Masking location: Adjusting

SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048)
2.6.4 Summary of pharmacokinetic study

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Terms and abbreviations used in this section

Term / Abbreviation	Not abbreviated expressions or definitions
ALC-0159	PEG lipid added to this drug
ALC-0315	Amino lipid added to this drug
[3 H]-CHE	Radiolabeled [cholesteryl-1,2- 3 H (N)] - cholesteryl hexadecyl Ether: radiolabeled [cholesteryl Lil-1, 2-3 H (N)] Hexadecyl ether
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine: 1,2-distearoyl-sn-glycero-3-phosphocholine Rin
GLP	Good Laboratory Practice: Criteria for conducting non-clinical studies on drug safety
LNP	Lipid-nanoparticle: Lipid nanoparticle
modRNA	Nucleoside-modified mRNA: Modified nucleoside mRNA
mRNA	Messenger RNA: Messenger RNA
m / z	m / z (m over z): Obtained by dividing the mass of an ion by the unified atomic mass unit (= Dalton). The dimensionless quantity obtained by dividing the obtained dimensionless quantity by the absolute value of the number of charges of the ion.
PEG	Polyethylene glycol: Polyethylene glycol
PK	Pharmacokinetics: Pharmacokinetics
RNA	Ribonucleic acid: Ribonucleic acid
S9	Supernatant fraction obtained from liver homogenate by centrifuging at 9000 g: liver homogenate Supernatant fraction centrifuged at 9000 g
WHO	World Health Organization: World Health Organization

Masking location: Adjusting

SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048)
2.6.4 Summary of pharmacokinetic study

1. Summary

BNT162b2 (BioNTech code number: BNT162, Pfizer code number: PF-07302048) is a severe acute call.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) spike glycoprotein (S protein) full length

It is a modified nucleoside mRNA (modRNA) that encodes against SARS-CoV-2 infection.
Development is underway as the essence of the mRNA vaccine. When formulating BNT162b2, there are two

Functional lipids ALC-0315 (aminolipid) and ALC-0159 (PEG lipid) and two structural lipids

By mixing with DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) and cholesterol

Lipid nanoparticles (LNP) that encapsulate BNT162b2 are formed (hereinafter, "BNT162b2-encapsulated LNP").
ALC-0315 and ALC-0315 contained in LNP to evaluate the nonclinical pharmacokinetics of BNT162b2 encapsulated LNP
In vivo and in vitro studies assessing absorption (PK), metabolism and excretion of ALC-0159 and BNT162b2
Biodistribution studies using luciferase or radiolabeled lipids as an alternative reporter for
Was carried out.

Based on the fact that the development of vaccines aimed at preventing infectious diseases does not require evaluation of systemic exposure.
(WHO, 2005; Non-clinical study guidelines for infectious disease preventive vaccines) 1 , 2 , BNT162b2 Encapsulated LNP muscle
No internal PK study was performed. In addition, two other types of lipids (choleste) contained in this drug
Rolls and DSPCs) are naturally occurring lipids that are thought to be metabolized and excreted in the same way as endogenous lipids.
available. In addition, BNT162b2 is degraded by ribonucleases in the cells that have taken it up, resulting in nucleic acid charges.
Apologize, the S protein from BNT162b2 is expected to undergo proteolysis. From the above,
It was considered unnecessary to evaluate the metabolism and excretion of these components again.

LNP (Luciferase) encapsulating RNA encoding luciferase as an alternative reporter for BNT162b2
Lase RNA is encapsulated in an LNP having the same lipid composition as the BNT162b2-encapsulated LNP:
In a PK study in which ZeRNA-encapsulated LNP ") was intravenously administered to Wistar Han rats, plasma, urine, feces and
Liver samples were collected over time and the concentrations of ALC-0315 and ALC-0159 in each sample were measured. The conclusion
As a result, ALC-0315 and ALC-0159 were shown to be rapidly distributed from the blood to the liver. Also,
About 1% and about 50% of the doses of ALC-0315 and ALC-0159 are excreted in feces as unchanged drug, respectively.
All of them were below the detection limit in urine.

In the biodistribution test, luciferase RNA-encapsulated LNP was intramuscularly administered to BALB / c mice. That
As a result, the expression of luciferase was observed at the administration site, and the expression level was lower than that in the liver.
Was also recognized. Expression at the administration site of luciferase was observed from 6 hours after administration, and 9 days after administration.
Disappeared. Expression in the liver was also observed 6 hours after administration and disappeared by 48 hours after administration. Also,
Intramuscular administration of radiolabeled LNP containing luciferase RNA to rats to quantify biodistribution
Upon evaluation, the radioactivity concentration was the highest at the administration site. Liver is highest except at the administration site
It was good (up to 18% of the dose).

Metabolism of ALC-0315 and ALC-0159 in CD-1 / ICR mice, Wistar Han or Sprague Dawley rats,
In vitro using cynomolgus monkey or human blood, liver microsomes, liver S9 fraction and hepatocytes
evaluated. In addition, plasma, urine, feces and liver samples collected in the above rat intravenous administration PK test were used.
We also examined in vivo metabolism. From these in vitro and in vivo studies, ALC-0315 and
ALC-0159 was added to ester and amide bonds in all animal species tested.
The solution showed that it was slowly metabolized.

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From the above nonclinical pharmacokinetic evaluation, it was shown that LNP that reached the circulating blood is distributed in the liver.
In addition, metabolism and fecal excretion may be involved in the disappearance of ALC-0315 and ALC-0159, respectively.
It was suggested.

2. Analytical method

Report number: PF-07302048_06 _072424

Intravenous administration of rats without GLP PK test (M2.6.4.3), ALC-0315, which is a constituent lipid of LNP, and
ALC-0159 We have developed an LC / MS method with appropriate performance for quantifying the concentration. That is, 20 µL
Plasma, liver homogenate (homogenates are prepared using sections collected from three parts of the liver, and they are used.
Dilute with a blank matrix as appropriate), urine and fecal homogenate (as appropriate, bran)
Dilute with kumatrix) Divide each sample with acetonitrile containing an internal standard substance (PEG-2000)
After protein, it was centrifuged and the supernatant was subjected to LC-MS / MS measurement.

