A SINGLE DOSE PHARMACOKINETICS STUDY OF ALC-0315 AND ALC-0159 FOLLOWING INTRAVENOUS BOLUS INJECTION OF PF-07302048 NANOPARTICLE FORMULATION IN WISTAR HAN RATS

1. SUMMARY

Following a single IV bolus administration of a luciferase-encoding modRNA formulated in an LNP with an identical lipid composition as PF-07302048 (SARS-CoV-2 mRNA Vaccine; BioNTech code number BNT162) at 1 mg/kg to male Wistar Han rats, plasma concentrations of ALC-0315 and ALC-0159 (the novel excipients in PF-07302048) decreased rapidly, with initial $t_{½}$ of 1.62 and 1.72 h, respectively. ALC-0315 and ALC-0159 were then cleared from plasma, resulting in terminal elimination $t_{½}$ of 139 and 72.7 h, respectively.

The estimated percent of dose distributed to the liver was ~60% for ALC-0315 and ~20% for ALC-0159. The percent of dose excreted unchanged in feces was ~1% for ALC-0315 and ~50% for ALC-0159. The percent of dose excreted unchanged in the urine was not calculated due to all values being BLQ.

2. OBJECTIVE

The PF-07302048 vaccine LNP formulation contains two novel excipients, ALC-0315 and ALC-0159, in the nanoparticle. The objective of this study is to assess the pharmacokinetics and elimination of ALC-0315 and ALC-0159 following a single IV bolus administration of a luciferase-encoding modRNA with an identical nanoparticle lipid composition as PF-07302048 at 1 mg/kg to male Wistar Han rats.

3. MATERIALS AND METHODS

3.1. Preparation of Doses

	IV
Study ID	PF-07302048_06Jul20_072424
Compound lot number	FM-1261-A
Dose of modRNA (mg/kg)	1 (1.96 mg/kg ALC-0159, 15.3 mg/kg ALC-0315)
Formulation concentration (mg/mL)	1
Dose volume (mL/kg)	1

3.2. Study Conduct

	IV
In-life location	Pfizer[a]
Species (strain)	Rat (Wistar Han)
Sex/number of animals	Male/3 animals per time point[b]
Feeding Condition	Fasted
Administration type	IV bolus
Administration site	Lateral tail vein
Sampling site	Inferior vena cava
Blood and liver sampling time points (h postdose)	Predose, 0.1, 0.25, 0.5, 1, 3, 6, 24, 48, 96, 192, 336
Anticoagulant	K_2EDTA
Urine and feces sample collection interval (h postdose)[c]	Predose (-24-0), 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, 216-240, 240-264, 264-288, 288-312, 312-336

a. Pfizer Worldwide Research, Development and Medical, San Diego, CA.
b. Non-serial sampling, 36 animals total.
c. Urine and feces were collected from animals 34-36, placed in metabolism cages.

3.3. Bioanalytical Summary for Quantitation of ALC-0315 and ALC-0159 in Plasma, Liver Homogenates, Urine, and Feces Homogenates

Bioanalytical Platform (instrument)	LC-MS/MS (AB Sciex QTRAP 5500)		
Mobile phase	A: 0.1% formic acid with 10 mM ammonium formate B: Acetonitrile with 10 mM ammonium formate and 0.1% formic acid		
Flow rate	0.25 mL/min		
Gradient	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.1	0	100
	0.5	0	100
	2.5	55	45
	4.2	55	45
	4.5	0	100
	6.5	0	100
Column	Waters Atlantis HILIC Silica 2.1 × 100 mm, 3µ		
Detection mode	Positive selected reaction monitoring mode		
Data collection software/ version	Analyst Version 1.7.1		
Data analysis software/ version	Watson Version 7.5		
MRM transitions	766.9→510.7 (ALC-0315) 839.2→494.7 (ALC-0159) 837.1→264.6 (PEG-2000, ISTD)		

Additional conditions can be found in the analysis files referenced in Section 5 (Archiving).

3.4. Method Summary

1. In brief, 20 µL aliquots of plasma, liver homogenate (sections from 3 areas in the liver were homogenized, pooled, and diluted 1:20 or 1:100 with blank sample matrix when necessary), urine, and feces homogenate (diluted 1:10 or 1:20 with blank sample matrix when necessary) samples and standards were subjected to protein precipitation with acetonitrile containing an internal standard, PEG-2000. Samples were vortexed and centrifuged to obtain supernatant, which was analyzed using LC-MS/MS.

