlast} (ie, injection site, axillary lymph nodes, pooled inguinal/popliteal lymph nodes, and spleen) effective $T_{1/2}$ values were 16.2, 31.0, 14.6, and 39.5 hours, respectively (Table 10).

Tissue-to-serum ratios based on C_{max} and AUC_{tlast} for SM-102 lipid were ranked from the highest level observed to the lowest level observed on Days 1 and 28 and are presented in Table 8.

Table 9: SM-102 Lipid Tissue-to-Serum Ratios Ranked from High to Low Based on C_{max} and AUC_{tlast}

SM-102 Lipid:	Day	MTX Rank Order	
		F	M
C_{max} MTX	1	injection site > inguinal/popliteal lymph nodes > spleen > axillary lymph nodes > liver > lung > heart > brain	axillary lymph nodes > injection site > inguinal/popliteal lymph nodes > spleen > liver > lung > heart > brain (all tissue concentrations were not quantifiable)
	28	injection site > spleen > inguinal/popliteal lymph nodes > axillary lymph nodes > liver > lung > heart > brain (all tissue concentrations were not quantifiable)	injection site > inguinal/popliteal lymph nodes > axillary lymph nodes > spleen > liver > lung > heart > brain (all tissue concentrations were not quantifiable)
AUC_{tlast} MTX	1	injection site > inguinal/popliteal lymph nodes > spleen > axillary lymph nodes > liver > lung > heart > brain (AUC not reportable)	inguinal/popliteal lymph nodes > axillary lymph nodes > injection site > spleen > liver > lung > heart > brain (AUC not reportable)

SM-102 Lipid:	Day	MTX Rank Order	
		F	M
	28	injection site > inguinal/popliteal lymph nodes > axillary lymph nodes > spleen > liver > lung > heart > brain (AUC not reportable)	injection site > axillary lymph nodes > inguinal/popliteal lymph nodes > spleen > liver > lung > heart and brain (AUC not reportable)

Abbreviations: AUC_{last} = area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed; C_{max} = maximum observed concentration; MTX = matrix ratios (tissue-to-serum); SM-102 = ionizable lipid.

Source:

Table 10: Summary of SM-102 Lipid PK Parameters in Serum and Tissues Following IM Administration of 78 µg of mRNA-1273 (0.98 mg of SM-102 Lipid) on Day 1 and Day 28 in Male and Female Sprague Dawley Rats

Matrix	Day	T _{max} (h)		Effective T _{1/2} (h)		C _{max} MTX		AUC _{last} MTX	
		F	M	F	M	F	M	F	M
Plasma	1	4	1	4.83	7.02	NA	NA	NA	NA
	28	1	0.133	4.88	6.34	NA	NA	NA	NA
Liver	1	1	4	6.76	5.43	3.98	14.9	9.22	19.2
	28	10	4	9.13	5.51	6.62	12.7	25.6	30.6
Brain	1	4	NC	NC	NC	0.0651	NC	NC	NC
	28	NC	NC	NC	NC	NC	NC	NC	NC
Heart	1	1	0.183	1.21	0.990	0.381	0.648	0.132	0.132
	28	1	0.133	1.23	NC	0.537	0.0518	0.243	NA
Lung	1	4	4	5.30	4.98	2.66	1.47	2.30	1.93
	28	1	0.133	4.12	5.57	0.974	0.836	1.72	1.15
Lymph nodes (inguinal/popliteal)	1	4	24	43.7	27.1	105	285	448	1970
	28	24	10	32.2	14.6	72.6	153	613	540
Lymph node (axillary)	1	10	10	34.2	11.7	29.5	609	122	1100
	28	24	24	23.4	31.0	63.6	150	426	1190
Injection site	1	10	4	16.2	15.5	198	325	1140	577
	28	4	24	15.0	16.2	159	796	902	6000
Spleen	1	24	10	24.4	30.0	78.0	51.0	432	270
	28	10	4	15.6	39.5	88.4	50.2	246	223

Abbreviations: AUC_{last} = area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed; C_{max} = maximum observed concentration; effective T_{1/2} = half-life determination using mean residence time; MTX = matrix ratios

(tissue-to-plasma); NA = not applicable; NC = not calculable (insufficient data points above the lower limit of quantification); T_{max} = time after dosing at which the maximum concentration was observed.

Source:

Expressed SARS-CoV-2 S Protein

Following a single IM administration of 78 µg/dose mRNA-1273, systemic exposure to SARS-CoV-2 S protein generally appeared to be sex dependent for serum and tissues with females having higher exposure for serum and tissues compared with males (Table 12); therefore, data below are discussed in terms of males and females separately. Where quantifiable, exposure in tissues was generally greater than exposure in serum for males and females. Exposures and kinetics of SARS-CoV-2 S protein, where quantifiable, were generally similar between Day 1 and Day 28.

