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2.4.1. Overview of Nonclinical Testing Strategy

To fulfill the commitment to the EMA on mRNA-1273, the 2 biodistribution studies in rats using mRNA-1273 and NPI-luciferase mRNA encapsulated [REDACTED] are complete and final reports are provided in Module 4 ([Report 20456513 Amendment 1](#) and [Report 2308-582 Amendment 1](#), respectively).

A summary of these data is provided in [Section 2.4.3](#) and aggregated with the rat biodistribution study conducted with mRNA-1647 ([Report 5002121 Amendment 2](#)), which was included in the original dossier for mRNA-1273.

All 3 mRNA-LNP Drug Products (DPs) used in these biodistribution studies were formulated in the same 4 lipids (SM-102, cholesterol, DSPC, and PEG2000-DMG) with generally similar lipid contents and mRNA ratios ([Table 1](#) below) and were administered via intramuscular (IM injection), which is the clinical route of administration.

Table 1: Comparison of the mRNA-LNP Drug Products Used in the IM Biodistribution Studies in Rats Supporting mRNA-1273

Drug Product	Study Type (Report No.)	Lot Number	mRNA Content (mg/mL)	mRNA Purity	Lipid Composition and Lipid Content (mg/mL)	Buffer Constituents
mRNA-1647	Single dose IM tissue distribution study in rats (5002121 Amendment 2)	MTDP17048	1.9	91.1%	SM-102: 18.0 Cholesterol: 7.8 DSPC: 4.0 PEG-DMG: 1.9	100 mM Tris 7% PG, 1 mM DTPA, pH 7.75
mRNA-1273	Single or repeat dose IM tissue distribution study in rats (20456513 Amendment 1)	DH-80430.1	0.39	76%	SM-102: 4.9 Cholesterol: 2.2 DSPC: 1.2 PEG2000-DMG: 0.5	20 mM Tris, 87 g/L sucrose, 2.0 mM sodium acetate, pH 7.5
NPI luciferase mRNA [REDACTED]	Single dose IM tissue distribution study in rats (2308-582 Amendment 1)	DH-79692.1	0.486	81%	SM-102: 4.1 Cholesterol: 1.9 DSPC: 1.1 PEG2000-DMG: 0.9	20 mM Tris, 87 g/L sucrose

Nonclinical PK evaluations included in vivo distribution, persistence, and clearance of mRNAs, SM-102 lipid, and/or expressed proteins using other representative mRNA-LNP drug products (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 by 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. These results demonstrate that the tissue distribution of mRNA in vaccines formulated in SM-102-containing LNPs is consistent with administration of DPs IM, and, specifically, distribution via the lymphatic system.

Results of 3 biodistribution studies using mRNA-LNP DPs comprised of the same 4 lipids (ie, SM 102, cholesterol, DSPC, and PEG2000-DMG) and generally similar lipid:mRNA ratios as mRNA-1273 were used to characterize the kinetics and tissue distribution of mRNA 1273 (see [Table 1](#)). 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 itself, and (3) mRNA-1647. The reporter mRNA (NPI-Luc) in the first aforementioned study encodes an intracellularly expressed NPI-Luc protein. mRNA-1647 is an mRNA-based novel mRNA-based cytomegalovirus (CMV) vaccine that contains 6 distinct mRNA sequences in 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.

2.4.2. Pharmacology

Not relevant for this Type II Variation submission.

2.4.3. Pharmacokinetics

[Table 2](#) lists the nonclinical distribution used in support of mRNA-1273. Results of these studies are summarized in [Module 2.6.4](#); tabulated summaries of results are presented in [Module 2.6.5](#).

Table 2: Summary of Nonclinical Biodistribution Studies Supporting mRNA-1273

Type of Study	Test Article; Dose	Test System	Method of Admin.	GLP	Report Number
Distribution					
Single-dose biodistribution study of NPI-Luc mRNA in SM-102/PEG2000-DMG	NPI-Luc mRNA in SM-102/PEG2000-DMG-containing LNPs: 100 µg/dose ^a	Sprague Dawley rats	Single IM dose	No	2308-582 Amendment 1
Single- or Repeat-dose biodistribution study of mRNA-1273	mRNA-1273: 78 µg/dose ^a	Sprague Dawley rats	Single- or Repeat IM dose on Days 1 and 28	No	20456513 Amendment 1
Single-dose tissue distribution study of mRNA-1647	mRNA-1647: 100 µg/dose ^a	Sprague Dawley rats	Single IM dose	No	5002121 Amendment 2

Abbreviations: Admin = administration; GLP = Good Laboratory Practice; IM = intramuscular; IV = intravenous; LNP=lipid nanoparticle; mRNA=messenger ribonucleic acid; NT-FIX=non-translating Factor IX; PK=pharmacokinetic; SM-102=an ionizable lipid.

