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LIST OF FIGURES

Terms and abbreviations used in this section

Terms and abbreviations not omitted or defined	ALC-0159 Added
to this drug.	PEG lipid
ALC-0315.	Aminoolipids added to this drug
[3h] -the	RadioLabeled [Cholesteryl-1,2-3H (N)] -Cholestryl Hexadecyl Ether: Radioactive Signs [Cholester Lil -1, 2-3H (N)] Hexadecyl ether
DSPC	1,2-Distearoyl-Sn-Glycero-3-Phosphocholine: 1,2-Jistealoyl-Sn-Glycero-3-Phosphoco Rin
GLP	Good Laboratory Practice: Standard of implementation of non-clinical trials on drug safety
LNP	Lipid-nanoparticle: Lipid nanoparticles
modrna	Nucleoside-Modified mRNA: Modified nucleoside mRNA
mRNA	Messenger RNA: Messenger RNA
m/z	M / Z (M Over Z): Give the weight of ions by unified atomic mass unit (= Dalton) A dimensionless amount obtained by dividing the amount of the number of ions by the absolute value of the number of ions.
PEG	Polyethylene Glycol: Polyethylene glycol
PK	Pharmacokinetics: Pharmacokinetics
Rna	Ribonucleic Acid: ribonucleic acid
There	Supernatant fraction obtained from liver homogenate by centrifuging at 9000 g□□□□□□□□ To A supernatant dispatched with 9000 g centrifuged
WHO	World Health Organization: World Health Organization

1. Summary

BNT162B2 (BionTech Code Number: BNT162, PFIZER Code Number: PF-07302048) is a heavy acute call
Susing syndrome coronavirus 2 (SARS-COV-2) spike glycoprotein (S protein) total length
Code modified nucleoside MRNA (MODRNA) and for infectious diseases with SARS-COV-2
Development has been developed as the essence of mRNA vaccines.In formulation of BNT162B2, two
Functional lipid ALC-0315 (amino lipid) and ALC-0159 (PEG lipid) and two structural lipids
As By mixing with DSPC (1,2-Distearoyl-Sn-Glycero-3-Phosphocholine) and cholesterol
Lipid nanoparticles (LNP) which encapsulate BNT162B2 are formed (hereinafter, "BNT162B2 encapsulated LNP").

ALC-0315 contained in LNP and ALC-0315 and
In vivo and in vitro tests and BNT162B2 to evaluate ALC-0159 absorption (PK), metabolism and excretion
In-vivo distribution test using luciferase or radiolabeled lipid as an alternative reporter
Conducted.

Based on the development of vaccines for the prevention of infections, based on the need to evaluate systemic exposure
(WHO, 2005; Infectious disease prevention vaccine non-clinical trial guidelines) 1, 2, BNT162B2 Encapsulated LNP muscles
By admission PK test did not conduct.Also, the other he contained in this drug is two lipids (cholester
Roll and DSPC is a naturally occurring lipid, and is considered to be metabolism as well as endogenous lipids.
available.in addition, BNT162B2 is degraded by ribonuclease in captured cells and nucleic acid
Thank you, S-protein derived from BNT162B2 is expected to be subject to proteolysis.From the above,
It was thought that no need to evaluate metabolism and excretion of these components.

LNP enclosed RNA encoding luciferase as an alternative reporter of BNT162B2 (Lucife
Laze RNA is enclosed in LNP with the same lipid configuration as BNT162B2 encapsulated LNP: Since then, "Lucifer
Zeros In the PK test, which was administered intravenously to Wistar Han rats), plasma, urine, feces and
Collect liver samples over time and in each sample ALC-0315 and ALC-0159 concentrations were measured.That
fruit, ALC-0315 and ALC-0159 have been shown to be promptly distributed from blood to the liver.Also,
ALC-0315 and ALC-0159 excreted about 1% and about 50% of doses as unchanged
In urine, all were less than the detection limit.

In vivo distribution test, luciferase RNA encapsulated LNP was intramuscularly administered to BALB / C mice.That
As a result, the expression of luciferase was found at the site of administration, and the expression level was low in the liver.
Also recognized.Expression at the administration site of luciferase is after administration It is recognized from 6 hours, and after administration 9 days
Was disappeared.After administration of the liver expression It was observed for 6 hours and disappeared by 48 hours after administration.Also,
Luciferase RNA encapsulated LNP radiolabeled body is intramuscularly administered into rats to quantitatively in vivo distribution.
When evaluated, the radioactivity concentration was the highest at the site of administration.The liver is the highest outside the administration site
It was (maximum of dose 18%).

