MODULE 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
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)azanediyl)bis(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 $t_{½}$ (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 (%)[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)azanediyl)bis(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; $t_{½}$ = 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

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:		Stability of ALC-0315 In Vitro	
Study System:	Liver Microsomes + NADPH	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
$t_{½}$ (min)	>120	>120	>120	>120	>120	>120	>120	>120	>120	>240	>240	>240	>240	>240

-- = Data not available; ALC-0315 = (4-hydroxybutyl)azanediyl)bis(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; $t_{½}$ = 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:		Stability of ALC-0159 In Vitro	
Study System:	Liver Microsomes + NADPH	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
$t_{½}$ (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
O-Demethylation, *O*-dealkylation	107.0703[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
O-Demethylation, *O*-dealkylation	151.0965[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
O-Demethylation, *O*-dealkylation	195.1227[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis, *N*-Dealkylation	214.2529[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-Dealkylation, oxidation	227.2017[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (amine)	410.4720[b]	+	+	ND	ND	+	+	+	+	+	+	+	+
N,*N*-Didealkylation	531.5849[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-Dealkylation	580.6396[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
O-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.