



## CMC REVIEW MEMORANDUM

**Date:** December 17, 2020

**To:** The Emergency Use Authorization (EUA) File STN 27034

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**Sponsor:** Pfizer Inc.

**Subject:** CMC Review of Original EUA STN 27034.0;  
Human Coronavirus mRNA Vaccine for the Prevention of Coronavirus  
Disease 2019 (COVID-19)

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The following abbreviations are used throughout the memorandum:

ATM	Animal Trial Material
BNT	BioNTech Manufacturing GmbH
CoA	Certificate of Analysis
CPP	Critical Process Parameter
CQA	Critical Quality Attribute
CTM	Clinical Trial Material
DS	Drug Substance
DP	Drug Product
EUA	Emergency Use Authorization
GMP	Good Manufacturing Practice
IPT-C	In-process Tests for Control
IPT-M	In-process Tests for Monitoring
IVT	<i>In vitro</i> Transcription
LNP	Lipid Nanoparticle
modRNA	Nucleoside-modified Messenger RNA
PPQ	Process Performance Qualification

(b) (4)

(b) (4)

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## 1. Product Name/Proprietary Name/Product Type

**Product name:** Pfizer-BioNTech COVID-19 Vaccine

**Proprietary name:** COMIRNATY

**Product Type:** Human Coronavirus mRNA vaccine expressing SARS-CoV-2 spike glycoprotein (BioNTech code number BNT162b2, Pfizer code number PF-07302048); the mRNA (variant RBP020.2) is formulated with lipids, including ALC-0315, ALC-0159, DSPC, and cholesterol to generate lipid nanoparticles (LNPs).

## 2. General Description

The BNT162b2 COVID-19 vaccine, developed under a collaboration agreement between Pfizer and BioNTech (BNT), is a nucleoside-modified messenger RNA (modRNA)-based vaccine candidate indicated for active immunization for the prevention of coronavirus disease 2019 (COVID-19). The single-stranded mRNA encodes the SARS-CoV-2 full-length spike (S) glycoprotein, which is codon-optimized and modified to express the P2 mutant, a pre-fusion S protein (P2 S; version 9). The modRNA is stabilized by formulation with lipids consisting of DSPC, cholesterol, ALC-0315, and ALC-0159 to generate LNPs. Other ingredients in the BNT162b2 vaccine include potassium chloride, monobasic potassium phosphate, sodium chloride, dibasic sodium phosphate dihydrate, and sucrose.

The final vaccine product is a white to off-white, sterile, preservative-free, multi-dose frozen suspension to be diluted with 0.9% sodium chloride injection, USP, for intramuscular injection. The vaccine is filled in a 2 mL clear glass vial with a rubber stopper (not made of natural rubber latex) as a multi-dose concentrate (after dilution each vial contains 5 doses of 0.3 mL) and is administered as a series of two 30-µg doses of diluted vaccine. The second dose is administered 21 days after the first dose. The undiluted vaccine should be stored frozen between -90°C to -60°C. According to the EUA Prescribing Information, thawed vials can be stored between 2°C to 8°C for up to 5 days prior to dilution; the diluted vaccine must be stored between 2°C to 25°C and used within 6 hours from the time of dilution. The vaccine is indicated for use in individuals 16 years of age or older.

## 3. Executive Summary and Recommendations

This review encompasses all quality-related information in Module 3 of IND19736, nonclinical studies relevant to evaluating the risk for enhanced respiratory disease (Module 4), validation of clinical diagnostic assays supporting clinical efficacy endpoints, and relevant CMC-related information submitted in the following IND amendments: IND19736/161, 160, 159, 151, 149, 138, 122, 119, 115, 112, 104, 103, 100, 96, 82, 79, 72, 68, 62, 59, 53, 49, 46, 43, 31, 29, 21, 12, 11, 3, 1.

The manufacturing process for the BNT162b2 drug substance (DS) consists of two major steps: *in vitro* transcription and purification of the modRNA. The BNT162b2 drug product (DP) is manufactured by mixing the modRNA DS with lipid excipients during LNP formulation followed by fill/finish. To support the EUA request, in-process, release,

and characterization data for a minimum of three process performance qualification (PPQ) DS batches for each DS manufacturing facility (Pfizer Andover and (b) (4) (b) (4) were provided. Certificates of Analysis (CoAs) for a minimum of three GMP commercial-scale DP lots from each DP manufacturing node were requested from the Sponsor to demonstrate DP process performance and consistency. DP data from four manufacturing nodes (Pfizer Andover/Polymun/Pfizer Puurs, Pfizer Andover/Kalamazoo/Kalamazoo, (b) (4) /Polymun/Pfizer Puurs, and (b) (4) (b) (4) /DermaPharm/Pfizer Puurs) were available during the EUA review. In addition, to support vaccine supply and availability, data from two additional nodes (Pfizer Andover/Puurs/Puurs and (b) (4) /Pfizer Puurs/Puurs) will be submitted to the EUA between December 17 and December 23, 2020. Once authorized, the Sponsor will submit the CoAs of DP lots to be distributed under the EUA for review at least 48 hours prior to lot distribution.

The DS manufacturing process underwent changes during vaccine development. DS (b) (4) was used for Phase 1/2/3 clinical trial material (CTM), while DS (b) (4) was used for a portion of Phase 3 CTM and will be used in the manufacture of vaccine intended for emergency use. A comprehensive analytical comparability assessment has been performed and the submitted data support the comparability of (b) (4) and (b) (4) for the manufacture of BNT162b2 DS. In addition, to support product comparability, a small cohort (approximately 250 subjects) was enrolled in the Phase 3 clinical trial (protocol C4591001) to evaluate the safety and immunogenicity of DP formulated with (b) (4) DS; clinical data from this trial will be submitted to the EUA upon completion. For DP, the manufacturing process was changed from a Classical process to an Upscale process involving an increase in batch size sufficient to meet commercial need (capable of accommodating larger RNA input). A comparison of available DP batch release data and an in-depth analytical comparability assessment between six representative Classical process DP batches and one Upscale process DP batch support the use of the Upscale process for DP manufacture under emergency use. A more comprehensive comparability assessment encompassing additional lots from multiple DP manufacturing nodes is ongoing and the results will be provided to the EUA upon completion of the study.

Stability studies have been designed to support the use of vaccine under the EUA. All available stability data generated using the BNT162b2 DS and DP lots support the emergency deployment of the Pfizer-BioNTech COVID-19 vaccine. All stability studies of the DS and DP lots are ongoing and will continue to be monitored. Data will be submitted to the EUA as they become available.

The analytical procedures developed and used for the release and stability monitoring of BNT162b2 DS and DP include tests to ensure their identity, purity, quality, and potency. The assays are appropriate and acceptable to be used for the control of DS/DP quality. All analytical procedures used for the release of emergency supply DS and DP have been adequately qualified. The summaries of the qualification results

demonstrate precision, accuracy, sensitivity, specificity, and reproducibility for each evaluated analytical assay, indicating that they are suitable for the intended use.

There were two notable issues identified during the EUA review. The first issue involves the occurrence of (b) (4) in DP lots produced for emergency use, and the second issue involves the occasional observation of visible intrinsic particles detected during visual inspection of filled DP vials. Extensive characterization demonstrated that (b) (4) consists of (b) (4) represents a (b) (4) in the DP (typically (b) (4) and the anticipated impact on safety/efficacy is minimal. A possible root cause of (b) (4) has been connected to the source of the (b) (4) (b) (4) and investigation on possible corrective actions is ongoing. Regarding the intrinsic particles, the frequency of occurrence is low and DP vials containing intrinsic particles can be detected and discarded through 100% automated/manual visual inspection. The intrinsic particles appear to consist of (b) (4) components used for (b) (4) (i.e., they are not foreign particles) and the available data suggest that they have minimal potential to impact product safety and quality.