3. Absorption

Report number: PF-07302048_06 _072424 , Summary table: 2.6.5.3

Male luciferase RNA-encapsulated LNP to study the pharmacokinetics of ALC-0315 and ALC-0159
A single intravenous dose of 1 mg RNA / kg was administered to Wistar Han rats over time (pre-dose, post-dose 0.1, 0.25,

Sparse plasma and liver 0.5, 1, 3, 6 and 24 hours and 2, 4, 8 and 14 days after dosing)

Sampling was performed (3 animals / time point). ALC-0315 and ALC-0159 in plasma and liver

The concentration was measured and the PK parameters were calculated (Table 1). ALC-0315 and ALC-0159 in the blood are thrown

It was promptly distributed to the liver by 24 hours after administration. In addition, the plasma concentration 24 hours after administration is the highest in plasma.

It was less than 1% of the concentration (Figure 1). The apparent terminal phase elimination half-life (t½) is in plasma and liver

At the same level, ALC-0315 took 6 to 8 days and ALC-0159 took 2 to 3 days. From the results of this test, the liver is in the blood

It was suggested that it is one of the major organizations that take up ALC-0315 and ALC-0159 from.

Results of examination of urinary and fecal concentrations of ALC-0315 and ALC-0159 conducted in this study

Is M2.6.4.Described in Section 6 .

Table 1 Intravenous injection of luciferase **RNA-** encapsulated **LNP** into **Wistar Han** rats at a dose of **1 mg RNA / kg**

Pharmacokinetics of **ALC-0315** and **ALC-0159** when given

Analytical material	Dosage of analyte **(mg / kg)**	Gender / **N**	**t½ (h)**	**AUC inf (μg • h / mL)**	**AUC last (Mg • h / mL)**	To the liver Distribution ratio **(%) a**
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

Calculated as [maximum liver distribution (μg)] / [dose (μg)].

b. 3 animals at each time point. Sparse sampling.

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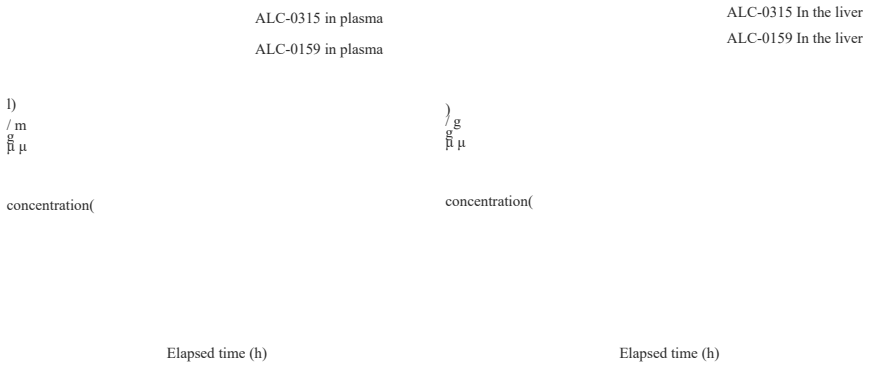
SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048)
2.6.4 Summary of pharmacokinetic study

Figure 1 Intravenous injection of luciferase **RNA-** encapsulated **LNP** into **Wistar Han** rats at a dose of **1 mg RNA / kg**

Plasma and liver concentrations of **ALC-0315** and **ALC-0159** when given



4. Distribution

Report number: R- -0072 , 185350, Summary table: 2.6.5.5A, 2.6.5.5B

Female BALB / c mice (3 mice) were administered luciferase RNA-encapsulated LNP to emit luciferase luminescence.

The biodistribution of BNT162b2 was examined as an alternative marker. That is, luciferase RNA inclusion
LNP was intramuscularly administered to the left and right hind limbs of mice at a dose of 1 μg RNA (2 μg RNA in total). After that, Le

Intraperitoneal administration of luciferin, a luminescent substrate, 5 minutes before detection of cipherase luminescence, isoflurane hemp

Intoxication, in vivo luminescence 6 and 24 hours after administration using Xenogen IVIS Spectrum and 2,

By measuring on days 3, 6 and 9, the expression of luciferase protein in the same individual was estimated over time.

Evaluated the transfer. As a result, expression of luciferase at the administration site was observed from 6 hours after administration, and it was administered.

It disappeared 9 days after giving. Expression in the liver was also observed 6 hours after administration and disappeared by 48 hours after administration. It was. Regarding the distribution to the liver, a part of locally administered luciferase RNA-encapsulated LNP reaches the circulating blood, and the liver

It was thought to indicate that it was taken up by the viscera. M2.6.4.Lucife in rats, as detailed in Section 3.

When intravenously administered with Lase RNA-encapsulated LNP, the liver is the major ALC-0315 and ALC-0159.

It has been suggested that it is a distributed organ, which is the finding of the results of this study, which was intramuscularly administered to mice.

It was a match. Toxicity findings indicating liver damage were observed in the rat repeated-dose toxicity test.

Not available (M2.6.6.3).

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

Male and female Wistar Han rats labeled with [3 H] -cholesteryl hexadecyl ether ([3 H] -CHE) LNP

Luciferase RNA-encapsulated LNP using luciferase RNA was intramuscularly administered at a dose of 50 μg RNA, and 15 minutes after administration.

Blood, plasma and tissue were collected from 3 males and 3 females at 1, 2, 4, 8, 24 and 48 hours each.

The biodistribution of LNP is evaluated by measuring the radioactivity concentration by the liquid scintillation counting method.

Worth it. In both males and females, the radioactivity concentration was highest at the administration site at all measurement points.

The radioactivity concentration in plasma was the highest 1 to 4 hours after administration. Also, mainly the liver, spleen, adrenal glands and

Distribution to the ovaries was observed, and the highest radioactivity concentration in these tissues was 8 to 48 after administration.

It was time. The total radioactivity recovery rate for doses other than the administration site is the highest in the liver (up to 18%).

Significantly lower in the spleen (1.0% or less), adrenal gland (0.11% or less) and ovary (0.095% or less) compared to the liver

won. In addition, the average concentration of radioactivity and the tissue distribution pattern were generally similar between males and females.

The in vivo expression distribution of the antigen encoded by BNT162b2 is considered to depend on the LNP distribution. For this test

Is the lipid composition of the luciferase RNA-encapsulated LNP the same as that of the submitted preparation of BNT162b2?

Therefore, the results of this test are considered to indicate the distribution of BNT162b2-encapsulated LNP.