Quantifiable SARS-CoV-2 S protein was observed in injection sites, liver, lymph nodes axillary, inguinal/popliteal lymph nodes pooled and spleen postdose with exposures above that of serum. Some PD parameters were not able to be estimated due to insufficient number of quantifiable concentration-time data values.

Tissue-to-serum ratios based on C_{max} and AUC_{tlast} for SM-102 lipid were ranked from the highest level observed to the lowest level observed on Days 1 and 28 and are presented in Table 11.

Table 11: SARS-CoV-2 S Protein Tissue-to-Serum Ratios Ranked from High to Low based on C_{max} and AUC_{tlast} .

SARS-CoV-2 S Protein:	Day	MTX Rank Order	
		F	M
E_{max} MTX	1	injection site > inguinal/popliteal lymph nodes > spleen > liver > axillary lymph nodes > all other tissues were not quantifiable	inguinal/popliteal lymph nodes > axillary lymph nodes > injection site > all other tissues were not quantifiable
	28	spleen > injection site > liver > all other tissues were not quantifiable	all tissues were not quantifiable
$AUEC_{tlast}$ MTX	1	injection site > inguinal/popliteal lymph nodes > spleen > axillary lymph nodes > liver > all other tissue AUC values were not reportable	axillary lymph nodes > inguinal/popliteal lymph nodes > injection site > all other tissue AUC values were not reportable
	28	spleen > liver > injection site > all other tissue AUC values were not reportable	all tissue AUC values were not reportable

Abbreviations: $AUEC_{tlast}$ = area under the response curve versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed;

E_{max} = maximum observed effect; MTX = matrix ratios (tissue-to-serum) SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Source:

1.

Table 12: Summary of Expressed SARS-CoV-2 S Protein PK Parameters in Serum and Tissues Following IM Administration of 78 µg of mRNA-1273 on Day 1 and Day 28 in Male and Female Sprague Dawley Rats

Matrix	Day	TE _{max} (h)		Effective T _{1/2} (h)		E _{max} MTX		AUEC _{tlast} MTX	
		F	M	F	M	F	M	F	M
Serum	1	24	24	18.1	NC	NA	NA	NA	NA
	28	168	NC	NC	NC	NA	NA	NA	NA
Liver	1	10	NC	8.79	NC	1120	NC	411	NC
	28	24	NC	NA	NC	833	NC	NC	NC
Brain	1	NC	NC	NC	NC	NC	NC	NC	NC
	28	NC	NC	NC	NC	NC	NC	NC	NC
Heart	1	NC	NC	NC	NC	NC	NC	NC	NC
	28	NC	NC	NC	NC	NC	NC	NC	NC
Lung	1	NC	NC	NC	NC	NC	NC	NC	NC
	28	NC	10	NC	NC	NC	NC	NC	NC
Lymph nodes (inguinal/popiteal)	1	24	4	NC	NC	2810	31100	NC	NC
	28	NC	NC	NC	NC	NC	NC	NC	NC
Lymph node (axillary)	1	72	10	NC	NC	588	10600	NC	NC
	28	NC	NC	NC	NC	NC	NC	NC	NC
Injection site	1	10	24	15.2	NC	5600	3300	2150	NC
	28	10	NC	NC	NC	1650	NC	NC	NC
Spleen	1	10	NC	10.3	NA	1600	NC	712	NC
	28	10	NC	NC	NC	4560	NC	NC	NC

Abbreviations: AUEC_{tlast} = area under the response curve versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed; E_{max} = maximum observed effect; effective T_{1/2} = half-life determination using mean residence time; MTX = matrix ratios (tissue-to-serum); NA = not applicable; NC = not calculable (insufficient data points above the lower limit of quantification); TE_{max} = time after dosing at which the maximum effect was observed.

Source:

Anti-SARS-CoV-2 S Antibody Response

All animals were positive for anti-SARS-CoV-2 S protein antibodies following a single dose (measured 336 hours post-first dose administered on Day 1) with titers ranging from 18,020 to 59,578. Antibodies were sustained and present prior to the second dose on Day 28 with titers ranging from 11,027 to 27,552. There was a further increase in antibody titers following the second dose (measured 336 hours post-second dose administered on Day 28) with titers ranging from 85,007 to 363,244. Over time, titer values for the positive anti-SARS-CoV-2 S protein antibodies increased and was generally associated with lower exposure to SARS-CoV-2 S protein in all matrices on Day 28.

