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Terms and abbreviations used in this section

Terms and abbreviations	Unabridged expressions or definitions
ALC-0159	PEG lipids added to the drug
ALC-0315	Aminolipids added to this product
[$_3$H]-CHE	Radiolabeled [Cholesteryl-1,2-$_3$H(N)]-Cholesteryl Hexadecyl Ether
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
GLP	Good Laboratory Practice
LNP	Lipid-nanoparticle
modRNA	Nucleoside-modified mRNA
mRNA	Messenger RNA
m/z	m/z
PEG	Polyethylene glycol
PK	Pharmacokinetics
RNA	Ribonucleic acid
S9	Supernatant fraction obtained from liver homogenate by centrifuging at 9000 g
WHO	World Health Organization

1. Summary

BNT162b2 (BioNTech code: BNT162, Pfizer code: PF-07302048) is a modified nucleoside mRNA (modRNA) encoding the full-length spike glycoprotein (S protein) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is being developed as the essence of an mRNA vaccine against infections caused by SARS-CoV-2. To formulate BNT162b2, it was mixed with two functional lipids, ALC-0315 (aminolipid) and ALC-0159 (PEG-lipid), and two structural lipids, DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) and cholesterol. (1,2-distearoyl-sn-glycero-3-phosphocholine) as two structural lipids, and cholesterol to form lipid nanoparticles (LNPs) that encapsulate BNT162b2 (henceforth, "BNT162b2 encapsulated LNPs").

To evaluate the non-clinical pharmacokinetics of BNT162b2-encapsulated LNPs, we performed evaluation studies both in vivo and in vitro to see the absorption (PK), metabolism, and excretion of ALC-0315 and ALC-0159 in LNPs, as well as biodistribution studies using luciferase or radioactively labeled lipid as alternative reporters for BNT162b2.

Based on the fact that the development of vaccines for the prevention of infectious diseases does not require evaluation of systemic exposure (WHO, 2005; Guidelines for Non-clinical Studies of Vaccines for the Prevention of Infectious Diseases) [1,2], we did not conduct a PK study using intramuscular administration of BNT162b2-encapsulated LNP. The other two lipids contained in the drug (cholesterol and DSPC) are naturally occurring lipids and are considered to be metabolized and excreted in the same manner as endogenous lipids. In addition, it is expected that BNT162b2 is degraded by ribonuclease in uptake cells and metabolized by nucleic acid, and that BNT162b2-derived S protein undergoes proteolysis. Based on the above, it was not considered necessary to evaluate the metabolism and excretion of these components again.

As an alternative reporter to BNT162b2, luciferase RNA-encapsulated LNPs (luciferase RNA encapsulated in LNPs with the same lipid composition as BNT162b2-encapsulated

LNPs; hereafter referred to as "luciferase RNA-encapsulated LNPs") were intravenously administered to Wistar Han rats. In the study, plasma, urine, feces, and liver samples were collected over time, and the concentrations of ALC-0315 and ALC-0159 were measured in each sample. The results showed that ALC-0315 and ALC-0159 were rapidly distributed from the blood to the liver. About 1% and 50% of the dose of ALC-0315 and ALC-0159, respectively, were excreted in the feces as unchanged drug, and both were below the detection limit in the urine.

In the biodistribution study, luciferase RNA-encapsulated LNP was intramuscularly administered to BALB/c mice. In the biodistribution study, luciferase RNA-encapsulated LNP was intramuscularly administered to BALB/c mice, and the expression of luciferase was observed at the site of administration and also in the liver where the level of expression was even lower. Expression of luciferase at the site of administration was observed at 6 hours post-dose and disappeared by 9 days post-dose. Expression of luciferase in the liver was also observed at 6 hours post-dose and disappeared by 48 hours post-dose. The radioactivity of the radioactively labeled luciferase RNA-encapsulated LNPs was intramuscularly administered to rats, and the biodistribution of the radioactivity was quantitatively evaluated. The highest non-dose site was the liver (up to 18% of dose).