2. Method information was recorded and archival records are available as described in Section 5 (Archiving).

3. Analyst® was used to measure peak areas and peak area ratios of analyte to internal standard were calculated. A calibration curve was constructed from the peak area ratios (analyte to internal standard) with a quadratic (1/x) regression using Watson LIMS. The linear dynamic ranges of the standard curves for ALC-0315 and ALC-0159 were 4.88 to 2500 ng/mL for plasma, 19.53 to 10000 ng/g for liver, 4.88 to 2500 ng/mL for urine, and 6.592 to 3375 ng/g for feces.

3.5. Data Analysis

Generation and analyses of pharmacokinetic data were conducted at Pfizer Inc. The pharmacokinetic parameters were determined from pooled animal data using non-compartmental analysis in Watson LIMS 7.5. For PK calculations, 0 µg/ml was used for C_0 for both ALC-0315 and ALC-0159. For pharmacokinetic definitions and calculations, see Appendix 8.1. BLQ concentrations were not used in the PK calculations.

4. RESULTS AND DISCUSSION

Mean pharmacokinetic parameters of ALC-0315 and ALC-0159 following administration of a single IV dose of a luciferase-encoding modRNA formulated in an LNP with an identical lipid composition as PF-07302048 to male Wistar Han rats at 1 mg/kg are shown in Supportive Table 6.1. Mean concentration-time data of ALC-0315 and ALC-0159 from plasma, liver homogenates, urine, and feces are shown in Supportive Tables 6.2, 6.3, 6.5, and 6.6. The ratio of plasma concentrations of ALC-0315:ALC-0159 is shown in Supportive Table 6.4. The concentration-time profiles of plasma, liver, and feces following IV administration of the LNP are shown in Supportive Figures 7.1, 7.2, and 7.3, respectively.

Following a single IV dose of a luciferase-encoding modRNA formulated in an LNP with an identical lipid composition as PF-07302048 at 1 mg/kg to Wistar Han rats, plasma concentrations of ALC-0315 and ALC-0159 decreased rapidly, with initial $t_{½}$ values of 1.62 and 1.72 h, respectively. ALC-0315 and ALC-0159 were then cleared from plasma, resulting in terminal elimination $t_{½}$ of 139 and 72.7 h, respectively.

The estimated percent of dose distributed to the liver was ~60% for ALC-0315 and ~20% for ALC-0159. The percent of dose excreted unchanged in feces was ~1% for ALC-0315 and ~50% for ALC-0159. The percent of dose excreted unchanged in the urine was not calculated due to values being BLQ.

5. ARCHIVING

Data presented in this report can be found in the following locations:

	Experimental Data
E-Workbook	/Root/PDM/ (b) (6) VBN#00701419/VR_LNP/20200801 PF-07302048_06Jul20_072424_PLM /Root/PDM/ (b) (6) VBN#00701419/VR_LNP/20200805 PF-07302048_06Jul20_072424_liver /Root/PDM/ (b) (6) VBN#00701419/VR_LNP/20200806 PF-07302028_06Jul20_072424_Urine /Root/PDM/ (b) (6) VBN#00701419/VR_LNP/20200809 PF-07302048_06Jul20_072424_Feces
Watson LIMS	PROJECT ID: PF-07302048 STUDY ID: Covidvac_072414PK

	Bioanalytical Data
E-Workbook	/Root/PDM/ (b) (6) VBN#00701419/VR_LNP/Assay Development/LC-MSMS method for COVID-19 Excipients
OpenLAB LAJ_PDM	\COMPOUND\PF-07302048\Covidvac_072424PK\PLM.wiff