**Recommendation:** We recommend approval of the EUA request.

Distribution from two of the DP manufacturing nodes (Andover/Puurs/Puurs and (b) (4) (b) (4) /Puurs /Puurs) awaits submission of GMP DP lot data by the Sponsor and review by CBER/FDA.

#### 4. Manufacturers

Facilities and manufacturing sites involved in drug substance and drug product manufacturing and testing for EUA supplies for use in the US are presented in Table 1 below (latest version submitted in IND19736/149).

Table 1. Emergency Supply Chain Manufacturing Nodes

Emergency Supply						
<b>Drug Substance</b>	Pfizer Andover			(b) (4) BNT Mainz, Germany (Purification)		
<b>DS Testing</b>	Pfizer <sup>5</sup> Andover, <sup>1,2</sup> Chesterfield <sup>2</sup>			(b) (4) BNT Mainz, Germany <sup>4</sup>		
<b>LNP, DP</b>	Polymun	Pfizer Puurs	Pfizer Kalamazoo	Polymun	DermaPharm	Pfizer Puurs
<b>Fill/Finish</b>	Pfizer Puurs (Lines (b) (4))		Pfizer Kalamazoo (Lines (b) (4))	Pfizer Puurs (Lines (b) (4))		
<b>DP Release and Stability Testing</b>	Pfizer Andover, <sup>2</sup> Chesterfield, <sup>2</sup> Puurs <sup>3</sup>		Pfizer Andover, <sup>2</sup> Chesterfield, <sup>2</sup> Kalamazoo <sup>3</sup>	Pfizer Andover, <sup>2</sup> Chesterfield, <sup>2</sup> Puurs <sup>3</sup>		

1 Microbial Tests: Endotoxin, Bioburden.

2 Release and Stability Testing for Identity, Composition, Strength, Product Purity and/or Process Related Impurities.

3 Microbial Tests: Endotoxin, Sterility. Back-up sterility test sites may be employed.

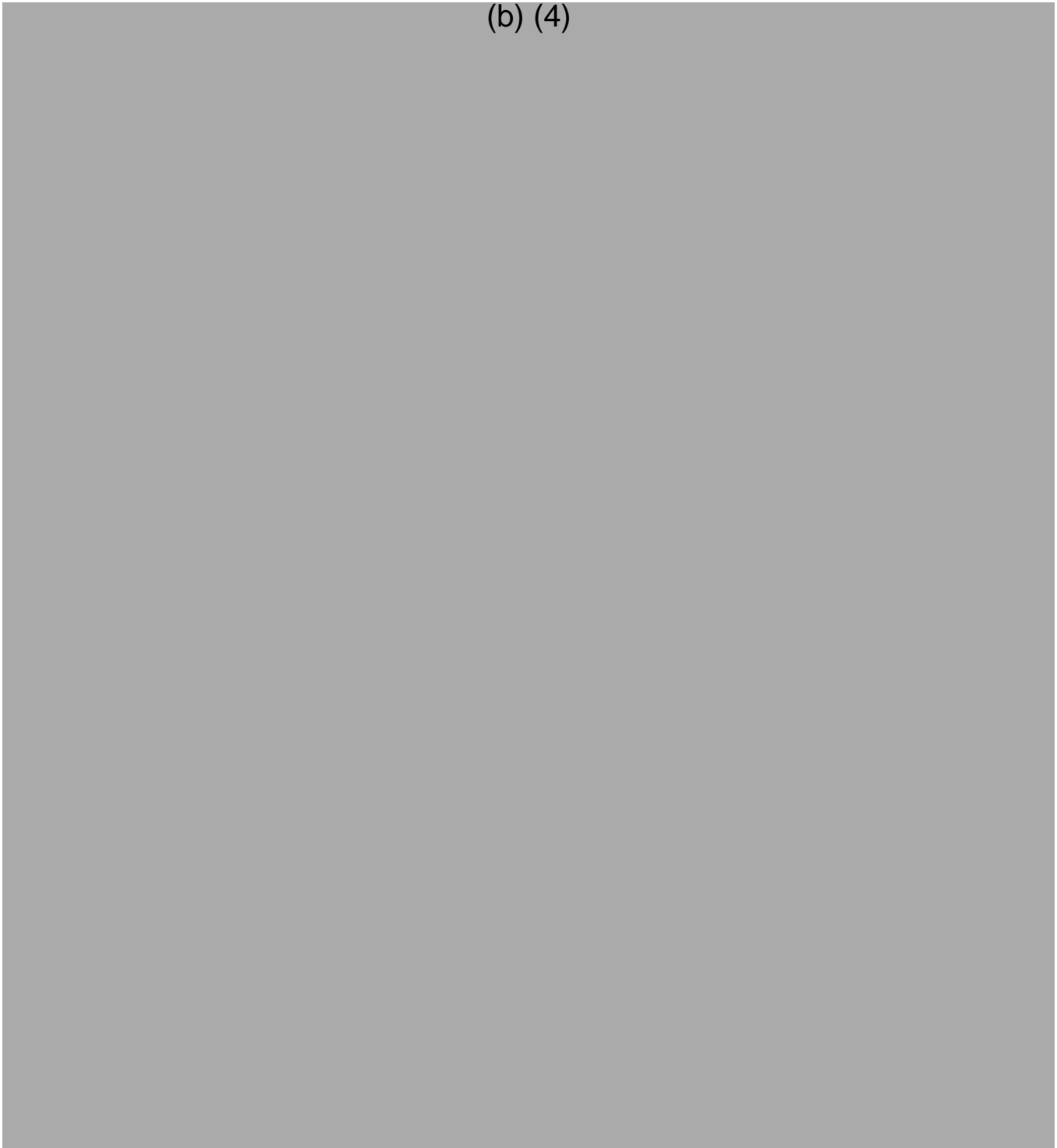
4 Poly(A) Tail and 5'-Cap (Composition) Tests may be performed for EUA supplies at Pfizer Andover or Pfizer Chesterfield.

5 (b) (4) (Product-related impurity) Test may be performed for EUA supplies at (b) (4) .

## **5. BNT162b2 Drug Substance**

### **5.1 Description of Drug Substance Manufacturing Process and Process Controls**

(b) (4)



**FOIA FDA/CBER #2021-5683**

**Pages 5-15 of the CMC Review Memorandum are being  
Withheld in Full as exempt from disclosure pursuant to  
FOIA Exemption 4**

**Bates Numbers:**

**FDA-CBER-2021-5683-1804616 – 1804626**

## 6. BNT162b2 Drug Product

### 6.1 Composition of the Drug Product

The BNT162b2 DP is a sterile dispersion of RNA-containing lipid nanoparticles (LNPs) in aqueous cryoprotectant buffer. The components of the DP, including the BNT162b2 DS, lipid excipients, buffer, and cryoprotectant, are listed in Table 9 below. In addition, the concentration and amount of each component per DP container, and the corresponding amount per dose are also listed.