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2.6.4 Summary of pharmacokinetic study

5. Metabolism

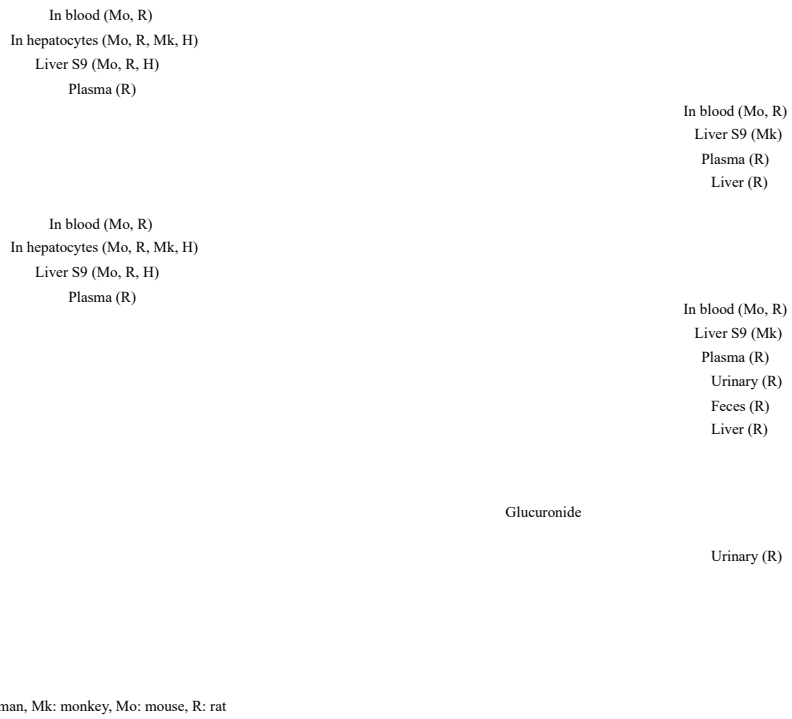
Report number: 01049-008 , 01049-009 , 01049- 010 , 01049- 020 , 01049-021 , 01049-022 , PF-07302048_05 _043725 , Summary table: 2.6.5.10A , 2.6.5.10B , 2.6.5.10C , 2.6.5.10D

CD-1 / ICR mouse, Wistar Han or Sprague Dawley rat, cynomolgus monkey and human liver mi

In vitro metabolic stabilization of ALC-0315 and ALC-0159 using crosome, liver S9 fraction and hepatocytes
Gender was evaluated. Liver microsomes or liver S9 fractions of each animal species with ALC-0315 or ALC-0159 (120)
Incubate) or add to hepatocytes (240 minutes incubation) and incubate
The proportion of unchanged drug after vation was measured. As a result, which of ALC-0315 and ALC-0159
It was also metabolically stable in animal species and test systems, with the final proportion of unchanged drug being over 82%.

Furthermore, the metabolic pathways of ALC-0315 and ALC-0159 were evaluated in vitro and in vivo. this
In these studies, CD-1 mouse, Wistar Han rat, cynomolgus monkey and human blood, liver S9 fractions
And hepatocytes were used to evaluate metabolism in vitro. In addition, plasma, urine, and feces collected in the rat PK test.
And liver samples were used to evaluate metabolism in vivo (M2.6.4.Item 3). From the test results, ALC-0315
And ALC-0159 are both slowly metabolized, with hydrolysis of ester and amide bonds, respectively.
It was revealed that it was metabolized by.Hydrolytic metabolism shown in Figures 3 and 4
Was found in all the animal species evaluated.

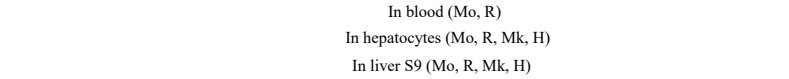
Figure 3 Estimated in vivo metabolic pathway of **ALC-0315** in various animal species



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

ALC-0315 is metabolized by undergoing ester hydrolysis twice in a row. These two hydrolysiss
First produces a monoester metabolite (*m / z* 528) and then a double deesterified metabolite (*m / z* 290).
Will be done. This double deesterified metabolite is further metabolized to the glucuronide conjugate (*m / z* 466).
However, this glucuronic acid conjugate was detected only in urine in the rat PK test. Also, two hydrolysiss
It was also confirmed that all of the acidic products of were 6-hexyldecanoic acid (*m / z* 255).

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



N, N-ditetradecylamine
m / z 410

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

In ALC-0159, *N* , *N* -ditetradecylamine (*m / z* 410) is produced by hydrolysis of the amide bond.

The pathway was the main metabolic pathway. This metabolite is found in mouse and rat blood as well as in mouse and rat.

It was detected in monkey and human hepatocytes and liver S9 fractions. Metabolites of ALC-0159 from in vivo samples

Not confirmed.

6. Excretion

PK study of intravenous luciferase RNA-encapsulated LNP in rats at a dose of 1 mg RNA / kg

(M2.6.4.The concentrations of ALC-0315 and ALC-0159 in urine and feces collected over time were measured in (3).

Neither ALC-0315 nor ALC-0159 unchanged form was detected in urine. On the other hand, in the feces

Unaltered forms of ALC-0315 and ALC-0159 were detected, at a rate of approximately 1% per dose, respectively.

It was about 50%. Also,Figure 3 As shown in, a metabolite of ALC-0315 was detected in urine.

7. Pharmacokinetic drug interactions

No pharmacokinetic drug interaction studies have been conducted with this vaccine.

8. Other pharmacokinetic studies

No other pharmacokinetic studies of this vaccine have been conducted.

9. Discussion and conclusion

Plasma and liver ALC-0315 levels were highest in rat PK studies by 2 weeks post-dose

It is reduced to about 1/7000 and about 1/4, respectively, and the ALC-0159 concentration is about 1/8000, respectively.

And reduced to about 1/250. t½ is comparable in plasma and liver, ALC-0315 is 6-8 days,

ALC-0159 was 2-3 days. The plasma t½ value is that each lipid is distributed in the tissue as LNP.

After that, it is considered to indicate that it was redistributed in plasma during the disappearance process.

Little unchanged form of ALC-0315 was detected in either urine or feces, but in the rat PK study

Monoester metabolites, double deesterified metabolites and 6-hexy from fecal and plasma samples collected in

Ludecanoic acid was detected in urine, and a glucuronic acid conjugate, a double deesterified metabolite, was detected in urine. This metabolism

The process is thought to be the major disappearance mechanism of ALC-0315, but quantitative data have been obtained to test this hypothesis.