The metabolism of ALC-0315 and ALC-0159 was evaluated in vitro using blood, liver microsomes, liver S9 fractions and hepatocytes from CD-1/ICR mice, Wistar Han or Sprague Dawley rats, crab-eating macaques or humans. In vivo metabolism was also investigated using plasma, urine, feces, and liver samples collected from the intravenous PK study in rats. These in vitro and in vivo studies showed that ALC-0315 and ALC-0159 were slowly metabolized by hydrolysis of ester and amide bonds, respectively, in all animal species tested.

These non-clinical pharmacokinetic evaluations indicated that LNP that reached the circulation was distributed to the liver. In addition, the disappearance of ALC-0315 and ALC-0159 was suggested to be related to metabolism and fecal excretion, respectively.

2. Analysis method

Report Number: **PF-07302048_06 _072424**

An LC/MS method with appropriate performance was developed to quantify the concentrations of ALC-0315 and ALC-0159, the constituent lipids of LNP, in the non-GLP rat intravenous PK study (Section M2.6.4.3). In other words, 20 μL of plasma, liver homogenate (homogenates were prepared from sections taken from three different locations of the liver and pooled and diluted with blank matrix as appropriate), urine and fecal homogenate (diluted with blank matrix as appropriate) samples were each deproteinized in acetonitrile containing an internal standard (PEG-2000). The samples were centrifuged and the supernatant was used for LC-MS/MS measurements.

3. Absorption

Report Number: **PF-07302048_06 _072424, Summary Table: 2.6.5.3**

To investigate the pharmacokinetics of ALC-0315 and ALC-0159, luciferase RNA-encapsulated LNPs were administered intravenously to male Wistar Han rats at a single dose of 1 mg RNA/kg, and plasma and liver samples were collected by sparse sampling over time (0.1, 0.25, 0.5, 1, 3, 6, and 24 h before, and 2, 4, 8, and 14 days after administration). Plasma and liver samples were collected by sparse sampling (3 animals/time point). Plasma and liver concentrations of ALC-0315 and ALC-0159 were measured and PK parameters were calculated (Table 1). ALC-0315 and ALC-0159 in blood were promptly distributed to the liver by 24 hours after administration. Plasma concentrations of ALC-0315 and ALC-0159 at 24 hours post-dose were less than 1% of the maximum plasma concentration (Figure 1). The apparent terminal phase elimination half-lives (t½) were similar in plasma and liver, 6-8 days for ALC-0315 and 2-3 days for ALC-0159. The results of this study suggest that the liver is one of the major tissues that take up ALC-0315 and ALC-0159 from the blood.

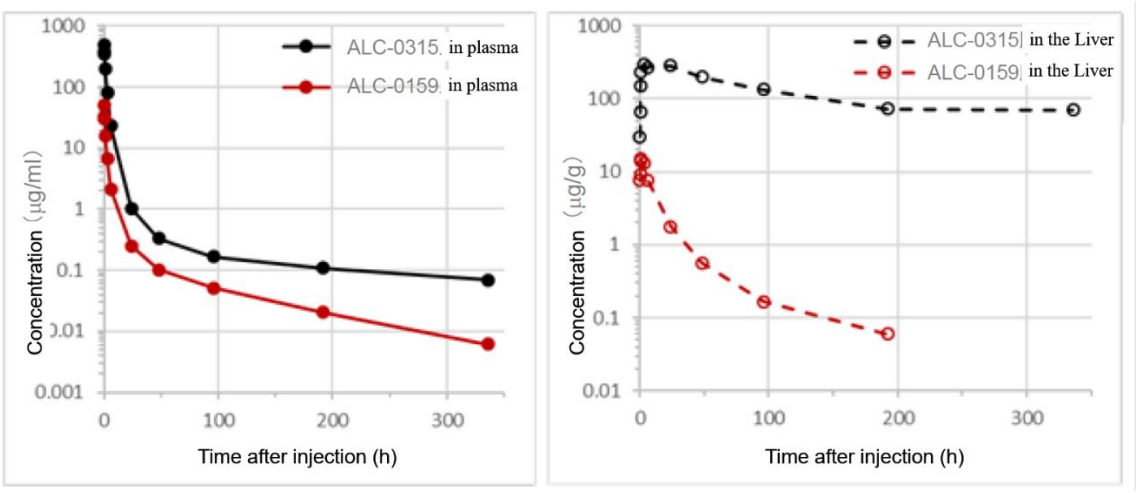
The results of the investigation of urinary and fecal concentrations of ALC-0315 and ALC-0159 conducted in this study are described in section M2.6.4.6.

