MODULE 2.6.4. PHARMACOKINETICS WRITTEN SUMMARY

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	Absorption, distribution, metabolism, excretion
ALC-0159	Proprietary PEG-lipid included as an excipient in the LNP formulation used in BNT162b2
ALC-0315	Proprietary amino-lipid included as an excipient in the LNP formulation used in BNT162b2
[^{3}H]-CHE	Radiolabeled [Cholesteryl-1,2-^{3}H(N)]-Cholesteryl Hexadecyl Ether
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
GLP	Good Laboratory Practice
H	Human (in metabolite scheme)
IM	Intramuscular(ly)
IV	Intravenous(ly)
LNP	Lipid-nanoparticle
Luc	Luciferase (from firefly *Pyractomena lucifera*)
Mk	Monkey (in metabolite scheme)
Mo	Mouse (in metabolite scheme)
modRNA	Nucleoside-modified mRNA
mRNA	Messenger RNA
PEG	Polyethylene glycol
PK	Pharmacokinetics
R	Rat (in metabolite scheme)
RNA	Ribonucleic acid
S9	Supernatant fraction obtained from liver homogenate by centrifuging at 9000 g
WHO	World Health Organization

2.6.4. PHARMACOKINETICS WRITTEN SUMMARY

2.6.4.1. Brief Summary

The ADME profile of BNT162b2 (BioNTech code number BNT162, Pfizer code number PF-07302048) included evaluation of the PK and metabolism of two novel lipid excipients (ALC-0315 and ALC-0159) in the LNP and potential biodistribution using luciferase expression as a surrogate reporter or a radiolabeled lipid marker. The PK study showed the LNP distributes from the blood to the liver, ~1% of ALC-0315 and ~50% of ALC-0159 were excreted unchanged in feces, and there was no detectable excretion of unchanged ALC-0315 and ALC-0159 in the urine.

In a mouse biodistribution study, protein expression was demonstrated at the site of injection and to a lesser extent in the liver after BALB/c mice received an IM injection of modRNA encoding luciferase in an LNP formulation, with the identical lipid composition as BNT162b2. Luciferase expression was identified at the injection site at 6 hours after injection and was not detected after 9 days. Liver expression was also present at 6 hours after injection and was no longer detected by 48 hours after injection. A quantitative biodistribution study was also carried out in Wistar Han rats using a radiolabeled lipid marker and a luciferase modRNA in the same LNP formulation as BNT162b2. Following IM administration, the greatest mean concentration remained at the injection site, while up to 18% of the administered dose was found in the liver.

The metabolism of ALC-0315 (aminolipid) and ALC-0159 (PEG-lipid) was evaluated in vitro using blood, liver microsomes, S9 fractions, and hepatocytes from mice, rats, monkeys, and humans. The in vivo metabolism was examined in rat plasma, urine, feces, and liver samples collected during the PK study. In vitro and in vivo studies indicated ALC-0315 and ALC-0159 are metabolized slowly by hydrolytic metabolism of the ester and amide functionalities, respectively, across the species evaluated.

2.6.4.2. Methods of Analysis

No methods of analysis have been validated to support GLP TK studies of components of BNT162b2; however, a qualified LC/MS method was developed to support quantitation of the two novel LNP excipients for the non-GLP IV PK study in rats (PF-07302048_06Jul20_072424). Methods for immunogenicity and efficacy studies are described in Section 2.6.2.12.