6. SUPPORTIVE TABLES

6.1. Summary of Mean Plasma Pharmacokinetic Parameters of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

Species (Strain)	Rat (Wistar Han)	
Sex/Number of Animals	Male/ 3 animals per timepoint[a]	
Feeding Condition	Fasted	
Method of Administration	IV	
Dose modRNA (mg/kg)	1	
Dose ALC-0159 (mg/kg)	1.96	
Dose ALC-0315 (mg/kg)	15.3	
Sample Matrix	Plasma	
Sampling Time Points (h post dose):	Predose, 0.1, 0.25, 0.5, 1, 3, 6, 24, 48, 96, 192, 336	
Analyte	ALC-0315	ALC-0159
PK Parameters:	Mean[b]	Mean[b]
AUC_{inf} (µg•h/mL)[c]	1030	99.2
AUC_{last} (µg•h/mL)	1020	98.6
Initial $t_{½}$ (h)[d]	1.62	1.74
Terminal elimination $t_{½}$ (h)[e]	139	72.7
Estimated fraction of dose distributed to liver (%)[f]	59.5	20.3
Dose in Urine (%)	NC[g]	NC[g]
Dose in Feces (%)	1.05	47.2

a. Non-serial sampling, 36 animals total.
b. Only mean PK parameters are reported due to non-serial sampling.
c. Calculated using the terminal log-linear phase (determined using 48, 96, 192, and 336 h for regression calculation).
d. ln(2)/initial elimination rate constant (determined using 1,3, and 6 h for regression calculation).
e. ln(2)/terminal elimination rate constant (determined using 48, 96, 192, and 336 for regression calculation).
f. Calculated as follows: highest mean amount in the liver (µg)/total mean dose (µg) of ALC-0315 or ALC-0159.
g. Not calculated due to BLQ data.

6.2. Summary of Mean and Individual Plasma and Liver Concentrations of ALC-0315 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

Animal Number	Animal weight (g)	Time (h postdose)	Plasma (µg/mL)	Liver (µg/g)	Liver weight (g)	Total amount in liver (µg)
1	--	Predose	BLQ	BLQ	5.78	BLQ
2	--	Predose	BLQ	BLQ	5.57	BLQ
3	--	Predose	BLQ	BLQ	5.89	BLQ
Mean ± SD	**--**	**Predose**	**BLQ ± NA**	**BLQ ± NA**	**--**	**BLQ ± NA**
4	221	0.1	456	28.5	5.74	163
5	225	0.1	564	30.2	5.62	170
6	220	0.1	435	30.9	5.52	171
Mean ± SD	**--**	**0.1**	**485 ± 69.2**	**29.9 ± 1.23**	**--**	**168 ± 3.93**
7	222	0.25	418	74.1	5.94	440
8	227	0.25	451	90.6	5.91	536
9	218	0.25	191	29.0	5.54	161
Mean ± SD	**--**	**0.25**	**353 ± 142**	**64.6 ± 31.9**	**--**	**379 ± 195**
10	228	0.5	356	164	6.49	1060
11	217	0.5	333	143	5.87	839
12	224	0.5	423	140	5.03	705
Mean ± SD	**--**	**0.5**	**371 ± 46.8**	**149 ± 13.1**	**--**	**869 ± 182**
13	212	1	204	228	6.04	1380
14	221	1	208	228	6.29	1430
15	238	1	172	240	6.40	1540
Mean ± SD	**--**	**1**	**195 ± 19.7**	**232 ± 6.93**	**--**	**1450 ± 80.3**
16	220	3	85.3	282	6.45	1820
17	209	3	71.2	318	5.63	1790
18	210	3	83.1	282	5.62	1580
Mean ± SD	**--**	**3**	**79.9 ± 7.59**	**294 ± 20.8**	**--**	**1730 ± 129**
19	222	6	24.2	270	6.04	1630
20	222	6	20.2	279	5.50	1530
21	222	6	24.4	256	6.56	1680
Mean ± SD	**--**	**6**	**22.9 ± 2.37**	**268 ± 11.6**	**--**	**1620 ± 74.4**
22	228	24	1.13	277	7.26	2010
23	229	24	1.11	281	7.48	2100
24	231	24	0.861	285	7.52	2140
Mean ± SD	**--**	**24**	**1.03 ± 0.150**	**281 ± 4.00**	**--**	**2090 ± 68.4**
25	222	48	0.253	218	7.59	1650
26	228	48	0.339	206	7.90	1630
27	223	48	0.399	166	7.39	1230
Mean ± SD	**--**	**48**	**0.330 ± 0.0734**	**197 ± 27.2**	**--**	**1500 ± 240**
28	216	96	0.188	187	7.84	1470
29	224	96	0.122	114	8.81	1000
30	242	96	0.192	97.1	9.86	957
Mean ± SD	**--**	**96**	**0.167 ± 0.0393**	**133 ± 47.8**	**--**	**1140 ± 282**
31	216	192	0.0812	66.9	9.10	609
32	212	192	0.135	60.7	8.34	506
33	233	192	0.110	90.3	9.59	866
Mean ± SD	**--**	**192**	**0.109 ± 0.0269**	**72.6 ± 15.6**	**--**	**660 ± 185**
34	210	336	0.0538	53.6	10.1	541
35	213	336	0.0724	95.3	8.13	774
36	219	336	0.0801	62.2	11.0	682
Mean ± SD	**--**	**336**	**0.0688 ± 0.0135**	**70.4 ± 22.0**	**--**	**666 ± 118**