Table 1 Pharmacokinetics of ALC-0315 and ALC-0159 in LNP included with luciferase labeled RNA injected intravenously in Wistar Han Rat at 1mg RNA/kg

component	Amount of component (mg/kg)	Sex/N	t½ (h)	AUC_{inf} (µg•h/mL)	AUC_{last} (µg•h/mL)	Distribution in the Liver (%)
ALC-0315	15.3	Male/3[b]	139	1030	1020	60
ALC-0159	1.96	Male/3[b]	72.7	99.2	98.6	20

a. Calculated by [Max amount of distribution in the Liver]/[Total amount injected]
b. 3 animals in each point. Spars sampling

Figure 1 Plasma and liver concentrations of ALC-0315 and ALC-0159 after intravenous administration of luciferase RNA-encapsulated LNPs at a dose of 1 mg RNA/kg to Wistar Han rats



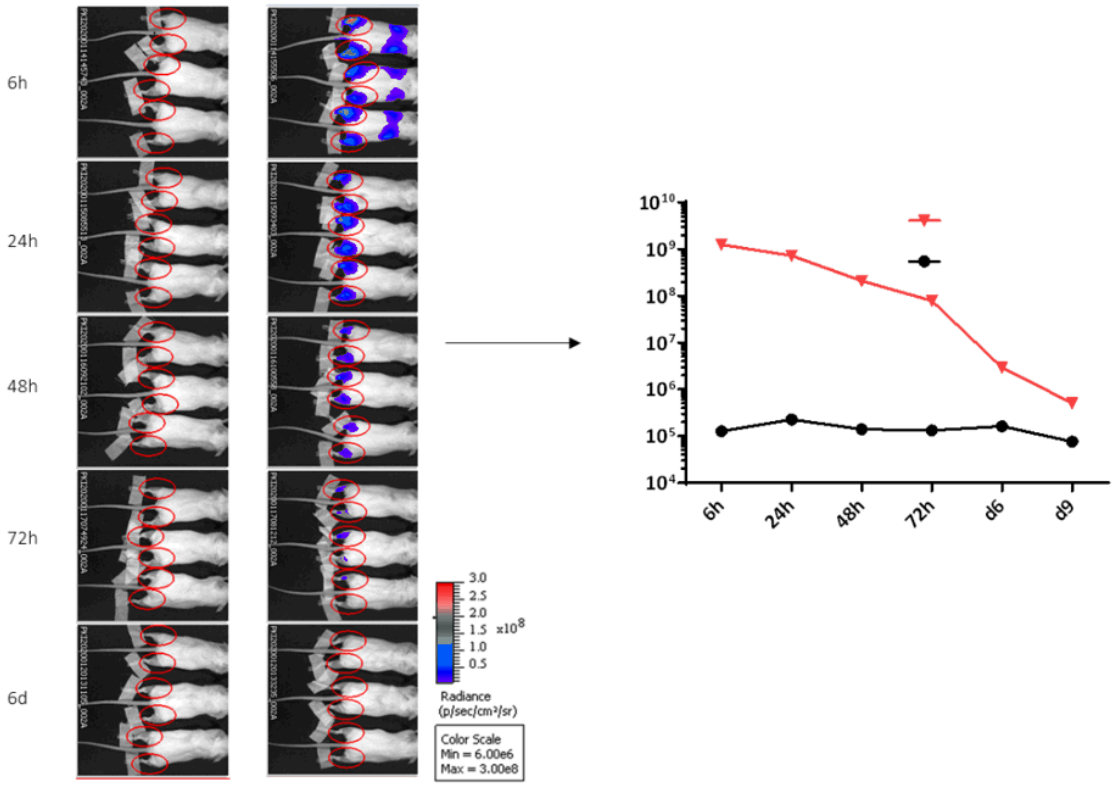
4. Distribution

Report No.: R[redacted]0072, 185350, Summary Table: 2.6.5.5A, 2.6.5.5B

Luciferase RNA-encapsulated LNPs were administered to three female BALB/c mice, and the biodistribution of BNT162b2 was examined using luciferase luminescence as a surrogate marker. In other words, luciferase RNA-encapsulated LNPs were intramuscularly administered to the left and right hind paws of mice at a dose of 1 μg RNA each (2 μg RNA in total). Luciferin, a luminescent substrate, was then administered intraperitoneally 5 min before detection of luciferase luminescence, and in vivo luminescence was measured under isoflurane anesthesia at 6 and 24 hours after administration and at 2, 3, 6, and 9 days using Xenogen IVIS Spectrum. The expression of luciferase protein was evaluated over time in the same individual. The expression of luciferase at the site of administration was observed from 6 hours post-dose and disappeared by 9 days post-dose. The expression of luciferase in the liver was also observed from 6 hours post-dose and disappeared by 48 hours post-dose. The