2.6.4.3. Absorption

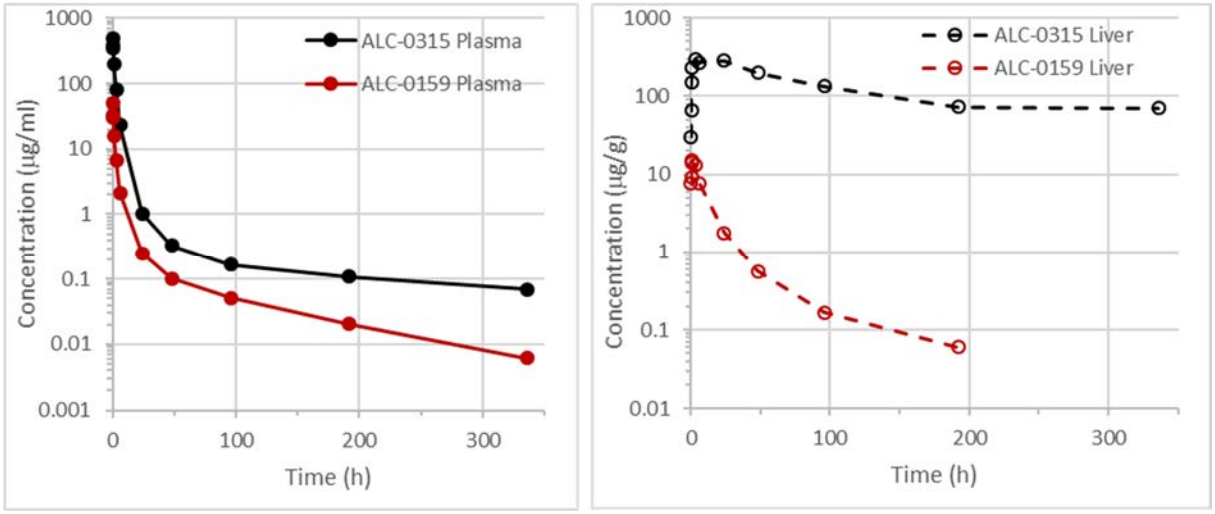
An intravenous rat PK study was performed using LNPs containing surrogate luciferase RNA, with the identical lipid composition as BNT162b2, to explore the disposition of ALC-0159 and ALC-0315 (Table 2.6.4-1, Study PF-07302048_06Jul20_072424; Tabulated Summary 2.6.5.3). The distribution of the LNP from the blood to the liver was rapid and essentially complete by 24 h, with <1% of the maximum observed plasma concentrations remaining (Figure 2.6.4-1). The liver appears to be the major site of drug uptake from the blood.

Table 2.6.4-1. PK of ALC-0315 and ALC-0159 in Wistar Han Rats After IV Administration of LNPs Containing Surrogate Luciferase RNA at 1 mg/kg

Analyte	Dose of Analyte (mg/kg)	Gender /N	t½ (h)	AUC_{inf} (µg•h/mL)	AUC_{last} (µg•h/mL)	Estimated fraction of dose distributed to liver (%)[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

a. Calculated as highest mean amount in the liver (µg)/total mean dose (µg) of ALC-0315 or ALC-0159.
b. 3 animals per timepoint; non-serial sampling.

Figure 2.6.4-1. Plasma and Liver Concentrations of ALC-0315 and ALC-0159 in Wistar Han Rats After IV Administration of LNPs Containing Surrogate Luciferase RNA at 1 mg/kg



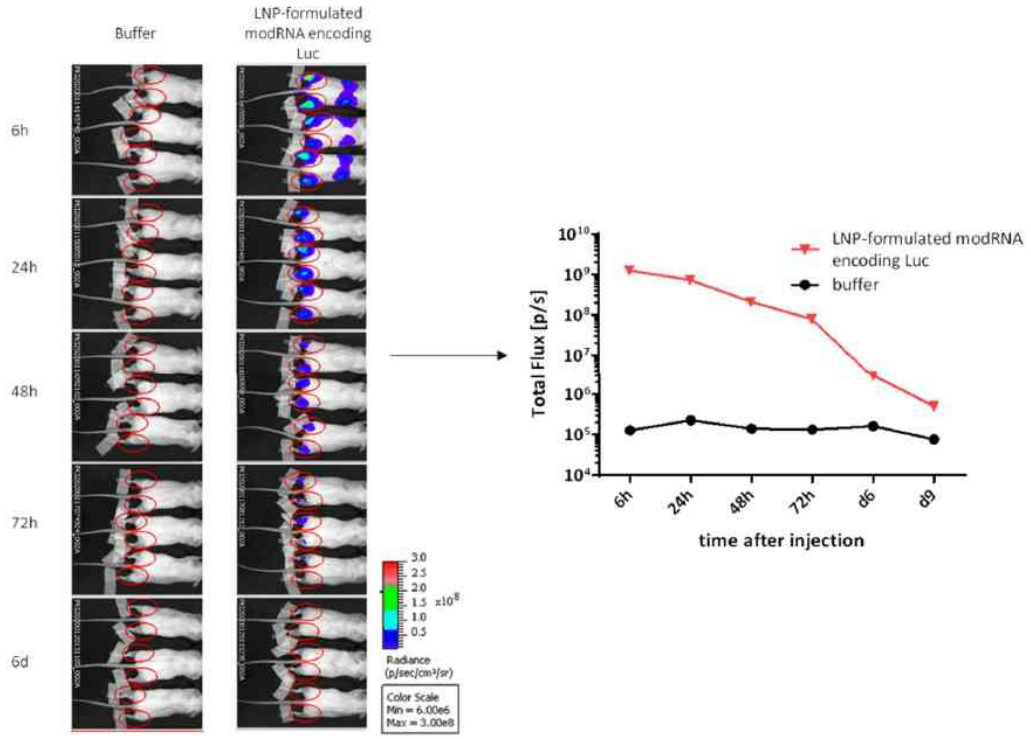
No absorption studies were conducted for BNT162b2, as the administration route is IM. Pharmacokinetic studies have not been conducted with BNT162b2 and are generally not considered necessary to support the development and licensure of vaccine products for infectious diseases (WHO, 2005; WHO, 2014).