The limit of quantitation was 0.00488 µg/mL for plasma and 0.01953 µg/g for liver.

6.3. Summary of Mean and Individual Plasma and Liver Concentrations of ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

Animal Number	Animal weight (g)	Time (h postdose)	Plasma (µg/mL)	Liver (µg/g)	Liver weight (g)	Total amount in liver (µg)
1	--	Predose	BLQ	BLQ	5.78	BLQ
2	--	Predose	BLQ	BLQ	5.57	BLQ
3	--	Predose	BLQ	BLQ	5.89	BLQ
Mean ± SD	**--**	**Predose**	**BLQ ± NA**	**BLQ ± NA**	**--**	**BLQ ± NA**
4	221	0.1	48.8	7.07	5.74	40.6
5	225	0.1	57.9	8.03	5.62	45.1
6	220	0.1	43.9	7.65	5.52	42.3
Mean ± SD	**--**	**0.1**	**50.2 ± 7.10**	**7.58 ± 0.483**	**--**	**42.7 ± 2.32**
7	222	0.25	37.9	10.8	5.94	64.1
8	227	0.25	41.9	12.5	5.91	73.9
9	218	0.25	18.6	4.48	5.54	24.8
Mean ± SD	**--**	**0.25**	**32.8 ± 12.5**	**9.26 ± 4.23**	**--**	**54.3 ± 26.0**
10	228	0.5	33.8	15.0	6.49	97.3
11	217	0.5	28.3	14.4	5.87	84.5
12	224	0.5	29.0	16.2	5.03	81.5
Mean ± SD	**--**	**0.5**	**30.4 ± 2.99**	**15.2 ± 0.917**	**--**	**87.8 ±8.40**
13	212	1	14.1	15.2	6.04	91.8
14	221	1	18.5	13.3	6.29	83.6
15	238	1	15.2	14.3	6.40	91.5
Mean ± SD	**--**	**1**	**15.9 ± 2.29**	**14.3 ± 0.950**	**--**	**89.0 ± 4.63**
16	220	3	7.67	12.6	6.45	81.3
17	209	3	5.55	12.9	5.63	72.6
18	210	3	6.64	13.6	5.62	76.4
Mean ± SD	**--**	**3**	**6.62 ± 1.06**	**13.0 ± 0.513**	**--**	**76.8 ± 4.35**
19	222	6	1.94	7.74	6.04	46.8
20	222	6	1.98	7.12	5.50	39.1
21	222	6	2.50	7.80	6.56	51.2
Mean ± SD	**--**	**6**	**2.14 ± 0.312**	**7.55 ± 0.376**	**--**	**45.7 ± 6.09**
22	228	24	0.270	2.14	7.26	15.5
23	229	24	0.251	1.58	7.48	11.8
24	231	24	0.223	1.64	7.52	12.3
Mean ± SD	**--**	**24**	**0.248 ± 0.0236**	**1.79 ± 0.307**	**--**	**13.2 ± 2.01**
25	222	48	0.113	0.565	7.59	4.29
26	228	48	0.105	0.593	7.90	4.69
27	223	48	0.0842	0.546	7.39	4.03
Mean ± SD	**--**	**48**	**0.101 ± 0.0149**	**0.568 ± 0.0236**		**4.34 ± 0.329**
28	216	96	0.0631	0.216	7.84	1.69
29	224	96	0.0385	0.138	8.81	1.22
30	242	96	0.0524	0.148	9.86	1.46
Mean ± SD	**--**	**96**	**0.0513 ± 0.0123**	**0.167 ± 0.0424**	**--**	**1.46 ± 0.239**
31	216	192	0.0182	0.0647	9.10	0.589
32	212	192	0.0204	0.0553	8.34	0.461
33	233	192	0.0226	0.0619	9.59	0.593
Mean ± SD	**--**	**192**	**0.0204 ± 0.00220**	**0.0606 ± 0.00483**	**--**	**0.548 ± 0.0750**
34	210	336	0.00568	BLQ	10.1	BLQ
35	213	336	0.00619	BLQ	8.13	BLQ
36	219	336	0.00639	BLQ	11.0	BLQ
Mean ± SD	**--**	**336**	**0.00609 ± 0.000366**	**BLQ ± NA**	**--**	**BLQ ± NA**

