

## MODULE 2.6.5. PHARMACOKINETICS TABULATED SUMMARY

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### 2.6.5.1. PHARMACOKINETICS OVERVIEW

Test Article: BNT162b2

Type of Study	Test System	Test item	Method of Administration	Testing Facility	Report Number
<b>Single Dose Pharmacokinetics</b>					
Single Dose Pharmacokinetics and Excretion in Urine and Feces of ALC-0159 and ALC-0315	Rat (Wistar Han)	modRNA encoding luciferase formulated in LNP comparable to BNT162b2	IV bolus	Pfizer Inc <sup>a</sup>	<a href="#">PF-07302048_06Jul20_072424</a>
<b>Distribution</b>					
In Vivo Distribution	Mice (BALB/c)	modRNA encoding luciferase formulated in LNP comparable to BNT162b2	IM Injection	BioNTech <sup>b</sup>	<a href="#">R-20-0072</a>
In Vivo Distribution	Rat (Wistar Han)	modRNA encoding luciferase formulated in LNP comparable to BNT162b2 with trace amounts of [ <sup>3</sup> H]-CHE as non-diffusible label	IM Injection	Charles River <sup>c</sup>	<a href="#">185350</a>
In Vivo Distribution and Protein Expression	Mice (BALB/c)	modRNA encoding luciferase formulated in LNP comparable to BNT162b2	IM Injection	BioNTech <sup>b</sup>	<a href="#">R-23-0355</a>
In Vivo Distribution of mRNA and Protein Expression	Mice (BALB/cJRj)	BNT162b2 modRNA formulated in LNP	IM Injection	BioNTech <sup>b</sup>	<a href="#">R-23-0475</a>
In Vivo Cellular Uptake and Distribution	Mice (CD-1)	modRNA encoding GFP formulated in LNP comparable to BNT162b2	IM Injection	Pfizer Inc <sup>c</sup>	<a href="#">22GR161</a>

**2.6.5.1. PHARMACOKINETICS OVERVIEW****Test Article: BNT162b2**

Type of Study	Test System	Test item	Method of Administration	Testing Facility	Report Number
<b>Metabolism</b>					
<b>In Vitro and In Vivo Metabolism</b>					
In Vitro Metabolic Stability of ALC-0315 in Liver Microsomes	Mouse (CD-1/ICR), rat (Sprague Dawley and Wistar Han), monkey (Cynomolgus), and human liver microsomes	ALC-0315	In vitro	Medicilon Preclinical Research LLC <sup>d</sup>	<a href="#">01049-20008</a>
In Vitro Metabolic Stability of ALC-0315 in Liver S9	Mouse (CD-1/ICR), rat (Sprague Dawley), monkey (Cynomolgus), and human S9 liver fractions	ALC-0315	In vitro	Medicilon Preclinical Research LLC <sup>d</sup>	<a href="#">01049-20009</a>
In Vitro Metabolic Stability of ALC-0315 in Hepatocytes	Mouse (CD-1/ICR), rat (Sprague Dawley and Wistar Han), monkey (Cynomolgus), and human hepatocytes	ALC-0315	In vitro	Medicilon Preclinical Research LLC <sup>d</sup>	<a href="#">01049-20010</a>
In Vitro Metabolic Stability of ALC-0159 in Liver Microsomes	Mouse (CD-1/ICR), rat (Sprague Dawley and Wistar Han), monkey (Cynomolgus), and human liver microsomes	ALC-0159	In vitro	Medicilon Preclinical Research LLC <sup>d</sup>	<a href="#">01049-20020</a>
In Vitro Metabolic Stability of ALC-0159 in Liver S9	Mouse (CD-1/ICR), rat (Sprague Dawley), monkey (Cynomolgus), and human S9 fractions	ALC-0159	In vitro	Medicilon Preclinical Research LLC <sup>d</sup>	<a href="#">01049-20021</a>

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**2.6.5.1. PHARMACOKINETICS OVERVIEW****Test Article: BNT162b2**

