Amsterdam, <insert full date>
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Committee for Medicinal Products for Human Use (CHMP)

Rapporteur's Rolling Review assessment report

Overview and list of questions

COVID-19 mRNA Vaccine BioNTech

BNT162b2, 5'capped mRNA encoding full length SARS-CoV-2 Spike protein

Procedure No. EMEA/H/C/005735/RR

Applicant: BioNTech Manufacturing GmbH

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Start of the procedure:	**RR1 2020-10-06** **RR2 2020-11-07**
Date of this report:	**2020-11-19**
Deadline for comments:	**2020-11-23**
Date of the revised report	**2020-11-25**
Date of final report	

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Administrative information

Invented name of the medicinal product:	**COVID-19 mRNA Vaccine BioNTech**
INN (or common name) of the active substance(s):	BNT162b2, 5'capped mRNA encoding full length SRAS-CoV-2 Spike protein
Applicant:	BioNTech Manufacturing GmbH
Applied Indication(s):	TBD
Pharmaco-therapeutic group (ATC Code):	J07BX
Pharmaceutical form(s) and strength(s):	Concentrate for suspension for injection 0,225 mg
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In accordance with Article 6(3) of Regulation (RC) No 726/2004, I the Rapporteur hereby declare that I have completed my assessment report in less than 80 days.	**Date 2020-11-19** **Signature Filip Josephson**

Declarations

This application includes an Active Substance Master File (ASMF):

☐ Yes

☒ No

☒ The assessor confirms that proprietary information on, or reference to, third parties (e.g. ASMF holder) or products are not included in this assessment, including the Product Information, unless there are previous contracts and/or agreements with the third party(ies).

☒ The assessor confirms that reference to ongoing assessments or development plans for other products is not included in this assessment report.

Whenever the above box is un-ticked please indicate section and page where confidential information is located (including the Product Information document) here:

List of abbreviations

5’ cap	5’ capping structure, (m27,3’-OGppp(m12’-O)ApG)
AF4-MALS-QELS	Asymmetric Flow Field-Flow Fractionation (AF4) Multi-Angle Static and Quasi-Elastic Light Scattering
ALC-0159	PEG-lipid, 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide
ALC-0315	Cationic lipid, ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)
AQL	Acceptance Quality Limit
ATP	adenosine triphosphate
AUC	Area under the curve
BNT162b2	Vaccine candidate encoding the SARS-CoV-2 full-length spike protein, modified by 2 proline mutations (P2 S)
BSE	bovine spongiform encephalopathies
C&E	Cause and Effect Matrices
C&E	Cause and Effects
CAD	Charged Aerosol Detection
CCI	Container Closure Integrity
CDI	N,N-carbonyldiimidazole
CGE	Capillary Gel Electrophoresis
CMA	Conditional marketing authorization
CoA	Certificate of Analysis
COVID-19	Coronavirus disease 2019
CPP	Critical process parameter
CQA	Critical Quality Attribute
CRM	Clinical Reference Material
CTM	Clinical Trial Material
ddPCR	Droplet digital PCR
DL	Detection Limit
DLS	Dynamic Light Scattering
DOE	Design of experiments
DP	Drug Product
DS	Drug Substance
DSC	Differential Scanning Calorimetry

DSPC	Phospholipid, (1,2-distearoyl-sn-glycero-3-phosphocholine)
DVS	Dynamic Vapor sorption
EDTA	Ethylenediaminetetraacetic acid
ELSD	Evaporative Light Scattering Detection
EVA	Ethylene Vinyl Acetate
EVA	Ethylene vinyl acetate
FID	Flame Ionization Detector
FMEA	Failure Modes and Effects Analysis
FTIR	Fourier-Transform Infrared
GC	Gas Chromatography
GMP	Good Manufacturing Practice
GTP	Guanidine triphosphate
HEPA filter	High Efficiency Particulate Arresting filter
HEPES	N-(2-hydroxyethyl)-piperazine-N-(2-ethanesulfonic acid)
HPLC	High Performance Liquid Chromatography
HTF	Heat Transfer Fluid
ICH	International Council for Harmonisation
ICP	Inductively Coupled Plasma
IP-RP-HPLC	ion-paired reversed-phase high performance liquid chromatography
IPT-C	In-Process Tests for Control
IPT-M	In-Process Tests for Monitoring
IR	Infrared spectroscopy
IVE	In-Vitro Expression
IVT	In vitro transcription
JP	Japanese Pharmacopeia
LC	Liquid Chromatography
LNP	Lipid nanoparticle
MAA	Marketing authorization application
MFAT	Multi-factor-at-a-time
MBC	Master cell bank
mRNA	Messenger RNA
MS	Mass Spectroscopy