Table 9. Emergency Use Supply BNT162b2 Drug Product Configuration

Name of Ingredients	Reference to Standard	Function	Concentration (mg/mL)	Amount per vial	Amount per dose
BNT162b2 DS	In-house specification	Active ingredient	0.5	225 µg	30 µg
ALC-0315	In-house specification	Functional lipid	7.17	3.23 mg	0.43 mg
ALC-0159	In-house specification	Functional lipid	0.89	0.4 mg	0.05 mg
DSPC	In-house specification	Structural lipid	1.56	0.7 mg	0.09 mg
Cholesterol	Ph. Eur.	Structural lipid	3.1	1.4 mg	0.2 mg
Sucrose	USP-NF, Ph. Eur. <sup>a</sup>	Cryoprotectant	103	46 mg	6 mg
Sodium chloride	USP-NF, Ph. Eur. <sup>a</sup>	Buffer component	6	2.7 mg	0.36 mg
Potassium chloride	USP-NF, Ph. Eur. <sup>a</sup>	Buffer component	0.15	0.07 mg	0.01 mg
Dibasic sodium phosphate, dihydrate	USP-NF, Ph. Eur. <sup>a</sup>	Buffer component	1.08	0.49 mg	0.07 mg
Monobasic potassium phosphate	USP-NF, Ph. Eur. <sup>a</sup>	Buffer component	0.15	0.07 mg	0.01 mg
Water for injection	USP-NF, Ph. Eur. <sup>a</sup>	Solvent/vehicle	q.s. <sup>b</sup>	q.s. <sup>b</sup>	q.s. <sup>b</sup>

a. Grades of incoming materials are the same across sites as confirmed by the supplier CoA. However, incoming testing at each manufacturing site may initially be performed only in accordance with each site's local compendia.

b. q.s. = quantum satis (as much as may suffice)

#### **(b) (4)** Lipid - ALC-0315

ALC-0315 (((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)), a novel lipid excipient, serves as the functional **(b) (4)** lipid component, **(b) (4)**

#### PEGylated Lipid - ALC-0159

The novel polyethylene glycol (PEG) lipid ALC-0159 (2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide) is another functional lipid excipient. The primary function of ALC-0159 is to **(b) (4)**



The PEG in ALC-0159 is linked to the diacyl lipid

(b) (4)

(b) (4)

#### Phospholipid - DSPC

The phospholipid component DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) provides (b) (4)

#### Sterol Lipid - Cholesterol

Cholesterol also serves as a structural lipid to support (b) (4)

### **6.2 Control of Lipid Excipients**

Lipid excipients used for LNP formulation of the clinical lots were sourced from (b) (4) (b) (4) for ALC-0315 and ALC-0159, (b) (4) for DSPC, and (b) (4) for cholesterol. For emergency use supplies, additional lipid suppliers were added including (b) (4) for ALC-0315 and (b) (4) for DSPC and Cholesterol. For all four lipid excipients, the supplier's release specifications include testing for (b) (4)

Additional control tests are performed in-house for all four lipids to ensure the quality of the lipids, including (b) (4), (b) (4) test by (b) (4), and a (b) (4) test.

Additional information regarding the lipid excipients is provided by cross-reference to the following DMFs:

- DMF# (b) (4) for ALC-0159, (b) (4)
- DMF# (b) (4) for ALC-0315, (b) (4)
- DMF# (b) (4) for DSPC, (b) (4)
- DMF# (b) (4) for cholesterol, (b) (4)
- DMF# (b) (4) for DSPC, (b) (4)

#### Introduction of Additional Lipid Suppliers and Update of Lipid Specifications

If lipid suppliers used for the manufacture of commercial DP need to be added or changed and are different from the ones used for the manufacture of DP for use in clinical supplies, a comparability study will be conducted. The lipid comparability assessment includes side-by-side testing of lipid (b) (4) (b) (4), as well as evaluation of LNPs formulated with lipid from the new supplier. At the time of EUA request, batch release data from six emergency supply DP lots were submitted. These six DP lots were manufactured using (b) (4) ALC-0315 lipid vendors, (b) (4). The manufacturer's release specifications for ALC-0315

from (b) (4) are the same. The CoAs for the lots used in the formulation of LNP/DP for emergency use supply, including (b) (4) (lot # (b) (4)) and (b) (4) lots (lot # (b) (4)), are provided. All tested quality attributes met the pre-defined acceptance criteria and the results suggest that ALC-0315 sourced from (b) (4) are comparable (however, please see additional discussion below regarding (b) (4)).

### 6.3 Description of Drug Product Manufacturing Process and Process Controls

The manufacturing process for the BNT162b2 DP includes the following major steps:

(b) (4)

(b) (4)

#### 3) Bulk DP Formulation

The bulk DP is formulated by (b) (4)

(b) (4)

#### 4) Fill and Finish

Following shipment of the (b) (4) bulk DP to the fill and finish site, (b) (4)

(b) (4)

At the end of filling, each vial is stoppered, capped, and crimped. For the emergency supply configuration, filling volume was set to a target of (b) (4) mL per vial. After labeling and packaging, BNT162b2 DP vials are frozen and stored in a freezer at -90°C to -60°C.

#### Critical Process Parameters and In-Process Controls

To ensure DP manufacturing process consistency and the quality of the BNT162b2 DP, process parameters have been established as follows in Table 10.

Table 10. Process Parameters for the Manufacture of BNT162b2 Bulk DP

Unit Operation	Process Parameter	Acceptable Range		
		Kalamazoo and Puurs	(b) (4)	DermaPharm

(b) (4)

For LNP formulation, the process parameters were set to achieve a reasonable balance among the following parameters: (b) (4)

(b) (4). Additionally, IPT-C and IPT-M are being used throughout the DP manufacturing process. IPT-Cs include (b) (4)

(b) (4). Quality attributes tested for monitoring purposes during the manufacturing process include (b) (4)

. These IPT-Ms are evaluated (b) (4)

#### Hold Times During DP Manufacturing Process

(b) (4)

### Hold Time for the Diluted BNT162b2 Vaccine Prior to Administration

The BNT162b2 final vaccine product is a concentrated suspension prepared in a multidose vial free of preservatives. Following dilution with saline, the in-use hold period and storage condition for the vaccine product is set to be up to 6 hours at 2°C to 25°C. To support this hold condition, an in-vial dilution hold-time study was conducted (submitted in IND19736/79), in which the physicochemical stability of the BNT162b2 DP, held in glass vials and diluted with saline as intended, was evaluated at 2-8°C, 25°C, and (b) (4) °C. The results indicate that the diluted vaccine can be stored at ambient temperature (25°C) for (b) (4) hours and can be exposed to elevated ambient temperature (b) (4) °C) with no impact on product quality. In addition, a microbial challenge with selected model diluents and surrogate DP solutions (reported in IND19736/43) as well as a BNT162b2-specific microbial challenge (reported in IND19736/79) were conducted to evaluate the potential for microbial growth in a matrix representative of saline-diluted vaccine. In both studies, (b) (4) (b) (4) microorganisms listed in (b) (4) and growth of the microorganisms was monitored. The results demonstrate that at 12 hours, which is (b) (4) the proposed in-use time, no increase in growth was observed for any of the organisms with storage at 20-25°C

### **Reviewer's Comments:**

The result was close to the acceptance criterion of (b) (4)  $\log_{10}$  increase from  $T_0$  for (b) (4) (b) (4) at the 12 hour time point ((b) (4) cfu at  $T_0$  vs (b) (4) cfu at 12 hours). Overall, the results support the proposed in-use period of 6 hours at ambient temperature after dilution with saline.

### **6.4 Process Validation**

The DP manufacturing process has not been validated at all manufacturing sites at the time of the EUA request. Prior to providing the PPQ (process validation) reports, the release data from a minimum of three GMP DP lots at each manufacturing node have been or will be submitted for review. All emergency supply lots are executed according to defined batch records and evaluated with predetermined acceptance criteria. At least three lots at each manufacturing site will be tested for stability. In the absence of PPQ data for DP manufactured at multiple sites, it will be necessary for the Sponsor to submit final CoAs for lots to be distributed under the EUA at least 48 hours prior to distribution. The Sponsor responded on 25 November 2020 (IND19736/149) with a commitment to do so with a submission schedule anticipated to be twice per week.

