Supplementary Information

Characterizing the Mechanism of Action for mRNA Therapeutics for the Treatment of Propionic Acidemia, Methylmalonic Acidemia, and Phenylketonuria

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Supplementary Table 1. Summary of plasma hPCCA mRNA and hPCCB mRNA PK parameters in male and female *Pcca*$^{-/-}$ (A138T) mice and male and females combined following a single IV bolus injection of mRNA-3927.

Analyte	**hPCCA**			**hPCCB**		
Sex	Male (n=9)	Female (n=9)	Combined (n=18)	Male (n=9)	Female (n=9)	Combined (n=18)
Dose	1.0 mg/kg					
AUC_{tlast} (hr·ng/mL)	8600	6290	7400	7180	4720	5880
C_{max} (ng/mL)	1080	1080	1080	1010	776	892
$T_{1/2}$ (hr)	5.37	4.99	5.39	4.45	4.47	4.92
CL (mL/hr/kg)	116	159	135	139	212	170
V_z (mL/kg)	900	1140	1050	894	1370	1210
Dose	2.0 mg/kg					
AUC_{tlast} (hr·ng/mL)	29400	18800	24000	20700	9280	14900
C_{max} (ng/mL)	2530	1930	2180	1710	1080	1350
$T_{1/2}$ (hr)	6.13	4.07	5.50	5.72	4.40	5.47
CL (mL/hr/kg)	67.6	107	83.0	96.5	215	134
V_z (mL/kg)	598	626	659	797	1370	1060

AUC_{tlast}, area under the concentration versus time curve from the last start of dose administration to the time after dosing; C_{max}, maximum observed concentration; CL, clearance; hPCCA, human propionyl-CoA carboxylase α subunit; hPCCB, human propionyl-CoA carboxylase β subunit; IV, intravenous; mRNA, messenger RNA; PK, pharmacokinetic; $T_{1/2}$, terminal half-life; V_z, volume of distribution based on the terminal elimination phase.

Supplementary Table 2. Summary of plasma disease biomarker AUC below baseline in mRNA-3927 or control treated *Pcca*$^{-/-}$ (A138T) mice.

	Control (Tris-sucrose) n=6	**mRNA-3927 (0.5 mg/kg) n=5~6**
AUC_below_B (mean ± SD)		
2-MC (week·μM)	2.37 ± 2.32	8.71 ± 3.49
3-HP (week·μM)	37.7 ± 37.0	170 ± 163
C3/C2 ratio (week·ratio)	0.124 ± 0.187	1.23 ± 0.916
PCC Activity (mean ±SD)	2.72 ± 2.26	7.77 ± 4.27

2-MC; 2-methylcitrate; 3-HP, 3-hydroxypropionate; AUC_below_B; area under the curve that is below the baseline and above the response curve; C3/C2, ratio of propionylcarnitine to acetylcarnitine; mRNA, messenger ribonucleic acid; PCC, propionyl-CoA carboxylase; SD, standard deviation.

Supplementary Table 3. Summary of plasma hMUT mRNA PK parameters following a single IV bolus administration of mRNA-3705 in CD1 mice.

Sex	**Male n=12**	**Female n=12**	**Combined[a] n=24**
Dose[b]	0.1 mg/kg		
AUC_{tlast} (hr·ng/mL)	1340	662	1010
C_{max} (ng/mL)	517	232	375
$T_{1/2}$ (hr)	4.05	18.7[c]	6.42
CL (mL/hr/kg)	74.3	149[c]	99.2
V_z (mL/kg)	434	4020[c]	919
Dose	0.5 mg/kg		
AUC_{tlast} (hr·ng/mL)	13900	8290	11100
C_{max} (ng/mL)	3750	1480	2620
$T_{1/2}$ (hr)	4.90	4.43	4.93
CL (mL/hr/kg)	35.9	60.2	45.0
V_z (mL/kg)	253	385	320
Dose	1.0 mg/kg		
AUC_{tlast} (hr·ng/mL)	36600	26100	31200
C_{max} (ng/mL)	6140	4740	5150
$T_{1/2}$ (hr)	8.57	4.77	7.33
CL (mL/hr/kg)	27.1	38.2	31.8
V_z (mL/kg)	335	263	337

AUC_{tlast}, area under the concentration versus time curve from the last start of dose administration to the time after dosing; CL, clearance; C_{max}, maximum observed concentration; hMUT, human methylmalonyl-CoA mutase; PK, pharmacokinetic; IV, intravenous; mRNA, messenger RNA; $T_{1/2}$, terminal half-life; V_z, volume of distribution based on the terminal elimination phase.