Conclusions:

In conclusion, administration of 78 µg/dose mRNA-1273 by IM bolus injection once or every 4 weeks on Days 1 and/or 28 to Sprague Dawley rats was clinically tolerated. Clinical observations were limited to transient findings of fur erect, hindlimb swelling and decreased activity that were observed within 24 hours postdose and resolved within a few days of the respective dose. There were no differences in exposure of mRNA from mRNA-1273, SM-102 lipid, or SARS-CoV-2 S protein following a single or two dose administration in any plasma or tissue matrix. Across matrices and analytes, exposures were generally sex-dependent, where female exposures were often higher than males. Because the dose level administered was not adjusted for body weight (fixed dose) and male body weights were higher than female body weights, the dose administered per body weight (g) was higher for females than males which likely contributed to the observation of higher exposure. Compared with serum/plasma, mRNA from mRNA-1273 and SM-102 lipid and exposures were generally higher in lymph nodes (inguinal/popliteal and axillary), the injection site, and spleen, whereas exposures were lower in brain, heart, liver, and lung. Expressed SARS-CoV-2 S protein was also generally higher in tissues than in serum, where lymph nodes (inguinal/popliteal), axillary lymph nodes, injection site, spleen and/or liver had highest exposures, and brain, heart, and lung had E_{max} values that were BQL or had unreportable AUEC values. All animals were positive for anti-SARS-CoV-2 S protein antibodies at 336 hours after each dose with increasing titer values after the second dose that were generally associated with lower exposure to SARS-CoV-2 S protein in all matrices.

2.6.4.4.3. Single Dose Intramuscular Injection Tissue Distribution Study of mRNA-1647 in Sprague Dawley Rats (Report 5002121 Amendment 2)

The objective of this non-GLP study was to determine the tissue distribution of mRNA-1647 when given once by IM injection to rats. The PK characteristics of mRNA-1647 were determined in plasma and tissue. A group of 35 male Sprague Dawley rats was given a single IM injection of 100 µg of mRNA-1647 in a dose volume of 200 µL (dose concentration of 0.5 mg/mL) on Day 1. Subgroups of 5 rats each were sacrificed pre-dose and 2, 8, 24, 48, 72, and 120 hours after IM dosing. Blood and tissues were collected and processed for quantitation of the 6 mRNA constructs (gB, gH, gL, UL128, UL130, and UL131A) present in mRNA-1647 using a qualified bDNA multiplex method (Section 2.6.4.2). The overall design of this study is presented in Table 13.

Table 13: A Single-Dose IM Pharmacokinetic and Biodistribution Study of mRNA-1647 in Sprague Dawley Rats

Group Number	Test Article (Method of Administration)	Species/Strain	Number of Animals/Sex	Dose Level (µg)	Dose Volume (µL)	Dose Concentration (mg/mL)	Sample Collection Time Points (h)
XDDDD DDDD.3 2221	mRNA-1647 (single IM injection)	Rats/Sprague Dawley	35/male	100	200	0.5	0 (pre-dose), 2, 8, 24, 48, 72, and 120

Abbreviations: IM = intramuscular.

Source: Report 5002121 Amendment 2 (Text Table 14 and Text Table 15).

No quantifiable concentrations for any of the mRNA constructs were observed in plasma or tissue in pre-dose samples, with the exception of 2 plasma samples for which the gH construct concentration was slightly above the LLOQ. For all 6 mRNA constructs present in mRNA-1647, post-dose levels were detectable in plasma and tissues in a 1:1:1:1:1:1 ratio. Mean plasma concentrations were quantifiable up to 24 hours with an inter-animal coefficient of variation from 21.8% and 79.8%. The only quantifiable plasma samples beyond 24 hours were 6 gH constructs that were slightly above the LLOQ.

After a single IM dose in male rats, the T_{\max} for all 6 mRNA constructs was 2 hours, followed by a rapid elimination phase. Mean concentrations became undetectable for all constructs after 24 hours with the exception of gH, which was detectable up to the last time point of 120 hours. Due to the lack of a distinct elimination phase, the $T_{1/2}$ of the mRNA constructs could not be calculated; however, the $T_{1/2}$ was estimated to range from 2.7 to 3.8 hours. The C_{\max} and $AUC_{(0-t)}$ ranged from 1.60 to 2.30 ng/mL and from 22.7 to 25.5 ng \times h/mL, respectively (Table 14).

