

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
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
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 ^c	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 ^c	01049-20009
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 ^c	01049-20010

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 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 ^c	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 ^c	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 ^c	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 ^d	PF-07302048_05Aug20_043725

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; IM = Intramuscular; IV = Intravenous; LNP = lipid nanoparticles; S9 = Supernatant fraction obtained from liver homogenate by centrifuging at 9000 g.

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

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	
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.

- 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

2.6.5.5. 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	modRNALuciferase in LNP	modRNALuciferase 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	1.32×10 ⁵	7.87×10 ⁷	Below detection ^a
6 days	1.62×10 ⁵	2.02×10 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.

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 urine, 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.

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)azanediyldis(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_{1/2} = 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 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)	NC	>120	NC	>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
N-dealkylation, oxidation	102.0561 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-Dealkylation, oxidation	104.0706 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-dealkylation, oxidation	130.0874 ^a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-Dealkylation, oxidation	132.1019 ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-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.

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.