Absent. On the other hand, about 50% of the dose of ALC-0159 was excreted in feces as unchanged drug. In vitro metabolism experiment

In, it was slowly metabolized by hydrolysis of the amide bond.

Since the in vivo expression distribution of the antigen encoded by BNT162b2 is considered to depend on the LNP distribution,

Intramuscularly administered luciferase RNA-encapsulated LNP to BALB / c mice as an alternative reporter protein

The biodistribution was examined. As a result, expression of luciferase was observed at the administration site, and more than that.

Although the expression level was low, it was also observed in the liver. Expression at the administration site of luciferase is post-administration

It was observed from 6 hours and disappeared 9 days after administration. Expression in the liver was observed from 6 hours after administration, and it was administered.

It disappeared by 48 hours after giving. Locally administered luciferase RNA-encapsulated LNP circulates in the liver

It was considered to indicate that it reached the ring blood and was taken up by the liver. Also, Luciferer on rats

When the radioactivity-labeled body of ZeRNA-encapsulated LNP was intramuscularly administered, the radioactivity concentration was the highest at the administration site.

Indicated. Other than the site of administration, it was highest in the liver, followed by the spleen, adrenal glands and ovaries.

Total radioactivity recovery for doses in these tissues was significantly lower than in the liver. This result is

This was consistent with the expression of luciferase in the liver in the mouse biodistribution test. In addition, it should be noted.

No toxic findings indicating liver damage were found in the rat repeated-dose toxicity test (M2.6.6.3).

From the above nonclinical pharmacokinetic evaluation, it was shown that LNP that reached the circulating blood is distributed in the liver.

In addition, metabolism and fecal excretion may be involved in the disappearance of ALC-0315 and ALC-0159, respectively.

It was suggested.

10. Chart

Charts are shown in the text and in the summary table.

References

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

2 Non-clinical study guidelines for infectious disease preventive vaccines (No. 0527 from Yaksik Examination) No. 1, May 27, 2010)

Masking location: Adjusting

SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048)
2.6.5 Pharmacokinetic study summary table

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	_072424
Distribution						
In Vivo Distribution	Mice BALB / c	modRNA encoding luciferase formulated in LNP comparable to BNT162b2	IM Injection	b b	R- -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	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	d	01049- 008	
In Vitro Metabolic Stability	Mouse (CD-1 / ICR), rat	ALC-0315	In vitro		01049-009	

of ALC-0315 in Liver S9	(Sprague Dawley), monkey (Cynomolgus), and human S9 liver fractions			d	

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2.6.5.1. PHARMACOKINETICS OVERVIEW — Test Article: BNT162b2

Type of Study	Test System	Test item	Method of Administration	Testing Facility	Report Number	
In Vitro Metabolic Stability of ALC-0315 in Hepatocytes	Mouse (CD-1 / ICR), rat (Sprague Dawley and Wistar Han), monkey (Cynomolgus), and human hepatocytes	ALC-0315	In vitro	d	01049- 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	d	01049- 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	d	01049-021	
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	d	01049- 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	_043725

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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

ALC-0159 = 2-[(polyethylene glycol)-2000]-N, N-ditetradecylacetamide), a proprietary polyethylene glycol-lipid included as an preferably 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 preferably 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. , Germany.
c. , UK.
d. , China.
e. Groton, Connecticut.

Masking location: Adjusting

SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048)
2.6.5 Pharmacokinetic study summary table

2.6.5.3. PHARMACOKINETICS: PHARMACOKINETICS AFTER A SINGLE DOSE

Test Article: modRNA encoding luciferase in LNP
Report Number: PF-07302048_06 _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 preferably 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 preferably 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- -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 / hidden 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.

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 preferably 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 preferably in the LNP formulation used in BNT162b2; LNP = Lipid nanoparticle; mRNA = messenger RNA.
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 _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- 008
01049-009
01049- 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 preferably 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- 020
01049-021
01049- 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 preferably 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 _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 _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.

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本項で使用する用語・略語

用語・略号	省略していない表現または定義
ALC-0159	本剤に添加される PEG 脂質
ALC-0315	本剤に添加されるアミノ脂質
[^{3}H]-CHE	Radiolabeled [Cholesteryl-1,2-^{3}H(N)]-Cholesteryl Hexadecyl Ether : 放射性標識 ［コレステリル-1, 2-^{3}H(N)］ヘキサデシルエーテル
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine：1,2-ジステアロイル-sn-グリセロ-3-ホスホコリン
GLP	Good Laboratory Practice：医薬品の安全性に関する非臨床試験の実施の基準
LNP	Lipid-nanoparticle：脂質ナノ粒子
modRNA	Nucleoside-modified mRNA：修飾ヌクレオシド mRNA
mRNA	Messenger RNA：メッセンジャーRNA
m/z	m/z（m・オーバー・z）：イオンの質量を統一原子質量単位（＝ダルトン）で割って得られた無次元量をさらにイオンの電荷数の絶対値で割って得られる無次元量
PEG	Polyethylene glycol：ポリエチレングリコール
PK	Pharmacokinetics：薬物動態
RNA	Ribonucleic acid：リボ核酸
S9	Supernatant fraction obtained from liver homogenate by centrifuging at 9000 g：肝ホモジネートを 9000 g で遠心分離した上清画分
WHO	World Health Organization：世界保健機関

1. まとめ

BNT162b2（BioNTech コード番号：BNT162，Pfizer コード番号：PF-07302048）は，重症急性呼吸器症候群コロナウイルス 2（SARS-CoV-2）のスパイク糖タンパク質（S タンパク質）全長体をコードする修飾ヌクレオシド mRNA（modRNA）であり，SARS-CoV-2 による感染症に対する mRNA ワクチンの本質として開発が進められている。BNT162b2 の製剤化にあたっては，2 つの機能脂質である ALC-0315（アミノ脂質）および ALC-0159（PEG 脂質）ならびに 2 つの構造脂質として DSPC（1,2-distearoyl-sn-glycero-3-phosphocholine）およびコレステロールと混合することで BNT162b2 を封入する脂質ナノ粒子（LNP）が形成される（以降，「BNT162b2 封入 LNP」）。

BNT162b2 封入 LNP の非臨床薬物動態を評価するために，LNP に含まれる ALC-0315 および ALC-0159 の吸収（PK），代謝および排泄を評価する in vivo および in vitro 試験ならびに BNT162b2 の代替レポーターとしてルシフェラーゼまたは放射能標識した脂質を利用した生体内分布試験を実施した。