[a]Sex differences were observed, values presented for information purpose only.

[b]Dose level refers to the average total amount of mRNA in the administered LNPs.

[c]Values presented for information purpose only.

Supplementary Table 4. Descriptive statistics of liver hMUT protein concentrations, liver MUT enzyme activity, and tissue methylmalonic acid concentrations in mRNA-3705 or control treated *Mut*$^{-/-}$;Tg$^{INS\text{-}CBA\text{-}G715V}$ mice at the end of the single-dose study.

	Control n=4	**mRNA-3705 (0.2 mg/kg) n=5**	**mRNA-3705 (0.5 mg/kg) n=4[a]**
Liver hMUT protein concentrations (ng/mg protein)			
Mean ± SD	0.0 ± 0.0	45.0 ± 42.2	70.4 ± 24.2
P value	-	0.0836	0.0136
Liver MUT enzyme activity (nmol/min/mg protein)			
Mean ± SD	2.8 ± 0.2	5.7 ± 2.8	10.7 ± 6.5
P-value	-	0.4577	0.0317
Plasma methylmalonic acid concentrations (µM)			
Baseline (Day -7)	892 ± 241	614 ± 190	589 ± 206
Day 1	1260 ± 280	425 ± 422	114 ± 20
P value	0.0732	0.4085	0.0201
Tissue methylmalonic acid concentrations 24 hours post-dose (nmol/g tissue)			
Liver			
Mean ± SD	4662.5 ± 1752.2	1091.9 ± 1457.5	7.9 ± 15.8
P value	-	0.0046	0.0011
Kidney			
Mean ± SD	3480.0 ± 1612.1	1094.0 ± 1245.6	189.7 ± 128.9
P value	-	0.0240	0.0052
Heart			
Mean ± SD	124.1 ± 37.1	43.6 ± 61.5	8.1 ± 16.3
P value	-	0.0411	0.0080

hMUT, human methylmalonyl-CoA mutase; mRNA, messenger RNA; SD, standard deviation.

[a]One animal in the mRNA-3705 mg/kg group received only a partial dose; therefore, the sample was removed from the analysis. For plasma methylmalonic acid, a two-way repeated measure ANOVA followed by Šídák's multiple comparisons test was used. For tissue methylmalonic acid concentrations, liver MUT protein concentrations, and liver protein activity, one-way ANOVAs followed by Dunnett's pairwise comparison tests were performed. For all statistical tests, two-tailed p-values <0.05 were considered statistically significant.

Supplementary Table 5. Summary of serum mRNA from mRNA-3210 PK parameters in male or female PAH^{enu2} mice following a single IV dose of mRNA-3210.

Sex	**Male n=4**	**Female n=4**	**Combined n=8**
Dose	0.25 mg/kg		
$AUC_{0\text{-}168}$ (hr·ng/mL)	2450	1780	2115
C_{max} (ng/mL)	453	434	444
Eff $T_{1/2}$ (hr)	4.89	3.46	4.18
CL (mL/hr/kg)	NA	141	-
V_z (mL/kg)	NA	3210	-
Dose	0.5 mg/kg		
$AUC_{0\text{-}168}$ (hr·ng/mL)	5880	4620	5250
C_{max} (ng/mL)	876	959	918
Eff $T_{1/2}$ (hr)	4.80	3.64	4.22
CL (mL/hr/kg)	85.1	108	96.6
V_z (mL/kg)	2360	2560	2460
Dose	1.0 mg/kg		
$AUC_{0\text{-}168}$ (hr·ng/mL)	11,200	31,700	21,450
C_{max} (ng/mL)	1640	1850	1750
Eff $T_{1/2}$ (hr)	5.29	19.9	12.6
CL (mL/hr/kg)	89.5	31.6	60.6
V_z (mL/kg)	2480	395	1438

$AUC_{0\text{-}168}$, area under the concentration-time curve from 0 to 168 hours; CL, clearance; C_{max}, maximum observed concentration; PK, pharmacokinetic; IV, intravenous; mRNA, messenger RNA; Eff $T_{1/2}$, Effective terminal half-life; V_z, volume of distribution based on the terminal elimination phase.