Table 14: Plasma Pharmacokinetic Parameters for a Single IM Dose of 100 μ g of mRNA-1647 in Male Sprague-Dawley Rats

Matrix	Construct	T_{\max} (h) ^a	C_{\max} (ng/mL) ^a	(ng \times h/mL) ^a	$T_{1/2}$ (h) ^b
Plasma	gB	2.0	2.02 \pm 0.181	22.7 \pm 3.77	NC
	gH	2.0	1.91 \pm 0.187	24.9 \pm 4.49	NC
	gL	2.0	1.74 \pm 0.177	23.4 \pm 4.07	NC
	UL128	2.0	1.66 \pm 0.151	24.1 \pm 4.44	NC
	UL130	2.0	2.30 \pm 0.621	25.5 \pm 4.65	NC
	UL131A	2.0	1.60 \pm 0.153	24.8 \pm 4.59	NC

Abbreviations: gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; IM = intramuscular; NC = not calculable (insufficient data points above the lower limit of quantification).

^a T_{\max} data reported as the mean; C_{\max} and $AUC_{(0-t)}$ data reported as the mean \pm standard error.

^b Due to the lack of a distinct elimination phase, the $T_{1/2}$ of the mRNA constructs could not be calculated; however, the $T_{1/2}$ was estimated to range from 2.7 to 3.8 hours.

Source: Report 5002121 Amendment 2 (Appendix 8, Table 2).

All constructs of mRNA-1647 were quantifiable in most tissues analyzed, except for the kidney, where all levels were below the LLOQ. For highly exposed tissues (injection site [muscle], lymph nodes [proximal and distal], and spleen), the C_{\max} was observed between 2- and 24-hours post-dose. The $T_{1/2}$ was calculated using the average tissue $T_{1/2}$ values for all 6 constructs. The results were 14.9 hours injection site (muscle), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

As observed with other IM delivered vaccines, the highest mRNA concentrations were observed at the injection site (muscle) followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0 .

The persistence of mRNA in tissues was evaluated in Sprague Dawley rats and indicated that for the majority of tissues, administered mRNA was not detectable after 1-3 days, the only

exceptions were at the site of injection (muscle) and within the draining lymph nodes and spleen where the mRNA had a calculated half-life of 14.9-63.0 hrs ([Table 15](#)).

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Table 15: Tissue Pharmacokinetic Parameters for a Single IM Dose of 100 µg of mRNA-1647 in Male Sprague-Dawley Rats

Matrix	Construct	T _{max} (h) ^a	C _{max} (ng/mL) ^a	AUC _(0-t) (ng × h/mL) ^{a,b}	T _{1/2} (h) ^a	AUC _(0-t) Ratio (Tissue/Plasma) ^c	AUC _(0-t) Ratio (Tissue/Plasma) Average
Bone marrow	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.254 ± 0.0871	7.85 ± 2.03	NC	0.316	
	gL	8.0	0.224 ± 0.0920	2.78 ± 1.03	NC	0.119	
	UL128	8.0	0.292 ± 0.120	3.53 ± 1.33	NC	0.147	
	UL130	NC	NC	NC	NC	NC	
	UL131A	8.0	0.186 ± 0.0829	2.05 ± 0.912	NC	0.0825	
Brain	gB	NC	NC	NC	NC	NC	NR
	gH	24.0	0.0800 ± 0.0491	2.19 ± 1.08	NC	0.0880	
	gL	2.0	0.0360 ± 0.0360	0.144 ± 0.144	NC	0.00615	
	UL128	2.0	0.0340 ± 0.0340	0.136 ± 0.136	NC	0.00564	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Distal lymph node	gB	8.0	108 ± 101	1,460 ± 1,110	31.6	64.1	62.8
	gH	8.0	110 ± 102	1,490 ± 1,130	36.2	59.8	
	gL	8.0	117 ± 109	1,460 ± 1,200	30.6	62.6	
	UL128	8.0	125 ± 117	1,620 ± 1,290	32.1	67.1	
	UL130	8.0	129 ± 121	1,630 ± 1,330	27.9	64	
	UL131A	8.0	114 ± 108	1,470 ± 1,190	28.5	59.2	
Eye	gB	2.0	4.72 ± 2.77	26.7 ± 13.6	NC	1.18	1.24
	gH	2.0	3.92 ± 2.19	37.6 ± 11.0	NC	1.51	
	gL	2.0	3.23 ± 1.84	29.2 ± 9.75	NC	1.25	