感染症予防を目的としたワクチンの開発では全身曝露量の評価を必要としないことを踏まえ（WHO, 2005；感染症予防ワクチンの非臨床試験ガイドライン）[1,2]，BNT162b2 封入 LNP の筋肉内投与による PK 試験は実施しなかった。また，本剤に含有される他の 2 種類の脂質（コレステロールおよび DSPC）は天然に存在する脂質であり，内在性脂質と同様に代謝，排泄されると考えられる。加えて，BNT162b2 は取り込んだ細胞中のリボヌクレアーゼにより分解されて核酸代謝され，BNT162b2 由来の S タンパク質はタンパク分解を受けると予想される。以上のことから，あらためてこれらの成分の代謝および排泄を評価する必要はないと考えられた。

BNT162b2 の代替レポーターとしてルシフェラーゼをコードする RNA を封入した LNP（ルシフェラーゼ RNA を BNT162b2 封入 LNP と同一の脂質構成を持つ LNP に封入：以降，「ルシフェラーゼ RNA 封入 LNP」）を Wistar Han ラットに静脈内投与した PK 試験では，血漿，尿，糞および肝臓試料を経時的に採取して，各試料中の ALC-0315 および ALC-0159 濃度を測定した。その結果，ALC-0315 および ALC-0159 は血中から肝臓にすみやかに分布することが示された。また，ALC-0315 および ALC-0159 はそれぞれ投与量の約 1%および約 50%が未変化体として糞中に排泄され，尿中においてはいずれも検出限界未満であった。

生体内分布試験では，ルシフェラーゼ RNA 封入 LNP を BALB/c マウスに筋肉内投与した。その結果，ルシフェラーゼの発現が投与部位でみられ，それより発現量は低値であったものの肝臓でも認められた。ルシフェラーゼの投与部位での発現は投与後 6 時間から認められ，投与後 9 日には消失した。肝臓での発現も投与後 6 時間に認められ，投与後 48 時間までに消失した。また，ルシフェラーゼ RNA 封入 LNP の放射能標識体をラットに筋肉内投与して生体内分布を定量的に評価したところ，放射能濃度は投与部位で最も高値であった。投与部位以外では肝臓が最も高かった（投与量の最大 18%）。

ALC-0315 および ALC-0159 の代謝を CD-1/ICR マウス，Wistar Han または Sprague Dawley ラット，カニクイザルもしくはヒトの血液，肝ミクロソーム，肝 S9 画分および肝細胞を用いて in vitro で評価した。また，上記のラット静脈内投与 PK 試験で採取した血漿，尿，糞および肝臓試料を用いて in vivo 代謝についても検討した。これら in vitro および in vivo 試験から，ALC-0315 および ALC-0159 は，試験したいずれの動物種でも，それぞれエステル結合およびアミド結合の加水分解により緩徐に代謝されることが示された。

以上の非臨床薬物動態評価より，循環血中に到達した LNP は肝臓に分布することが示された。また，ALC-0315 および ALC-0159 の消失には，それぞれ代謝および糞中排泄が関与することが示唆された。

2. 分析法

報告書番号：PF-07302048_06[redacted]_072424

GLP 非適用のラット静脈内投与 PK 試験（M2.6.4.3 項）で LNP の構成脂質である ALC-0315 および ALC-0159 濃度を定量するために適切な性能を有する LC/MS 法を開発した。すなわち，20 µL の血漿，肝ホモジネート（肝臓の 3 箇所から採取した切片を用いてホモジネートを調製し，それらをプールしたものを適宜，ブランクマトリクスで希釈），尿および糞ホモジネート（適宜，ブランクマトリクスで希釈）試料をそれぞれ内部標準物質（PEG-2000）を含有するアセトニトリルで除タンパクした後，遠心分離し，その上清を LC-MS/MS 測定に供した。

3. 吸収

報告書番号：PF-07302048_06[redacted]_072424，概要表：2.6.5.3

ALC-0315 および ALC-0159 の体内動態を検討するため，ルシフェラーゼ RNA 封入 LNP を雄性 Wistar Han ラットに 1 mg RNA/kg の用量で単回静脈内投与し，経時的（投与前，投与後 0.1，0.25，0.5，1，3，6 および 24 時間ならびに投与後 2，4，8 および 14 日）に血漿および肝臓をスパースサンプリングにより採取（3 匹/時点）した。血漿中および肝臓中の ALC-0315 および ALC-0159 濃度を測定し，PK パラメータを算出した（Table 1）。血中の ALC-0315 および ALC-0159 は，投与後 24 時間までにすみやかに肝臓へ分布した。また，投与後 24 時間の血漿中濃度は最高血漿中濃度の 1%未満であった（Figure 1）。見かけの終末相消失半減期（t½）は血漿中および肝臓中で同程度で，ALC-0315 は 6～8 日，ALC-0159 は 2～3 日であった。本試験の結果から，肝臓が血中からの ALC-0315 および ALC-0159 を取り込む主要組織の 1 つであることが示唆された。

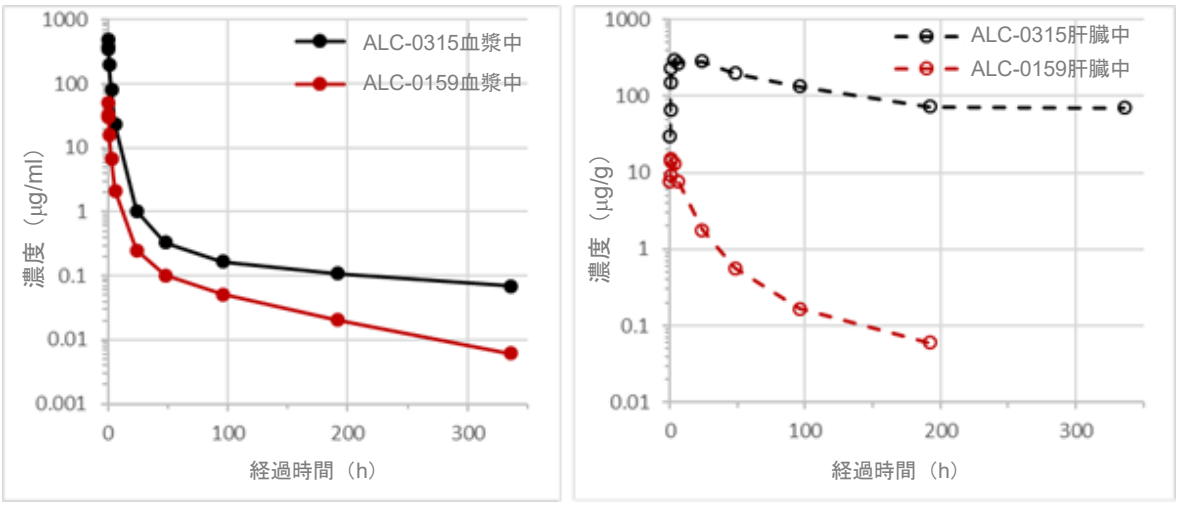
本試験において実施した ALC-0315 および ALC-0159 の尿中および糞中濃度の検討結果については M2.6.4.6 項で述べる。

