

TABLE OF CONTENTS

TABLE OF CONTENTS.....	1
LIST OF TABLES	2
2.6.5.1 PHARMACOKINETICS: OVERVIEW	3
2.6.5.2 ANALYTICAL METHODS AND VALIDATION REPORTS.....	4
2.6.5.3 PHARMACOKINETICS: ORGAN DISTRIBUTION.....	5
2.6.5.4 IN VITRO METABOLITE IDENTIFICATION	16
2.6.5.5 IN VIVO METABOLITE IDENTIFICATION.....	17

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LIST OF TABLES

Table 1:	Summary of metabolite profiling of SM-102 in Human, Rat and NHP hepatocytes	16
Table 2:	Identification of Metabolites.....	17
Table 3:	Metabolites of SM102 in Plasma.....	19
Table 4:	Metabolites of SM-102 in Urine Pools.....	20
Table 5:	Metabolites of SM-102 in Bile	21
Table 6:	Quantitation of Parent SM102 in Plasma, Urine and Bile Samples using LC-QQQ*	22

2.6.5.1 PHARMACOKINETICS: OVERVIEW

Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location in eCTD
Distribution					
A non-GLP biodistribution study of NPI-Luc mRNA in SM-102/PEG2000-DMG following single intramuscular injection in Sprague Dawley rats	Sprague Dawley rats	Single IM dose	Charles River Laboratories, Sherbrooke, QC, Canada	2308-582 Amendment 1	Section 4.2.2.3
A single or repeat dose biodistribution study of mRNA-1273 by intramuscular administration in Sprague Dawley rats	Sprague Dawley rats	Single and repeat IM dose	Charles River Laboratories, Mattawan, MI, USA	20456513 Amendment 1	Section 4.2.2.3
A single dose IM tissue distribution study using mRNA-1647 in male Sprague Dawley rats	Sprague Dawley rats	Single IM dose	Charles River Laboratories, Sherbrooke, QC, Canada	5002121 Amendment 2	Section 4.2.2.3
Metabolism and excretion					
Identification and profiling of metabolites of SM-102 in rat, monkey, and human hepatocytes	Cryopreserved hepatocytes from Sprague Dawley rats, cynomolgus monkey, and human	Incubation of SM-102-containing LNPs with rat, monkey, and human hepatocytes	ModernaTX, Inc., Cambridge, MA, USA	NCS-BA-2022-010	Section 4.2.2.4
An assessment of the metabolism of SM-102 following intravenous infusion dose administration of SM-102-containing lipid nanoparticles to male Sprague-Dawley rats	Sprague Dawley rats	Single IV dose	Charles River Laboratories, Worcester, MA, USA	QV-0236-DA-RE	Section 4.2.2.4

Abbreviations: eCTD = electronic common technical document; GLP = Good Laboratory Practice; IV = intravenous; Luc = luciferase; mRNA = messenger RNA; NPI = nascent peptide imaging; PEG2000 DMG = 1 monomethoxypolyethyleneglycol2,3dimyristylglycerol with polyethylene glycol of average molecular weight 2000; PK = pharmacokinetic; SM 102 = an ionizable lipid.

2.6.5.2 ANALYTICAL METHODS AND VALIDATION REPORTS

Analyte	Analytical Method	Species	Matrix	Assay Rigor	Calibration Range	Study Number (Non-GLP)
NPI-Luc mRNA	bDNA singleplex	Rats	Serum, tissues	Qualified	0.125 to 8,000 ng/mL	2308-582 Amendment 1
mRNA from mRNA-1273	RT-qPCR	Rats	Serum, tissues	Qualified	0.850 to 170,000 pg/mL for serum 0.850 to 170,000 pg/g for tissue	20456513 Amendment 1
mRNAs from mRNA-1647 (gB, gH, gL, UL128, UL130, and UL131A)	bDNA multiplex	Rats	Plasma, tissues	Fit for purpose	0.10-50.00 pg/mL for gH, gL, UL128, and UL131A ^b 0.50-50.00 pg/mL for gB and UL130 ^b	5002121 Amendment 2
SM-102	LC-MS/MS	Rats	Plasma, tissues	Qualified	0.500 to 500 ng/mL	2308-582 Amendment 1 20456513 Amendment 1
			Plasma, bile, urine	Fit for purpose	0.2 to 500 ng/mL	QV-0236-DA-RE-RPT-01
NPI-Luc protein	ECL	Rats	Tissue	Qualified	200 to 16,000 ng/g	2308-582 Amendment 1
SARS-CoV-2 S protein ^a	LBA	Rats	Serum	Validated	4.57 to 10,000 ng/mL	20456513 Amendment 1
			Tissues		91.4 to 100,000 ng/mL	20456513 Amendment 1
Anti-SARS-CoV-2 S protein IgG antibody	ELISA	Rats	Serum	Qualified	0.059 AU/mL	20456513 Amendment 1
SM-102 metabolite identification	LC-HRMS	Rat	Plasma, bile, urine	Fit for purpose	NA	QV-0236-DA-RE
SM-102 metabolite identification	LC-HRMS	In vitro	Cryopreserved hepatocytes from Sprague Dawley rats, cynomolgus monkey, and human	Fit for purpose	NA	BCS-BA-2022-010