The limit of quantitation was 0.00488 µg/mL for plasma and 0.01953 µg/g for liver.

6.4. Summary of the Ratio of ALC-0315:ALC-0159 in Plasma of Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

Time postdose (h)	Mean plasma concentration ALC-0315 (µg/mL)	Mean plasma concentration ALC-0159 (µg/mL)	Ratio (ALC-0315)/(ALC-0159)[a]
0	BLQ	BLQ	NA
0.1	485	50.2	9.66
0.25	353	32.8	10.8
0.5	371	30.4	12.2
1	195	15.9	12.3
3	79.9	6.62	12.1
6	22.9	2.14	10.7
24	1.03	0.248	4.15
48	0.330	0.101	3.27
96	0.167	0.0513	3.26
192	0.109	0.0204	5.34
336	0.0688	0.00609	11.3

The limit of quantitation was 0.00488 µg/mL.
a. Ratio prior to injection is 7.8 (15.3 mg/kg/1.96 mg/kg)

6.5. Summary of Mean and Individual Urine Concentrations of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

	ALC-0315				ALC-0159			
	Individual Animal			Mean ± SD	Individual Animal			Mean ± SD
	Animal No. 34	Animal No. 35	Animal No. 36		Animal No. 34	Animal No. 35	Animal No. 36	
Animal Weight (g)	210	213	219	--	210	213	219	--
Time (h postdose)	Urine Concentration (ng/mL)							
Predose	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
0-24	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
24-48	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
48-72	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
72-96	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
96-120	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
120-144	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
144-168	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
168-192	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
192-216	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
216-240	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
240-264	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
264-288	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
288-312	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
312-336	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
Time (h)	Urine Volume (mL)							
Predose	22.0[a]	17.0	15.0[a]	--	22.0[a]	17.0	15.0[a]	--
0-24	11.0	10.0	15.0	--	11.0	10.0	15.0	--
24-48	9.00	7.50	12.0	--	9.00	7.50	12.0	--
48-72	10.0	10.0	10.0	--	10.0	10.0	10.0	--
72-96	12.0	10.0	9.00	--	12.0	10.0	9.00	--
96-120	15.0	12.0	12.5	--	15.0	12.0	12.5	--
120-144	14.5	11.5	12.5	--	14.5	11.5	12.5	--
144-168	13.0	9.00	10.0	--	13.0	9.00	10.0	--
168-192	15.0[b]	12.5	14.5	--	15.0[b]	12.5	14.5	--
192-216	13.0	8.00	12.5	--	13.0	8.00	12.5	--
216-240	12.0	8.00	12.0	--	12.0	8.00	12.0	--
240-264	15.0	14.0	16.0	--	15.0	14.0	16.0	--
264-288	9.50	8.00	12.0	--	9.50	8.00	12.0	--
288-312	13.0	10.0	15.0	--	13.0	10.0	15.0	--
312-336	16.0	10.0	15.0	--	16.0	10.0	15.0	--

The limit of quantitation was 0.00488 µg/mL
a. Possible water contamination.
b. Urine overflowed.