Type of Study	Test System	Test item	Method of Administration	Testing Facility	Report Number
In Vitro Metabolic Stability of ALC-0159 in Hepatocytes	Mouse (CD-1/ICR), rat (Sprague Dawley and Wistar Han), monkey (Cynomolgus), and human hepatocytes	ALC-0159	In vitro	Medicilon Preclinical Research LLC <sup>d</sup>	<a href="#">01049-20022</a>
Biotransformation of ALC-0159 and ALC-0315 In Vitro and In Vivo in Rats	In vitro: CD-1 mouse, Wistar Han rat, cynomolgus monkey, and human blood, liver S9 fractions and hepatocytes In vivo: male Wistar Han rats	ALC-0315 and ALC-0159	In vitro or IV (in vivo in rats)	Pfizer Inc <sup>e</sup>	<a href="#">PF-07302048_05Aug20_043725</a>

ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, a proprietary polyethylene glycol-lipid included as an excipient in the LNP formulation used in BNT162b2; ALC-0315 = (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate), a proprietary aminolipid included as an excipient in the LNP formulation used in BNT162b2; GFP = green fluorescent protein; [<sup>3</sup>H]-CHE = radiolabeled [cholesteryl-1,2-<sup>3</sup>H(N)]-cholesteryl hexadecyl ether; IM = Intramuscular; IV = Intravenous; LNP = lipid nanoparticles; modRNA = nucleoside modified messenger RNA; S9 = Supernatant fraction obtained from liver homogenate by centrifuging at 9000 g.

- a. La Jolla, California.  
b. Mainz, Germany.  
c. Tranent, UK.  
d. Shanghai, China.  
e. Groton, Connecticut.

### 2.6.5.3. PHARMACOKINETICS: PHARMACOKINETICS AFTER A SINGLE DOSE

Test Article: modRNA encoding luciferase in LNP

Report Number: **PE-07302048\_06Jul20\_072424**

Species (Strain)	Rat (Wistar Han)	
Sex/Number of Animals	Male/ 3 animals per timepoint <sup>a</sup>	
Feeding Condition	Fasted	
Method of Administration	IV	
Dose modRNA (mg/kg)	4	
Dose ALC-0159 (mg/kg)	1.96	
Dose ALC-0315 (mg/kg)	15.3	
Sample Matrix	Plasma, liver, urine and feces	
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 <sup>b</sup>	Mean <sup>b</sup>
AUC <sub>inf</sub> (µg•h/mL) <sup>c</sup>	1030	99.2
AUC <sub>last</sub> (µg•h/mL)	1020	98.6
Initial t <sub>1/2</sub> (h) <sup>d</sup>	1.62	1.74
Terminal elimination t <sub>1/2</sub> (h) <sup>e</sup>	439	72.7
Estimated fraction of dose distributed to liver (%) <sup>f</sup>	59.5	20.3
Dose in Urine (%)	NC <sup>g</sup>	NC <sup>g</sup>
Dose in Feces (%) <sup>h</sup>	1.05	47.2

ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide), a proprietary polyethylene glycol-lipid included as an excipient in the LNP formulation used in BNT162b2; ALC-0315 = (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate), a proprietary aminolipid included as an excipient in the LNP formulation used in BNT162b2; AUC<sub>inf</sub> = Area under the plasma drug concentration-time curve from 0 to infinite time; AUC<sub>last</sub> = Area under the plasma drug concentration-time curve from 0 to the last quantifiable time point; BLQ = Below the limit of quantitation; LNP = Lipid nanoparticle; modRNA = Nucleoside modified messenger RNA; PK = Pharmacokinetics; t<sub>1/2</sub> = Half-life.

- Non-serial sampling, 36 animals total.
- Only mean PK parameters are reported due to non-serial sampling.
- Calculated using the terminal log-linear phase (determined using 48, 96, 192, and 336 h for regression calculation).
- ln(2)/initial elimination rate constant (determined using 1, 3, and 6 h for regression calculation).
- ln(2)/terminal elimination rate constant (determined using 48, 96, 192, and 336 h for regression calculation).
- Calculated as follows: highest mean amount in the liver (µg)/total mean dose (µg) of ALC-0315 or ALC-0159.
- Not calculated due to BLQ data.
- Fecal excretion, calculated as: (mean µg of analyte in feces/ mean µg of analyte administered) × 100

**2.6.5.5A. PHARMACOKINETICS: ORGAN DISTRIBUTION****Test Article: modRNA encoding luciferase in LNP****Report Number: R-20-0072**