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

2.4.3.1 Distribution

To support the development of mRNA-1273, 3 biodistribution 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:mRNA ratios as mRNA-1273 (Table 1). The biodistribution and kinetics of SM-102 lipid, mRNA, and/or expressed protein(s) following a single IM administration were evaluated using NPI-Luc mRNA encapsulated in SM-102/PEG2000-DMG-containing LNPs and mRNA-1647. In the study with mRNA-1273, the biodistribution and kinetics of SM-102 lipid, mRNA, and SARS-CoV-2 S protein were evaluated following a single or 2-dose IM administration to assess potential accumulation and effect of an immune response following repeat dosing. Results from the biodistribution studies demonstrated that:

- There are general similarities in exposure tissue rank order and kinetics across mRNA-LNP DPs (highest concentrations of mRNA [Table 3] and SM-102 lipid [Table 4] were observed at the injection site, spleen, and lymph nodes), substantiating that mRNA cargo and the presence or absence of an immune response do not alter tissue distribution of mRNA-LNP DPs.
- Tissue distribution of mRNA and SM-102 lipid is similar following 1 or 2 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.
- While expressed NPI-Luc protein in tissues was largely not quantifiable (likely limited by the analytical method), expressed SARS-CoV-2 protein was also generally higher in tissues than in serum, where lymph nodes (inguinal/popliteal and axillary), injection site, spleen, and/or liver had highest exposures. Exposures of expressed SARS-CoV-2 protein in brain, heart, and lung were generally undetectable or unreportable because of limited numbers of samples with quantifiable levels.

The results from the individual studies supporting these findings are summarized below:

After a single IM injection of NPI-Luc mRNA encapsulated in CCI to rats, the systemic exposure of NPI-Luc mRNA and SM-102 lipid appeared to be independent of sex for serum and tissues (Report 2308-582 Amendment 1). 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 hours (serum) and 8.44 hours (plasma), respectively. 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 limited by the analytical method, the expressed NPI-Luc protein 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).

When comparing a single or repeat-dose (2 doses, Day 1 and Day 28) of 0.78 µg/dose mRNA-1273 to rats, there were no differences in exposure for mRNA from mRNA-1273, SM-102 lipid, or SARS-CoV-2 S protein expression in any plasma or tissue matrix (Report 20456513 Amendment 1). 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 hours (serum) and 4.83 to 7.02 hours (plasma), respectively. 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. 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.

To add to the body of evidence around the distribution of mRNA-LNP vaccines of the same lipid composition, another single-dose biodistribution study was conducted using mRNA-1647 in male rats ([Report 5002121 Amendment 2](#)). After administering a single IM dose of mRNA-1647, all 6 mRNA constructs contained within mRNA-1647 were detected in plasma and tissues, with the highest concentrations observed at the injection site, lymph nodes, and spleen. Only a small fraction of the administered dose reached distant tissues, and mRNA concentrations in most tissues became undetectable within 1 to 3 days, except at the injection site, lymph nodes, and spleen.

Table 3: Summary of mRNA AUC Tissue:Serum or Tissue:Plasma Ratios and Effective Half-Life in Sprague Dawley Rats

Matrix	AUC MTX						Effective T _{1/2} (h)					
	mRNA-1647	NPI-Luc mRNA in SM-102- containing LNPs	mRNA-1273				mRNA-1647	NPI-Luc mRNA in SM-102- containing LNPs	mRNA-1273			
			Day 1		Day 28				Day 1		Day 28	
			Male	Combined	Female	Male			Female	Male	Male	Combined
Plasma	NA	–	–	–	–	–	NC	–	–	–	–	–
Serum	–	NA	NA	NA	NA	NA	–	2.95	2.90	1.88	3.89	1.77
Bone marrow	NR	1.32	–	–	–	–	NC	7.59				
Brain	NR	NC	0.0773	NC	NC	NC	NC	NC	1.68	NC	NC	NC
Eye	1.24	NC	–	–	–	–	NC	NC	–	–	–	–
Heart	NR	NC	8.14	5.97	18.0	7.73	NC	NC	2.84	2.40	2.51	21.2
Injection site	1010	2250	2210	2410	514	9470	13.5-17.1 ^a	4.88	14.8	15.5	9.36	10.8
Liver	0.499	31.3	69.3	47.2	67.7	90.7	NC	2.98	8.68	5.26	7.48	5.61
Lung	NR	4.59 ^b	16.6	62.6	15.0	24.4	NC	3.07 ^b	5.66	0.905	7.95	7.28
Lymph node (axillary)	–	686	918	1710	1560	4260	–	24.1	45.5	50.4	41.5	33.4
Lymph node (distal)	62.8	–	–	–	–	–	27.9-36.2 ^a	–	–	–	–	–
Lymph node (inguinal)	–	NC	–	–	–	–	–	NC	–	–	–	–
Lymph node (inguinal/popiteal)	–		1550	5760	1540	8920	–	–	62.3	23.3	52.2	40.6
Lymph node (popiteal)	–	NC	–	–	–	–	–	NC	–	–	–	–
Lymph node (proximal)	201	–	–	–	–	–	32.2-38.2 ^a	–	–	–	–	–