The initial EUA request included data from six (6) DP GMP commercial-scale lots for which LNP production and fill and finish processes were performed at Polymun and Puurs (b) (4) line), respectively (data submitted in IND19736/96 and IND19736/138). The processes used for production of these 6 lots, including process parameters and in-process controls, are highly aligned with the expected commercial production parameters to be validated through execution of PPQ studies.

## Reviewer's Comments:

Available release data from 6 GMP commercial-scale DP lots suggest consistent process performance and are supportive of the EUA request for the Polymun (LNP formulation)/Puurs (fill and finish) manufacturing node. However, for each of the other DP manufacturing nodes, data from at least three GMP commercial-scale DP lots will be required prior to allowing distribution of EUA supplies from that node. This requirement was communicated to the Sponsor on 20 November 2020, and an acknowledgement was returned in IND19736/149.

For each manufacturing node, GMP batch release data, interim comparability analysis, and the final PPQ report will be submitted for review with a proposed timeline as shown below in Table 11. The schedule was submitted on 16 November 2020 in IND19736/138 and updated (in red) in IND19736/149 (submitted on 25 November, 2020) and IND19736/160 (submitted on 8 December, 2020).

Table 11. Draft Timeline for PPQ Campaigns and Availability of PPQ Reports

Site of Manufacture	1 <sup>st</sup> GMP Lot	Planned PPQ Campaign Start	Release & In-process Data (CoA available)			Interim Consistency/ Comparability Data Analysis	Full PPQ Report Availability
			From 3 GMP lots	From 1 <sup>st</sup> PPQ lot	From last (3+) PPQ lot		
Polymun LNP/ Pfizer, Puurs fill line (b) (4)	August 10	Nov 23	Nov 16	Dec 19	Feb 13, 2020	Dec 11, 2020 (3 GMP)	March 2021
Pfizer, Puurs LNP/ Pfizer, Puurs Fill line (b) (4)	Nov 2	Nov 23	Dec 17	Dec 22	Jan 26, 2021	Jan 16, 2021 (3 GMP)	March 2021
Pfizer, Puurs LNP/ Pfizer, Puurs fill line (b) (4)	NA	Dec 21	NA	Jan 23, 2021	Jan 29, 2021	NA	March 2021
Kalamazoo LNP/ Kalamazoo fill line (b) (4)	Oct 5	Nov 23	Dec 29 <sup>a</sup>	Dec 19	Jan 23, 2021	Jan 22, 2021 (3 GMP)	March 2021
Kalamazoo LNP/ Kalamazoo fill line (b) (4)	Sept 21	Nov 23	Dec 15 <sup>b</sup>	Dec 19	Jan 23, 2021	Jan 15, 2021 (3 GMP)	March 2021
DermaPharm LNP/ Pfizer, Puurs fill line (b) (4)	Oct 5	Dec 21	Dec 8	Jan 9, 2021	Jan 29, 2021	Jan 11, 2021 (3 GMP)	March 2021

a. Data from two GMP lots are available on 8 December, 2020, and data from the third GMP lot will be available on December 29, 2020.

b. Data from one GMP lot is available on 8 December, 2020, and data from two other GMP lots will be available 14 December, 2020, and 15 December, 2020.

## 6.5 Major Changes to the Manufacturing Process for Emergency Supply

### Introduction of a Scaled-up Process for the Production of LNPs

The manufacturing process for BNT162b2 DP was initially developed by (b) (4) based on general principles, experience, and historical data for other LNP manufacturing processes. A stage-appropriate scaled process was developed in two phases, the Phase 1 “Classical process” and the Phase 2 “Upscale process”. The

clinical lots were manufactured using the Classical process at a batch size of up to (b) (4) (based on (b) (4)). For emergency supply material, the Upscale process was implemented to increase the batch size to (b) (4) or larger by (b) (4).

. Comparability and suitability of the scaled manufacturing process for BNT162b2 DP was demonstrated by an in-depth comparability assessment including (b) (4) representative clinical lots (Classical process; Table 12) and one emergency supply lot (EE8493; Upscale process; Table 12). The assessment included both release and heightened characterization testing with some quality attributes evaluated side-by-side. The comparability data shown in Table 12 below support the BNT162b2 DP comparability between the Upscale process and the Classical process.

Table 12. Comparability Data for BNT162b2 Drug Product Lots

Quality Attribute	Analytical Procedure	Drug Product Lot Number						
		BCV40420-A	BCV40620-A	BCV40620-D	BCV40720-A	BCV40720-P	BCV40820-P	EE8493
Appearance	Appearance (Visual)	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension
Appearance (visible particulates)	Appearance (Particles)	Free from observable particles	Free from observable particles	Free from observable particles	Free from observable particles	Free from observable particles	Free from observable particles	Free from observable particles
Subvisible particles	Subvisible particulate matter	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
pH	(b) (4)	(b) (4)						
Osmolality	Osmometry							
LNP Size	DLS							
LNP polydispersity	DLS							
RNA encapsulation	Fluorescence assay							
RNA content	Fluorescence assay							
ALC-0315 content	HPLC-CAD							
ALC-0159 content	HPLC-CAD							
DSPC content	HPLC-CAD							
Cholesterol content	HPLC-CAD							
(b) (4)	(b) (4)							
(b) (4)	(b) (4)							
(b) (4)	(b) (4)							
(b) (4)	(b) (4)							
Lipid identities	HPLC-CAD	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Identity of encoded RNA sequence <sup>a</sup>	RT-PCR	Identity confirmed	Identity confirmed	Identity confirmed	Identity confirmed	Identity confirmed	Identity confirmed	Identity confirmed
IVE (% cells positive) <sup>a</sup>	Cell-base flow cytometry	(b) (4)						
RNA integrity <sup>a</sup>	CGE							
(b) (4)	(b) (4)							
(b) (4)	(b) (4)							
(b) (4)	(b) (4)							

a. Tested side-by-side. Abbreviations: DLS = dynamic light scattering; HPLC-CAD = high performance liquid chromatographyv-charged aerosol detector; CGE = capillary gel electrophoresis; RP-HPLC = reverse phase high performance liquid chromatography; (b) (4)

## Reviewer's Comments:

Introduction of the Upscale process (b) (4) ) for the production of LNPs at Polymun was submitted in IND19736/96. In IND19736/115, the scaled-up processes at the Pfizer Puurs and Kalamazoo sites were introduced; this amendment described a “scale out” (rather than a scale up) approach for processing (b) (4) levels (b) (4) ). For this scale change, (b) (4) ; all other process parameters and in-process controls remain the same. The Upscale process, including the scale out approach, is now being implemented for LNP production across all four manufacturing sites: Polymun, Pfizer Puurs, Dermapharm, and Pfizer Kalamazoo.

A comparison of the available batch-release data for the six emergency use batches with LNP production at Polymun and fill and finish at Puurs revealed no significant differences from clinical lots made by the Classical process, supporting the capability of the Upscale process to produce scaled-up LNP batches while still maintaining appropriate product quality.

Data obtained from the in-depth comparability assessment (Table 12) also support the comparable process performance for Upscale process vs. Classical process. However, this assessment is limited, with data derived from only one Upscale process DP lot (EE8493) manufactured at one manufacturing node (LNP manufactured at Polymun/fill and finish at Puurs). A more comprehensive comparability study evaluating DP lots manufactured at the four LNP production sites (Polymun, Pfizer Puurs, Dermapharm and Pfizer Kalamazoo) and the two fill and finish sites (Puurs and Kalamazoo) is planned. At least three EUA DP lots from each manufacturing site will be assessed for comparability. Representative clinical DP lots will be included in side-by-side testing to bridge the study to the previous comparability assessment. The data will be available post-EUA.

