

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

MODULE 2.6.5. PHARMACOKINETICS TABULATED SUMMARY

This document contains confidential information belonging to BioNTech/Pfizer. Except as may be otherwise agreed to in writing, by accepting or reviewing these materials, you agree to hold such information in confidence and not to disclose it to others (except where required by applicable law), nor to use it for unauthorized purposes. In the event of actual or suspected breach of this obligation, BioNTech/Pfizer should be promptly notified.

CONFIDENTIAL

Page 1

FDA-CBER-2021-4379-0001116

090177e196137069\Approved\Approved On: 21-Jan-2021 23:22 (GMT)

BNT162b2
2.6.5 Pharmacokinetics Tabulated Summary

2.6.5.1. PHARMACOKINETICS OVERVIEW

Test Article: BNT162b2

Type of Study	Test System	Test item	Method of Administration	Testing Facility	Report Number
Single Dose Pharmacokinetics					
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 ^a	PF-07302048_06Jul20_072424
Distribution					
In Vivo Distribution	Mice (BALB/c)	modRNA encoding luciferase formulated in LNP comparable to BNT162b2	IM Injection	BioNTech ^b	R-20-0072
In Vivo Distribution	Rat (Wistar Han)	modRNA encoding luciferase formulated in LNP comparable to BNT162b2 with trace amounts of [³ H]-CHE as non-diffusible label	IM Injection	Charles River ^c	185350
Metabolism					
In Vitro and In Vivo Metabolism					
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 ^d	01049-20008
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 ^d	01049-20009

090177e196137069\Approved\Approved On: 21-Jan-2021 23:22 (GMT)

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

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-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 ^d	01049-20010
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 ^d	01049-20020
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 ^d	01049-20021
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 ^d	01049-20022
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 ^e	PF-07302048_05Aug20_043725

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

2.6.5.1. PHARMACOKINETICS OVERVIEW

Test Article: BNT162b2

Type of Study	Test System	Test item	Method of Administration	Testing Facility	Report Number
<p>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; [³H]-CHE = radiolabeled [cholesteryl-1,2-³H(N)]-cholesteryl hexadecyl ether; IM = Intramuscular; IV = Intravenous; LNP = lipid nanoparticles; S9 = Supernatant fraction obtained from liver homogenate by centrifuging at 9000 g.</p> <p>a. La Jolla, California. b. Mainz, Germany. c. Tranent, UK. d. Shanghai, China. e. Groton, Connecticut.</p>					

090177e196137069\Approved\Approved On: 21-Jan-2021 23:22 (GMT)

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

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

**Test Article: modRNA encoding luciferase in LNP
Report Number: PF-07302048_06Jul20_072424**

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, 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 ^b	Mean ^b
AUC _{inf} (µg•h/mL) ^c	1030	99.2
AUC _{last} (µg•h/mL)	1020	98.6
Initial t _{1/2} (h) ^d	1.62	1.74
Terminal elimination t _{1/2} (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 (%) ^h	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_{inf} = Area under the plasma drug concentration-time curve from 0 to infinite time; AUC_{last} = 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_{1/2} = Half-life.

- 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 h 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.
- h. Fecal excretion, calculated as: (mean µg of analyte in feces/ mean µg of analyte administered) × 100

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

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	1.28×10 ⁵	1.26×10 ⁹	4.94×10 ⁷
24 hours	2.28×10 ⁵	7.31×10 ⁸	2.4×10 ⁶
48 hours	1.40×10 ⁵	2.10×10 ⁸	Below detection ^a
72 hours	1.33×10 ⁵	7.87×10 ⁷	Below detection ^a
6 days	1.62×10 ⁵	2.92×10 ⁶	Below detection ^a
9 days	7.66×10 ⁴	5.09×10 ⁵	Below detection ^a

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

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

090177e196137069\Approved\Approved On: 21-Jan-2021 23:22 (GMT)

BNT162b2
2.6.5 Pharmacokinetics Tabulated Summary

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

**Test Article: [³H]-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 [³H]-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 min	1 h	2 h	4 h	8 h	24 h	48 h	0.25 min	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

090177e196137069Approved\Approved On: 21-Jan-2021 23:22 (GMT)

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

**Test Article: [³H]-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 min	1 h	2 h	4 h	8 h	24 h	48 h	0.25 min	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 ^a	0.815	0.515	0.550	0.510	0.555	0.530	0.540	--	--	--	--	--	--	--

090177e196137069Approved\Approved On: 21-Jan-2021 23:22 (GMT)

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

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

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

-- = Not applicable, partial tissue taken; [³H]-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)azanediyl)bis(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.