Metabolism of ALC-0315 and ALC-0159 CD-1 / ICR mouse, Wistar Han or Sprague Dawley rats,
Cynomolgus monkeys or human blood, liver microsomes, liver In vitro using S9 fractions and hepatocytes
evaluated.Also, the above-mentioned rat intravenous administration For plasma, urine, feces and liver samples collected in PK test
In IN VIVO metabolism was also examined.From these in vitro and in vivo tests, ALC-0315 and
ALC-0159 is an ester bond and an amide bond hydration, respectively, in any animal species of testing
It has been shown to be slowly metabolized by solution.

From the above non-clinical pharmacokinetic evaluation, the circulating blood was reached LNP was shown to be distributed in the liver.
Also, Metabolism and feces excretion is involved in the disappearance of ALC-0315 and ALC-0159, respectively.
It was suggested.

2. Analysis Method

Report number: PF-07302048_06 [redacted]_072424

ALC-0315 and ALC-0315, which is a LNP constituent lipid in rat intravenous administration PK test (M2.6.4.3) of GLP non-application
ALC-0159 Developed LC / MS method with appropriate performance to quantify concentrations.That is, 20 μl
Plasma, liver homogenate (liver A homogenate is prepared using sections collected from three places.
Suitable for pooling, dilute with blank matrix), urine and feces homogenate (as appropriate, Blanc
Cumatrix diluted) Samples Internal standards (Removed by acetonitrile containing PEG-2000)
After protein, centrifuge and the supernatant We subjected to LC-MS / MS measurement.

3. Absorption

Report number: PF-07302048_06 [redacted]_ 072424, Overview Table: 2.6.5.3

Luciferase RNA encapsulated LNP is male to consider the in-vibration condition of ALC-0315 and ALC-0159
Wistar Han rats are administered in a single intravenous administration at a dose of 1 mg RNA / kg, with time (before administration, 0.1, 0.25,
Sparse plasma and liver on 0.5, 1, 3, 6 and 24 hours and 2, 4, 8 and 14 days after administration.
Collected by sampling Three / time pointed).ALC-0315 and ALC-0159 in plasma and liver
Measure the concentration, PK parameters were calculated (Table 1).Blood ALC-0315 and ALC-0159
After giving Slightly distributed to the liver by 24 hours.Also, 24 hours plasma concentration after administration is in the highest plasma
Density It was less than 1% (Figure 1).Close-end phase disappearance half-life (T2) is in plasma and in liver
The same level, ALC-0315 was 6 to 8 days, and ALC-0159 was 2-3 days.From the results of this test, the liver is in blood
from It was suggested that it is one of the major organizations that take ALC-0315 and ALC-0159.

Conducted in this study On the examination results of Urinary and feces concentration of ALC-0315 and ALC-0159
It is Section M2.6.4.6.

Table 1 luciferase RNA encapsulated LNP in Wistar Han rats at a dose of 1 mg RNA / kg

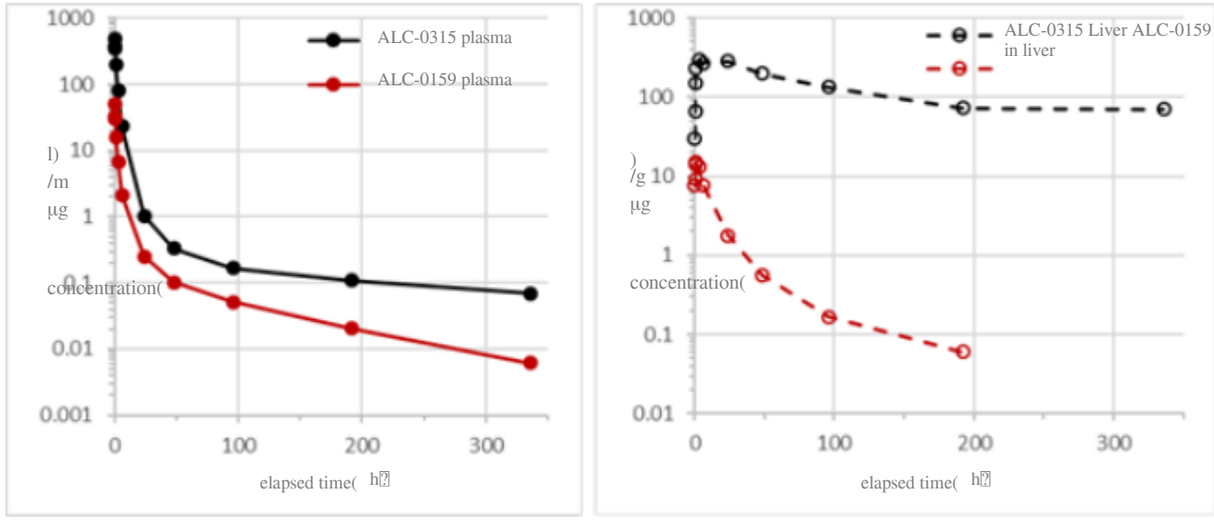
When given Pharmacokinetics of ALC-0315 and ALC-0159

Analyte	Analyze dose (mg/kg)	sex	/Nt½ (h)	AUCinf (μg•h/mL)	AUClast (μg•h/mL)	To the liver Distribution ratio (%)a
ALC-0315.	15.3	Male	3b 139	1030	1020	60
ALC-0159.	1.96	Male	3b 72.7	99.2	98.6	20

a. Calculated as the highest liver distribution amount (μg) / [dose (μg)].b. Each time point.Sparse sampling.