Abbreviations: bDNA = branched DNA; ECL = electrochemiluminescence ligand binding assay; ESI = electrospray ionization; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; HRMS = high-resolution mass spectrometry; LBA = ligand binding assay; LC = liquid chromatography; LLOQ = lower limit of quantification; Luc = luciferase; mRNA = messenger RNA; MS = mass spectrometry; MS/MS = tandem mass spectrometry; NPI = nascent peptide imaging; NA = not available; SM 102 = an ionizable lipid.

2.6.5.3 PHARMACOKINETICS: ORGAN DISTRIBUTION

2.6.5.3.1 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)

Test Article: NPI-Luc mRNA encapsulated in SM-102/PEG2000 DMG-containing LNP												
Study title: A non-GLP biodistribution study of NPI-Luc mRNA in SM-102/PEG2000-DMG following single intramuscular injection in Sprague Dawley rats												
Species/strain/sex: Rat/Sprague Dawley/male and female						Location in eCTD: Section 4.2.2.3						
Number per group: 6 animals/timepoint (3/sex)						Study number: 2308-582 Amendment 1				GLP study: No		
Feeding condition: Ad libitum		Dose level: NPI-Luc mRNA (100 µg/dose); SM-102 lipid (0.84 mg/dose)				Vehicle/formulation: Sodium chloride 0.9% (saline), sodium heparin (1000 IU/mL) and 1% sodium nitrite in deionized water				Sample matrix: Serum/plasma and tissues		
Plasma and tissue collection timepoints: Predose (0), 0.167, 1, 4, 10, 24, 72, 120, 168 h after single IM dose										Analytes: NPI-Luc mRNA and SM-102 lipid		
Matrix	T_{max} (h)			Effective T_{1/2} (h)			C_{max} MTX			AUC_{tlast} MTX		
	F	M	Combined	F	M	Combined	F	M	Combined	F	M	Combined
NPI-Luc mRNA												
Serum	3.23	2.03	1	3.23	2.03	2.95	NA	NA	NA	NA	NA	NA
Axillary lymph node	24	10	13.4	82.0	NA	24.1	82.0	70.1	84.6	163	NC	686
Bone marrow	10	0.167	10	7.58	7.60	7.59	0.270	0.985	0.461	0.714	3.39	1.32
Brain	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Eye	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Heart	1	4	1	NC	NC	NC	3.28	1.85	2.54	NC	NC	NC
Inguinal lymph node	120	168	120	NC	NC	NC	93.3	15.3	72.2	NC	NC	NC
Injection site	0.167	0.167	0.167	4.57	5.24	4.88	1390	8930	4220	1280	5570	2250
Jejunum	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Kidney (left)	1	NC	NC	NC	NC	NC	0.848	NC	0.848	NC	NC	NC
Liver	4	1	4	3.28	1.45	2.98	25.4	18.9	25.2	30.9	18.6	31.3
Lung	1	NC	NC	3.07	NC	NC	3.18	NC	NC	4.59	NC	NC