Matrix	Construct	T _{max} (h) ^a	C _{max} (ng/mL) ^a	AUC _(0-t) (ng × h/mL) ^{a,b}	T _{1/2} (h) ^a	AUC _(0-t) Ratio (Tissue/Plasma) ^c	AUC _(0-t) Ratio (Tissue/Plasma) Average
	UL128	2.0	3.91 ± 2.19	34.5 ± 12.2	NC	1.43	
	UL130	2.0	3.61 ± 2.14	21.3 ± 11.0	NC	0.838	
	UL131A	2.0	3.43 ± 1.96	31.1 ± 10.2	NC	1.26	
Heart	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.548 ± 0.107	9.94 ± 1.85	NC	0.400	
	gL	8.0	0.220 ± 0.0907	2.96 ± 1.05	NC	0.127	
	UL128	8.0	0.276 ± 0.113	4.49 ± 1.51	NC	0.186	
	UL130	NC	NC	NC	NC	NC	
	UL131A	8.0	0.312 ± 0.0896	3.71 ± 1.02	NC	0.150	
Injection site muscle	gB	2.0	1,770 ± 803	27,100 ± 4,880	13.5	1190	1010
	gH	2.0	1,720 ± 828	26,100 ± 4,700	17.1	1050	
	gL	2.0	1,310 ± 638	20,900 ± 3,720	15.2	893	
	UL128	2.0	1,620 ± 720	25,300 ± 4,090	14.9	1050	
	UL130	2.0	1,630 ± 777	24,500 ± 4,240	13.8	961	
	UL131A	2.0	1490 ± 729	23000 ± 4000	15.0	927	
Jejunum	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.0800 ± 0.0490	2.06 ± 1.04	NC	0.0827	
	gL	2.0	0.0700 ± 0.0429	0.720 ± 0.472	NC	0.0308	
	UL128	NC	NC	NC	NC	NC	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Kidney	gB	NC	NC	NC	NC	NC	NR
	gH	NC	NC	NC	NC	NC	

Matrix	Construct	T _{max} (h) ^a	C _{max} (ng/mL) ^a	AUC _(0-t) (ng × h/mL) ^{a,b}	T _{1/2} (h) ^a	AUC _(0-t) Ratio (Tissue/Plasma) ^c	AUC _(0-t) Ratio (Tissue/Plasma) Average
	gL	NC	NC	NC	NC	NC	
	UL128	NC	NC	NC	NC	NC	
	UL130	NC	NC	NC	NC	NC	
	UL131A	NC	NC	NC	NC	NC	
Liver	gB	2.0	2.16 ± 1.21	8.65 ± 4.83	NC	0.381	0.49 9
	gH	2.0	2.12 ± 0.982	16.8 ± 4.15	NC	0.674	
	gL	2.0	1.30 ± 0.432	11.0 ± 2.37	NC	0.470	
	UL128	2.0	2.00 ± 0.814	13.7 ± 3.72	NC	0.570	
	UL130	2.0	1.87 ± 1.01	7.46 ± 4.04	NC	0.293	
	UL131A	2.0	1.99 ± 0.928	13.9 ± 4.04	NC	0.562	
Lung	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.442 ± 0.130	8.04 ± 1.96	NC	0.323	
	gL	8.0	0.274 ± 0.0984	3.45 ± 1.12	NC	0.148	
	UL128	8.0	0.340 ± 0.129	5.40 ± 1.74	NC	0.224	
	UL130	8.0	0.188 ± 0.188	2.07 ± 2.07	NC	0.0812	
	UL131A	8.0	0.310 ± 0.111	4.86 ± 1.49	NC	0.196	
Proximal lymph nodes	gB	2.0	260 ± 121	5,850 ± 949	33.5	257	201
	gH	8.0	206 ± 51.6	4,860 ± 722	38.2	195	
	gL	2.0	175 ± 81.9	3,460 ± 538	36.3	148	
	UL128	8.0	246 ± 66.6	5,190 ± 875	32.8	215	
	UL130	8.0	252 ± 67.2	5,240 ± 881	35.7	206	
	UL131A	2.0	225 ± 106	4,600 ± 719	32.2	185	
Spleen	gB	2.0	7.36 ± 3.81	460 ± 52.9	46.9	20.2	13.4
	gH	24.0	5.63 ± 1.28	371 ± 39.5	83.0	14.9	