Figure 1 luciferase RNA encapsulated LNP in Wistar Han rats at a dose of 1 mg RNA / kg

When given Plasma and liver concentrations of ALC-0315 and ALC-0159

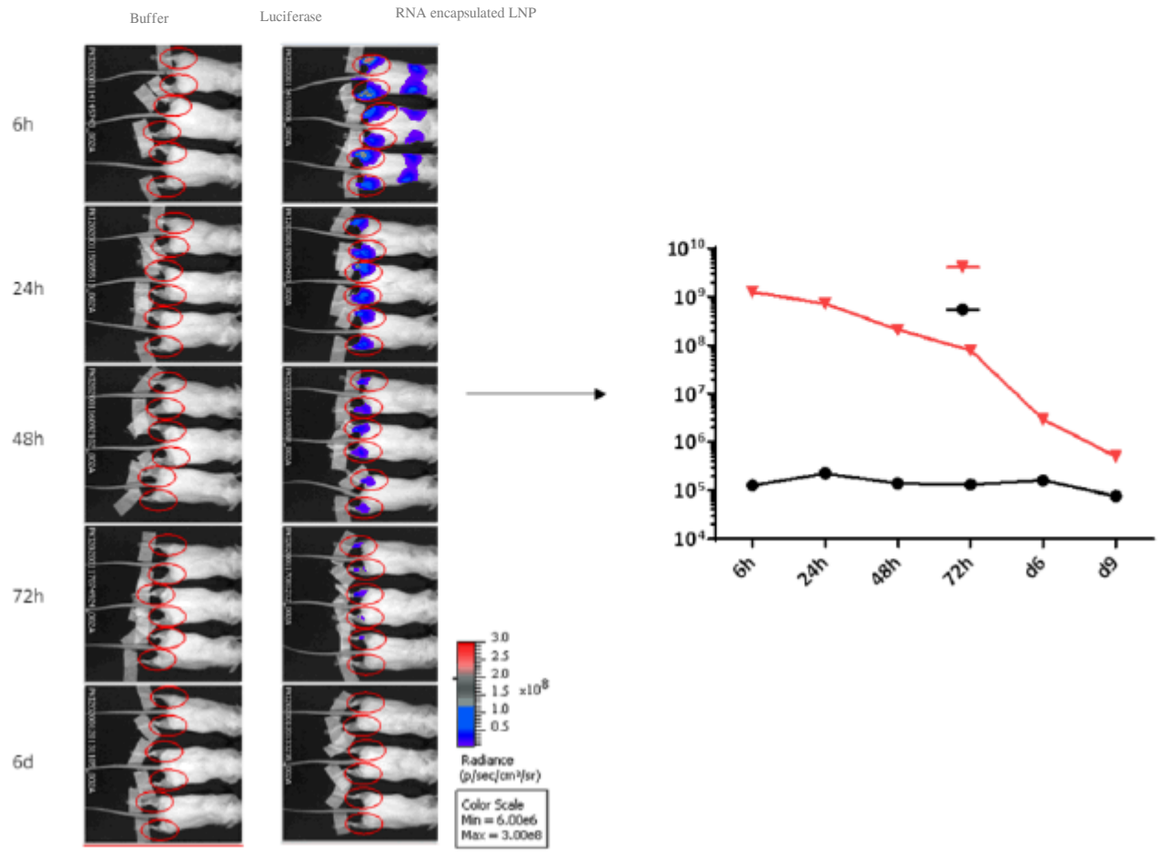


4. Distribution

Report number: R-[redacted]72, 185350, Overview Table: 2.6.5.5a, 2.6.5.5b

female Administer luciferase RNA encapsulated LNP to BALB / C mice (3 animals) and luciferase emission
As an alternative marker The vivo distribution of BNT162B2 was examined.That is, luciferase RNA encapsulation
LNP was administered intramuscularly at a dose of 1 μg RNA (total 2 μg RNA) in the left and right hindlimbs of mice.Then
Cypherase emission detection Luciferin, which is a light emitting substrate 5 minutes ago, is administered intraperitoneally, isoflurane hemp
Downward 6 and 24 hours after administration using Xenogen IVIS Spectrum in vivo, 6 and 24 hours and 2,
By measuring it on 3, 6 and 9 days, it is recommended with time with the same individual of luciferase protein
I was evaluated.As a result, expression at the site of administration of luciferase is administered Recognized from 6 hours,
After giving It disappeared on the 9th.Liver expression was also from 6 hours after administration, and disappeared by 48 hours after administration
I was.Distribution to the liver is a luciferase where topically administered Some of the RNA encapsulated LNP reaches circulating blood and liver
It was considered to indicate that it was incorporated in the needs. As detailed in M2.6.4.3, rats are
Laze When RNA encapsulated LNP is administered intravenously, the liver is the main of ALC-0315 and ALC-0159
It is suggested that it is a distributed organ, this is the finding of the test results that were intramuscularly administered to mice
The mixture was.In addition, a toxic finding finding of liver disorder is recognized in rat repeated dose toxicity test
Absent(M2.6.6.3).

Figure 2 Luciferase RNA encapsulated LNP in vivo luminescence in BALB / C mice administered intramuscularly



male and female Wistar Han rats, LNP labeled with [3H] -colesteryl hexadecyl ether ([3H] -CHE)

Luciferase using The RNA encapsulated LNP is intramuscularly administered at a dose of 50 μg RNA and 15 minutes after administration

Atmosphere Help plasma and tissues from 3 males and 3 males at each time of 1, 2, 4, 8, 24 and 48 hours