Matrix	T _{max} (h)			Effective T _{1/2} (h)			C _{max} MTX			AUC _{last} MTX		
	F	M	Combined	F	M	Combined	F	M	Combined	F	M	Combined
Pancreas	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Popliteal lymph node	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Spleen	24	10	24	47.7	40.4	45.8	265	262	277	4590	5220	4800
Stomach	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Testis (left)	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Thymus	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Uterus	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
SM-102 lipid												
Plasma	0.167	0.167	0.167	8.29	8.72	8.44	NA	NA	NA	NA	NA	NA
Axillary lymph node	1	0.167	4	61.0	53.1	60.5	48.8	32.3	25.7	308	30.2	195
Bone marrow (femur)	1	0.167	1	9.29	7.20	7.94	4.93	16.0	10.1	5.03	13.5	8.44
Brain	NC	1	1	NC	NC	NC	NC	0.265	0.128	NC	NC	NC
Eye	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Heart	1	NC	1	NC	NC	NC	1.22	NC	0.630	NC	NC	NC
Inguinal lymph node	10	168	10	29.2	73.5	37.5	886	92.1	478	1630	544	1190
Injection site	24	0.167	24	14.6	12.6	14.4	1040	371	588	3260	593	2170
Jejunum	10	4	4	13.5	2.90	10.9	1.91	2.37	2.03	2.95	1.29	2.33
Kidney (left)	4	1	4	NC	NC	1.73	0.586	0.580	0.425	NC	NC	0.115
Kidney (right)	1	4	4	NC	NC	1.85	0.955	0.796	0.847	NC	NC	0.208
Liver	4	4	4	5.66	5.18	6.33	34.1	16.4	25.5	39.7	13.3	31.0
Lung	1	1	1	5.13	2.33	4.80	4.02	1.38	2.74	4.01	0.957	2.84
Ovaries (females)	10	NC	NC	27.8	NC	NC	2.72	NC	NC	5.51	NC	NC
Pancreas	10	NC	NC	NC	NC	NC	0.246	NC	NC	NC	NC	NC

Matrix	T _{max} (h)			Effective T _{1/2} (h)			C _{max} MTX			AUC _{last} MTX		
	F	M	Combined	F	M	Combined	F	M	Combined	F	M	Combined
Popliteal lymph node	1	10	1	12.9	23.6	18.9	1770	350	969	875	1240	1020
Spleen	10	4	10	22.1	25.5	22.8	53.2	24.7	33.5	134	50.4	99.6
Stomach	1	NC	1	NC	NC	NC	0.408	NC	0.210	NC	NC	NC
Testis (right) (males)	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Thymus	NC	0.167	NC	NC	NC	NC	NC	0.163	NC	NC	NC	NC
Uterus (females)	0.167	NC	NC	NC	NC	NC	0.331	NC	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; h = hour(s); 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.

Note: 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.

Source: [Report 2308-582 Amendment 1](#).

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

Test Article: mRNA-1273									
Study title: A single or repeat dose biodistribution study of mRNA-1273 by intramuscular administration in Sprague Dawley rats									
Species/strain/sex: Rat/Sprague Dawley/male and female				Location in eCTD: Section 4.2.2.3					
Number per group: 8 animals/timepoint (4/sex)				Study number: 20456513 Amendment 1				GLP study: No	
Feeding condition: Ad libitum		Dose level: mRNA-1273 (78 µg/dose); SM-102 lipid (0.98 mg/dose)		Vehicle/formulation: PBS (20mM Tris, 2.0mM acetate, 87g/L sucrose, pH 7.5)				Sample matrix: Serum/plasma and tissues	
Plasma and tissue collection timepoints: 0 (predose), 0.16, 1, 4, 10, 24, 72, 120, 168, and 336 h after Dose 1 (Day 1) and Dose 2 (Day 28)								Analyte: mRNA-1273, SM-102, and SARS-CoV-2 S protein	
Matrix	Day	T _{max} (h)		Effective T _{1/2} (h)		C _{max} MTX		AUC _{last} MTX	
		F	M	F	M	F	M	F	M
mRNA from mRNA-1273									
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/popiteal)	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

Matrix	Day	T _{max} (h)		Effective T _{1/2} (h)		C _{max} MTX		AUC _{last} MTX	
		F	M	F	M	F	M	F	M
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
Matrix	Day	T _{max} (h)		Effective T _{1/2} (h)		C _{max} MTX		AUC _{last} MTX	
		F	M	F	M	F	M	F	M
SM-102 lipid									
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	NC
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/popiteal)	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