Matrix	Construct	T _{max} (h) ^a	C _{max} (ng/mL) ^a	AUC _(0-t) (ng × h/mL) ^{a,b}	T _{1/2} (h) ^a	AUC _(0-t) Ratio (Tissue/Plasma) ^c	AUC _(0-t) Ratio (Tissue/Plasma) Average
	gL	8.0	3.83 ± 1.04	196 ± 21.0	68.2	8.36	
	UL128	24.0	4.87 ± 1.22	297 ± 34.8	68.8	12.3	
	UL130	8.0	5.03 ± 1.41	288 ± 33.0	64.9	11.3	
	UL131A	2.0	5.10 ± 2.64	277 ± 33.1	46.2	11.2	
Stomach	gB	NC	NC	NC	NC	NC	NR
	gH	8.0	0.110 ± 0.0696	3.49 ± 1.59	NC	0.140	
	gL	8.0	0.0800 ± 0.0499	2.07 ± 1.19	NC	0.0886	
	UL128	24.0	0.102 ± 0.0648	2.85 ± 1.47	NC	0.118	
	UL130	NC	NC	NC	NC	NC	
	UL131A	24.0	0.0980 ± 0.0634	2.53 ± 1.39	NC	0.102	
Testes	gB	2.0	1.16 ± 0.719	4.64 ± 2.88	NC	0.204	0.20 9
	gH	2.0	1.11 ± 0.480	5.52 ± 2.20	NC	0.222	
	gL	8.0	0.420 ± 0.335	6.08 ± 3.73	NC	0.260	
	UL128	2.0	0.946 ± 0.397	4.73 ± 1.85	NC	0.196	
	UL130	2.0	0.682 ± 0.442	2.73 ± 1.77	NC	0.107	
	UL131A	2.0	0.872 ± 0.380	4.54 ± 1.85	NC	0.183	

Abbreviations: gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; IM = intramuscular; NC = not calculable (insufficient data points above the lower limit of quantitation); NR = not reported (some constructs measured all samples as below limit of quantitation).

^a T_{max} and T_{1/2} data reported as the mean; C_{max} and AUC data reported as the mean ± standard error.

^b For the bone marrow, brain, jejunum, heart, liver, lung, stomach, and testes, AUC was calculated using less than 3 quantifiable mean concentrations and therefore is an estimate.

^c For AUC Ratio, samples listed as NC were not calculable because all samples were below limit of quantitation. Source: Report 5002121 Amendment 2 (Appendix 8, T 2 and 9)

2.6.4.5 METABOLISM

No metabolism studies with mRNA-1273 or mRNA-1273 variant vaccines have been performed. However, the metabolism and elimination of the novel amino-lipid component in mRNA-1273, SM-102, have been examined in vivo (Study QV-0236-DA-RE) and in vitro (Study NCS-BA-2022-010).

SM-102 is metabolized primarily by hydrolysis of the ester groups followed by β -oxidation of the resulting aliphatic acidic linkers in vivo in Sprague-Dawley rats after IV administration of SM-102/DMG-containing LNPs (0.7 mg/kg of encapsulated mRNA). In vitro incubation of the same LNPs with rat, non-human primate (NHP), and human hepatocytes yielded identical ester-hydrolyzed and β -oxidized metabolites with no human-specific metabolites detected (Study NCS-BA-2022-010).

In addition to SM-102, low abundances of eight metabolites appeared in plasma from 2 to 6 hours. These included multiple species of ester-hydrolyzed, β -oxidized and hydroxylated metabolites. By 24 hours post-dose, unchanged SM-102, one mono acidic ester hydrolysis metabolite, one di-acidic ester hydrolysis metabolite, and their corresponding β -oxidation products were detected in plasma as well as a metabolite resulting from N-dealkylation of the straight chain ester linker of SM-102.

2.6.4.6 EXCRETION

No excretion studies with mRNA-1273 or mRNA-1273 variant vaccines have been performed. However, the metabolism and elimination of the novel amino-lipid component in mRNA-1273, SM-102, have been examined in vivo (Study QV-0236-DA-RE) and in vitro (Study NCS-BA-2022-010).

After IV administration **CCI** to Sprague-Dawley rats in Study QV-0236-DA-RE (approximately 2100 ug/animal of SM-102), the predominant species in plasma was intact SM-102 (approximately 22.5 ug/mL) at 2 hours, declining to <10% and <1% of C_{max} by 6 and 24 hours, respectively. Intact SM-102 was detected in bile at 14% and 9% of plasma concentrations at 2- and 6-hours post-dose, respectively, dropping to 1.2% at 24 hours. Intact SM-102 was not detected in urine above the limit of quantitation (0.2 ng/mL) at any time tested (2-24 hours post-dose).

Four metabolites and trace levels of SM-102 appear in the urine including diacids and a monoacidic secondary amine that are relatively more hydrophilic and lower molecular weight compared to those in bile and plasma. Unchanged SM-102 and 12 metabolites were identified in the bile. In addition to those metabolites found in urine and plasma, multiple-step β -oxidation products, a β -oxidation+glutathione conjugation product, and hydroxylated metabolites were identified in bile.

2.6.4.7 PHARMACOKINETIC DRUG INTERACTIONS

No PK drug interaction studies with mRNA-1273 have been performed.

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2.6.4.8 OTHER PHARMACOKINETIC STUDIES

No other PK studies with mRNA-1273 have been performed.