## 6.6 Control of Drug Product

### Specifications

The release specifications for emergency supply and PPQ DP are shown in Table 13 below.

Table 13. Specifications for Emergency Supply and PPQ Drug Product

Quality Attribute	Analytical Procedure <sup>a</sup>	Acceptance Criteria
<b>Composition and Strength</b>		
Appearance	Appearance	White to off-white suspension
Appearance (Visible Particulates)	Appearance (Particles) <sup>b</sup>	May contain white to off-white opaque, amorphous particles
Subvisible Particles	Subvisible Particulate Matter <sup>b, c</sup>	(b) (4)



pH	(b) (4) <sup>b</sup>	7.4 ± 0.5
Osmolality	Osmometry <sup>b, d, e</sup>	(b) (4)
LNP Size	Dynamic Light Scattering (DLS)	(b) (4)
LNP Polydispersity	Dynamic Light Scattering (DLS)	(b) (4)
RNA Encapsulation	Fluorescence Assay	(b) (4)
RNA Content	Fluorescence Assay	(b) (4)
ALC-0315 Content	HPLC-CAD	(b) (4)
ALC-0159 Content	HPLC-CAD	(b) (4)
DSPC Content	HPLC-CAD	(b) (4)
Cholesterol Content	HPLC-CAD	(b) (4)
Container Content for Injections	Volume of Injections in Containers <sup>e, f</sup>	Not less than the sum of the nominal values of (b) (4)
<b>Identity</b>		
Lipid Identities	HPLC-CAD <sup>e</sup>	Retention times consistent with references
Identity of Encoded RNA Sequence	RT-PCR <sup>e</sup>	Identity confirmed
<b>Potency</b>		
<i>In Vitro</i> Expression	Cell-based Flow Cytometry	(b) (4)
<b>Purity</b>		
RNA Integrity	Capillary Gel Electrophoresis	(b) (4)
<b>Adventitious Agents</b>		
Bacterial Endotoxins	Endotoxin (LAL) <sup>b</sup>	(b) (4)
Sterility	Sterility <sup>b</sup>	No growth detected
Container Closure Integrity	Dye Incursion <sup>g</sup>	Pass

- All assays performed on stability unless otherwise noted
- Compendial
- USP<787> (obscuration method), and aligned with upcoming (Jan 2021) revision of Ph. Eur. 2.9.19
- USP<785>; also in accordance with Ph. Eur. 2.2.35, with minor difference in instrument calibration
- Assay not performed on stability
- Procedure is aligned with Test for Extractable Volume of Parenteral Preparation
- Tested at release and on stability for stability batches only

### Batch Analyses

At the time of the EUA request, batch release data were submitted for six (6) DP lots for emergency supply. The manufacturing nodes for the 6 DP lots include Pfizer Andover-DS/Polymun-LNP production/Pfizer Puurs-fill and finish for DP lots EE8492, EE8493, EJ0553, and EK1768, and (b) (4) -DS/Polymun-LNP production/Pfizer Puurs-fill and finish for DP lots EJ1685 and EJ1686. DP Lots EE8492 and EE8493 were manufactured using the Upscale process with (b) (4). The other (b) (4) also used the Upscale process but with an (b) (4). All tested attributes listed in the specification table for all 6 DP lots met the acceptance criteria. The results support the use of the 6 DP lots for emergency supply. Batch EE8493 was used in the ongoing clinical study C4591001 to evaluate the safety and immunogenicity of BNT162b2 DP manufactured with (b) (4) DS. In IND19736/151, batch EJ1865 was also introduced for possible use in C4591001.

On 8 December 2020 (IND19736/160), CoAs of additional GMP DP lots, including EH9899, EK5730, and EL1284 manufactured at Andover-DS/Kalamazoo-LNP production/Kalamazoo-fill and finish, and EJ0724, EJ1688, EL0140, EL0142, and EK4175 manufactured at (b) (4) -DS/DermaPharm-LNP production/Puurs-fill and finish, were submitted. In addition, the CoA of the third DP lot EL0141 manufactured at (b) (4) -DS/Polymun-LNP production/Pfizer Puurs-fill and finish manufacturing node was also submitted. The batch release data for all DP lots intended for use under the EUA met the acceptance criteria.

## 6.7 Characterization Studies on Drug Product

The DP characterization assays were developed to further describe and demonstrate the structure and physicochemical properties of the DP. The analytical characterization assays employed for BNT162b2 DP and the testing results are briefly described as follows:

(b) (4)

## 6.8. Stability of Drug Product

Materials used for the DP stability studies include four emergency supply lots (EE8492, EE8493, EJ0553, and EJ1685), representative CTM, and animal trial material (ATM) lots including the lots used for nonclinical and toxicology studies. The overall stability plan for the DP lots is presented in Table 14 below.

Table 14. Summary of DP Stability Plan

Stability study	Batch No.	Use of batch	Storage condition	Duration (months)
Emergency supply stability study	EE8492, EE8493, EJ0553, EJ1685	Emergency supply	Long-term (-60 to -90°C) Accelerated (5 ± 3°C) Stressed (25 ± 2°C/60 ± 5%RH)	24M 24 or 6M <sup>a</sup> 1M <sup>b</sup>
Primary stability study I	BCV10320-A <sup>c</sup> , BCV40420-A,	Clinical	Long-term (-70 ± 10°C) Long-term (-40 ± 5°C)	24M 24M

	BCV50620-B <sup>d</sup>		Accelerated (5 ± 3°C) Stressed (25 ± 2°C)	6M 3M
Primary stability study II	BCV10420-A <sup>c</sup>	Clinical	Long-term (-70 ± 10°C)	12M
Primary stability study III	BCV40620-A, -E BCV10720-A <sup>c</sup> , -E <sup>c</sup> BCV40720-A, -C, -P BCV40820-P	Clinical	Long-term (-70 ± 10°C) Accelerated (5 ± 3°C)	6M 3M
Supportive stability study I	RBP020.3LNP <sup>c</sup> , RBP020.2LNP, RBP020.1LNP, RBP020.1LNP	ATM, Toxicology	Long-term (-70 ± 10°C) Accelerated (5 ± 3°C)	6M 6M
Supportive stability study II	RMAB/150319	ATM	Long-term (-70 ± 10°C)	6M
Supportive stability study III	FM-0595D	R&D grade	Long-term (-70 ± 10°C) Accelerated (5 ± 3°C) Stressed (25 ± 2°C)	24M 12M 12M

<sup>a</sup> Lot EE8492 will be evaluated for 24 months and the other 3 DP lots will be evaluated for 6 months.

<sup>b</sup> Lots EE8493, EJ0553, and EJ1685 will be evaluated at the stress condition for 1 month.

<sup>c</sup> BNT162b1 DP is a LNP-formulated modRNA encoding the RBD (V5)

<sup>d</sup> BNT162b3 DP is a LNP-formulated modRNA encoding the membrane-anchored RBD

The major quality attributes being tested in the stability studies include Appearance, ALC-0315 Content, ALC-0159 Content, DSPC Content, Cholesterol Content, RNA Content, RNA Integrity, RNA Encapsulation, and LNP Size/Polydispersity. *In Vitro* Expression (IVE), Endotoxin, and Container Closure Integrity are additionally included in the emergency supply stability study. The analytical procedures used for stability testing are the same as those used for batch release testing.