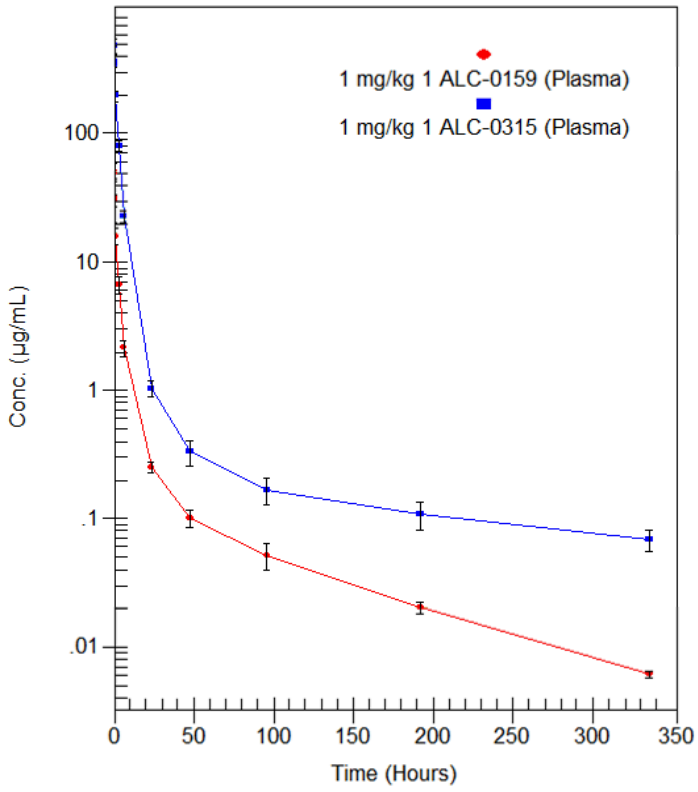
6.6. Summary of Mean and Individual Fecal Concentrations of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