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2.6.4.9 DISCUSSION AND CONCLUSION

Distribution, metabolism, and excretion studies were conducted using mRNA-LNP DPs comprised of the same 4 lipids (ie, SM-102, cholesterol, DSPC, and PEG2000-DMG) and generally similar lipid to mRNA ratios as the mRNA-1273. The biodistribution studies are considered sufficient to support the characterization of mRNA-1273 because the data demonstrate that the outcomes of these studies are driven primarily by the LNP and route of administration, with no apparent impact from mRNA cargo, expressed protein, or immune response on the results. Collectively, the biodistribution studies with NPI-Luc mRNA, mRNA-1273, and mRNA-1647 provide insights into the tissue distribution and kinetics of mRNA-1273, while metabolism and excretion studies demonstrate the extensive clearance of SM-102. The in vivo distribution, persistence, and clearance of the mRNAs, SM-102 lipid, and expressed proteins from NPI-Luc mRNA, mRNA-1273, and/or mRNA-1647 are considered to be representative of mRNA-1273 given that the LNP composition are the same.

Collectively, data derived from the 3 biodistribution studies provide evidence that LNP composition and route of administration drive tissue distribution. Specifically, the general similarities in exposure tissue rank order and kinetics across DPs demonstrate that mRNA cargo and presence or absence of an immune response do not alter tissue distribution of mRNA-LNP DPs. Furthermore, tissue distribution is similar following single or two dose administration(s) of mRNA-1273, demonstrating that there is low to no risk of accumulation and no differences in distribution of DP components with repeat dosing.

The PK of NPI-Luc mRNA and SM-102 lipid and the PD of expressed NPI-Luc protein were evaluated following a single IM injection of 100 µg NPI-Luc mRNA encapsulated in SM-102/PEG2000-DMG-containing LNPs in male and female Sprague Dawley rats (). The systemic exposure of NPI-Luc mRNA and SM-102 appeared to be independent of sex for serum and tissues. Tissues with the highest NPI Luc mRNA and SM-102 exposures were generally similar for both analytes with highest exposures in the injection site, axillary lymph nodes, and spleen; and all of these had exposures greater than serum or plasma. Effective $T_{1/2}$ of systemic mRNA and SM-102 was 2.95 (serum) and 8.44 (plasma) hours. Across both analytes, the effective $T_{1/2}$ in tissues with higher exposures than systemic (based on AUC in serum or plasma) ranged from 2.98 to 60.5 hours. Likely due to the limitations of the analytical method, the expressed NPI-Luc was only quantifiable in 5 of 769 female samples, including 3 in the liver and 2 in the injection site (concentrations ranged from 211 to 339 ng/g).

To evaluate the distribution after a single or two dose administrations of mRNA-1273, the PK of mRNA from mRNA-1273 and SM-102 lipid and the PD of expressed SARS-CoV-2 S protein were evaluated following IM injections of 78 µg/dose mRNA-1273 on Day 1 and Day 28 in male and female Sprague Dawley rats (). There were no differences in exposure for mRNA from mRNA-1273, SM-102 lipid, or SARS-CoV-2 S protein expression when comparing exposures after single or two dose administrations in any plasma or tissue matrix. Across matrices and analytes, exposures were generally sex dependent, where female exposures were often higher than males. Since the dose level administered was not adjusted for body weight (fixed dose) and male body weights were higher than female body weights in this study, the dose administered per body weight (g) was higher for females than males, which likely contributed to the observation of higher exposure in females. Compared with

serum/plasma, mRNA from mRNA-1273 and SM-102 lipid exposures were generally higher in lymph nodes (inguinal/popliteal and axillary), the injection site, and spleen. Effective $T_{1/2}$ of systemic mRNA and SM-102 ranged from 1.77 to 3.89 (serum) and 4.83 to 7.02 hours (plasma). Across both analytes, the effective $T_{1/2}$ in tissues with higher exposures than systemic (based on AUC) ranged from 2.49 to 64.4 hours. Expressed SARS-CoV-2 protein was also generally higher in tissues than in serum, where lymph nodes (inguinal/popliteal), axillary lymph nodes, injection site, spleen and/or liver had highest exposures. Exposures of expressed SARS-CoV-2 protein in brain, heart, and lung had E_{max} values that were below the limit of quantification or had unreportable AUEC values due to limited number of samples with quantifiable values. All animals were positive for anti-SARS-CoV-2 S protein antibodies at 336 hours after each dose with increasing titer values after the second dose that were generally associated with lower exposure to SARS-CoV-2 S protein in all matrices, demonstrating a robust immunological response to the vaccine.