By measuring the radioactivity concentration by liquid scintillation counting method Review the vivo distribution of LNP

It was reported.Both male and female, the radioactivity concentration was the highest dosing site at any measurement.

After administration of radioactivity concentration in plasma The highest value was shown for 1 to 4 hours.In addition, liver, spleen, adrenal and

Distribution to the ovary was observed, and after administration that the radioactivity was the highest in these tissues 8 to 48

It was time.Total radiation recovery rate for doses other than the site of administration is the highest in the liver (maximum 18%)

spleen(1.0% or less), adrenal (less than 0.11%) and ovary (0.095% or less) significantly lower than the liver

won.In addition, the average concentration and tissue distribution pattern of radioactivity were roughly similar to male and female.

It is believed that the in vivo expression distribution of the antigen encoded by BNT162B2 depends on the LNP distribution.For this test

Luciferase Is the lipid configuration of RNA encapsulated LNP be identical to the application formulation of BNT162B2

The results of this test It is believed that the distribution of BNT162B2 encapsulated LNP is shown.

5. Metabolism

Report number: 01049-0[redacted]49-01049-020,0[redacted]49-021,01049-[redacted]2, [redacted] [redacted] [redacted]
PF-07302048_05 [redacted]_043725, Overview 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 rats, cynomolgus monkeys and humans

Chrome, liver In vitro metabolic stability of ALC-0315 and ALC-0159 using S9 fractions and hepatocytes

The sex was evaluated. ALC-0315 or ALC-0159 for each animal species Microsomer or liver S9 fraction (120)

Intercarding incubation) or hepatocytes (Add to 240 minutes incubation)

The proportion of unconstructed unaccurations after bath was measured.resulting in, ALC-0315 and ALC-0159

It is metabolically stable in animal species and test systems, and the ultimate percentage of unaccurate is More than 82%.

further Metabolic pathways of ALC-0315 and ALC-0159 were evaluated in vitro and in vivo.this

In the test, CD-1 mouse, Wistar Han rats, cynomolgus monkey and human blood, liver S9 fraction

And using hepatocytes IN Vitro metabolism was evaluated.In addition, plasma, urine, feces collected in rat PK test