Supplementary Table 6. Summary of liver mRNA from mRNA-3210 PK parameters in male and female mice combined following a single IV dose of mRNA-3210

PK parameter	**mRNA-3210 (0.25 mg/kg) n=8**	**mRNA-3210 (0.5 mg/kg) n=8**	**mRNA-3210 (1.0 mg/kg) n=8**
$AUC_{0\text{-}168}$ (hr·ng/mg/mg/kg)	1.03	1.96	6.28
C_{max} (ng/mg)	0.133	0.178	0.504
T_{max} (hr)	2	2	2
Eff $T_{1/2}$ (hr)	10.7	10.5	11.1

$AUC_{0\text{-}168}$, area under the concentration-time curve from 0 to 168 hours; C_{max}, maximum observed concentration; PK, pharmacokinetic; IV, intravenous; mRNA, messenger RNA; Eff $T_{1/2}$, Effective terminal half-life; T_{max}, time to maximum observed concentration.

Supplementary Table 7. Summary statistics for PD Parameters of liver hPAH protein and blood phenylalanine in from mRNA-3210 treated PAH^{enu2} mice.

	mRNA-3210 (0.25 mg/kg) n=8	**mRNA-3210 (0.5 mg/kg) n=8**	**mRNA-3210 (1.0 mg/kg) n=8**
hPAH Protein[a]			
TE_{max} (hr)	24	24	8
E_{max} (ng/g)	6950	16,700	35,600
TE_{last} (h), median	96	168	168
$AUEC_{0\text{-}168}$ (h·ng/g), mean	498,000	1,130,000	2,090,000
Phenylalanine			
TE_{max} (h), median (min-max)	12 (1-24)	24 (24-24)	48 (12-48)
E_{max} (µmol/L), mean ± SD	1105 ± 209	561 ± 279	138 ± 78
TE_{last} (h), median (min-max)	168 (168-168)	168 (168-168)	168 (168-168)
AUC_below_B, mean ± SD	21,502 ± 15,533	64,960 ± 25,013	150,044 ± 52,699

$AUC_{0\text{-}168}$, area under the concentration-time curve from 0 to 168 hours; AUC_below_B; area under the curve that is below the baseline and above the response curve; C_{max}, maximum observed concentration; E_{max}, maximum effect; hPAH, human phenylalanine hydroxylase; mRNA, messenger ribonucleic acid; PD, pharmacodynamics; PK, pharmacokinetic; TE_{last}, time to last measurable effect; TE_{max}, time to maximum effect; T_{last}, time to last measurable concentration.

[a]PD parameters generated using sparse samples.

Supplementary Table 8. List of studies and analytes used for development of PK/PD the mRNA-3927 model.

Study	Test article	IV dose (mg/kg)	Dosing frequency	Species	Strain	Analytes
2308-060	mRNA-3927	1.0 and 2.0	Single dose	Mouse	*Pcca*$^{-/-}$ (A138T)	Plasma hPCCA and hPCCB mRNA
01070005	mRNA-3927	1.0, 3.0, and 9.0	Q2W × 3	Rat	Sprague-Dawley	Plasma hPCCA and hPCCB mRNA
5002694	mRNA-3927	1.0, 3.0, and 5.0	Q2W × 3	Monkey	Cynomolgus	Plasma hPCCA and hPCCB mRNA
2357, 2514, 2524	hPCCA/hPCCB mRNA[a]	1.0	Single dose	Mouse	*Pcca*$^{-/-}$ (A138T)	Liver hPCCA and hPCCB protein
3037	mRNA-3927	0.2, 0.5, 1.0, and 2.0	Single dose	Mouse	*Pcca*$^{-/-}$ (A138T)	Plasma 2-MC, 3-HP, and C3/C2
3082	mRNA-3927	0.5 and 2.0	Q3W × 4	Mouse	*Pcca*$^{-/-}$ (A138T)	Plasma 2-MC, 3-HP, and C3/C2

