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2.6.5.1 PHARMACOKINETICS: OVERVIEW

Type of Study	Test Article	Test System	Method of Administration	Testing Facility	Report Number	Location in eCTD
Distribution						
Single Dose IM tissue distribution study in male Sprague Dawley rats	mRNA-1647 ^a	Rat, Sprague Dawley	Single IM Dose	Charles River Laboratories, Sherbrooke, QC, Canada	5002121 Amendment 1	4.2.2.3

Abbreviations: CMV = cytomegalovirus; gB = glycoprotein B; gH = glycoprotein; gL = glycoprotein L; eCTD = electronic common technical document; 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.

2.6.5.2 ANALYTICAL METHODS AND VALIDATION REPORTS

Species/ Sample Matrix	Analyte	Calibration Range	Type of Assay	Method Utilized	Noteworthy Details (Qualification ^a)	GLP Compliance	Report Number
Rat/ plasma and tissues	mRNA-1647	The LLOQs for plasma and tissues were 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.	bDNA multiplex assay	QuantiGene 2.0 Plex assay	A multiplex bDNA 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.	No	5002121 Amendment 1

Abbreviations: bDNA = branched DNA; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; LLOQ = lower limit of quantification.

^a The method was not formally validated.

Source: Report [5002121 Amendment 1](#).

2.6.5.5 PHARMACOKINETICS: ORGAN DISTRIBUTION

Test Article: mRNA-1647

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

Number per Group: 5 animals terminated per time point (35 total)

Report Number: 5002121 Amendment 1

GLP Study: No

Feeding Condition: Ad libitum

Dose Level: 100 µg

Vehicle/Formulation: PBS, pH 7.2

Sample Matrix: Plasma and tissues

Plasma and Tissue Collection Time Points: 0 (pre-dose), 2, 8, 24, 48, 72, and 120 h post-dose

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	
	UL130	NC	NC	NC	NC	NC	
	UL131A	8.0	0.186 ± 0.0829	2.05 ± 0.912	NC	0.0825	

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
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	
	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	

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
Injection site, muscle	gB	2.0	1,770 ± 803	27,100 ± 4,880	13.5	1190	939
	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	8.0	427 ± 210	12,100 ± 2,830	15.0	487	
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-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
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 1 ([Appendix 8](#), [Table 2](#) and [Table 3](#)).