2.6.4.4. Distribution

In an in vivo study in BALB/c mice (Study R-20-0072; Tabulated Summary 2.6.5.5A), the biodistribution of BNT162b2 was assessed using luciferase as a surrogate marker protein. RNA encoding luciferase was formulated like BNT162b2, with the identical lipid composition, and mice received IM injections of 1 µg each in the right and left hind leg (for a total of 2 µg) of LNP-formulated modRNA encoding luciferase. Luciferase protein expression was detected at different timepoints, by measuring the in vivo bioluminescence (Figure 2.6.4-2) after injection of luciferin substrate, at the site of injection and to a lesser extent in the liver. Distribution to the liver is likely mediated by LNPs entering the blood stream. The repeat-dose toxicity studies in rats showed no evidence of liver injury (Section 2.6.6.3). The luciferase expression at the injection site, the tissue with the highest

bioluminescence, dropped to background levels after 9 days. As detailed in Section 2.6.4.3, following systemic (IV) administration the liver appears to be the major organ into which the LNPs distribute, this is consistent with the observations made following IM administration.

Figure 2.6.4-2. Bioluminescence Emission in BALB/c Mice after IM Injection of an LNP Formulation of modRNA Encoding Luciferase



These qualitative data are supported by a biodistribution study (Study 185350; Tabulated Summary 2.6.5.5B) carried out with LNPs with a comparable lipid composition as BNT162b2 but with a luciferase mRNA and a [^{3}H]-CHE lipid radiolabel. Following IM administration to male and female Wistar Han rats at a dose of 50 µg (1.29 mg total lipid), the greatest mean concentration was found remaining in the injection site at each time point in both sexes. Outside the injection site, the highest levels of radioactivity were observed in plasma at 1-4 hours post-dose. Over 48 hours, the radiolabel distributed mainly to the liver, adrenal glands, spleen and ovaries, with maximum concentrations observed at 8-48 hours post-dose. Total recovery of radioactivity (% of injected dose) outside the injection site was greatest in the liver (up to 18%) and was much less in the spleen (≤1.0%), adrenal glands (≤0.11%) and ovaries (≤0.095%). The mean concentrations and tissue distribution pattern were broadly similar between sexes.