Species (Strain):	Mice (BALB/c)		
Sex/Number of Animals:	Female/3 per group		
Feeding Condition:	Fed ad libitum		
Vehicle/Formulation:	Phosphate-buffered saline		
Method of Administration:	Intramuscular injection		
Dose (mg/kg):	1 µg/hind leg in gastrocnemius muscle (2 µg total)		
Number of Doses:	1		
Detection:	Bioluminescence measurement		
Sampling Time (hour):	6, 24, 48, 72 hours; 6 and 9 days post-injection		
Time point	Total Mean Bioluminescence signal (photons/second)		Mean Bioluminescence signal in the liver (photons/second)
	Buffer control	modRNA Luciferase in LNP	modRNA Luciferase in LNP
6 hours	$1.28 \times 10^5$	$1.26 \times 10^9$	$4.94 \times 10^7$
24 hours	$2.28 \times 10^5$	$7.31 \times 10^8$	$2.4 \times 10^6$
48 hours	$1.40 \times 10^5$	$2.10 \times 10^8$	Below detection <sup>a</sup>
72 hours	$1.33 \times 10^5$	$7.87 \times 10^7$	Below detection <sup>a</sup>
6 days	$1.62 \times 10^5$	$2.92 \times 10^6$	Below detection <sup>a</sup>
9 days	$7.66 \times 10^4$	$5.09 \times 10^5$	Below detection <sup>a</sup>

LNP = Lipid nanoparticle; modRNA = Nucleoside modified messenger RNA.

a. At or below the background level of the buffer control.

**2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED**

**Test Article: [<sup>3</sup>H]-Labelled LNP-mRNA formulation containing ALC-0315 and ALC-0159**  
**Report Number: 185350**

Species (Strain): Rat (Wistar Han)  
Sex/Number of Animals: Male and female/3 animals/sex/timepoint (21 animals/sex total for the 50 µg dose)  
Feeding Condition: Fed ad libitum  
Method of Administration: Intramuscular injection  
Dose: 50 µg [<sup>3</sup>H]-08-A01-C0 (lot # NC-0552-1)  
Number of Doses: 1  
Detection: Radioactivity quantitation using liquid scintillation counting  
Sampling Time (hour): 0.25, 1, 2, 4, 8, 24, and 48 hours post-injection

Sample	Mean total lipid concentration (µg lipid equivalent/g (or mL) (males and females combined))							% of administered dose (males and females combined)						
	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h
Adipose tissue	0.057	0.100	0.126	0.128	0.093	0.084	0.181	--	--	--	--	--	--	--
Adrenal glands	0.271	1.48	2.72	2.89	6.80	13.8	18.2	0.001	0.007	0.010	0.015	0.035	0.066	0.106
Bladder	0.041	0.130	0.146	0.167	0.148	0.247	0.365	0.000	0.001	0.001	0.001	0.001	0.002	0.002
Bone (femur)	0.091	0.195	0.266	0.276	0.340	0.342	0.687	--	--	--	--	--	--	--
Bone marrow (femur)	0.479	0.960	1.24	1.24	1.84	2.49	3.77	--	--	--	--	--	--	--
Brain	0.045	0.100	0.138	0.115	0.073	0.069	0.068	0.007	0.013	0.020	0.016	0.011	0.010	0.009
Eyes	0.010	0.035	0.052	0.067	0.059	0.091	0.112	0.000	0.001	0.001	0.002	0.002	0.002	0.003
Heart	0.282	1.03	1.40	0.987	0.790	0.451	0.546	0.018	0.056	0.084	0.060	0.042	0.027	0.030
Injection site	128	394	311	338	213	195	165	19.9	52.6	31.6	28.4	21.9	29.1	24.6
Kidneys	0.391	1.16	2.05	0.924	0.590	0.426	0.425	0.050	0.124	0.211	0.109	0.075	0.054	0.057
Large intestine	0.013	0.048	0.093	0.287	0.649	1.10	1.34	0.008	0.025	0.065	0.192	0.405	0.692	0.762
Liver	0.737	4.63	11.0	16.5	26.5	19.2	24.3	0.602	2.87	7.33	11.9	18.1	15.4	16.2
Lung	0.492	1.21	1.83	1.50	1.15	1.04	1.09	0.052	0.101	0.178	0.169	0.122	0.101	0.101

**2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED**

Test Article: [<sup>3</sup>H]-Labelled LNP-mRNA formulation containing  
ALC-0315 and ALC-0159  
Report Number: **185350**