Matrix	Day	T _{max} (h)		Effective T _{1/2} (h)		C _{max} MTX		AUC _{last} MTX	
		F	M	F	M	F	M	F	M
	28	10	4	15.6	39.5	88.4	50.2	246	223
Matrix	Day	TE _{max} (h)		Effective T _{1/2} (h)		E _{max} MTX		AUEC _{last} MTX	
		F	M	F	M	F	M	F	M
SARS-CoV-2 protein									
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	NC	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	NC	1600	NC	712	NC
	28	10	NC	NC	NC	4560	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; AUEC_{last} = 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); PBS = phosphate-buffered saline; T_{max} = time after dosing at which the maximum concentration was observed; TE_{max} = time after dosing at which the maximum effect was observed.

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

2.6.5.3.3 A Single Dose Intramuscular Injection Tissue Distribution Study of mRNA-1647 in Male Sprague Dawley Rats (Report 50002121 Amendment 2)

Study Title: A single dose intramuscular injection tissue distribution study of mRNA-1647 in male Sprague-Dawley rats							
Species/Strain/Sex: Rat/Sprague-Dawley/male				Location in eCTD: 4.2.2.3		Test Article: mRNA-1647	
Number per Group: 5 animals terminated per time point (35 total)				Report Number: 5002121 Amendment 2		GLP Study: No	
Feeding Condition: Ad libitum				Dose Level: 100 µg		Sample Matrix: Plasma and tissues	
Plasma and Tissue Collection Time Points: 0 (pre-dose), 2, 8, 24, 48, 72, and 120 h post-dose				Vehicle/Formulation: PBS, pH 7.2		Analyte: mRNA-1647 (6 mRNA constructs)	
Pharmacokinetic Parameters							
Sample Matrix	mRNA Construct	T _{max} (h) ^a	C _{max} (ng/mL) ^a	AUC _(0-t) (ng × h/mL) ^{a,b}	T _{1/2} (h) ^c	AUC _(0-t) Ratio (Tissue/Plasma) ^d	AUC _(0-t) Ratio (Tissue/Plasma) Average
Plasma	gB	2.0	2.02 ± 0.181	22.7 ± 3.77	NC	NA	NA
	gH	2.0	1.91 ± 0.187	24.9 ± 4.49	NC	NA	
	gL	2.0	1.74 ± 0.177	23.4 ± 4.07	NC	NA	
	UL128	2.0	1.66 ± 0.151	24.1 ± 4.44	NC	NA	
	UL130	2.0	2.30 ± 0.621	25.5 ± 4.65	NC	NA	
	UL131A	2.0	1.60 ± 0.153	24.8 ± 4.59	NC	NA	
Tissue							
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	

Pharmacokinetic Parameters							
Sample Matrix	mRNA Construct	T _{max} (h) ^a	C _{max} (ng/mL) ^a	AUC ₍₀₋₄₎ (ng × h/mL) ^{a,b}	T _{1/2} (h) ^c	AUC ₍₀₋₄₎ Ratio (Tissue/Plasma) ^d	AUC ₍₀₋₄₎ Ratio (Tissue/Plasma) Average
	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	
	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	NR
	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	
	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	

Pharmacokinetic Parameters							
Sample Matrix	mRNA Construct	T _{max} (h) ^a	C _{max} (ng/mL) ^a	AUC _(0-t) (ng × h/mL) ^{a,b}	T _{1/2} (h) ^c	AUC _(0-t) Ratio (Tissue/Plasma) ^d	AUC _(0-t) Ratio (Tissue/Plasma) Average
	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	
	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	1010
	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	
	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	0.499
	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	

Pharmacokinetic Parameters							
Sample Matrix	mRNA Construct	T _{max} (h) ^a	C _{max} (ng/mL) ^a	AUC ₍₀₋₄₎ (ng × h/mL) ^{a,b}	T _{1/2} (h) ^c	AUC ₍₀₋₄₎ Ratio (Tissue/Plasma) ^d	AUC ₍₀₋₄₎ Ratio (Tissue/Plasma) Average
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 node	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	
	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	

Pharmacokinetic Parameters							
Sample Matrix	mRNA Construct	T _{max} (h) ^a	C _{max} (ng/mL) ^a	AUC _(0-t) (ng × h/mL) ^{a,b}	T _{1/2} (h) ^c	AUC _(0-t) Ratio (Tissue/Plasma) ^d	AUC _(0-t) Ratio (Tissue/Plasma) Average
Testes	gB	2.0	1.16 ± 0.719	4.64 ± 2.88	NC	0.204	0.209
	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: eCTD = electronic common technical document; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; mRNA = messenger RNA; NA = not applicable; NC = not calculable (insufficient data points above lower limit of quantitation); NR = not reported (some constructs measured all samples as below limit of quantitation); PBS = phosphate-buffered saline.