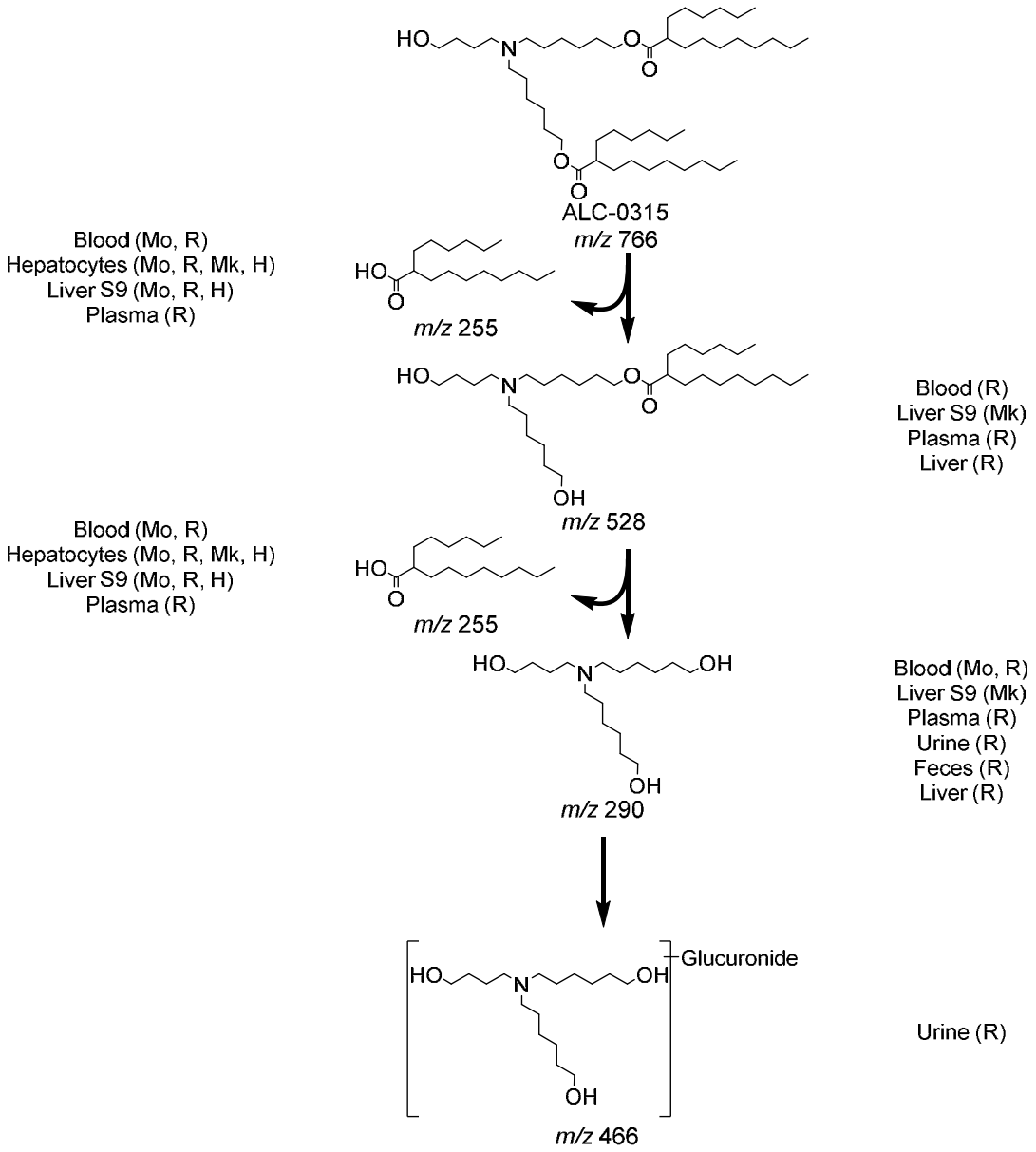
The biodistribution of the expression of the antigen encoded by the RNA component of BNT162b2 is expected to be dependent on the LNP distribution. Therefore, results of these biodistribution studies should be representative for BNT162b2, as the LNP-formulated luciferase-encoding modRNA had the same lipid composition.

2.6.4.5. Metabolism

Metabolism studies were conducted to evaluate ALC-0315 (aminolipid) and ALC-0159 (PEG-lipid). These novel lipids were evaluated for in vitro metabolic stability in CD-1/ICR mouse, Wistar Han and/or Sprague Dawley rat, cynomolgus monkey, and human liver microsomes, S9 fractions, and hepatocytes. ALC-0315 and ALC-0159 were stable (>82% remaining) over 120 min in liver microsomes and S9 fractions and over 240 min in hepatocytes in all species and test systems (Studies 01049-20008, 01049-20009, 01049-20010, 01049-20020, 01049-20021, and 01049-20022; Tabulated Summaries 2.6.5.10A and 2.6.5.10B).

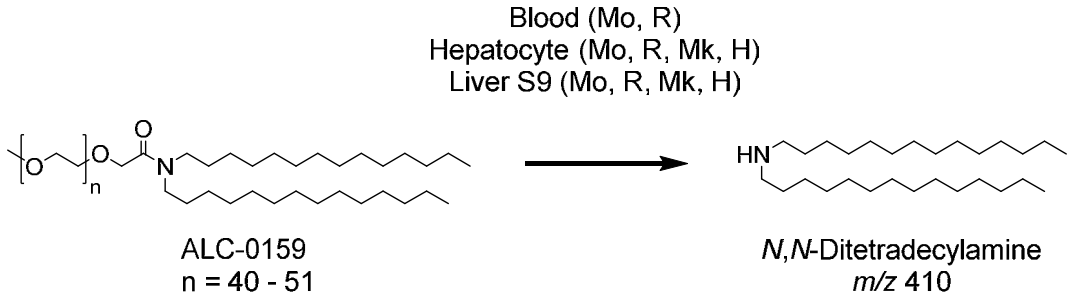
The metabolism of ALC-0315 and ALC-0159 was further evaluated (Study PF-07302048_05Aug20_043725; Tabulated Summaries 2.6.5.9, 2.6.5.10C, and 2.6.5.10D) in vitro using blood, liver S9 fractions, and hepatocytes from CD-1 mice, Wistar Han rats, cynomolgus monkeys, and humans and in vivo using the rat plasma, urine, feces, and liver from the PK study (Section 2.6.4.3). This study determined ALC-0315 and ALC-0159 are metabolized slowly and undergo hydrolytic metabolism of the ester and amide functionalities, respectively. This hydrolytic metabolism was observed across the species evaluated, as shown in Figure 2.6.4-3 and Figure 2.6.4-4.