	ALC-0315				ALC-0159			
	Individual Animal			Mean ± SD	Individual Animal			Mean ± SD
	No. 34	No. 35	No. 36		No. 34	No. 35	No. 36	
Animal Weight (g)	210	213	219	--	210	213	219	--
Time (h postdose)	Fecal Concentration (µg/g)							
Predose	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
0-24	1.50	0.474	0.397	0.790 ± 0.616	38.9	28.0	15.2	27.4 ± 11.9
24-48	0.972	0.432	0.778	0.727 ± 0.274	2.17	5.64	4.79	4.20 ±1.81
48-72	0.355	0.572	1.33	0.752 ± 0.512	0.710	0.952	1.33	0.997 ± 0.312
72-96	0.167	0.294	0.450	0.304 ± 0.142	0.401	0.389	0.421	0.404 ± 0.0162
96-120	0.369	0.114	0.173	0.219 ± 0.133	0.332	0.269	0.217	0.273 ± 0.0576
120-144	0.239	0.0881	0.113	0.147 ± 0.0809	0.375	0.171	0.143	0.230 ± 0.127
144-168	0.0888	0.100	0.175	0.121 ± 0.0469	0.300	0.157	0.228	0.228 ± 0.0715
168-192	0.110	0.0783	0.129	0.106 ± 0.0256	0.145	0.124	0.147	0.139 ± 0.0127
192-216	0.0790	0.0809	0.183	0.114 ± 0.0595	0.0904	0.163	0.146	0.133 ± 0.0380
216-240	0.142	0.152	0.101	0.132 ± 0.0270	0.155	0.182	0.116	0.151 ± 0.0332
240-264	0.0781	0.0764	0.135	0.0965 ± 0.0334	0.109	0.0783	0.142	0.110 ± 0.0319
264-288	0.0947	0.0635	0.122	0.0934 ± 0.0293	0.0754	0.0641	0.109	0.0828 ± 0.0234
288-312	0.0457	0.0758	0.107	0.0762 ± 0.0307	0.106	0.0580	0.0982	0.0874 ± 0.0258
312-336	0.0290	0.0641	0.0993	0.0641 ± 0.0352	0.0605	0.0494	0.0854	0.0651 ± 0.0184
Time (h)	Feces weight (g)							
Predose	4.80	4.40	6.40	--	4.80	4.40	6.40	--
0-24	2.50	7.90	5.50	--	2.50	7.90	5.50	--
24-48	8.90	5.50	8.70	--	8.90	5.50	8.70	--
48-72	11.5	8.60	13.1	--	11.5	8.60	13.1	--
72-96	11.8	13.1	10.5	--	11.8	13.1	10.5	--
96-120	13.9	10.6	13.2	--	13.9	10.6	13.2	--
120-144	10.9	12.9	12.6	--	10.9	12.9	12.6	--
144-168	13.5	15.3	8.20	--	13.5	15.3	8.20	--
168-192	8.40	15.4	10.5	--	8.40	15.4	10.5	--
192-216	13.0	11.3	8.00	--	13.0	11.3	8.00	--
216-240	10.1	11.1	9.80	--	10.1	11.1	9.80	--
240-264	10.4	11.4	9.40	--	10.4	11.4	9.40	--
264-288	11.1	11.2	8.40	--	11.1	11.2	8.40	--
288-312	11.7	11.6	9.70	--	11.7	11.6	9.70	--
312-336	12.0	11.5	7.20	--	12.0	11.5	7.20	--
Time (h)	Amount excreted in feces (µg)							
Predose	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
0-24	3.75	3.74	2.18	3.23 ± 0.903	97.3	221	83.6	134± 75.8
24-48	8.65	2.38	6.77	5.93 ± 3.22	19.3	31.0	41.7	30.7 ± 11.2
48-72	4.08	4.92	17.4	8.81 ± 7.47	8.17	8.19	17.4	11.3 ± 5.34
72-96	1.97	3.85	4.73	3.52 ± 1.41	4.73	5.10	4.42	4.75 ± 0.338
96-120	5.13	1.21	2.28	2.87 ± 2.03	4.61	2.85	2.86	3.44 ± 1.01
120-144	2.61	1.14	1.42	1.72 ± 0.778	4.09	2.21	1.80	2.70 ± 1.22
144-168	1.20	1.53	1.44	1.39 ± 0.171	4.05	2.40	1.87	2.77 ± 1.14
168-192	0.924	1.21	1.35	1.16 ± 0.219	1.22	1.91	1.54	1.56 ± 0.346
192-216	1.03	0.914	1.46	1.14 ± 0.290	1.18	1.84	1.17	1.40 ± 0.387
216-240	1.43	1.69	0.990	1.37 ± 0.353	1.57	2.02	1.14	1.57 ± 0.442
240-264	0.812	0.871	1.27	0.984 ± 0.249	1.13	0.893	1.33	1.12 ± 0.221
264-288	1.05	0.711	1.02	0.929 ± 0.189	0.837	0.718	0.916	0.823 ±0.100
288-312	0.535	0.879	1.04	0.817 ± 0.257	1.24	0.673	0.953	0.955 ± 0.284
312-336	0.348	0.737	0.715	0.600 ± 0.219	0.726	0.568	0.615	0.636 ± 0.0811

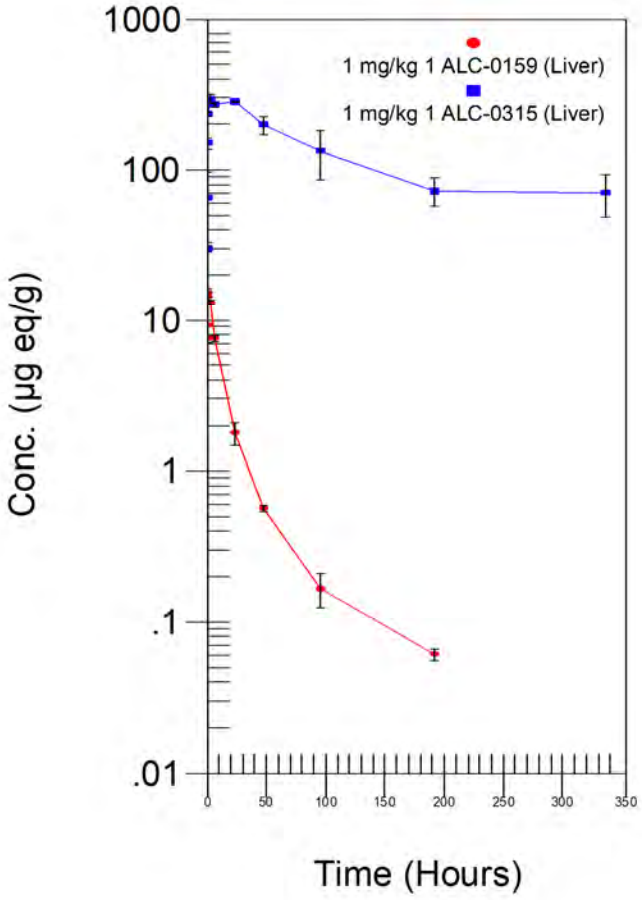
The limit of quantitation was 0.006592 µg/mL.