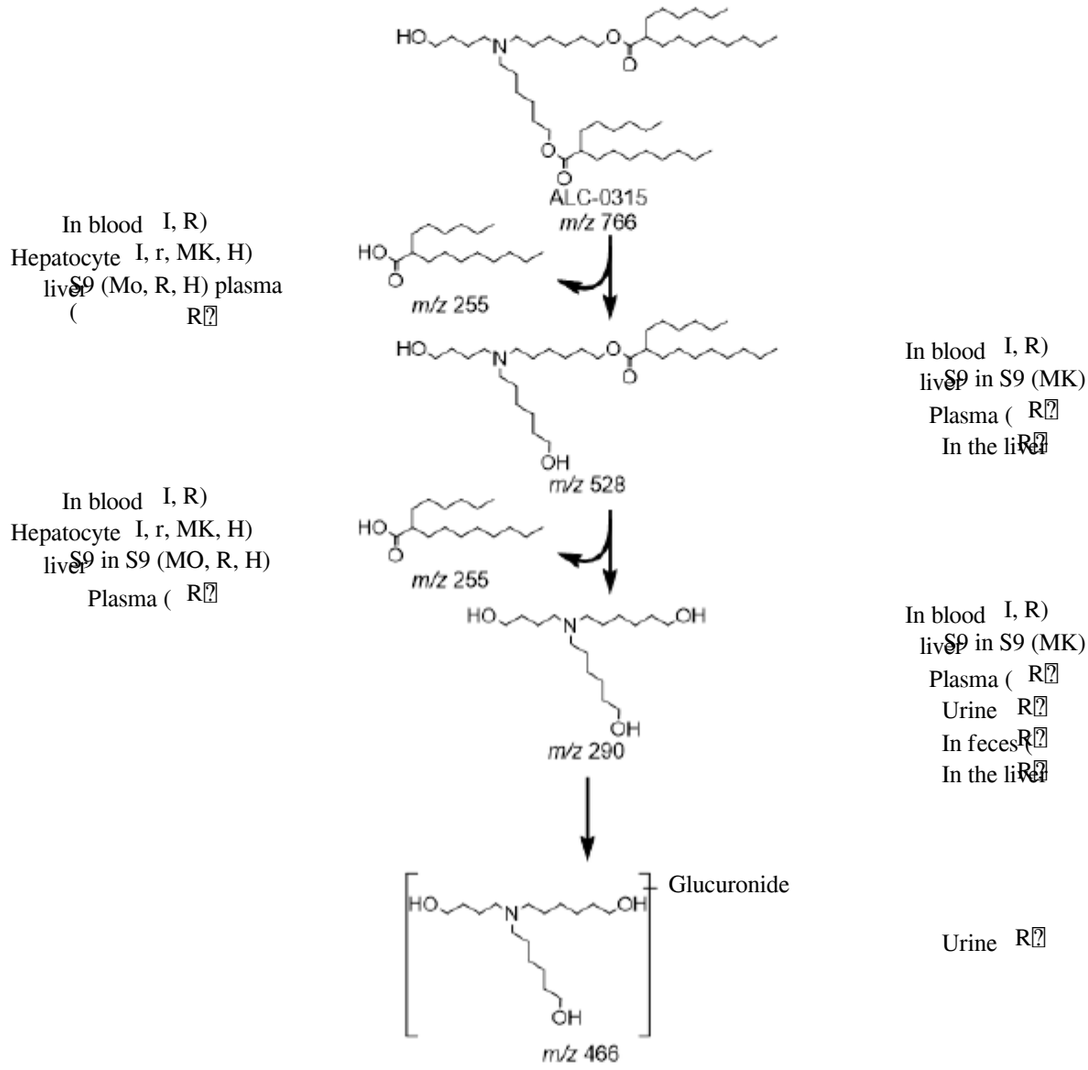
And liver samples, IN VIVO metabolism was evaluated (M2.6.4.3).From the test results, ALC-0315

When Metabolism of ALC-0159 is all slowly slow, and hydrolysis of ester bonds and amide bonds, respectively

It became clear that it is metabolized by. Metabolism by hydrolysis shown in Figure 3 and Figure 4

Was found in all 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 receiving ester hydrolysis twice in succession.This two hydrolysis

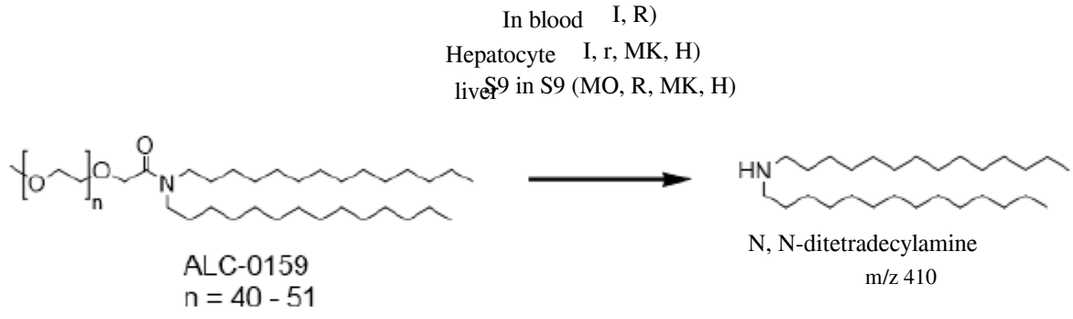
By first, monoester metabolites (M / Z 528), then a dual-dose esterification metabolite (M / z 290) is formed

It is done.This double-dose esterification metabolite is further metabolized and glucuronic acid conjugate (M / Z 466)

However, this glucuronic acid conjugate is rats PK test was only detected in urine.In addition, two hydrolysis

Any acidic product of It was also confirmed that 6-hexyl decanoic acid (m / z 255).