^a T_{max} and T_{1/2} data reported as the mean; C_{max} and AUC_(0-t) data reported as the mean ± standard error.

^b For the bone marrow, brain, jejunum, heart, liver, lung, stomach, and testes, AUC_(0-t) was calculated using less than 3 quantifiable mean concentrations and therefore is an estimate.

^c Due to the lack of a distinct elimination phase in plasma, 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.

^d For AUC_(0-t) Ratio, samples listed as NC were not calculable because all samples were below limit of quantitation. Source: Report 5002121 Amendment 2 (Appendix 8, Table 2, and Table 3).

2.6.5.4 IN VITRO METABOLITE IDENTIFICATION

Study Title: Identification and profiling of metabolites of SM-102 In Rat, Monkey and Human Hepatocytes	
Report Number: NCS-BA-2022-010	Location in eCTD: 4.2.2.3
Test Article: SM-102	GLP Study: No

Table 1: Summary of metabolite profiling of SM-102 in Human, Rat and NHP hepatocytes

Metabolite ID	RT	Observed m/z	Theoretical m/z	Error	Elemental Composition	Proposed Biotransformation	Human	Rat	NHP
M1	3.2	204.1591	204.1592	-0.48	C ₁₀ H ₂₂ NO ₃	N Dealkylation + Hydrolysis	Y	Y	Y
M3	6.2	290.1961	290.1962	0.34	C ₁₄ H ₂₈ NO ₅	Ester Hydrolysis (2X)+ B-oxidation(2X)	Y*	N	Y
M4	6.9	318.2269	318.2275	1.8	C ₁₆ H ₃₂ NO ₅	Ester Hydrolysis (2X)	Y	Y	Y
M6	15.1	528.4614	528.4623	1.7	C ₃₁ H ₆₂ NO ₅	Ester Hydrolysis(1X) + B-oxidation (1X)	Y	Y	Y
M7	16.4	556.4934	556.4936	0.35	C ₃₃ H ₆₆ NO ₅	Ester Hydrolysis(1X)	Y	Y	Y
Parent	19.2	710.6660	710.6657	0.42	C ₄₄ H ₈₈ NO ₅	NA	Y	Y	Y

Labels for metabolites are consistent with In vivo Rat Met ID – Y = Detected, N = Below Detection limit

M2, M5, M8-M12(In vivo) were not detected in vitro samples

Confirmed based on RT and HRMS – Low signal for MS/MS

Abundances (EIC signal) of all the Metabolites are at least similar or less in Human hepatocytes compared to Rat and NHP hepatocytes

2.6.5.5 IN VIVO METABOLITE IDENTIFICATION

Study Title: 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		
Species/Strain/Sex: Rat/Sprague-Dawley/male	Location in eCTD: 4.2.2.3	Test Article: SM-102 with NPI-luciferase mRNA
Number per Group: 3 plus 1 spare	Report Number: QV 0236-DA-RE	GLP Study: No
Feeding Condition: Ad libitum	Dose Level: 0.7 mg/kg	Sample Matrix: Plasma, urine, bile
Plasma and Tissue Collection Time Points: 0hr (pre-dose), 2hr, 6hr and 24 hr. Samples from group 1 animals (n=3) were pooled before analysis		
Urine and Bile Time Points: samples from group 1 animals (n=3) were collected between 0-2hr, 2-6 hr. and 6-24hr and pooled for analysis. Control urine and bile from the spare animal was used as a pre-dose reference		