Sample	Total Lipid concentration (µg lipid equivalent/g [or mL]) (males and females combined)							% of Administered Dose (males and females combined)						
	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h
Lymph node (mandibular)	0.064	0.189	0.290	0.408	0.534	0.554	0.727	--	--	--	--	--	--	--
Lymph node (mesenteric)	0.050	0.146	0.530	0.489	0.689	0.985	1.37	--	--	--	--	--	--	--
Muscle	0.021	0.061	0.084	0.103	0.096	0.095	0.192	--	--	--	--	--	--	--
Ovaries (females)	0.104	1.34	1.64	2.34	3.09	5.24	12.3	0.001	0.009	0.008	0.016	0.025	0.037	0.095
Pancreas	0.081	0.207	0.414	0.380	0.294	0.358	0.599	0.003	0.007	0.014	0.015	0.015	0.011	0.019
Pituitary gland	0.339	0.645	0.868	0.854	0.405	0.478	0.694	0.000	0.001	0.001	0.001	0.000	0.000	0.001
Prostate (males)	0.061	0.091	0.128	0.157	0.150	0.183	0.170	0.001	0.001	0.002	0.003	0.003	0.004	0.003
Salivary glands	0.084	0.193	0.255	0.220	0.135	0.170	0.264	0.003	0.007	0.008	0.008	0.005	0.006	0.009
Skin	0.013	0.208	0.159	0.145	0.119	0.157	0.253	--	--	--	--	--	--	--
Small intestine	0.030	0.221	0.476	0.879	1.28	1.30	1.47	0.024	0.130	0.319	0.543	0.776	0.906	0.835
Spinal cord	0.043	0.097	0.169	0.250	0.106	0.085	0.112	0.001	0.002	0.002	0.003	0.001	0.001	0.001
Spleen	0.334	2.47	7.73	10.3	22.1	20.1	23.4	0.013	0.093	0.325	0.385	0.982	0.821	1.03
Stomach	0.017	0.065	0.115	0.144	0.268	0.152	0.215	0.006	0.019	0.034	0.030	0.040	0.037	0.039
Testes (males)	0.031	0.042	0.079	0.129	0.146	0.304	0.320	0.007	0.010	0.017	0.030	0.034	0.074	0.074
Thymus	0.088	0.243	0.340	0.335	0.196	0.207	0.331	0.004	0.007	0.010	0.012	0.008	0.007	0.008
Thyroid	0.155	0.536	0.842	0.851	0.544	0.578	1.00	0.000	0.001	0.001	0.001	0.001	0.001	0.001
Uterus (females)	0.043	0.203	0.305	0.140	0.287	0.289	0.456	0.002	0.011	0.015	0.008	0.016	0.018	0.022
Whole blood	1.97	4.37	5.40	3.05	1.31	0.909	0.420	--	--	--	--	--	--	--
Plasma	3.97	8.13	8.90	6.50	2.36	1.78	0.805	--	--	--	--	--	--	--
Blood:Plasma ratio <sup>a</sup>	0.815	0.515	0.550	0.510	0.555	0.530	0.540	--	--	--	--	--	--	--



### 2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

Test Article: [<sup>3</sup>H]-Labelled LNP-mRNA formulation containing  
ALC-0315 and ALC-0159  
Report Number: 185350

-- = Not applicable, partial tissue taken; [<sup>3</sup>H]-08-A01-C0 = An aqueous dispersion of LNPs, including ALC-0315, ALC-0159, distearoylphosphatidylcholine, cholesterol, mRNA encoding luciferase and trace amounts of radiolabeled [Cholesteryl-1,2-3H(N)]-Cholesteryl Hexadecyl Ether, a nonexchangeable, non-metabolizable lipid marker used to monitor the disposition of the LNPs; ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N--ditetradecylacetamide, a proprietary polyethylene glycol-lipid included as an excipient in the LNP formulation used in BNT162b2; ALC-0315 = (4--hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate), a proprietary aminolipid included as an excipient in the LNP formulation used in BNT162b2; LNP = Lipid nanoparticle; mRNA = messenger RNA.

a. The mean male and female blood:plasma values were first calculated separately and this value represents the mean of the two values.