Matrix	AUC MTX						Effective T _{1/2} (h)					
	mRNA-1647	NPI-Luc mRNA in SM-102- containing LNPs	mRNA-1273				mRNA-1647	NPI-Luc mRNA in SM-102- containing LNPs	mRNA-1273			
	Day 1	Day 1	Day 1		Day 28		Day 1	Day 1	Day 1		Day 28	
	Male	Combined	Female	Male	Female	Male	Male	Combined	Female	Male	Female	Male
Spleen	13.4	4800	4600	12000	6120	30900	46.2-83.0 ^a	48.5	37.7	62.1	51.4	64.4
Testes	0.209	NC	–	–	–	–	NC	NC	–	–	–	–

Abbreviations: – = not evaluated; AUC = area under the concentration versus time curve.; effective T_{1/2} = half-life determination using mean residence time; IM = intramuscular; Luc = luciferase; MTX = matrix ratios (tissue-to-serum or plasma); NA = not applicable; NC = not calculable (insufficient data points above the lower limit of quantification); NPI = nascent peptide imaging; NR = not reported (some constructs measured all samples as below the limit of quantitation).

Notes: In [Report 5002121 Amendment 2](#), Sprague Dawley rats were administered a single IM injection of 100 µg of mRNA-1647 on Day 1. Blood and tissues were collected predose and through 120 hours postdose. Tissues evaluated included bone marrow, brain, distal lymph node, eye, heart, injection site muscle, jejunum, kidney, liver, lung, proximal lymph nodes, spleen, stomach, and testes. Note, AUC₍₀₋₁₂₀₎ was used in the table and defined as “area under the concentration versus time curve from time zero to the last collection point”

In [Report 2308-582 Amendment 1](#), Sprague Dawley rats were administered a single IM injection of 100 µg of NPI-Luc mRNA encapsulated in SM-102 (0.84 mg)/PEG2000-DMG-containing LNPs on Day 1. Tissues evaluated included brain, eye, femur bone marrow, heart, jejunum, kidney, liver, lung, pancreas, lymph nodes (axillary, inguinal and popliteal), spleen, stomach, testes, ovary, uterus, thymus, and injection site. For NPI-Luc mRNA, right kidney, right testis, and ovaries tissue samples were not analyzed due to small tissue sample size but were analyzed for SM-102 lipid. Note, AUC_{last} was used in the table and defined as “area under the concentration versus time curve from time zero to the last quantifiable concentration”

In [Report 20456513 Amendment 1](#), Sprague Dawley rats were administered an IM injection of 78 µg of mRNA-1273 (0.98 mg/dose SM-102) on Day 1 and Day 28. Blood and tissue samples were collected predose and through 336 hours postdose on Day 1 and Day 28. Tissues evaluated included brain, heart, liver, lung, lymph nodes (inguinal and popliteal pooled and axillary), injection site, and spleen. Note, AUC_{last} was used in the table and defined as “area under the concentration versus time curve from time zero to the last quantifiable concentration”

^a. Range reported across 6 mRNA constructs.

^b. Results reported for females only.