At the time of the EUA request, stability data for emergency supply DP lot EE8492 stored at -70°C (-90 to -60°C) and 5 ± 3°C were available for two weeks. Data were also available for lot EE8493 for two weeks under the stress condition of 25 ± 2°C/60 ± 5% RH. All data generated to date are within the specifications for DP lots intended for emergency supply. For lot EE8493, there is a decrease in the IVE result when stored at the stress condition.

Based on the available stability data from the primary stability studies I and II and the supportive stability study I, long-term storage (-70 ± 10°C) has been demonstrated for 3 months. A decrease in RNA integrity was observed in lot BCV40420-A stored at 25 ± 2°C for two months and in lot RBP020.1LNP stored at 5 ± 3°C for three months. Additionally, an increase in particle size/polydispersity was observed at the two-month time point under accelerated storage (5 ± 3°C) in lot BCV40420-A and in all tested ATM lots included in the supportive stability study I.

Related stability data were presented for a different clinical product produced by BioNTech that was manufactured with a comparable process but included a different (b) (4), although similar (b) (4). The stability data obtained with this product, ATM DP batch RMAB/150319, supported the long-term

storage ( $-70 \pm 10^{\circ}\text{C}$ ) of LNP for six months. The stability study results with the R&D grade material (batch FM-0595D), which has a different (b) (4) composition identical to BNT162 DP, demonstrate stability for 24 months under the long-term storage condition ( $-70 \pm 10^{\circ}\text{C}$ ).

## 6.9 Impurity Profile of the Drug Product

Possible process-related impurities include (b) (4), from the DP manufacturing process, and (b) (4), which are part of the (b) (4). All of these impurities are present in low amounts and are further reduced during the DP manufacturing process by (b) (4) step.

The Sponsor noted the potential for (b) (4) as reported in published studies. (b) (4)

## 7. Analytical Assays and Assay Validation

### Description of Analytical Assays for Both DS and DP

(b) (4)

(b) (4)

(b) (4)

### Description of Analytical Assays for DS Only

(b) (4)

#### Description of Analytical Assays for DP Only

##### *Fluorescence Assay for RNA Content and Encapsulation*

This is a fluorescence-based (b) (4) assay using (b) (4)

The acceptance criteria for RNA content and RNA encapsulation in the final DP vial is (b) (4) respectively.

##### *Cell-based Flow Cytometry for In Vitro Spike Protein Expression (IVE)*

(b) (4)

The DP release acceptance criterion for IVE is currently set as (b) (4)

(b) (4) only as negative control (NC) are included in each assay run. Key reagents, including (b) (4) reagents. Any new reagent lots introduced in the future will be qualified prior to use.

(b) (4)

(b) (4)

**Reviewer's Comments:**

Based on the available IVE data from clinical lots (results ranging from 50 -71%) and emergency supply lots (> 60%), the stringency of the specification could be tightened; this should continue to be evaluated and revised, if appropriate, to better align with manufacturing process and assay capabilities.

Validation of Analytical Procedures for Emergency Supply

Compendial procedures were qualified for use in accordance with the applicable pharmacopeias, excluding endotoxin and sterility. Endotoxin, in alignment with USP<85> and Ph. Eur. 2.6.14, and sterility in alignment with USP<71> and Ph. Eur. 2.6.1, were validated in the course of release testing of the first three CTM batches.


For non-compendial analytical methods, due to the urgent need to provide a vaccine in response to the pandemic, initial analytical procedure validations were conducted evaluating each method performance characteristic described in ICH Q2(R1) without test method-specific acceptance criteria as suggested in ICH Q7. In accordance with ICH Q7 and ICH Q2(R1), the validation of methods was guided by a standard operating procedure protocol on what elements of the method should be evaluated and this was reviewed and approved by the quality units at Pfizer. The initial absence of validation acceptance criteria is the reason why Pfizer described the assay assessment as “qualification” rather than “validation”. All test methods applicable to emergency supply DS and DP release testing have been qualified according to approved test method SOPs. The precision, accuracy, sensitivity, specificity, linearity, range, and robustness of test methods were established under actual conditions of use. For the EUA request, tabular summaries of qualification results were provided. The following sections focus on the qualification results of selected key assays.

*Qualification of the Capillary Gel Electrophoresis (CGE) for RNA Integrity*

(b) (4)

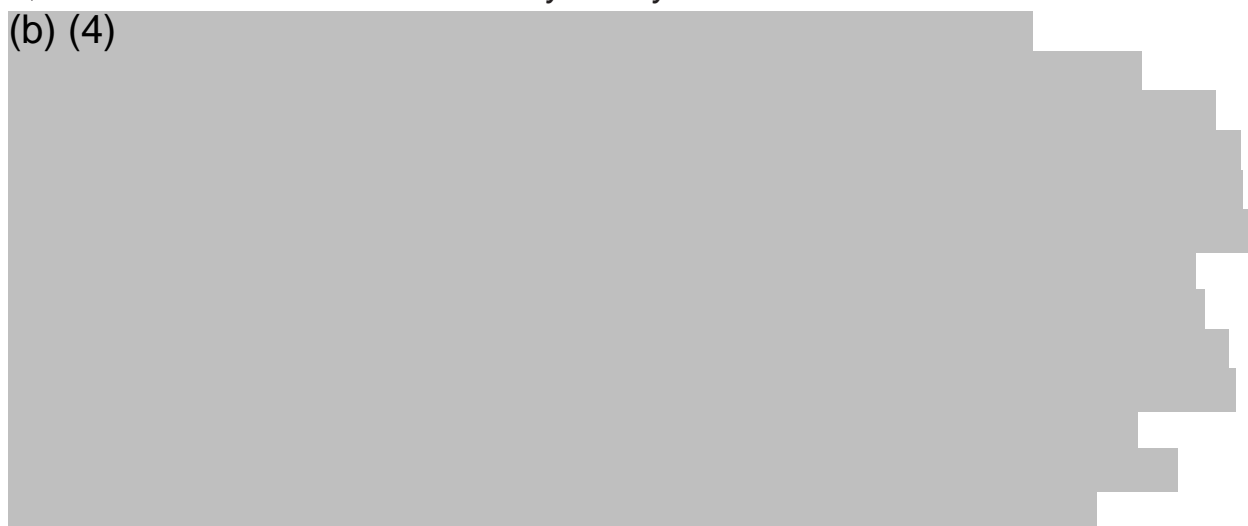
#### *Qualification of the Fluorescence Assay for RNA Content and Encapsulation*

(b) (4)



#### *Qualification of the Cell-based Flow Cytometry for IVE*

(b) (4)



#### **Reviewer's Comments:**

The qualification result summaries for the DS and DP release tests demonstrate the suitability of the analytical procedures for their intended use.

There are multiple testing sites involved in release and stability testing for DS (3 main sites: Pfizer Andover, Pfizer Chesterfield, and (b) (4)) and DP (2 main sites: Pfizer Andover and Pfizer Chesterfield). To demonstrate comparable assay performance between testing sites, site-specific qualification information was requested. In response to the request, the Sponsor reported in IND19736/149 that Andover and Chesterfield were involved in co-qualification of the analytical methods. Consistent assay performance was demonstrated based on intermediate precision determined through the reproducibility studies. The Sponsor also confirmed that for methods requiring critical reagents, the same lot or an alternative qualified lot is used across testing sites. The full validation/qualification report for each analytical method at each testing site will be required prior to the BLA.