### 2.6.5.5C. PHARMACOKINETICS: ORGAN DISTRIBUTION

Test Article: modRNA encoding Luciferase in LNP  
Report Number: R-23-0355

Species (Strain):	Mice (BALB/c)
Sex/Number of Animals:	Female/6 per dose/3 per timepoint
Vehicle/Formulation:	Sterile saline (0.9% NaCl)
Method of Administration:	Intramuscular injection
Dose:	0.2 or 1 µg (20 µL) in right gastrocnemius muscle
Number of Doses:	1
Detection:	Bioluminescence measurement and luciferase assay
Sampling Time (hour):	1 (24 hours) and 6 days post-injection

Sample	Percentage of luciferase signal (%)											
	0.2 µg						1 µg					
	Day 1 (24 hours)			Day 6			24 hours			Day 6		
	Animal #											
Mouse 1	Mouse 2	Mouse 3	Mouse 1	Mouse 2	Mouse 3	Mouse 1	Mouse 2	Mouse 3	Mouse 1	Mouse 2	Mouse 3	
Muscle – Right (injection site)	87.80	78.17	86.76	98.93	57.84	100.00	27.00	64.64	86.62	74.97	33.97	10.94
Popliteal LN – Right	10.40	16.80	8.23	0.00	42.16	0.00	71.57	34.96	13.23	0.00	66.01	71.04
Axillary LN – Right	0.00	2.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Liver	0.33	0.93	3.81	0.00	0.00	0.00	0.88	0.25	0.05	0.00	0.02	0.02
Inguinal LN – Right	0.00	1.31	0.00	0.00	0.00	0.00	0.00	0.00	0.03	25.03	0.00	18.00
Axillary LN – Left	0.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spleen	0.07	0.11	1.21	1.07	0.00	0.00	0.44	0.15	0.06	0.00	0.00	0.00
Inguinal LN – Left	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00
Muscle – Left	0.01	0.05	0.00	0.00	0.00	0.00	0.03	0.00	0.01	0.00	0.00	0.00
Mesenteric LN	0.21	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Brain (Cerebellum)	0.27	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Note: All tissues analyzed and not reported produced percentages of luciferase signal of 0.00%.  
HPI = Hours post-injection; LN = Lymph node.

**2.6.5.5D. PHARMACOKINETICS: OTHER - ORGAN DISTRIBUTION**

Test Article: BNT162b2

Report Number: **R-23-0475**

Species (Strain):	Mice (BALB/cJRj)		
Sex/Number of Animals:	Female/3		
Vehicle/Formulation:	Sterile saline (0.9% NaCl)		
Method of Administration:	Intramuscular injection		
Dose:	1 µg (20 µL) in one hind leg		
Number of Doses:	1		
Detection:	Anti-spike IHC; RNAscope-based ISH-IF		
Sampling Time (hour):	24 hours post-injection		
Organ	BNT162b2 mRNA Presence	Spike Protein Presence	Colocalization of spike & CD169 (macrophage marker)
Muscle (side of injection)	Present	Present	No
Popliteal lymph node (side of injection)	Present	Present	Yes
Inguinal lymph node (side of injection)	Present	Present <sup>a</sup>	Yes
Axillary lymph node (side of injection)	Present	Present <sup>a</sup>	Yes
Spleen	Present	Negative	No
Liver	Present	Negative	No
Lung	Negative	Negative	No
Heart	Negative	Negative	No
Brain	N/A	Negative	N/A

IF = Immunofluorescence; IHC = Immunohistochemistry; ISH = In situ hybridization; mRNA = Messenger RNA; N/A = Not applicable; RNA = Ribonucleic acid.

a. Present in 1 out of 3 animals.

**2.6.5.9. PHARMACOKINETICS: METABOLISM IN VIVO, RAT****Test Article: modRNA encoding luciferase in LNP****Report Number: PF-07302048\_05Aug20\_043725**

Species (Strain):	Rat (Wistar Han)
Sex/ Number of animals	Male/ 36 animals total for plasma and liver, 3 animals for urine and feces
Method of Administration:	Intravenous
Dose (mg/kg):	1
Test System:	Plasma, Urine, Feces, Liver
Analysis Method:	Ultrahigh performance liquid chromatography/ mass spectrometry