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



H: Human, MK: Monkey, MO: Mouse, R: Rat

ALC-0159 produces N, N-ditetradecylamine (M / Z 410) by hydrolysis of amide bonds

The pathway was the main metabolic pathway.This metabolite is blood and mice rats of mouse rats.

Sal-human hepatocytes and liver It was detected in the S9 fraction.Metabolites of ALC-0159 from in vivo samples

It was not confirmed.

6. Excretion

Luciferase PK test with intravenous administered intravenously to rats at a dose of 1 mg RNA / kg of RNA encapsulated LNP

(M2.6.4.3, ALC-0315 and ALC-0159 in urine and feces collected over time were measured.

None of the unchangeable bodies of ALC-0315 and ALC-0159 were not detected in urine.On the other hand, in the feces

ALC-0315 and ALC-0159 unchanged substances are detected, and the percentage per dose is about 1% and

about It was 50%.Also, as shown in Figure 3, the metabolites of ALC-0315 were detected in urine.

7. Pharmacokinetic drug interaction

The pharmacokinetic drug interaction test of this vaccine has not been conducted.

8. Other pharmacokinetic tests

Other pharmacokinetic tests of this vaccine have not been conducted.

9. Consideration and conclusion

Rats In the PK test, the concentration of ALC-0315 in plasma and liver is the highest concentration for 2 weeks after administration.

Every Decreased to 1/7000 and about 1/2-sq, and the ALC-0159 concentration is about 8000 minutes, respectively.

And about It decreased to one of 250 minutes.T-13 is the same in plasma and liver, ALC-0315, he is 6 to 8 days,

ALC-0159 was 2-3 days.Plasma T-13 values are distributed in tissues as LNP, each lipid.

It is then considered to indicate that it has been redistributed in plasma during the disappearance process.

Although the unchangeable body of ALC-0315 was hardly detected in any of urine and feces, rat PK test

Monomeric metabolites and dual esterification metabolites from feces and plasma samples collected 6-Hexy

Radecanoic acid detected glucuronic acid conjugate of dual-dose-esterified metabolites from urine.This metabolism

Process Although it is considered as the main loss mechanism of ALC-0315, quantitative data to verify this hypothesis is obtained

Absent.on the other hand, ALC-0159 was excreted in feces as an unchangeable body of dose.In vitro metabolic experiment

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

Because the in-vivo expression distribution of the antigen encoded by BNT162B2 is considered to depend on the LNP distribution,

BALB / C mice are intramuscularly administered luciferase RNA encapsulated LNP and alternative reporter protein

In-vivo distribution was examined.As a result, expression of luciferase is found at the site of administration,

The expression level was also observed in the liver but was also observed.Expression at the site of administration of luciferase was observed from 6 hours after administration and disappeared on 9 days after administration.The expression in the liver is observed from 6 hours after administration.

After giving It disappeared by 48 hours.Distribution to the liver is a circular luciferase RNA encapsulated LNP

It was considered to indicate that it was reached and taken up in the liver.Also, Lucifer in rats

Zeros When the radiolabel of RNA encapsulated LNP was administered intramuscularly, the radioactivity concentration is the highest value at the dosing site.

Indicated.Other than the site of administration, the liver was the highest and then detected in the spleen, adrenal and ovaries,

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

In-mouse biological distribution tests were encoded by luciferase expression in liver.In addition,

No toxic findings were observed showing liver injury in rat repeated dose toxicity tests (M2.6.6.3).

From the above non-clinical pharmacokinetic evaluation, the circulating blood was reached LNP was shown to be distributed in the liver.

Also, Metabolism and feces excretion is involved in the disappearance of ALC-0315 and ALC-0159, respectively.

It was suggested.

10. Charts

The chart is shown in the text and outline 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 trial guidelines for infection prevention vaccine (Medicine dike examination 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 yet	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 [3H]-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	01049-008
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	01049-009

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]
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]
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]
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]
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 thin	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. , Germany.
c. [redacted]
, U[redacted]
Ch[redacted]ton, Connecticut.

2.6.5.3. PHARMACOKINETICS: PHARMACOKINETICS AFTER A SINGLE DOSE

Test Article: modRNA encoding luciferase in LNP Report Number: PF-07302048_06 _072424 [redacted]

Species (Strain)	Rat (Wistar Han)	
Sex/Number of Animals	Male/ 3 animals per timepointa	
Feeding Condition	Fasted	
Method of Administration	IV	
Dose modRNA (mg/kg)	1	
How to LC-0159 (MG / KG)	1.96	
How do you have LC-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:	Meanb	Meanb
AUCinf (µg•h/mL)c	1030	99.2
Aaclast (µg • h / ml)	1020	98.6
Initial t½ (h)d	1.62	1.74
Terminal elimination t½ (h)e	139	72.7
Estimated fraction of dose distributed to liver (%)f	59.5	20.3
Dose in Urine (%)	Ncg	Ncg
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; AUCinf = Area under the plasma drug concentration-time curve from 0 to infinite time; AUClast = 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; t½ = 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 [redacted]