Figure 2.6.4-3. Proposed Biotransformation Pathway of ALC-0315 in Various Species



Metabolism of ALC-0315 occurs via two sequential ester hydrolysis reactions, first yielding the monoester metabolite (*m/z* 528) followed by the doubly deesterified metabolite (*m/z* 290). Subsequent metabolism of the doubly deesterified metabolite resulted in a glucuronide metabolite (*m/z* 466), which was only observed in urine from the rat PK study. The acid product of both hydrolysis reactions of ALC-0315, 6-hexyldecanoic acid (*m/z* 255), was also identified.

Figure 2.6.4-4. Proposed Biotransformation Pathway of ALC-0159 in Various Species



The primary route of metabolism identified for ALC-0159 involves amide bond hydrolysis yielding *N*,*N*-ditetradecylamine (*m/z* 410). This metabolite was identified in mouse and rat blood, as well as hepatocytes and liver S9 from mouse, rat, monkey, and human. No metabolites of ALC-0159 were identified from in vivo samples.

The other two lipids in the LNP are naturally occurring (cholesterol and DSPC) and will be metabolized and excreted like other endogenous lipids. As the protein encoded by the mRNA in BNT162b2 is expected to be proteolytically degraded and RNA is degraded by cellular RNases and subjected to nucleic acid metabolism, no RNA or protein metabolism or excretion studies will be conducted.

2.6.4.6. Excretion

In the rat PK study (Section 2.6.4.3), there was no detectable excretion ALC-0315 and ALC-0159 in urine after IV administration of LNPs containing surrogate luciferase RNA at 1 mg/kg. The percent excreted unchanged in feces was ~1% for ALC-0315 and ~50% for ALC-0159. Metabolites of ALC-0315 were detected in the urine of rats (Figure 2.6.4-3). No excretion studies have been conducted with BNT162b2 for the reasons described in Section 2.6.4.5.

2.6.4.7. Pharmacokinetic Drug Interactions

No PK drug interaction studies have been conducted with BNT162b2.

2.6.4.8. Discussion and Conclusions

In the rat PK study, concentrations of ALC-0159 dropped approximately 8000- and >250-fold in plasma and liver, respectively, during this 2-week study. For ALC-0315, the elimination of the molecule from plasma and liver was slower, but concentrations fell approximately 7000- and 4-fold in two weeks for plasma and liver, respectively. Overall, the apparent terminal $t_{½}$ in plasma and liver were similar in both tissues and were 2-3 and 6-8 days for ALC-0159 and ALC-0315, respectively. The apparent terminal $t_{½}$ in plasma likely represents the re-distribution of the respective lipids from the tissues into which they have distributed as the LNP back to plasma where they are eliminated.

Overall, it appears that 50% of ALC-0159 was eliminated unchanged in feces. Metabolism played a role in the elimination of ALC-0315, as little to no unchanged material was detected in either urine or feces. Investigations of urine, feces and plasma from the rat PK study

identified a series of ester cleavage products of ALC-0315; this likely represents the primary clearance mechanism acting on this molecule, although no quantitative data is available to confirm this hypothesis. In vitro, ALC-0159 was metabolized slowly by hydrolytic metabolism of the amide functionality.

The potential biodistribution of BNT162b2 was assessed using luciferase expression as a surrogate reporter. Protein expression was demonstrated at the site of injection and to a lesser extent, and more transiently, in the liver after BALB/c mice received an IM injection of RNA encoding luciferase in an LNP formulation like BNT162b2. Luciferase expression was identified at the injection site at 6 hours after injection and was not detected by 9 days. Expression in the liver was also present at 6 hours after injection and was not detected by 48 hours after injection. These findings are supported by a quantitative biodistribution study in Wistar Han rats. After IM administration of a radiolabeled lipid marker and a luciferase modRNA in the same LNP formulation as BNT162b2 to rats, the percent of administered dose was greatest at the injection site. Outside of the injection site, total recovery of radioactivity was highest in the liver and much lower in the spleen, with very little recovery in the adrenal glands and ovaries.

2.6.4.9. 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. World Health Organization. Annex 2. Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines. In: WHO Technical Report Series No. 987, Geneva, Switzerland. World Health Organization 2014:59-100.