Biotransformation	m/z	Metabolites of ALC-0315 Detected			
		Plasma	Urine	Feces	Liver
N-dealkylation, oxidation	102.0561 <sup>a</sup>	ND	ND	ND	ND
N-Dealkylation, oxidation	104.0706 <sup>b</sup>	ND	ND	ND	ND
N-dealkylation, oxidation	130.0874 <sup>a</sup>	ND	ND	ND	ND
N-Dealkylation, oxidation	132.1019 <sup>b</sup>	ND	ND	ND	ND
N-dealkylation, hydrolysis, oxidation	145.0506 <sup>a</sup>	ND	ND	ND	ND
Hydrolysis (acid)	255.2330 <sup>a</sup>	+	ND	ND	ND
Hydrolysis, hydroxylation	271.2279 <sup>a</sup>	ND	ND	ND	ND
Bis-hydrolysis (amine)	290.2690 <sup>b</sup>	+	+	+	+
Hydrolysis, glucuronidation	431.2650 <sup>a</sup>	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	464.2865 <sup>a</sup>	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	466.3011 <sup>b</sup>	ND	+	ND	ND
Hydrolysis (amine)	528.4986 <sup>b</sup>	+	ND	ND	+
Hydrolysis (amine), Glucuronidation	704.5307 <sup>b</sup>	ND	ND	ND	ND
Oxidation to acid	778.6930 <sup>a</sup>	ND	ND	ND	ND
Oxidation to acid	780.7076 <sup>b</sup>	ND	ND	ND	ND
Hydroxylation	782.7232 <sup>b</sup>	ND	ND	ND	ND
Sulfation	844.6706 <sup>a</sup>	ND	ND	ND	ND
Sulfation	846.6851 <sup>b</sup>	ND	ND	ND	ND
Glucuronidation	940.7458 <sup>a</sup>	ND	ND	ND	ND
Glucuronidation	942.7604 <sup>b</sup>	ND	ND	ND	ND

Note: Both theoretical and observed metabolites are included.

m/z = mass to charge ratio; ND = Not detected; + = minor metabolite as assessed by ultraviolet detection.

a. Negative ion mode.

b. Positive ion mode.

## 2.6.5.10A. PHARMACOKINETICS: METABOLISM IN VITRO

**Test Article: ALC-0315**  
**Report Numbers: 01049-20008**  
**01049-20009**  
**01049-20010**

Type of Study:	Liver Microsomes + NADPH					Stability of ALC-0315 In Vitro S9 Fraction + NADPH, UDPGA, and alamethicin					Hepatocytes				
Study System:															
ALC-0315	1 µM					1 µM					1 µM				
Concentration:															
Duration of	120 min					120 min					240 min				
Incubation (min):															
Analysis Method:	Ultra-high performance liquid chromatography-tandem mass spectrometry														
Incubation time (min)	Percent ALC-0315 remaining														
	Liver Microsomes					Liver S9 Fraction				Hepatocytes					
	Mouse (CD-1/ICR)	Rat (SD)	Rat (WH)	Monkey (Cyno)	Human	Mouse (CD-1/ICR)	Rat (SD)	Monkey (Cyno)	Human	Mouse (CD-1/ICR)	Rat (SD)	Rat (WH)	Monkey (Cyno)	Human	
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
15	98.77	94.39	96.34	97.96	100.24	97.69	98.85	99.57	95.99	--	--	--	--	--	
30	97.78	96.26	97.32	96.18	99.76	97.22	99.62	96.96	97.32	101.15	97.75	102.70	96.36	100.72	
60	100.49	99.73	98.54	100.00	101.45	98.61	99.62	99.13	94.98	100.77	98.50	102.32	97.82	101.44	
90	97.78	98.66	94.15	97.96	100.48	98.15	98.85	98.70	98.33	101.92	99.25	103.09	100.0	100.36	
120	96.54	95.99	93.66	97.71	98.31	96.76	98.46	99.57	99.33	98.85	97.38	99.61	96.36	100.72	
180	--	--	--	--	--	--	--	--	--	101.15	98.88	103.47	95.64	98.92	
240	--	--	--	--	--	--	--	--	--	99.62	101.12	100.00	93.82	99.64	
t½ (min)	>120	>120	>120	>120	>120	>120	>120	>120	>120	>240	>240	>240	>240	>240	

-- = Data not available; ALC-0315 = (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate), a proprietary aminolipid included as an excipient in the lipid nanoparticle formulation used in BNT162b2; Cyno = Cynomolgus; NADPH = Reduced form of nicotinamide adenine dinucleotide phosphate; NC = not calculated; SD = Sprague Dawley; t<sub>1/2</sub> = half-life; WH = Wistar-Han; UDPGA = uridine diphosphate-glucuronic acid trisodium salt.