Table 2: Identification of Metabolites

ID	Retention Time (min)	Observed m/z	Proposed Formula [M+H] ⁺	Theo. m/z [M+H] ⁺	Error (ppm)	Proposed Biotransformation
M1	3.5	204.1595	C ₁₀ H ₂₂ NO ₃	204.1594	-0.48	N Dealkylation + Ester Hydrolysis
M2	3.9	262.1654	C ₁₂ H ₂₄ NO ₅	262.1649	1.91	Ester Hydrolysis (2X) + B-oxidation(2X)
M3	5.3	290.1962	C ₁₄ H ₂₈ NO ₅	290.1963	0.68	Ester Hydrolysis (2X)+ B-oxidation(2X)
M4	6.1	318.2279	C ₁₆ H ₃₂ NO ₅	318.2275	1.25	Ester Hydrolysis (2X)
M5	15.98	442.4263	C ₂₇ H ₅₆ NO ₃	442.4255	1.8	N-Dealkylation of straight chain ester
M6	15.94	528.4633	C ₃₁ H ₆₂ NO ₅	528.4623	1.9	Ester Hydrolysis(1X) + B-oxidation (1X)
M7	16.00	556.4942	C ₃₃ H ₆₆ NO ₅	556.4936	1.1	Ester Hydrolysis(1X)
M8	13.92	572.4898	C ₃₃ H ₆₆ NO ₆	572.4885	2.3	Ester Hydrolysis(1X) + Aliphatic Hydroxylation
M9	13.75	544.4590	C ₃₁ H ₆₂ NO ₅	544.4572	3.3	Ester Hydrolysis(1X) + B-oxidation (1X)+ Hydroxylation
M10	13.83	542.4430	C ₃₁ H ₆₀ NO ₅	544.4430	2.7	Ester Hydrolysis(1X) + B-oxidation (1X)+ Hydroxylation + Dehydrogenation

ID	Retention Time (min)	Observed m/z	Proposed Formula [M+H] ⁺	Theo. m/z [M+H] ⁺	Error (ppm)	Proposed Biotransformation
M11	17.85	724.6461	C44H86NO5	724.6450	1.5	Aliphatic Hydroxylation + Dehydrogenation
M12	15.02	833.5324	C41H77N4O11S	833.5304	2.4	Beta-oxidation + GSH conjugation

Table 3: Metabolites of SM102 in Plasma

Metabolite ID	m/z	RT (min)	Plasma 2 hour		Plasma 6 hour		Plasma 24 hour	
			EIC Area (Counts)	% Area	EIC Area (Counts)	% Area	EIC Area (Counts)	% Area
M1	204.1594	3.51	4.29E+04	0.00%	2.37E+04	0.01%	0.00E+00	0.00%
M2	262.1649	3.92	ND	ND	ND	ND	ND	ND
M3	290.1962	5.1	4.07E+05	0.02%	1.18E+05	0.07%	5.66E+03	0.06%
M4	318.2275	6.1	2.30E+05	0.01%	1.30E+05	0.08%	3.76E+04	0.40%
M5	442.4255	15.98	8.91E+04	0.00%	6.74E+04	0.04%	3.92E+04	0.41%
M6	528.4623	15.91	4.75E+06	0.23%	2.29E+06	1.39%	6.81E+04	0.71%
M7	556.4936	16	6.49E+06	0.31%	2.21E+06	1.34%	6.62E+04	0.69%
M8	572.4885	13.91	2.10E+04	0.00%	1.52E+04	0.01%	ND	ND
M9	544.4572	13.73	ND	ND	ND	ND	ND	ND
M10	542.4415	13.83	ND	ND	ND	ND	ND	ND
M11	724.645	17.86	8.65E+04	< 0.01 %	8.13E+03	< 0.01%	4.84E+03	0.05%
M12	833.5304	15.02	ND	ND	ND	ND	ND	ND
Parent SM102	710.6657	19.3	2.09E+09	99.42%	1.60E+08	97.05%	9.30E+06	97.67%