#### **8. Recently Identified Ongoing Issues and Communications with the Sponsor**

##### RNA Integrity of BNT162b2 Drug Product – (b) (4)

In IND19736/138 submitted on 16 November 2020, the Sponsor stated that in recent DP batches, (b) (4) were observed during CGE

runs. These (b) (4)

An investigation was performed, and (b) (4) correlating with (b) (4) obtained from an orthogonal method (b) (4) were characterized by (b) (4)

(b) (4)

The results indicated that (b) (4) consists of (b) (4)

(b) (4) data confirmed that no unexpected modifications occurred to the (b) (4). IVE testing on the DP lots that contain (b) (4) produced results comparable with those of DP lots (b) (4). Based on the available data, the Sponsor concluded that (b) (4)

(b) (4) in the CGE method. In addition, a comparison of the batch release data and heightened characterization analysis on BNT162b2 DP lots (b) (4) revealed no significant differences (data submitted in IND19736/159).

In response to an Information Request (20 Nov 2020), the Sponsor provided data in IND19736/149 indicating that most recent lots (14 out of 20) have some level of (b) (4) and for lots that met release specification acceptance criteria, (b) (4) ranged from (b) (4). Three BNT162b2 DP lots that possess (b) (4) at release (EJ1685, EJ1688, and EH9899) were evaluated after simulating the maximum allowable storage and handling conditions prior to administration (held at 2-8°C for ≥ 5 days plus an additional 2 hours at (b) (4) °C, followed by dilution with saline, and held in the vial or syringe for an additional 6 hours at (b) (4) °C). The results indicated that RNA integrity and (b) (4) levels were maintained across the evaluated period.

In response to an Information Request (2 December 2020) with regard to the root cause of (b) (4) in the recent DP batches, the Sponsor stated in IND19736/159 that (b) (4) appeared to be correlated with certain ALC-0315 lipid lots supplied from (b) (4). The investigation into the ALC-0315 lipid source is ongoing, including an assessment of the differences in the manufacturing process, the impurity profile of the lipid lot, and the impact of certain impurities on (b) (4). Initial evaluations of planned improvements to the (b) (4) manufacturing operation at (b) (4) at laboratory scale have demonstrated that reduced (b) (4) levels can be achieved. A communication was issued to the Sponsor requesting the submission of detailed reports and a summary of corrective actions once the investigation is completed. Also, we advised the Sponsor to consider additional specifications for the ALC-0315 lipid excipient after receipt from the vendor to mitigate the occurrence of (b) (4) when new sources are introduced.

#### **Reviewer's Comments:**

The Sponsor, in collaboration with the ALC-0315 supplier ((b) (4)) appears to be actively investigating the root cause of (b) (4) and possible corrective actions. The identity of (b) (4) as consisting of RNA is unequivocal and there appears to be limited *a priori* foundation (experimental or theoretical) for associating the presence of (b) (4) with safety concerns. (b) (4) is also anticipated to have limited impact on DP functionality due to the



following reasons: (b) (4)

While the analytical data support the use DP lots with (b) (4) for EUA, it is important to note that associated clinical data are currently unavailable. The sole (b) (4) DP lot (EE8493) assessed in study C4591001 thus far was formulated with (b) (4) ALC-0315 and appears to be free of (b) (4). The Sponsor intends to initiate a clinical lot consistency study (C4591017) in Jan 2021 (described in IND19736/152). In response to an Information Request, the Sponsor indicated that DP lots selected for the clinical lot consistency study will include those that are representative in terms of levels of (b) (4) (within constraints allowable by the matrix approach for the study design encompassing the sites of DP manufacture). The Sponsor currently intends to supply data from the clinical lot consistency study as a post-BLA approval commitment.

#### Intrinsic Particles During Visual Inspection

In IND19736/138, the Sponsor reported that during the visual inspection step of the DP manufacturing process, white-colored particulate matter has been occasionally observed. Upon investigation by (b) (4), the visible particles were identified as product-related and most likely consisted of (b) (4) (b) (4) components. Upon addition of sterile 0.9% sodium chloride for injection and mixing, the particulate matter appeared to be dispersible. Based on these observations, the specification for appearance was updated from “(b) (4) (b) (4)” for clinical lots to “may contain white to off-white opaque, amorphous particles” for emergency supply lots.

In response to an Information Request (20 Nov 2020), the Sponsor reported in IND19736/149 that visible particles had been observed during 100% inspection in a small number of vials for nearly all DP lots (29 out of (b) (4) lots). The percentage of vials rejected due to particles during inspection ranged from (b) (4) %. Vials containing particles are rejected and discarded during the 100% automated or manual inspection and Acceptable Quality Limit (AQL) sampling procedure for the inspected vials are further conducted to assess the robustness of the inspection method. No specific factors have been identified as definitively correlating with or contributing to the formation of these intrinsic particles. Particles have been observed to a varying degree across many lots spanning multiple manufacturing sites, including four LNP production sites and two fill/finish sites and across different lipid sources (vendors and batches).

An additional Information Request was issued on 2 December 2020 with regard to the propensity for particle formation post-inspection and the impact of intrinsic particles on vaccine quality. In IND19736/159, the Sponsor stated that the incidence of particles has been low, and to date, visible particles have not been observed in vials during routine release or stability testing. Further evaluation of samples from DP lot EJ0553 with and

without visible particles demonstrated that product quality (RNA content and percentage of RNA encapsulation) was not impacted by the presence of visible particles.

#### **Reviewer's Comments:**

The impact of visible intrinsic particles appears to be minimal due to the following reasons: (1) the particles are intrinsic to the product (i.e., they are not foreign particles); (2) the frequency of occurrence is low; (3) the rare vials with intrinsic particles can be identified during 100% visual inspection and discarded; (4) the vials with intrinsic particles are not associated with changes in RNA content or (b) (4); (5) the intrinsic particles are dispersible upon dilution with saline; and (6) as a precautionary measure, the instruction for the preparation of vaccine directs the healthcare provider to withhold administration if visible particles are observed after dilution with saline.

### **9. Adventitious Agents Safety Evaluation**

The adventitious agent control program includes the engineering systems of the facility and vessels, the control of raw materials, various filtration steps to control microbial burden in buffers and the process stream, and in-process and environmental testing to monitor the level of adventitious agents in and around the process stream.

The only raw material of direct animal origin was identified to be (b) (4)

. Other materials of animal origin may be used in the production of polymer for filters, manifolds, containers, and/or filter components. These equipment components may contain traces of animal tallow derivatives. Based on the comprehensive adventitious-agent control program, all raw materials were found to be compliant with the EMA Note for Guidance (EMA/410/01 rev.3) and associated with minimal risk for transmissible spongiform encephalopathy (TSE)/bovine spongiform encephalopathy (BSE).

### **10. Nonclinical Studies**

This review focuses on selected studies that may be relevant to vaccine-associated enhanced disease and pharmacokinetics.

#### Immunogenicity in Mice

BNT162b2 was highly immunogenic in mice with strong antigen-binding IgG and high titer neutralizing antibody responses together with a Th1-phenotype CD4+ response as well as an IFN $\gamma$ +, IL-2+, CD8+ T-cell response after a single immunization. Total IgG ELISA showed that the vaccine induced a strong, dose-dependent IgG response that recognizes S1 and the RBD and elicited high neutralizing titers in a pseudotype neutralization assay. Stimulation of fresh splenocytes, collected 28 days after immunization, with an S protein-specific overlapping peptide pool demonstrated robust

CD4+ and CD8+ T-cell IFN $\gamma$  responses and a Th1-dominant profile was demonstrated in quantification of cytokines (IL-2 and IFN $\gamma$ ) in the corresponding culture supernatants.