## 2.6.5.10B. PHARMACOKINETICS: METABOLISM IN VITRO CONTINUED

Test Article: ALC-0159  
 Report Numbers: 01049-20020  
 01049-20021  
 01049-20022

Type of Study:	Liver Microsomes + NADPH					Stability of ALC-0159 In Vitro S9 Fraction + NADPH, UDPGA, and alamethicin					Hepatocytes				
Study System:															
ALC-0159	1 μM					1 μM					1 μM				
Concentration:															
Duration of	120 min					120 min					240 min				
Incubation (min):															
Analysis Method:	Ultra-high performance liquid chromatography-tandem mass spectrometry														
Incubation time (min)	Percent ALC-0159 remaining														
	Liver Microsomes					Liver S9 Fraction				Hepatocytes					
	Mouse (CD-1/ICR)	Rat (SD)	Rat (WH)	Monkey (Cyno)	Human	Mouse (CD-1/ICR)	Rat (SD)	Monkey (Cyno)	Human	Mouse (CD-1/ICR)	Rat (SD)	Rat (WH)	Monkey (Cyno)	Human	
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
15	82.27	101.24	112.11	100.83	99.59	98.93	84.38	91.30	106.73	--	--	--	--	--	
30	86.40	93.78	102.69	85.12	92.28	91.10	90.87	97.96	107.60	100.85	93.37	113.04	90.23	106.34	
60	85.54	98.34	105.38	86.36	95.53	102.85	97.97	105.56	104.97	94.92	91.81	105.07	92.93	101.58	
90	85.41	95.44	100.90	94.63	97.97	90.75	93.51	108.33	109.36	94.28	90.25	112.80	94.59	92.67	
120	95.87	97.10	108.97	93.39	93.09	106.76	92.70	105.74	119.59	87.08	89.47	104.11	97.51	96.04	
180	--	--	--	--	--	--	--	--	--	94.92	93.96	102.90	89.81	93.66	
240	--	--	--	--	--	--	--	--	--	102.75	94.93	98.79	92.93	102.57	
t½ (min)	>120	>120	>120	>120	>120	>120	>120	>120	>120	>240	>240	>240	>240	>240	

-- = Data not available; ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide), a proprietary polyethylene glycol-lipid included as an excipient in the lipid nanoparticle formulation used in BNT162b2; Cyno = Cynomolgus; NADPH = Reduced form of nicotinamide adenine dinucleotide phosphate; NC = not calculated; SD = Sprague Dawley; WH = Wistar-Han; UDPGA= uridine-diphosphate-glucuronic acid trisodium salt.

### 2.6.5.10C. PHARMACOKINETICS: METABOLISM IN VITRO CONTINUED

Test Article: ALC-0315  
Report Number: PF-07302048\_05Aug20\_043725

Type of study		Metabolism of ALC-0315 In Vitro											
Study system		Blood				Hepatocytes				Liver S9 Fraction			
ALC-0315 concentration		10 µM				10 µM				10 µM			
Duration of incubation		24 h				4 h				24 h			
Analysis Method:		Ultrahigh performance liquid chromatography/ mass spectrometry											
Biotransformation	m/z	Blood				Hepatocytes				Liver S9 Fraction			
		Mouse	Rat	Monkey	Human	Mouse	Rat	Monkey	Human	Mouse	Rat	Monkey	Human
<i>N</i> -dealkylation, oxidation	102.0561 <sup>a</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -Dealkylation, oxidation	104.0706 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -dealkylation, oxidation	130.0874 <sup>a</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -Dealkylation, oxidation	132.1019 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -dealkylation, hydrolysis, oxidation	145.0506 <sup>a</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (acid)	255.2330 <sup>a</sup>	+	+	ND	ND	+	+	+	+	+	+	ND	+
Hydrolysis, hydroxylation	271.2279 <sup>a</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bis-hydrolysis (amine)	290.2690 <sup>b</sup>	+	+	ND	ND	ND	ND	ND	ND	ND	ND	+	ND
Hydrolysis, glucuronidation	431.2650 <sup>a</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	464.2865 <sup>a</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	466.3011 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (amine)	528.4986 <sup>b</sup>	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	+	ND
Hydrolysis (amine), glucuronidation	704.5307 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxidation to acid	778.6930 <sup>a</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxidation to acid	780.7076 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydroxylation	782.7232 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfation	844.6706 <sup>a</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfation	846.6851 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Glucuronidation	940.7458 <sup>a</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Glucuronidation	942.7604 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: Both theoretical and observed metabolites are included.

m/z = mass to charge ratio; ND = Not detected; + = metabolite present.

a. Negative ion mode.

b. Positive ion mode.