N/P	Molar ratio of the amine in the cationic lipid (N) to the phosphate in anionic phosphodiester backbone of RNA (P)
NGS	Next Generation Sequencing
NMR	Nuclear Magnetic Resonance
NOR	Normal Operating Range
NTP	Nucleotide triphosphate
OFAT	One-factor-at-a-time
OQ	Operational Qualification
PAR	Proven acceptable ranges
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PEG	Polyethylene glycol
PES filter	Polyethersulfone filter
Ph. Eur.	European Pharmacopeia
poly(A)	polyadenosine
PPQ	Process Performance Qualification
PQ	Performance Qualification
PRM	Primary Reference Materials
PTFE	Polytetrafluoroethylene
PV	Process Validation
QA	Quality Attributes
QC	Quality Control
QL	Quantitation Limit
QTPP	Quality Target Product Profile
RPN	Risk Priority Number
RP-HPLC/UV-ESI MS	ion-pair reversed-phase high performance liquid chromatography- ultraviolet light detection at 260 nm and online electrospray ionization mass spectrometry
RR	Rolling review
RSF	Residual Seal Force
RT-PCR	Reverse Transcription Polymerase Chain Reaction
Rz/Rh	Overall ratio between root mean square radius (Rz) and hydrodynamic radius (Rh)
S	Spike glycoprotein

SARS	Severe acute respiratory syndrome
SARS-CoV-2	SARS Coronavirus-2; virus causing the disease COVID-19
TFF	Tangential Flow Filtration
TLC	Thin Layer Chromatography
TOC	Total Organic Carbon
TSE	transmitting transmissible spongiform encephalopathies
UFDF	ultrafiltration/diafiltration
USP	United States Pharmacopeia
UTP	uridine triphosphate
UTR	Untranslated region
UV	Ultraviolet
WCB	Working cell bank
WRM	Working Reference Materials
XRD	X-Ray Diffraction

1. Executive summary

1.1. Scope of the rolling review submission

Only the non-clinical dossier was submitted and assessed during the first rolling review cycle 1 (RR1).

For this second rolling review cycle 12(RR2) only Quality data has been submitted and assessed. This second rolling review cycle is the first rolling review that contains Quality documentation, RR2 (CMC1).

The applicant plans to update several sections in the Quality part of dossier and states the following: *"Data for this section is pending and will be updated once the data has been generated, analyzed, and verified"*. Until these data are available for assessment, no final conclusions can be drawn on the concerned sections.

New active substance status

Based on the review of the data the active substance BNT162b2, 5'capped mRNA encoding full length SRAS-CoV-2 Spike protein contained in the medicinal product COVID-19 mRNA Vaccine BioNTech is considered to be qualified as a new active substance in itself.

1.2. The development programme/compliance with CHMP guidance/scientific advice

N/A

1.3. General comments on compliance with GMP, GLP, GCP

Regarding the non-clinical dossier, the pivotal toxicological studies are stated to be GLP. There are some issues with repeat-dose toxicity study #38166 regarding the documentation which may lead to an GLP inspection to the laboratory site (under discussion at the CHMP).

Assessment of the documentation of GMP compliance is within the remit of the European Medicines Agency (EMA). The EMA Compliance and Inspection Service has reviewed the manufacturer information contained in the application form and available certificates from the EEA National Competent Authorities. EMA confirms that a GMP Distant Assessment (DA) of the US Andover and Chesterfield sites will be performed as soon as possible.

1.4. Type of application and other comments on the submitted dossier

Legal basis

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application.

New active substance status

The applicant requested the active substance BNT162b2, 5'capped mRNA encoding full length SRAS-CoV-2 Spike protein contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

2. Scientific overview and discussion on new data

2.1. Quality aspects

2.1.1. Introduction

The vaccine is based on the SARS CoV-2 spike glycoprotein (S) encoded in RNA and formulated in lipid nanoparticles (LNPs), referred to as COVID-19 Vaccine (BNT162b2).