Table 1 ルシフェラーゼ RNA 封入 LNP を Wistar Han ラットに 1 mg RNA/kg の用量で静脈内投与したときの ALC-0315 および ALC-0159 の薬物動態

分析物	分析物の投与量 (mg/kg)	性/N	t½（h）	AUC_{inf} (µg•h/mL)	AUC_{last} (µg•h/mL)	肝臓への分布割合 (%)[a]
ALC-0315	15.3	雄/3[b]	139	1030	1020	60
ALC-0159	1.96	雄/3[b]	72.7	99.2	98.6	20

a. ［最高肝臓分布量 (µg)］/［投与量 (µg)］として算出。
b. 各時点 3 匹。スパースサンプリング。

Figure 1 ルシフェラーゼ RNA 封入 LNP を Wistar Han ラットに 1 mg RNA/kg の用量で静脈内投与したときの ALC-0315 および ALC-0159 の血漿および肝臓中濃度

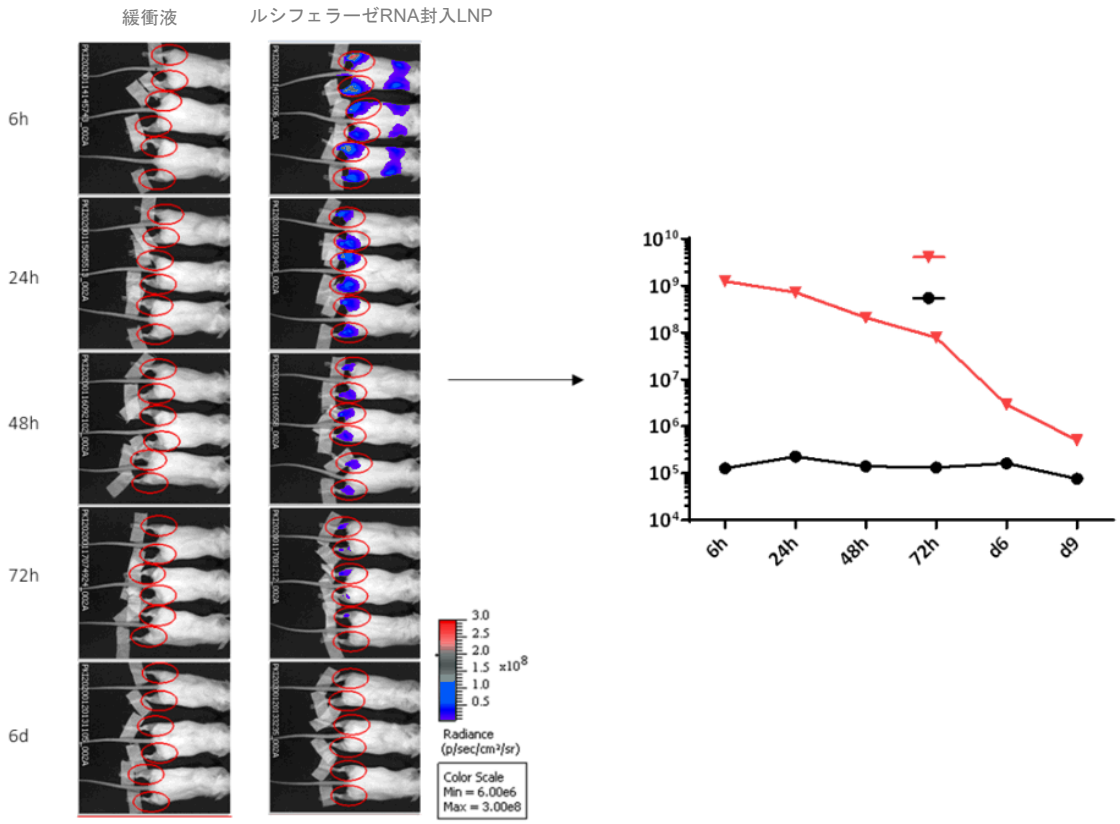


4. 分布

報告書番号：R-[redacted]-0072，185350，概要表：2.6.5.5A，2.6.5.5B

雌性 BALB/c マウス（3 匹）にルシフェラーゼ RNA 封入 LNP を投与し，ルシフェラーゼ発光を代替マーカーとして BNT162b2 の生体内分布を検討した。すなわち，ルシフェラーゼ RNA 封入 LNP をマウスの左右の後肢に各 1 μg RNA（計 2 μg RNA）の用量で筋肉内投与した。その後，ルシフェラーゼ発光検出の 5 分前に発光基質であるルシフェリンを腹腔内投与し，イソフルラン麻酔下, in vivo における発光を Xenogen IVIS Spectrum を用いて投与後 6 および 24 時間ならびに 2，3，6 および 9 日に測定することにより，ルシフェラーゼタンパクの同一個体での経時的な発現推移を評価した。その結果，ルシフェラーゼの投与部位での発現は投与後 6 時間から認められ，投与後 9 日には消失した。肝臓での発現も投与後 6 時間からみられ，投与後 48 時間までに消失した。肝臓への分布は局所投与したルシフェラーゼ RNA 封入 LNP の一部が循環血中に到達し，肝臓で取り込まれたことを示すものと考えられた。M2.6.4.3 項で詳述したように, ラットにルシフェラーゼ RNA 封入 LNP を静脈内投与した場合には，肝臓が ALC-0315 および ALC-0159 の主要な分布臓器であることが示唆されており，このことはマウスに筋肉内投与した本試験結果の所見と符合するものであった。なお，ラット反復投与毒性試験で肝障害を示す毒性所見は認められていない（M2.6.6.3 項）。