Species (Strain):	Mice (BALB/c)
Sex/Number of Animals:	Female/3 per group
Feeding Condition:	Fed adlibitum
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 x 105	1.26 × 10 9	4.94 × 107
24 hours	2.28 x 105	7.31 × 10 8	2.4 × 10 6
48 hours	1.40 × 10 5	2.10 × 10 8	Below detectiona
72 hours	1.33 × 10 5	7.87 × 107	Below detectiona
6 days	1.62 × 105	2.92 × 10 6	Below detectiona
9 days	7.66 × 10 4	5.09 x 105	Below detectiona

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: [3H]-Labelled LNP-mRNA formulation containing ALC-0315 and ALC-0159

Report Number: 185350

Species (Strain):	Rat (Wistar Han)
Sex/Number of Animals:	Male and female/3 animals/sex/timepoint (21 animals/sex total for the 50 µg dose)
Feeding Condition:	Fed adlibitum
Method of Administration:	Intramuscular injection
Please:	50 µg [3H]-08-A01-C0 (lot # NC-0552-1)
Number of Doses:	1
Detection:	Radioactivity quantitation using liquid scintillation counting
Sampling Time (hour):	0.25, 1, 2, 4, 8, 24, and 48 hours post-injection

Sample	Mean total lipid concentration (µg lipid equivalent/g (or mL) (males and females combined)							% of administered dose (males and females combined)						
	0.25 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: [3H]-Labelled LNP-mRNA formulation containing ALC-0315 and ALC-0159 Report Number: 185350

Sample	Total Lipid concentration (µg lipid equivalent/g [or mL]) (males and females combined)							% of Administered Dose (males and females combined)						
	0.25 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 (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
Tests (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	0.815	0.515	0.550	0.510	0.555	0.530	0.540	-	-	-	-	-	-	-

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

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

-- = Not applicable, partial tissue taken; [3H]-08-A01-C0 = An aqueous dispersion of LNPs, including ALC-0315, ALC-0159, distearoylphosphatidylcholine, cholesterol, mRNA encoding luciferase and trace amounts of radiolabeled [Cholesteryl-1,2-3H(N)]-Cholesteryl Hexadecyl Ether, a nonexchangeable, non-metabolizable lipid marker used to monitor the disposition of the LNPs; ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N--ditetradecylacetamide), a proprietary polyethylene glycol-lipid included as an excipient in the LNP formulation used in BNT162b2; ALC-0315 = (4--hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), a proprietary aminolipid included as an excipient in the LNP formulation used in BNT162b2; LNP = Lipid nanoparticle; mRNA = messenger RNA.
a. The mean male and female blood:plasma values were first calculated separately and this value represents the mean of the two values.

2.6.5.9. PHARMACOKINETICS: METABOLISM IN VIVO, RAT

Test Article: modRNA encoding luciferase in LNP Report Number: PF-07302048_05 _043725 [redacted]

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	102.0561a	ND	ND	ND	ND
N-Dealkylation, oxidation	104.0706 b	ND	ND	ND	ND
N-dealkylation, oxidation	130.0874	ND	ND	ND	ND
N-Dealkylation, oxidation	132.1019b	ND	ND	ND	ND
N-dealkylation, hydrolysis, oxidation	145.0506a	ND	ND	ND	ND
Hydrolysis (acid)	Brother .2330	+	ND	ND	ND
Hydrolysis, hydroxylation	271. Investing	ND	ND	ND	ND
Bis-Hydrolysis (Amine)	290.2690 b	+	+	+	+
Hydrolysis, glucuronidation	431.2650a	ND	ND	ND	ND
Bis-hydrolysis (amines), glucuronidation	464.2865a	ND	ND	ND	ND
Bis-hydrolysis (amines), glucuronidation	466.3011b	ND	+	ND	ND
Hydrolysis (amine)	528.4986 b	+	ND	ND	+
Hydrolysis (amine), Glucuronidation	704.5307 b	ND	ND	ND	ND
Otachi and Ashi D	778.6930a	ND	ND	ND	ND
Otachi and Ashi D	780.7076 b	ND	ND	ND	ND
Hydroxylation	Achieve.	ND	ND	ND	ND
Sulfation	844.6706	ND	ND	ND	ND
Sulfation	846.6851b	ND	ND	ND	ND
Glucuronidation	940.7458	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
01049-00[redacted]
01049-01[redacted]