2-MC, 2-methylcitrate; 3-HP, 3-hydroxypropionate; C3/C2, ratio of propionylcarnitine to acetylcarnitine; hPCCA, human propionyl-CoA carboxylase α subunit; hPCCB, human propionyl-CoA carboxylase β subunit; IV, intravenous; mRNA, messenger RNA; PD, pharmacodynamic; PK, pharmacokinetic; Q2W, every 2 weeks; Q3W, every 3 weeks.

[a]PCCA and PCCB mRNA co-dosed in a 1:1 ratio.

Supplementary Table 9. List of studies and analytes used for development of PK/PD the mRNA-3705 model.

Study	Test article	IV dose (mg/kg)	Dosing frequency	Species	Strain	Analytes
20238405	mRNA-3705	0.1, 0.5, and 1.0	Single dose	Mouse	CD1	Plasma hMUT mRNA Liver hMUT protein
	CX-020629 in SM86/DMG	1.0				
20238406	mRNA-3705	0.1, 0.5, and 1.0	Single dose	Rat	Sprague-Dawley	Plasma hMUT mRNA
2023408	mRNA-3705	0.2 and 1.0	Single dose	Monkey	Cynomolgus	Plasma hMUT mRNA
3767	mRNA-3705	0.2 and 0.5	QM × 2	Mouse	*Mut*$^{-/-}$;TgINS-CBA-G715V hypomorphic	Plasma methylmalonic acid
	CX-020629 in SM86/DMG	0.5				
3893	mRNA-3705	0.2, 0.5, and 1.0	6 doses administered on Day 0, Week 4, Week 6, Week 8, Week 10, and Week 12	Mouse	*Mut*$^{-/-}$;TgINS-CBA-G715V hypomorphic	Plasma methylmalonic acid
	CX-020629 in SM86/DMG	0.5				
3495	mRNA-3705	0.2 and 0.5	Single dose	Mouse	*Mut*$^{-/-}$;TgINS-CBA-G715V hypomorphic	Plasma methylmalonic acid

DMG, dimyristoyl glycerol; hMUT, human methylmalonyl-CoA mutase; IV, intravenous; LNP, lipid nanoparticle; mRNA, messenger RNA; PD, pharmacodynamic; PK, pharmacokinetic; QM, monthly.

Note: mRNA-3705 consists of CX-020629 mRNA formulated in SM-86/OL56 LNP; CX-020629 in SM-86/DMG consists of CX-020629 formulated in SM-86/DMG LNP.

Supplementary Table 10. List of studies and analytes used for development of PK/PD the mRNA-3210 model.

Study	Test article	IV dose (mg/kg)	Dosing frequency	Species	Strain	Analytes
5003937	mRNA-3210	0.25, 0.5, and 1.0	Single dose	Mouse	B6.BTRB-*Pah*^{enu2}	Blood Phe Serum and liver hPAH mRNA Liver hPAH protein
5003975	mRNA-3210	0.5	Single dose	Mouse	C57BL/6 B6.BTRB-*PAH*$^{enu2+/+}$ (wild-type)	Serum and liver hPAH mRNA Liver hPAH protein
5003976	mRNA-3210	0.25, 0.5, and 1.0	QW × 3	Mouse	B6.BTRB-*PAH*^{enu2}	Blood Phe Liver hPAH protein
20358989	mRNA-3210	0.5	Single dose	Monkey	Cynomolgus	Serum hPAH mRNA Liver hPAH protein
20358990	mRNA-3210	1.0, 3.0, and 6.0	QW × 3	Monkey	Cynomolgus	Serum hPAH mRNA

enu2, N-ethyl-N-nitrosourea, hPAH, human phenylalanine hydroxylase; mRNA, messenger RNA; PAH, phenylalanine hydroxylase; PD, pharmacodynamic; Phe, phenylalanine; PK, pharmacokinetic; QW, weekly.