In the non-GLP biodistribution study with mRNA-1647, an mRNA-based vaccine combined in SM-102-containing LNPs, was administered to male Sprague Dawley rats and is provided to support the development of mRNA-1273 and mRNA-1273 variant vaccines using the Sponsor's mRNA technology platform. The biodistribution of mRNA-based vaccines in LNPs is predicted to be driven by the LNP characteristics. Therefore, mRNAs that are within an LNP of the same composition (eg, mRNA-1273, mRNA-1273 variant vaccines, and mRNA-1647) are expected to distribute similarly. Results from the study with mRNA-1647 demonstrate that:

- Concentrations for mRNA constructs were detected at levels above the LLOQ in most tissues analyzed, except for the kidney, where all levels were below the LLOQ.
- As observed with other IM-delivered vaccines, the highest mRNA concentrations were observed at the injection site followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0.
- The T_{max} in plasma was achieved at 2 hours post-dose, with an estimated $T_{1/2}$ in plasma ranging from 2.7 to 3.8 hours. For highly exposed tissues, C_{max} was observed between 2- and 24-hours post-dose. The $T_{1/2}$ values, calculated using the average tissue $T_{1/2}$ values for all 6 constructs, were 14.9 hours for site of injection (muscle), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

The persistence of mRNA in tissues was evaluated in Sprague Dawley rats and indicated that for the majority of tissues, administered mRNA was not detectable after 1-3 days, the only exceptions were at the site of injection (muscle) and within the draining lymph nodes and spleen where the mRNA had a calculated half-life of 14.9-63.0 hrs. Therefore, mRNAs that are within an LNP of the same composition (eg, mRNA-1273 and mRNA-1647) are expected to distribute similarly.

Across the 3 biodistribution studies, the highest concentrations of SM-102 and mRNA were observed at the injection site, spleen, and lymph nodes in a manner similar to other IM delivered vaccines (Gómez-Mantilla et al 2016). The highest tissue-to-serum/plasma ratios based on C_{max} of mRNA and SM-102 lipid were observed in the injection site followed by the lymph nodes and spleen. The highest tissue-to-plasma ratios based on AUC were observed in the injection site

followed lymph nodes for SM-102 lipid. The mRNA tissue-to-serum ratios based on AUC in the spleen were higher in the studies with NPI-Luc mRNA and mRNA-1273 compared to the study with mRNA-1647, which is likely attributed to the wider range of collection times in the studies with NPI-Luc mRNA and mRNA-1273 (0.16 to 168 or 336 hours) compared to study with mRNA-1647 (2 to 120 hours). Results from these rat studies are corroborated by a published report by [Hassett et al \(2023\)](#), where NHPs were administered a single IM dose of a mRNA-LNP DP formulated in the same 4 lipids, and a select number of tissues (lymph nodes, spleen, liver, and injection site muscle) were evaluated for mRNA concentrations. Although the collection period in that publication was limited (ranging from 8 to 168 hours postdose), results were generally consistent with those observed in the rat studies. Specifically, mRNA in plasma and spleen were detected over 168 hours, and the spleen had the highest exposures, most likely from infiltrating cells. In that report, mRNA was not detected in the injection site, lymph nodes, and liver beyond 24 hours. Overall, the data derived from the rat studies confirms similarities in tissue distribution and kinetics, consistent with distribution via the lymphatic system and indicates cross-species similarities to NHPs.

Two non-GLP studies were completed with SM-102-containing lipid nanoparticles are also provided to support the development of mRNA-1273 and mRNA-1273 variant vaccines using the Sponsor's mRNA technology platform. The metabolic disposition of SM-102 in mRNA-1273 and mRNA-1273 variant vaccines is expected to be comparable to the SM-102 containing LNPs used in these studies.

- The primary circulating species following intravenous dosing of an SM-102 containing LNP to Sprague-Dawley rats was intact SM-102 (>95% of the EIC).
- The primary metabolites formed both in vitro and in vivo were principally ester hydrolysis and β -oxidation products and these metabolites were primarily excreted in the urine (>99% of TIC) and bile (approximately 70% of EIC).
- Low molecular weight, hydrophilic metabolites were detected in relatively higher amounts (approximately 10-fold) in urine compared to larger, more hydrophobic metabolites in plasma and bile.

SM-102 is extensively metabolized through multiple high-capacity systems leading to almost complete clearance of SM-102 within 24 hours. Intact SM-102 and metabolites account for <3.0% of the maximum level observed in the study, suggesting that SM-102 is unlikely to accumulate on repeat IM dosing or present an issue for elimination in patients with hepatic or renal insufficiency.

2.6.4.10 TABLES AND FIGURES

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

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2.6.4.11 REFERENCES

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