distribution in the liver was considered to indicate that a portion of the locally administered luciferase RNA-encapsulated LNP reached the circulating blood and was taken up by the liver. As described in detail in Section M2.6.4.3, when luciferase RNA-encapsulated LNPs were administered intravenously to rats, the liver was suggested to be the major organ of distribution for ALC-0315 and ALC-0159, which is consistent with the findings of this study in which ALC-0315 and ALC-0159 were administered intramuscularly to mice. No toxicity findings indicating hepatic injury were observed in the rat repeated-dose toxicity study (Section M2.6.6.3).

Figure 2 In vivo luminescence in BALB/c mice intramuscularly treated with luciferase RNA-encapsulated LNP



Luciferase RNA-encapsulated LNPs using [3H]-cholesteryl hexadecyl ether ([3H]-CHE)-labeled LNPs were administered intramuscularly to male and female Wistar Han rats at a dose of 50 μg RNA, and blood, plasma, and tissues were collected from three animals per sex at 15 min and at 1, 2, 4, 8, 24, and 48 h after administration. Blood, plasma, and tissue samples were collected from three animals each at 15 min and 1, 2, 4, 8, 24, and 48 h post-dose, and the biodistribution of LNPs was evaluated by measuring the radioactivity concentration by liquid scintillation counting. In both males and females, the

highest radioactivity levels were observed at the site of administration at all measurement time points. Plasma radioactivity levels were highest in the first 1 to 4 hours post-dose. The highest radioactivity levels in these tissues were observed 8 to 48 hours post-dose. Total recoveries of radioactivity relative to the dose outside of the dose site were highest in the liver (up to 18%) and were significantly lower in the spleen (≤1.0%), adrenal glands (≤0.11%), and ovaries (≤0.095%) than in the liver. The mean radioactivity concentrations and tissue distribution patterns were generally similar in males and females.

The distribution of the antigen encoded by BNT162b2 in vivo is considered to be dependent on the distribution of LNP. Since the lipid composition of the luciferase RNA-encapsulated LNP used in this study is identical to that of the application formulation of BNT162b2, the results of this study are considered to indicate the distribution of BNT162b2-encapsulated LNP.