ND = Below Detection Limit

Table 4: Metabolites of SM-102 in Urine Pools

Metabolite ID	m/z	RT (min)	Urine 0-2 hr		Urine 2-6 hr		Urine 6-24 hr	
			EIC Area (Counts)	% Area	EIC Area (Counts)	% Area	EIC Area (Counts)	% Area
M1	204.1594	3.51	3.89E+07	35.4%	1.90E+08	32.6%	1.23E+08	36.7%
M2	262.1649	3.92	1.52E+07	13.8%	7.80E+07	13.3%	3.64E+07	10.9%
M3	290.1962	5-5.5	1.36E+07	12.4%	2.59E+08	44.4%	1.36E+08	40.6%
M4	318.2275	6.1	4.24E+07	38.5%	5.62E+07	9.6%	3.93E+07	11.7%
M5	442.4255	15.98	Detected but no trend (Abundance similar to Predose samples)	NA	ND	NA	ND	NA
M6	528.4623	15.91	ND	NA	ND	NA	ND	NA
M7	556.4936	16	ND	ND	ND	ND	ND	ND
M8	572.4885	13.91	ND	ND	ND	ND	ND	ND
M9	544.4572	13.73	ND	ND	ND	ND	ND	ND
M10	542.4415	13.83	ND	ND	ND	ND	ND	ND
M11	724.645	17.86	ND	ND	ND	ND	ND	ND
M12	833.5304	15.02	ND	ND	ND	ND	ND	ND
Parent SM102	710.6657	19	1.04E+04	0.01%	3.64E+04	0.01%	9.25E+04	0.03%

ND = Below Detection Limit

Table 5: Metabolites of SM-102 in Bile

Metabolite ID	m/z	RT	Bile 0-2h		Bile 2-6h		Bile 6-24h	
			EIC Area (Counts)	% Area	EIC Area (Counts)	% Area	EIC Area (Counts)	% Area
M1	204.1594	3.51	1.45E+06	0.32%	1.28E+06	0.17%	1.17E+05	0.20%
M2	262.1649	3.92	1.74E+07	3.79%	7.46E+06	1.00%	1.00E+06	1.73%
M3	290.1962	5-5.5	1.89E+08	41.11%	4.60E+08	61.97%	2.37E+07	40.75%
M4	318.2275	6.1	3.05E+07	6.64%	3.84E+07	5.17%	3.84E+06	6.62%
M5	442.4255	15.98	4.58E+06	4.16%	1.01E+07	1.73%	6.13E+05	0.18%
M6	528.4623	15.91	2.38E+07	5.19%	3.06E+07	4.12%	2.25E+06	3.88%
M7	556.4936	16	2.65E+07	5.77%	2.47E+07	3.33%	1.46E+06	2.52%
M8	572.4885	13.91	4.46E+06	0.97%	1.18E+07	1.59%	1.51E+05	0.26%
M9	544.4572	13.73	5.17E+06	1.13%	1.34E+07	1.80%	3.63E+05	0.62%
M10	542.4415	13.83	4.67E+06	1.02%	1.69E+07	2.28%	4.90E+05	0.84%
M11	724.645	17.86	6.17E+06	1.34%	8.69E+06	1.17%	5.11E+04	0.09%
M12	833.5304	15.02	1.25E+06	0.27%	7.86E+06	1.06%	1.01E+06	1.73%
Parent SM102	710.6657	19	1.44E+08	31.46%	1.11E+08	14.97%	2.30E+07	39.69%

Table 6: Quantitation of Parent SM102 in Plasma, Urine and Bile Samples using LC-QQQ*

Matrix	Timepoint	Animal No.	Measured Concentration (ng/mL)
Plasma	Pre Dose	1	BQL
	Pre Dose	2	BQL
	Pre Dose	3	BQL
	2 hr	1	25334
	2 hr	2	23682
	2 hr	3	18609
	6 hr	1	1778
	6 hr	2	2945
	6 hr	3	474
	24 hr	1	25
	24 hr	2	17
	24 hr	3	83
Bile	Pre Dose	Pooled (1-3)	BQL
	2 hr	Pooled (1-3)	3140
	6 hr	Pooled (1-3)	2029
	24 hr	Pooled (1-3)	291
Urine	Pre	Pooled (1-3)	BQL
	2 hr	Pooled (1-3)	BQL
	6 hr	Pooled (1-3)	BQL
	24 hr	Pooled (1-3)	BQL

*Note

1. SM-102 Quantitation range in Rat Urine (0.2- 500 ng/mL) with LLOQ – 0.2 ng/mL
2. SM-102 Quantitation range in Rat Plasma and Bile (5- 500 ng/mL) with LLOQ – 5 ng/ml