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 Said Frazy				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	9.82	101.44	
90	97.78	98.66	94.15	97.96	100.48	98.15	98.85	98.70	98.33	101.92	99.25	103.09	100.0	100.36	
120	96.54	95.99	93.66	97.71	98.31	96.76	98.46	99.57	99.33	98.85	97.38	99.61	96.36	100.72	
180	-	-	-	-	-	-	-	-	-	101.15	98.88	103.47	95.64	98.92	
240	-	-	-	-	-	-	-	-	-	99.62	101.12	100.00	93.82	99.64	
t½ (min)	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 240	> 240	> 240	> 240	> 240	

-- = Data not available; ALC-0315 = (4-hydroxybutyl)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; t½ = 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-[redacted]21
01049-02[redacted]

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 Said Frazy				Hepatocytes				
	Mouse (CD-1/ICR)	Rat (SD)	Rat (WH)	Monkey (Cyno)	Human	Mouse (CD-1 / ICR)	Rat (SD)	Monkey (Cyno)	Human	Mouse (CD-1 / ICR)	Rat (SD)	Rat (WH)	Monkey (Cyno)	Human
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
15	82.27	101.24	112.11	100.83	99.59	98.93	84.38	91.30	106.73	-	-	-	-	-
30	86.40	93.78	102.69	85.12	92.28	91.10	90.87	97.96	107.60	100.85	93.37	113.04	90.23	106.34
60	85.54	98.34	105.38	86.36	95.53	102.85	97.97	105.56	104.97	94.92	91.81	105.07	92.93	101.58
90	85.41	95.44	100.90	94.63	97.97	90.75	93.51	108.33	109.36	94.28	90.25	112.80	94.59	92.67
120	95.87	97.10	108.97	93.39	93.09	106.76	92.70	105.74	119.59	87.08	89.47	104.11	97.51	96.04
180	-	-	-	-	-	-	-	-	-	94.92	93.96	102.90	89.81	93.66
240	-	-	-	-	-	-	-	-	-	102.75	94.93	98.79	92.93	102.57
t½ (min)	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 240	> 240	> 240	> 240	> 240

-- = Data not available; ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide), a proprietary polyethylene glycol-lipid included as an excipient in the lipid nanoparticle formulation used in BNT162b2; Cyno = Cynomolgus; NADPH = Reduced form of nicotinamide adenine dinucleotide phosphate; NC = not calculated; SD = Sprague Dawley; WH = Wistar-Han; UDPGA= uridine-diphosphate-glucuronic acid trisodium salt.

2.6.5.10C. PHARMACOKINETICS: METABOLISM IN VITRO CONTINUED

Test article: alc-0315

Report Number: OF-07302048_05 [redacted]_043725

Type of study		Metabolism of ALC-0315 In Vitro											
Study system		Blood				Hepatocytes				Liver Said Frazy			
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 Said Frazy			
		Mouse	Rat	Monkey	Human	Mouse		Rat	Monkey	Human	Mouse	Rat	Monkey	Human		
N-dealkylation, oxidation	102.0561a	ND	ND	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	ND	ND	
N-dealkylation, oxidation	130.0874	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
N-Dealkylation, oxidation	132.1019b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
N-dealkylation, hydrolysis, oxidation	145.0506a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Hydrolysis (acid)	Brother .2330	+	+	ND	ND	+	+	+	+	+	+	ND	+			
Hydrolysis, hydroxylation	271. Investing	ND	ND	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	+	ND		
Hydrolysis, glucuronidation	431.2650a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Bis-hydrolysis (amines), glucuronidation	464.2865a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Bis-hydrolysis (amines), glucuronidation	466.3011b	ND	ND	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	+	ND		
Hydrolysis (amine), glucuronidation	704.5307 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Otachi and Ashi D	778.6930a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Otachi and Ashi D	780.7076 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Hydroxylation	Achieve.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Sulfation	844.6706	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Sulfation	846.6851b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Glucuronidation	940.7458	ND	ND	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	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: OF-07302048_05 [redacted]_043725

Type of study		Metabolism of ALC-0159 In Vitro											
Study system		Blood				Hepatocytes				Liver Said Frazy			
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 Said Frazy			
		Mouse	Rat	Monkey	Human	Mouse	Rat	Monkey	Human	Mouse	Rat	Monkey	Human
Oh, it's THY ACON, LKY	107.0703 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oh, it's THY ACON, LKY	151.0965b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oh, it's THY ACON, LKY	195.1227 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis, N-Dealkylation	214. Stere	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-Dealkylation, oxidation	227.2017	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (amine)	410.4720b	+	+	ND	ND	+	+	+	+	+	+	+	+
N, Lky	531.5849 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-Dealkylation	580. Step	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oh, THY AICO, OY	629. Greatness	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	637.1880b	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.