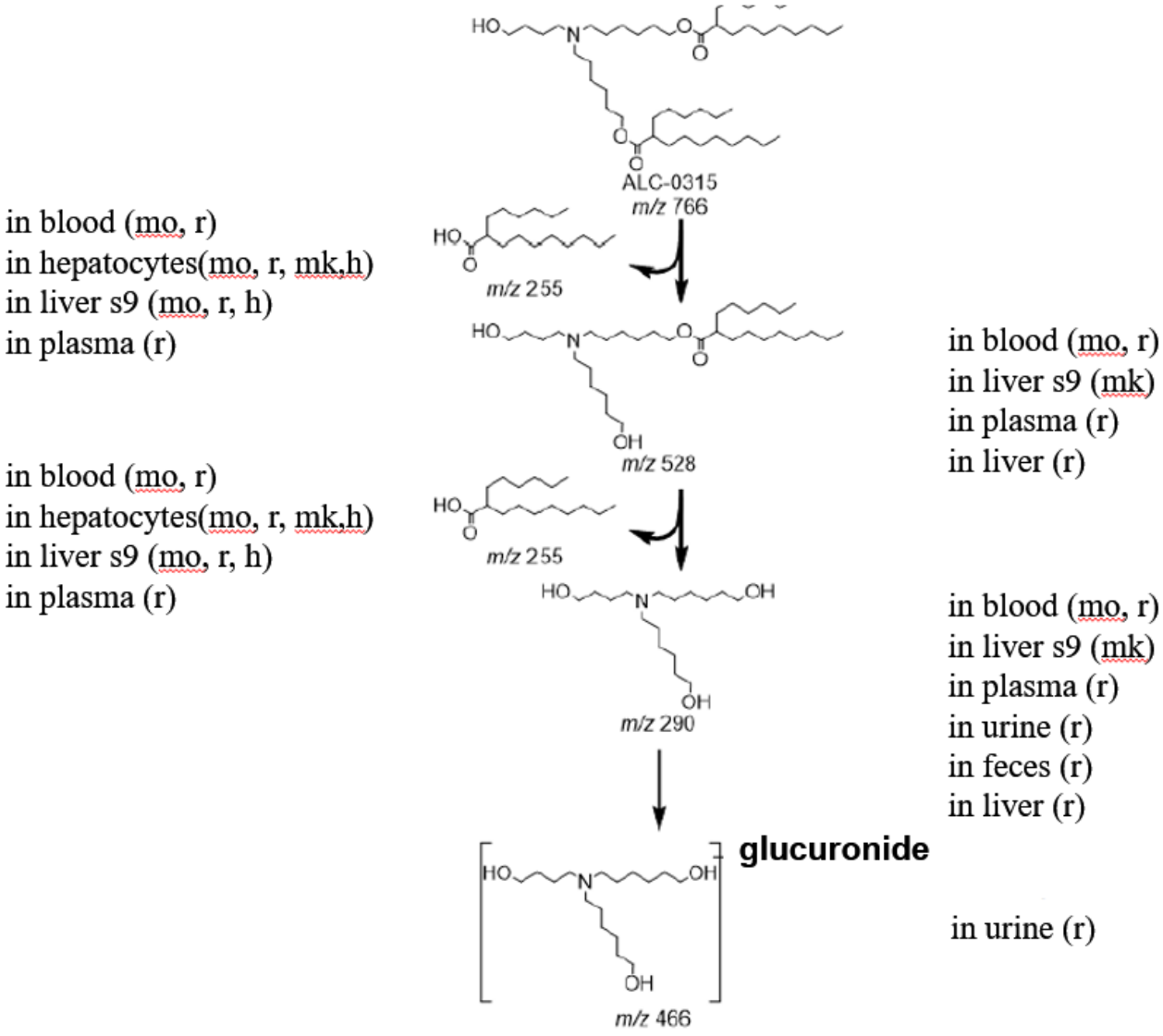
5. Metabolism

Report Number: **01049- 008, 01049- 009, 01049- 010, 01049- 020, 01049- 021, 01049- 022, PF-07302048_05 _043725,** Summary Table: **2.6.5.10A, 2.6.5.10B, 2.6.5.10C, 2.6.5.10 D**

The in vitro metabolic stability of ALC-0315 and ALC-0159 was evaluated using liver microsomes, liver S9 fractions, and hepatocytes from CD-1/ICR mice, Wistar Han or Sprague Dawley rats, crab-eating macaques, and humans. ALC-0315 or ALC-0159 were added to liver microsomes or liver S9 fractions (120 min incubation) or hepatocytes (240 min incubation) of each animal species, and the percentage of unchanged product after incubation was determined. The results showed that ALC-0315 and ALC-0159 were metabolically stable in both animal species and test systems, and the final percentage of unchanged product was over 82%.

In addition, the metabolic pathways of ALC-0315 and ALC-0159 were evaluated in vitro and in vivo. In these studies, in vitro metabolism was evaluated using blood, liver S9 fractions, and hepatocytes from CD-1 mice, Wistar Han rats, crab-eating macaques, and humans. In addition, plasma, urine, feces, and liver samples collected in the rat PK study were used to evaluate in vivo metabolism (Section M2.6.4.3). The test results showed that the metabolism of both ALC-0315 and ALC-0159 was slow and metabolized by hydrolysis of ester and amide bonds, respectively. Metabolism by hydrolysis, shown in Figure 3 and Figure 4, was observed in all animal species evaluated.