Table 4: Summary of SM-102 Lipid AUC Tissue:Plasma Ratios and Effective Half-Life in Sprague Dawley Rats

Matrix	AUC _{last} MTX					Effective T _{1/2} (h)				
	NPI-Luc mRNA in SM-102-containing LNPs		mRNA-1273			NPI-Luc mRNA in SM-102-containing LNPs		mRNA-1273		
	Day 1	Day 1		Day 28		Day 1	Day 1		Day 28	
	Combined	Female	Male	Female	Male	Combined	Female	Male	Female	Male
Plasma	NA	NA	NA	NA	NA	8.44	4.83	7.02	4.88	6.34
Bone marrow (femur)	8.44	–	–	–	–	7.94	–	–	–	–
Heart	NC ^a	0.132	0.132	0.243	NC	NC ^a	1.21	0.990	NC	0.537
Injection site	2170	1140	577	902	6000	14.4	16.2	15.5	15.0	16.2
Jejunum	2.33	–	–	–	–	10.9	–	–	–	–
Kidney (left)	0.115	–	–	–	–	1.73	–	–	–	–
Kidney (right)	0.208	–	–	–	–	1.85	–	–	–	–
Liver	31.0	9.22	19.2	25.6	30.6	6.33	6.76	5.43	5.51	6.62
Lung	2.84	2.30	1.93	1.72	1.15	4.80	5.30	4.98	4.12	5.57
Lymph node (axillary)	195	122	1400	426	1190	60.5	34.2	11.7	23.4	31.0
Lymph node (inguinal)	1190	–	–	–	–	37.5	–	–	–	–
Lymph node (inguinal/popiteal)	–	448	1970	613	540	–	43.7	27.1	32.2	14.6
Lymph node (popiteal)	1010	–	–	–	–	18.9	–	–	–	–
Ovaries (females)	5.51 ^a	–	–	–	–	27.8 ^a	–	–	–	–
Spleen	99.6	432	270	246	223	22.8	24.4	30.0	15.6	39.5

Abbreviations: – = not evaluated; 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; effective T_{1/2} = half-life determination using mean residence time; IM = intramuscular; MTX = matrix ratios (tissue-to-serum or plasma); NA = not applicable; NC = not calculable (insufficient data points above the lower limit of quantification); NR = not reported (some constructs measured all samples as below the limit of quantitation).

Notes: In [Report 2308-582 Amendment 1](#), Sprague Dawley rats were administered a single IM injection of 100 µg of NPI-Luc mRNA encapsulated in SM-102 (0.84 mg)/PEG2000-DMG-containing LNPs on Day 1. Tissues evaluated included brain, eye, femur bone marrow, heart, jejunum, kidney, liver, lung, pancreas, lymph nodes (axillary, inguinal and popliteal), spleen, stomach, testes, ovary, uterus, thymus, and injection site. For NPI-Luc mRNA, right kidney, right testis, and ovaries tissue samples were not analyzed due to small tissue sample size but were analyzed for SM-102 lipid.

In [Report 20456513 Amendment 1](#), Sprague Dawley rats were administered an IM injection of 78 µg of mRNA-1273 (0.98 mg/dose SM-102) on Day 1 and

Day 28. Blood and tissue samples were collected predose and through 336 hours postdose on Day 1 and Day 28. Tissues evaluated included brain, heart, liver, lung, lymph nodes (inguinal and popliteal pooled and axillary), injection site, and spleen.

^a. Results reported for females only.

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2.4.4. Toxicology

Not relevant for this Type II Variation submission.

2.4.5. Integrated Overview and Conclusions

Nonclinical PK studies consisted of distribution using mRNA-LNP DPs comprised of the same 4 lipids as mRNA-1273. 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 immune response do not alter tissue distribution of mRNA-LNP DPs. Furthermore, tissue distribution is similar following 1 or 2 administration(s) of mRNA-1273, demonstrating that there is low risk of accumulation and no differences in distribution of DP components with repeat dosing.

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 different range of collection times in the studies. Results from the rat biodistribution studies are corroborated by a published report by Hassett et al 2024 where a single IM dose of an mRNA-LNP DP formulated in the same 4 lipids was administered to NHPs, and mRNA concentrations were measurable in plasma and spleen over 168 hours. 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 biodistribution studies confirm similarities in tissue distribution and kinetics, consistent with distribution via the lymphatic system, and indicate cross-species similarities to NHP.

2.4.6. List of Literature Citations

Gómez-Mantilla JD, Trocóniz IF, Garrido MJ. ADME process in vaccines and PK/PD approaches for vaccination optimization. In: Honghui Z, Theil P, editors. ADME translational pharmacokinetics/pharmacodynamics of therapeutic proteins: applications in drug discovery and development. John Wiley and Sons, Inc; 2016. p. 1-22.

Hassett KJ, Rajlic IL, Bahl K, White R, Cowens K, Jacquinet E, et al. mRNA vaccine trafficking and resulting protein expression after intramuscular administration. Mol Ther Nucleic Acids. 2023;35(1):102083.