The finished product is presented as a preservative-free, multi-dose concentrate to be diluted for intramuscular injection, intended for 5 doses. The finished product is a sterile dispersion of RNA-containing lipid nanoparticles (LNPs) in aqueous cryoprotectant buffer containing 30 µg/dose of the active substance BNT162b2, 5'capped mRNA encoding full length SARS-CoV-2 Spike protein as active substance.

Other ingredients are: ALC-0315((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), ALC-0159 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide), DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), cholesterol, sucrose, sodium chloride, potassium chloride, disodium phosphate dihydrate, potassium dihydrogen phosphate and water.

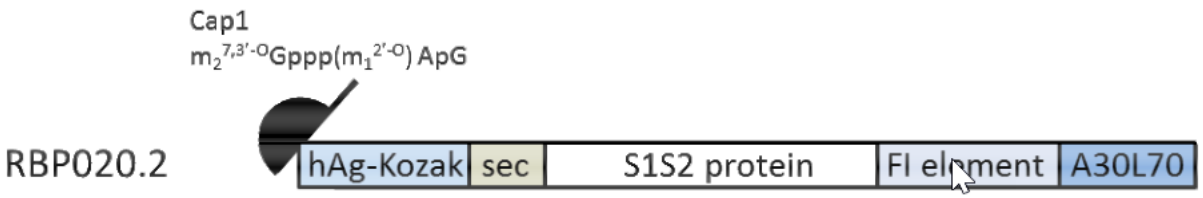
The product is available in glass vial sealed with a bromobutyl rubber stopper and an aluminium seal with flip-off plastic cap.

2.1.2. Active Substance

General Information

The active substance consists of a single-stranded, 5'-capped mRNA that is translated into a codon-optimized sequence encoding the spike antigen of SARS-CoV-2. Figure 1 illustrates the general structure of the antigen-encoding RNA: In addition to the codon-optimized sequence encoding the antigen, the RNA contains common structural elements optimized for mediating high RNA stability and translational efficiency (5'-cap, 5'-UTR, 3'-UTR, poly(A)-tail; see below). Furthermore, an intrinsic signal peptide (sec) is part of the open reading frame and is translated as an N-terminal peptide. The RNA does not contain any uridines; instead of uridine the modified N1-methylpseudouridine is used in RNA synthesis.

Figure 1. General structure of the RNA



Schematic illustration of the general structure of the BNT162b2 drug substance with 5'-cap, 5'- and 3'-untranslated regions (hAg-Kozak and FI element, respectively), coding sequence with mutations and intrinsic signal peptide (sec) as well as poly(A)-tail (A30L70). Individual elements are not drawn to scale compared to their respective sequence lengths.

Manufacture, process controls and characterisation

Manufacturers

The DS is manufactured and controlled by either Wyeth BioPharma Division, Andover, United States or by BioNTech Manufacturing GmbH, Mainz, Germany, (steps 1-3) and Rentschler Biopharma SE, Laupheim, Germany (steps 4 and 5). Of note, the manufacturing process at the European sites is not yet included in the application.

Release and stability testing sites are listed. As Mutual Recognition Agreement is not in force for human vaccines, the provided documentation for testing sites located in the USA is not considered sufficient **(MO)**.

Description of manufacturing process and process controls

Information on the manufacturing process and process controls for the manufacturing site BNT Mainz & Rentschler is not yet provided. Therefore, the comments below are related only to the Andover site. It is expected that no significant differences between the two processes are envisaged. However, minor process adaption could be accepted provided that they will be appropriately validated.