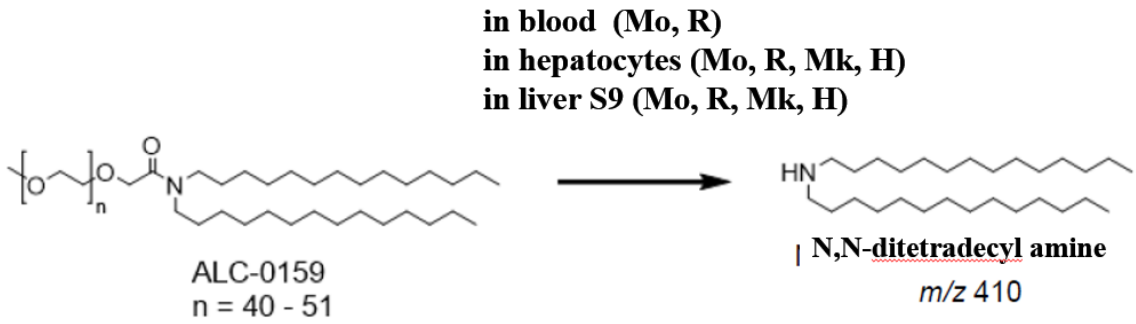
Figure 3 The estimated metabolic pathway in organisms of ALC-0315 in various spices of animals



H: Human, Mk: Monkey, Mo: Mouse, R: Rat

ALC-0315 is metabolized by undergoing two successive rounds of ester hydrolysis. These two hydrolysis events produce first the monoester metabolite (m/z 528) and then the double transesterification metabolite (m/z 290). The double transesterification metabolite was further metabolized to a glucuronide conjugate (m/z 466), which was detected only in urine in the rat PK study. It was also confirmed that the acidic products of the two hydrolyses were both 6-hexyl decanoic acid (m/z 255).

Figure 4 Estimated metabolic pathway of ALC-0159 in various animal species



H: human, Mk: monkey, Mo: mouse, R: rat

The major metabolic pathway of ALC-0159 was the hydrolysis of amide bonds to form N,N-ditetradecylamine (m/z 410). The metabolites were detected in mouse and rat blood, mouse, rat, monkey, and human hepatocytes and liver S9 fractions.

6. Excretion

The concentrations of ALC-0315 and ALC-0159 were measured in urine and feces collected over time in a PK study in which luciferase RNA-encapsulated LNPs were administered intravenously to rats at a dose of 1 mg RNA/kg (Section M2.6.4.3). None of the unchanged forms of ALC-0315 or ALC-0159 were detected in the urine. On the other hand, unchanged forms of ALC-0315 and ALC-0159 were detected in the feces, and the percentages per dose were about 1% and 50%, respectively. As shown in Figure 3, metabolites of ALC-0315 were detected in urine.

7. Pharmacokinetic Drug Interactions

Pharmacokinetic drug interaction studies have not been conducted for this vaccine.

8. Other pharmacokinetic studies

No other pharmacokinetic studies have been conducted for this vaccine.

9. Discussion and Conclusion

In the rat PK study, plasma and liver ALC-0315 concentrations decreased to approximately 1/7000 and 1/4 of the maximum concentrations, respectively, by 2 weeks post-dose, and ALC-0159 concentrations decreased to approximately 1/8000 and 1/250 of the maximum concentrations, respectively. t½ was similar in plasma and liver, 6-8 days for LC-0315 and 2-3 days for ALC-0159. The t½ was similar in plasma and liver, 6-8 days for LC-0315 and 2-3 days for ALC-0159. Plasma t½ values may represent the distribution of each lipid in the tissues as LNP and its subsequent redistribution in the plasma during the elimination process. The unchanged form of ALC-0315 was almost undetectable in both urine and feces, but monoester metabolites, double transesterified metabolites, and 6-hexyl decanoate were detected in feces and plasma samples collected in the rat PK study, and glucuronide conjugates of the double transesterified metabolite were detected in urine. This metabolic process is thought to be the major mechanism of ALC-0315 disappearance, but no quantitative data have been obtained to test this hypothesis. On the other hand, about 50% of the dose of ALC-0159 was excreted in feces as unchanged drug, and it was metabolized slowly by hydrolysis of amide bond in in vitro metabolism experiments.