Figure 2 ルシフェラーゼ RNA 封入 LNP を筋肉内投与した BALB/c マウスにおける生体内発光



雌雄 Wistar Han ラットに，[^{3}H]-コレステリルヘキサデシルエーテル（[^{3}H]- CHE）で標識した LNP を用いたルシフェラーゼ RNA 封入 LNP を 50 µg RNA の用量で筋肉内投与し，投与後 15 分ならびに 1，2，4，8，24 および 48 時間の各時点において雌雄各 3 匹から血液，血漿および組織を採取し，液体シンチレーション計数法により放射能濃度を測定することで LNP の生体内分布を評価した。雌雄ともに，放射能濃度はいずれの測定時点においても投与部位が最も高値であった。血漿中の放射能濃度は投与後 1～4 時間で最も高値を示した。また，主に肝臓，脾臓，副腎および卵巣への分布がみられ，これらの組織において放射能濃度が最も高くなったのは投与後 8～48 時間であった。投与部位以外での投与量に対する総放射能回収率は肝臓で最も高く（最大 18%），脾臓（1.0%以下），副腎（0.11%以下）および卵巣（0.095%以下）では肝臓と比較して著しく低かった。また，放射能の平均濃度および組織分布パターンは雌雄でおおむね類似していた。

BNT162b2 がコードする抗原の生体内発現分布は LNP 分布に依存すると考えられる。本試験で用いたルシフェラーゼ RNA 封入 LNP の脂質の構成は，BNT162b2 の申請製剤と同一であることから，本試験結果は BNT162b2 封入 LNP の分布を示すと考えられる。

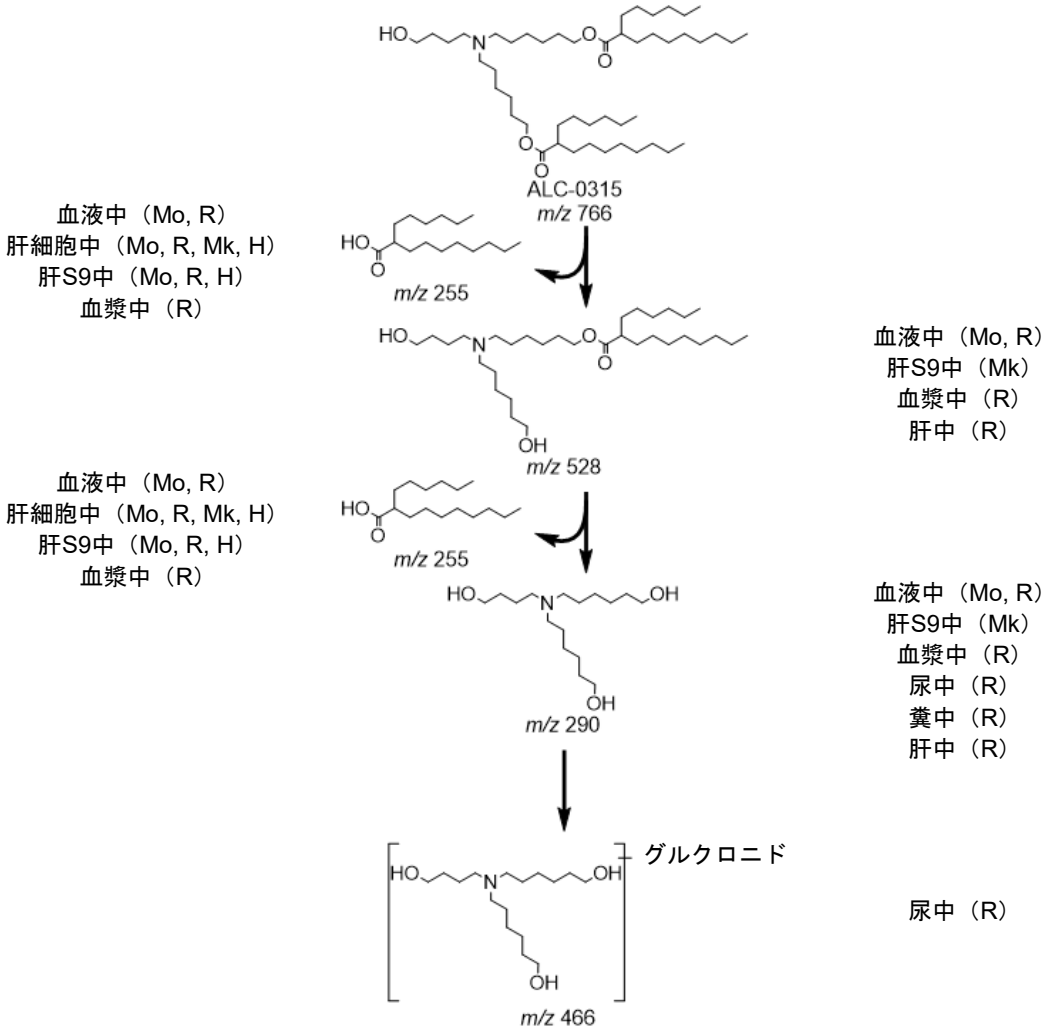
5. 代謝

報告書番号：01049-[redacted]008，01049-[redacted]009，01049-[redacted]010，01049-[redacted]020，01049-[redacted]021，01049-[redacted]022，PF-07302048_05[redacted]_043725，概要表：2.6.5.10A，2.6.5.10B，2.6.5.10C，2.6.5.10D

CD-1/ICR マウス，Wistar Han または Sprague Dawley ラット，カニクイザルならびにヒトの肝ミクロソーム，肝 S9 画分および肝細胞を用いて，ALC-0315 および ALC-0159 の in vitro 代謝安定性を評価した。ALC-0315 または ALC-0159 を各動物種の肝ミクロソームまたは肝 S9 画分（120 分間インキュベーション）もしくは肝細胞（240 分間インキュベーション）に添加して，インキュベーション後の未変化体の割合を測定した。その結果，ALC-0315 および ALC-0159 はいずれの動物種・試験系でも代謝的に安定であり，未変化体の最終的な割合は 82%超であった。