### 2.6.5.10D. PHARMACOKINETICS: METABOLISM IN VITRO CONTINUED

Test Article: ALC-0159  
Report Number: PF-07302048\_05Aug20\_043725

Type of study	Metabolism of ALC-0159 In Vitro												
Study system	Blood				Hepatocytes				Liver S9 Fraction				
ALC-0159 concentration	10 µM				10 µM				10 µM				
Duration of incubation	24 h				4 h				24 h				
Analysis Method:	Ultrahigh performance liquid chromatography/ mass spectrometry												
Biotransformation	m/z	Blood				Hepatocytes				Liver S9 Fraction			
		Mouse	Rat	Monkey	Human	Mouse	Rat	Monkey	Human	Mouse	Rat	Monkey	Human
<i>O</i> -Demethylation, <i>O</i> -dealkylation	107.0703 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>O</i> -Demethylation, <i>O</i> -dealkylation	151.0965 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>O</i> -Demethylation, <i>O</i> -dealkylation	195.1227 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis, <i>N</i> -Dealkylation	214.2529 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -Dealkylation, oxidation	227.2017 <sup>a</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (amine)	410.4720 <sup>b</sup>	+	+	ND	ND	+	+	+	+	+	+	+	+
<i>N,N</i> -Didealkylation	531.5849 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -Dealkylation	580.6396 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>O</i> -Demethylation, oxidation	629.6853 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydroxylation	633.6931 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ω-Hydroxylation, Oxidation	637.1880 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (acid)	708.7721 <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: Both theoretical and observed metabolites are included.

m/z = mass to charge ratio; ND = Not detected; + = metabolite present.

a. Negative ion mode.

b. Positive ion mode.



**2.6.5.16. PHARMACOKINETICS: OTHER - IMMUNOLOGY AND SERUM CONCENTRATIONS****Test Article: modRNA encoding GFP in LNP****Report Number: 22GR161**

Species (Strain):	Mice (CD-1)			
Sex/Number of Animals:	Male/6 per timepoint			
Vehicle/Formulation:	Sterile saline			
Method of Administration:	Intramuscular injection			
Dose (mg/kg):	1 µg/hind leg in quadriceps muscle (2 µg total)			
Number of Doses:	1			
Detection:	GFP ELISA (serum) or LEGENDplex™ Mouse Anti-Virus Response Panel (Cytokine/Chemokine)			
Sampling Time (hour):	6, 24, 72 and 168 hours post-injection			
Sample	6 h	24 h	72 h	168 h
<b>Mean GFP concentration (pg/mL)<sup>a</sup></b>				
Serum	356	196	15.1	68.0
<b>Mean Cytokine/Chemokine Concentration (pg/mL)<sup>a</sup></b>				
IFN-γ	19.1	37.6	3.08	2.08
CXCL1/KC	149	47.4	35.3	35.7
TNF-α	8.09	6.43	19.5	9.31
CCL2/MCP-1	118	128	45.7	29.5
IL-12 (p70)	2.95	2.04	1.93	1.87
CCL5/RANTES	57.0	19.3	25.0	61.1
IL-1β	1.95	6.53	3.97	1.24
CXCL10/IP-10	2340	899	496	220
GM-CSF	1.96	1.87	4.34	20.1
IL-10	87.1	118	41.2	48.0
IFN-β	32.7	0.88	0.49	0.49
IFN-α	186	14.2	0.68	0.98
IL-6	39.3	4.93	0.80	6.29

CCL2/MCP-1 = chemokine (C-C motif) ligand 2 (monocyte chemoattractant protein-1); CCL5 = Chemokine (C-C motif) ligand 5 (regulated on activation, normal T cell expressed and secreted); CXCL1/KC = chemokine (C-X-C motif) ligand 1 (keratinocyte chemoattractant); CXCL10 = C-X-C motif chemokine ligand 10; ELISA = enzyme-linked immunosorbent assay; GFP = green fluorescence protein; GM-CSF = granulocyte macrophage-colony stimulating factor; IFN = interferon; IL = interleukin; modRNA = nucleoside modified messenger RNA; TNF-α = tumor necrosis factor alpha.

a. Mean of n = 6.