Since the biodistribution of the antigen encoded by BNT162b2 depends on the distribution of LNP, we administered luciferase RNA-encapsulated LNP intramuscularly to BALB/c mice and examined the biodistribution of alternative reporter proteins. The results showed that luciferase was expressed at the site of administration, and was also observed in the liver, although at a lower level. The expression of luciferase at the site of administration was observed from 6 hours post-dose and disappeared by 9 days post-dose. The expression of luciferase in the liver was observed from 6 hours post-dose and disappeared by 48 hours post-dose. The distribution in the liver was considered to indicate that the locally administered luciferase RNA-encapsulated LNP reached the circulating blood and was taken up by the liver. When the radioactivity-conjugated form of luciferase RNA-encapsulated LNP was administered intramuscularly to rats, the highest radioactivity levels were observed at the site of administration. Outside of the dose site, the highest radioactivity was detected in the liver, followed by the spleen, adrenal glands, and ovaries, but the total radioactivity recovery relative to the dose in these tissues was significantly lower than in the liver. This result is consistent with the fact that luciferase expression was observed in the liver in the mouse biodistribution study. No toxicity findings indicative of hepatic injury was observed in repeated-dose toxicity studies in rats (see Section M2.6.6.3).

These non-clinical pharmacokinetic evaluations indicated that LNP reaching the circulation was distributed in the liver, and the disappearance of ALC-0315 and ALC-0159 was related to metabolism and fecal excretion, respectively.

10. Figures

Figures are shown in the text and in the summary tables.

References

1. World Health Organization. Annex 1. Guidelines on the non-clinical evaluation of vaccines. In: WHO Technical Report Series No. 927, Geneva, Switzerland. World Health Organization; 2005:31-63.

2. Guidelines on the non-clinical evaluation of vaccines for the prevention of infectious diseases (Pharmaceutical and Food Safety Inspection Service No. 0527-1, May 27, 2010).

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_06[redacted]_072424
Distribution					
In Vivo Distribution	Mice BALB/c	modRNA encoding luciferase formulated in LNP comparable to BNT162b2	IM Injection	[redacted][b]	R-[redacted]-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	[redacted][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	[redacted][d]	01049-[redacted]008
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	[redacted][d]	01049-[redacted]009

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	[redacted][d]	01049-[redacted]010
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	[redacted][d]	01049-[redacted]020
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	[redacted][d]	01049-[redacted]021
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	[redacted][d]	01049-[redacted]022
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_05[redacted]_043725

2.6.5.1. PHARMACOKINETICS OVERVIEW Test Article: BNT162b2

Type of Study	Test System	Test item	Method of Administration	Testing Facility	Report Number

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. [redacted], Germany.
c. [redacted], UK.
d. [redacted], China.
e. Groton, Connecticut.

2.6.5.3. PHARMACOKINETICS: PHARMACOKINETICS AFTER A SINGLE DOSE

Test Article: modRNA encoding luciferase in LNP
Report Number: PF-07302048_06[redacted]_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 $t_{½}$ (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

2.6.5.5A. PHARMACOKINETICS: ORGAN DISTRIBUTION

Test Article: modRNA encoding luciferase in LNP
Report Number: R-[redacted]-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	1.33×10^{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.

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

Test Article: [^{3}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 [^{3}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: [^{3}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[a]	0.815	0.515	0.550	0.510	0.555	0.530	0.540	--	--	--	--	--	--	--

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

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

-- = Not applicable, partial tissue taken; [^{3}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)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.

2.6.5.9. PHARMACOKINETICS: METABOLISM IN VIVO, RAT

Test Article: modRNA encoding luciferase in LNP
Report Number: PF-07302048_05[redacted]_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.

2.6.5.10A. PHARMACOKINETICS: METABOLISM IN VITRO

Test Article: ALC-0315
Report Numbers: 01049-[redacted]008
01049-[redacted]009
01049-[redacted]010

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-[redacted]020
01049-[redacted]021
01049-[redacted]022

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)	>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_05[redacted]_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_05[redacted]_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.