090177e196137069\Approved\Approved On: 21-Jan-2021 23:22 (GMT)

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

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 ^a	ND	ND	ND	ND
N-Dealkylation, oxidation	104.0706 ^b	ND	ND	ND	ND
N-dealkylation, oxidation	130.0874 ^a	ND	ND	ND	ND
N-Dealkylation, oxidation	132.1019 ^b	ND	ND	ND	ND
N-dealkylation, hydrolysis, oxidation	145.0506 ^a	ND	ND	ND	ND
Hydrolysis (acid)	255.2330 ^a	+	ND	ND	ND
Hydrolysis, hydroxylation	271.2279 ^a	ND	ND	ND	ND
Bis-hydrolysis (amine)	290.2690 ^b	+	+	+	+
Hydrolysis, glucuronidation	431.2650 ^a	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	464.2865 ^a	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	466.3011 ^b	ND	+	ND	ND
Hydrolysis (amine)	528.4986 ^b	+	ND	ND	+
Hydrolysis (amine), Glucuronidation	704.5307 ^b	ND	ND	ND	ND
Oxidation to acid	778.6930 ^a	ND	ND	ND	ND
Oxidation to acid	780.7076 ^b	ND	ND	ND	ND
Hydroxylation	782.7232 ^b	ND	ND	ND	ND
Sulfation	844.6706 ^a	ND	ND	ND	ND
Sulfation	846.6851 ^b	ND	ND	ND	ND
Glucuronidation	940.7458 ^a	ND	ND	ND	ND
Glucuronidation	942.7604 ^b	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.

CONFIDENTIAL

Page 10

090177e196137069Approved\Approved On: 21-Jan-2021 23:22 (GMT)

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

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:	Liver Microsomes + NADPH		Stability of ALC-0315 In Vitro S9 Fraction + NADPH, UDPGA, and alamethicin		Hepatocytes
ALC-0315 Concentration:	1 µM		1 µM		1 µM
Duration of Incubation (min):	120 min		120 min		240 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; t½ = half-life; WH = Wistar-Han; UDPGA= uridine-diphosphate-glucuronic acid trisodium salt.

090177e196137069\Approved On: 21-Jan-2021 23:22 (GMT)

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

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:	Liver Microsomes + NADPH		Stability of ALC-0159 In Vitro S9 Fraction + NADPH, UDPGA, and alamethicin		Hepatocytes
ALC-0159 Concentration:	1 µM		1 µM		1 µM
Duration of Incubation (min):	120 min		120 min		240 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.

090177e196137069\Approved On: 21-Jan-2021 23:22 (GMT)

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

**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 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -Dealkylation, oxidation	104.0706 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -dealkylation, oxidation	130.0874 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -Dealkylation, oxidation	132.1019 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -dealkylation, hydrolysis, oxidation	145.0506 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (acid)	255.2330 ^a	+	+	ND	ND	+	+	+	+	+	+	ND	+
Hydrolysis, hydroxylation	271.2279 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bis-hydrolysis (amine)	290.2690 ^b	+	+	ND	ND	ND	ND	ND	ND	ND	ND	+	ND
Hydrolysis, glucuronidation	431.2650 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	464.2865 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	466.3011 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (amine)	528.4986 ^b	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	+	ND
Hydrolysis (amine), glucuronidation	704.5307 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxidation to acid	778.6930 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxidation to acid	780.7076 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydroxylation	782.7232 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfation	844.6706 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfation	846.6851 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Glucuronidation	940.7458 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Glucuronidation	942.7604 ^b	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.

090177e196137069\Approved\Approved On: 21-Jan-2021 23:22 (GMT)

BNT162b2

2.6.5 Pharmacokinetics Tabulated Summary

**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 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>O</i> -Demethylation, <i>O</i> -dealkylation	151.0965 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>O</i> -Demethylation, <i>O</i> -dealkylation	195.1227 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis, <i>N</i> -Dealkylation	214.2529 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -Dealkylation, oxidation	227.2017 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (amine)	410.4720 ^b	+	+	ND	ND	+	+	+	+	+	+	+	+
<i>N,N</i> -Didealkylation	531.5849 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>N</i> -Dealkylation	580.6396 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>O</i> -Demethylation, oxidation	629.6853 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydroxylation	633.6931 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ω -Hydroxylation, Oxidation	637.1880 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (acid)	708.7721 ^b	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.

090177e196137069\Approved\Approved On: 21-Jan-2021 23:22 (GMT)