7. SUPPORTIVE FIGURES

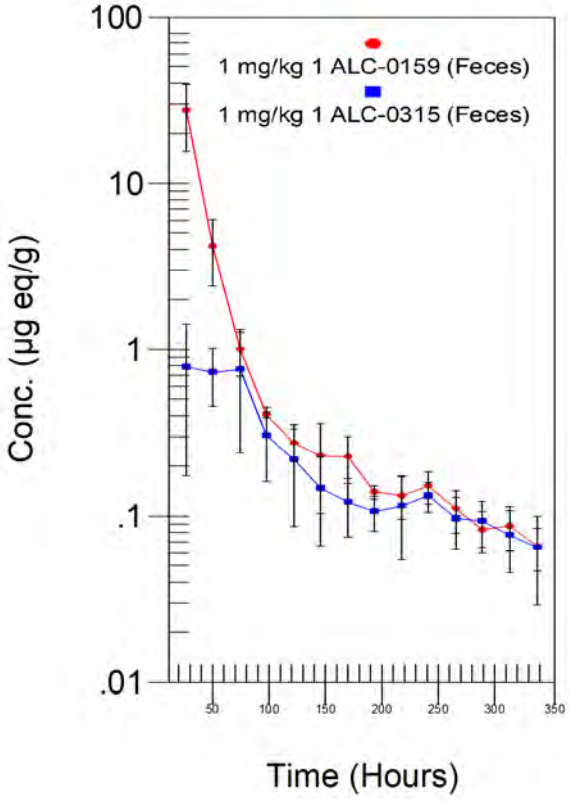
7.1. Mean Plasma Concentrations of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg



7.2. Mean Liver Concentrations of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg



7.3. Mean Fecal Concentrations of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg



8. SUPPORTIVE APPENDIX

8.1. Abbreviations and Pharmacokinetic Calculations

--	Data not available
AUC_{inf}	Area under the plasma drug concentration-time curve from 0 to infinite time. AUC_t plus extrapolated area determined by dividing plasma concentration at t by the slope of the terminal log-linear phase.
AUC_{last}	Area under the plasma drug concentration-time curve from 0 to the last quantifiable time point. Determined using the linear trapezoidal method.
BLQ	Below the limit of quantitation
K_2EDTA	Potassium ethylene diamine tetraacetic acid
ID	Identification
Initial $t_{½}$	Half-life. ln(2)/initial elimination rate constant
ISTD	Internal standard
IV	Intravenous
LNP	Lipid nanoparticle
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MRM	Multiple reaction monitoring
NA	Not applicable
NC	Not calculated
PEG	Polyethylene glycol
PK	Pharmacokinetic
SD	Standard deviation
Terminal elimination $t_{½}$	Half-life. ln(2)/terminal elimination rate constant
Dose in Feces (%)	Fecal excretion. (Mean µg of analyte in feces/ Mean µg of analyte administered) × 100
Dose in Urine (%)	Urinary excretion. (Mean µg of analyte in urine/ Mean µg of analyte administered) × 100

9. OTHER CONTRIBUTING SCIENTISTS

The following scientists were involved in the conduct of the PK portion of this study and are responsible for the scientific content of this research report.

ADME Associate/
Portfolio Support Group
Scientist

Bioanalytical Associate

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10. CHANGE HISTORY

Version	Sections	Revisions
1.0	NA	New document
2.0	Summary, Section 3.1, Results and Discussion, Supportive Table 6.1	Updated the doses of ALC-0159 and ALC-0315 and the percent of dose distributed to the liver and excreted in feces.

11. APPROVAL SIGNATURES

The author and approver are responsible for providing a true representation of the data.

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Signed By:	Date(GMT)	Signing Capacity
(b) (6)	11-Sep-2020 12:52:54	Author Approval
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