さらに ALC-0315 および ALC-0159 の代謝経路について in vitro および in vivo で評価した。これらの試験では，CD-1 マウス，Wistar Han ラット，カニクイザルおよびヒトの血液，肝 S9 画分および肝細胞を用いて in vitro での代謝を評価した。また，ラット PK 試験で採取した血漿，尿，糞および肝臓試料を用い，in vivo での代謝を評価した（M2.6.4.3 項）。試験結果から，ALC-0315 と ALC-0159 の代謝はいずれも緩徐であり，それぞれエステル結合およびアミド結合の加水分解により代謝されることが明らかになった。Figure 3 および Figure 4 に示した加水分解による代謝は，評価したすべての動物種でみられた。

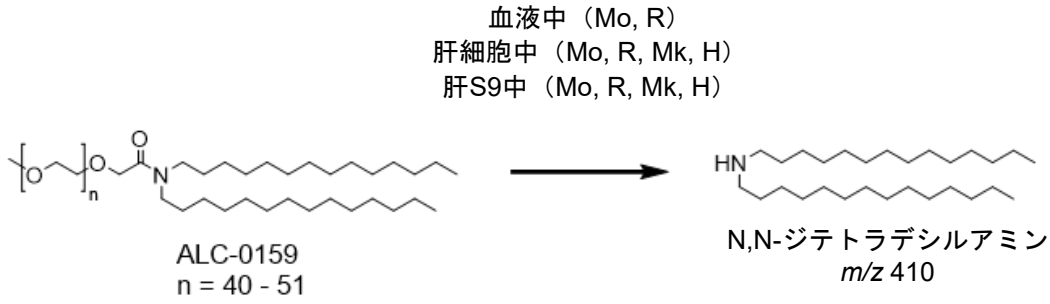
Figure 3 種々の動物種での ALC-0315 の推定生体内代謝経路



H：ヒト，Mk: サル，Mo：マウス，R：ラット

ALC-0315 はエステル加水分解を 2 回連続で受けることにより代謝される。この 2 回の加水分解により，最初，モノエステル代謝物（*m/z* 528），次に二重脱エステル化代謝物（*m/z* 290）が生成される。この二重脱エステル化代謝物はさらに代謝され，グルクロン酸抱合体（*m/z* 466）となるが，このグルクロン酸抱合体はラット PK 試験で尿中にのみ検出された。また，2 回の加水分解の酸性生成物がいずれも 6-ヘキシルデカン酸（*m/z* 255）であることも確認された。

Figure 4 種々の動物種での ALC-0159 の推定生体内代謝経路



H：ヒト，Mk: サル，Mo：マウス，R：ラット

ALC-0159 は，アミド結合の加水分解により *N,N*-ジテトラデシルアミン（*m/z* 410）が生成される経路が主要な代謝経路であった。この代謝物は，マウス・ラットの血液ならびにマウス・ラット・サル・ヒトの肝細胞および肝 S9 画分中に検出された。In vivo 試料からは ALC-0159 の代謝物は確認されなかった。

6. 排泄

ルシフェラーゼ RNA 封入 LNP を 1 mg RNA/kg の用量でラットに静脈内投与した PK 試験（M2.6.4.3 項）で経時的に採取した尿および糞中の ALC-0315 および ALC-0159 濃度を測定した。ALC-0315 および ALC-0159 の未変化体はいずれも尿中に検出されなかった。一方，糞中には ALC-0315 および ALC-0159 の未変化体が検出され，投与量当たりの割合はそれぞれ約 1%および約 50%であった。また，Figure 3 に示したように，ALC-0315 の代謝物が尿中で検出された。

7. 薬物動態学的薬物相互作用

本ワクチンの薬物動態学的薬物相互作用試験は実施していない。

8. その他の薬物動態試験

本ワクチンのその他の薬物動態試験は実施していない。

9. 考察および結論

ラット PK 試験において，血漿および肝臓中 ALC-0315 濃度は，投与後 2 週間までに最高濃度のそれぞれ約 7000 分の 1 および約 4 分の 1 に減少し，ALC-0159 濃度はそれぞれ約 8000 分の 1 および約 250 分の 1 に減少した。t½は血漿中および肝臓中で同程度で，ALC-0315 は 6～8 日，ALC-0159 は 2～3 日であった。血漿中 t½値は，それぞれの脂質が LNP として組織中に分布し，その後，消失過程で血漿中に再分布したことを表すと考えられる。

ALC-0315 の未変化体は尿中と糞中のいずれにもほとんど検出されなかったが，ラット PK 試験で採取した糞および血漿試料からモノエステル代謝物，二重脱エステル化代謝物および 6-ヘキシルデカン酸が，尿からは二重脱エステル化代謝物のグルクロン酸抱合体が検出された。この代謝過程が ALC-0315 の主要消失機序と考えられるが，この仮説を検証する定量データは得られていない。一方，ALC-0159 は投与量の約 50%が未変化体として糞中に排泄された。In vitro 代謝実験において，アミド結合の加水分解により緩徐に代謝された。

BNT162b2 がコードする抗原の生体内発現分布は LNP 分布に依存すると考えられることから，BALB/c マウスにルシフェラーゼ RNA 封入 LNP を筋肉内投与し，代替レポータータンパク質の生体内分布を検討した。その結果，ルシフェラーゼの発現が投与部位においてみられ，それより発現量は低値であったものの肝臓でも認められた。ルシフェラーゼの投与部位での発現は投与後 6 時間から認められ，投与後 9 日には消失した。肝臓での発現は投与後 6 時間から認められ，投与後 48 時間までに消失した。肝臓への分布は局所投与したルシフェラーゼ RNA 封入 LNP が循環血中に到達し，肝臓で取り込まれたことを示すものと考えられた。また，ラットにルシフェラーゼ RNA 封入 LNP の放射能標識体を筋肉内投与したところ，放射能濃度は投与部位で最も高値を示した。投与部位以外では，肝臓で最も高く，次いで脾臓，副腎および卵巣でも検出されたが，これらの組織における投与量に対する総放射能回収率は肝臓より著しく低かった。この結果は，マウス生体内分布試験において肝臓でルシフェラーゼ発現がみられたことと符合した。なお，ラット反復投与毒性試験で肝障害を示す毒性所見は認められなかった（M2.6.6.3 項）。

以上の非臨床薬物動態評価より，循環血中に到達した LNP は肝臓に分布することが示された。また，ALC-0315 および ALC-0159 の消失には，それぞれ代謝および糞中排泄が関与することが示唆された。

10. 図表

図表は本文中および概要表に示した。

参考文献

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

2 感染症予防ワクチンの非臨床試験ガイドラインについて（薬食審査発 0527 第 1 号，平成 22 年 5 月 27 日）

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.