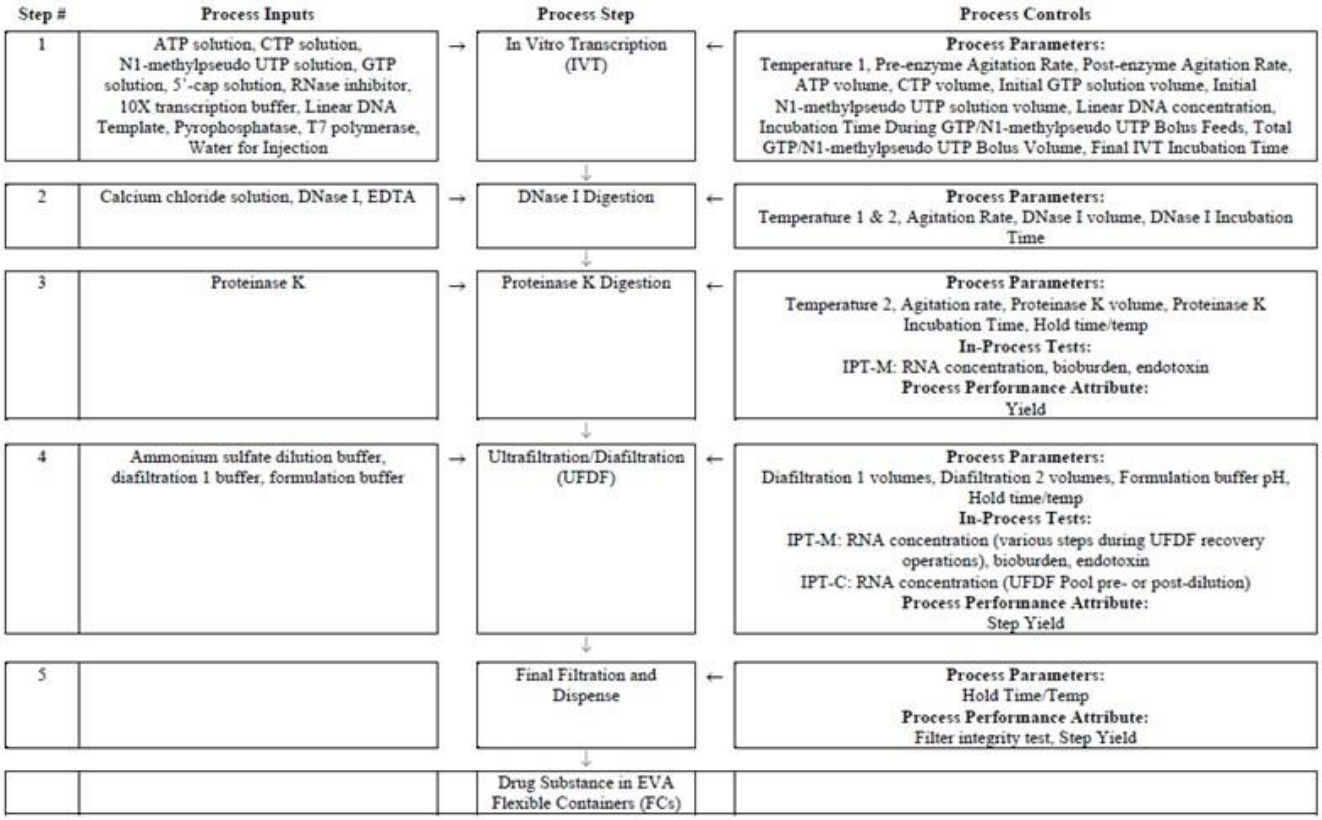
Overall description of the manufacturing process steps

The manufacturing process of BNT162b2 drug substance (DS) involves five major steps. The DS is produced at a scale of 37.6 L. The RNA is first synthesized from linear DNA via an in vitro transcription (IVT) step. It should be observed that the linear DNA template is defined as a starting material, and therefore manufacturing of the template via plasmid DNA is not included in the process. The IVT step is followed by two enzymatic steps, i.e. the DNase I (this reaction is stopped with EDTA addition) and proteinase K digestion steps, which aid in purification. The crude RNA is then purified through a two-stage ultrafiltration/diafiltration (UFDF) step. Lastly, the RNA undergoes a final filtration before being dispensed and stored frozen in EVA flexible containers.