#### Immunogenicity and SARS-CoV-2 Challenge in Nonhuman Primates

BNT162b2 was assessed for immunogenicity and for protection against an infectious SARS-CoV-2 challenge in rhesus macaques. Rhesus macaques immunized IM with 30  $\mu$ g or 100  $\mu$ g of BNT162b2 on Days 0 and 21 had readily detectable S1-binding IgG and SARS-CoV-2 neutralizing titers (NT50) as early as 14 days after a single immunization, with substantial increases following the second immunization. On Day 28, seven days after Dose 2, at the 30  $\mu$ g dose level, the neutralizing geometric mean titer (GMT) reached 8-fold the GMT of a 38 member panel of human convalescent sera (HCS); at the 100  $\mu$ g dose level, the neutralizing GMT was 18-fold the HCS GMT. The HCS sera were drawn from SARS-CoV-2 infected individuals 18 to 83 years of age, at least 14 days after PCR-confirmed diagnosis and at a time when individuals were asymptomatic. A decline of both S1-binding IgG levels and neutralizing titers was observed out to the latest measured time point (Day 56) but remained above the neutralizing GMT and the S1-binding geometric mean concentration (GMC) of the HCS. As seen following mouse immunization, strong S-specific Th1-dominant IFN $\gamma$ + T-cell responses were detected in all immunized rhesus macaques. By intracellular cytokine staining analysis, there was a dose-dependent increase in S-specific CD4+ T cell responses with a strong Th1-bias evidenced by high frequency of IFN $\gamma$ +, IL-2+, or TNF- $\alpha$ + cells. Groups of 2-4 year old male rhesus macaques that had received two IM immunizations with 100  $\mu$ g BNT162b2 V9 (n=6) or saline (Control; n=3) 21 days apart were challenged 55 days after the second immunization with  $1.05 \times 10^6$  plaque forming units of SARS-CoV-2 (strain USA-WA1/2020), split equally between the intranasal (IN) and intratracheal (IT) routes. The difference in viral RNA detection in BAL fluid between BNT162b2-immunized and control-immunized rhesus macaques after challenge was highly significant statistically (by a nonparametric test, p=0.0014). None of the challenged animals showed clinical signs of significant illness, indicating that the 2-4 years old male rhesus challenge model is primarily an infection model for SARS-CoV-2, not a COVID-19 disease model. No radiographic evidence of vaccine-elicited enhanced disease was observed.

#### Pharmacokinetics

Assessment included evaluating the PK and metabolism of two novel lipid excipients (ALC-0315 and ALC-0159) in the LNP and potential biodistribution of BNT162b2 using (b) (4) expression as a surrogate reporter. An intravenous rat PK study, using LNPs with the identical lipid composition as BNT162b2, demonstrated that ALC-0315 and ALC-0159 distribute from the plasma to the liver. While there was no detectable excretion of either lipid in the urine, the percent of dose excreted unchanged in feces was ~1% for ALC-0315 and ~50% for ALC-0159. The biodistribution of BNT162b2 was evaluated using (b) (4) expression as a surrogate reporter in BALB/c mice. Mice were administered a (b) (4) expressing modRNA formulated like BNT162b2 with the identical lipid composition. (b) (4) expression was measured *in vivo* following

(b) (4) application. (b) (4) expression was identified at the injection site at 6 hours after injection and was not detected after 9 days. Expression in the liver was also present to a lesser extent at 6 hours after injection and was not detected by 48 hours after injection. The *in vitro* metabolism of ALC-0315 and ALC-0159 was evaluated in 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 from the PK study. Metabolism of ALC-0315 and ALC-0159 appears to occur slowly *in vitro* and *in vivo*. ALC-0315 and ALC-0159 are metabolized by hydrolytic metabolism of the ester and amide functionalities, respectively, and this hydrolytic metabolism is observed across the species evaluated.

### Reviewer's Comments

Based on current hypotheses regarding the etiology of vaccine-associated enhanced disease, the provided data are reassuring due to: (1) the robust induction of functional (i.e., neutralizing) antibodies in mice and rhesus macaques; (2) the Th1 bias in T cell responses; and (3) the lack of disease in vaccinated rhesus macaques challenged with SARS-CoV-2. The nonclinical ADME studies indicate that the LNP mainly localizes to the site of injection and, to a lesser extent, distributes to the liver. Approximately 50% of ALC-0159 is excreted unchanged in feces, while metabolism appears to play a role in the elimination of ALC-0315.

## 11. Validation of Diagnostic Assays Used to Support Clinical Efficacy Endpoints

Information regarding the two diagnostic assays (Cepheid Xpert Xpress RT-PCR assay for the detection of SARS-CoV-2 in clinical specimens and Roche Elecsys Anti-SARS-CoV-2 assay for the evaluation of serostatus to SARS-CoV-2) was submitted in IND19736/49 and IND19736/100.

### Cepheid Xpert Xpress RT-PCR Assay

The Cepheid Xpert Xpress SARS-CoV-2 assay, which has received FDA approval under EUA, is a rapid, automated *in vitro* diagnostic test for the qualitative detection of the N and E gene sequences from nasopharyngeal, nasal, or mid-turbinate swab and/or nasal wash/ aspirate specimens collected from patients suspected of having COVID-19 disease. The Cepheid Xpert assay is used to assess for viral infection before vaccination and to confirm COVID-19 disease cases during study follow-up. Detection of RNA sequences for the N and E genes are carried out by real-time (b) (4) RT-PCR following a single-step sample processing protocol.

The data based on simulated samples (generated by spiking with the commercially available AccuPlex SARS-CoV-2 reference material or a live SARS-CoV-2 reporter virus) confirmed the detection limits of (b) (4) and (b) (4) established by Cepheid. (b) (4)

(b) (4)

A response to IRs were submitted in IND19736/100. An explanation was provided for the algorithm prioritizing a positive coronavirus result over the Sample Processing Control (SPC) result. The Sponsor received clarification from Cepheid that the SPC may fail to amplify in situations where N and E produce strong positives due to the N and E reactions consuming the available (b) (4). The Sponsor also communicated that (b) (4) external controls are run at the (b) (4); trend analyses of C<sub>t</sub> values for these controls were supplied for the period between Aug 10 and Sep 9.

#### Roche Elecsys Anti-SARS-CoV-2 Assay

The Roche Elecsys Anti-SARS-CoV-2 assay, which has received FDA approval under EUA, is a rapid, automated *in vitro* diagnostic test for the qualitative detection of SARS-CoV-2 nucleocapsid (N) protein-specific antibodies in serum or plasma samples. The assay is marketed as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, which would indicate a recent or prior infection. The Elecsys Anti-SARS-CoV-2 assay will be used to assess serostatus before vaccination. Detection of N protein antibodies to SARS-CoV-2 is carried out by a sandwich method in which biotin- and ruthenium-conjugates of N protein are incubated with human serum or plasma. Streptavidin coated paramagnetic beads are then added to isolate N protein/antibody complexes and chemiluminescent emissions are measured by a photomultiplier.

(b) (4)

All convalescent serum samples were obtained between  $\geq 14$ -30 days post-PCR diagnosis. The positive % agreement was (b) (4)

A response to IRs were submitted in IND19736/100. Included in this submission were trend analyses of Cutoff Index Values (COIs) generated during routine clinical testing using Positive Control and Negative Control supplied by Roche for the period between Aug 12 and Sep 7.

#### **Reviewer's Comments**

The submitted data are supportive of both the Cepheid Xpert Xpress assay and the Roche Elecsys Anti-SARS-CoV-2 assay being suitable for their intended use in Phase

2/3 clinical studies when performed at Pfizer's testing facility (Pfizer Vaccine Research and Development; Pearl River, NY). It is important to note that the validation studies relied on available information generated by the assay kit manufacturers (Cepheid and Roche) in support of assay EUA as well as academic studies assessing assay performance published in peer-reviewed publications; the validation results generated by the Sponsor were in general agreement with information from these external sources.