A flow diagram is provided (Figure 3.2.S.2.2-1), presenting the process inputs and the process controls for each step. The purpose of each step in the manufacturing process is sufficiently described. The hold times, process parameters and corresponding acceptance criteria are listed for each step. It is noted that not all process parameters are listed, but that the lists include all critical and several non-critical process parameters. In general, it is agreed that the key process parameters are described in section 3.2.S.2.2. However, for the IVT step, the added volumes of the enzymes T7 polymerase and pyrophosphatase should be regarded as critical, unless justified. It should also be noted that future changes to any of the process parameters listed in S.2.2, regardless of the classification as CPP or non-CPP, should be applied for as variation applications. A few concerns are raised regarding the incubation time during GTP/N1-methylpseudo UTP bolus feeds, the transfers of the UFDF pool into a single PE flexible container and DS filling volume range.

The Applicant explains that the UFDF membrane lifetime remain to be established and the concurrent validation plan is found adequately described in the dossier. The strategy could be found acceptable, provided that the Applicant will update the manufacturing process description with control of feed flow rates, transmembrane pressure and membrane surface area.

Figure 3.2.S.2.2-1. RNA Manufacturing Process



Drug substance transportation

The drug substance is stored between -15 °C and -25 °. Transportation using an insulated shipper is qualified for a shipping time up to 106 hours at ≤-15 °C.

Reprocessing

It is stated that if the post-use integrity test on the final 0.45/0.2 µm filter fails, refiltration is allowed. It is clearly defined that reprocessing at the final filtration step is only allowed once. This is found acceptable.

Batch scale and definition

It is explained that commercial scale drug substance batches are executed at a scale of 37.6 L starting volume for in vitro transcription (IVT). All material produced is purified by a single, two-stage ultrafiltration/diafiltration (UFDF) to produce drug substance. The batch numbering system is sufficiently described. Each batch is assigned one batch number for the entire process. This is found acceptable. However, in addition, information on the final DS volume should be provided.

Control of materials

An adequate overview of the raw materials and solutions used in the Drug Substance manufacturing process is provided. Limited acceptance criteria are included in a tabular format for all raw materials but representative CoAs should also be provided for the non-compendial materials. In general, the submitted information seem to support an appropriate quality of raw materials, however, several concerns are raised at this point.

Starting materials:

The listed starting materials include ATP solution, CTP solution, GTP solution, N1-methylpseudo UTP solution and 5'-cap solution and the linear DNA template. The approach is acceptable. As the 5'-cap structure is complex, additional information on its synthesis and discussion on its impurities are requested. Clarifications are also requested on materials testing.

Linear DNA template

BNT162b2 drug substance is manufactured by in vitro transcription using a linear DNA template, produced via plasmid DNA (pST4-1525) from transformed DH10B Escherichia coli cells.

The linear DNA template is not part of the final product but defines the sequence of the mRNA product and therefore it is fundamental to ensure its adequate control. Changes to the manufacturing process of the linear DNA template (e.g. change to plasmid host cell) may result in a different impurity profile in the active substance. Therefore, the level of details included in the dossier with respect to the manufacturing process and the control strategy for this starting material, although shortly described, is not yet considered adequate to allow for a proper assessment.

The functional elements of the pST4-1525 are sufficiently described in graphic and tabular formats and the sequence is included. However, details regarding the bacterial strain and the source and generation of the pST4-1525 plasmid used remain to be documented.

The cell banks involved in the plasmid manufacturing process are described. MCB and WCB qualification tests are listed and include morphologic and genotypic identity, restriction map analysis and DNA sequencing, absence of contaminating bacteriophages, viability, plasmid retention and plasmid copy number. Relevant specifications are set and data from the current MCB and WCB are provided. The plasmid MCBs and WCBs are enrolled in a cell bank stability program consisting of viability and plasmid retention assays conducted at all stability time points. The strategy is, in general, considered adequate, although some details are requested.

pST4-1525 is manufactured by a fed-batch fermentation process initiated from the bacterial working cell bank (WCB). Following fermentation, the cells are harvested and chemically lysed to recover the plasmid DNA. After this lysis step, the circular plasmid DNA is purified by ultrafiltration/diafiltration and anion exchange chromatography. The circular plasmid DNA is filtered via 0.2 µm filtration and stored frozen at -60 to -90 °C; the hold time for this intermediate is not defined. The filtrate is sampled for the circular plasmid DNA specifications. After thawing, the plasmid is linearized, concentrated, filtered and stored frozen at -15 to -25 °C. No additional information nor data are provided to support stability. The filtrate is sampled for the linear DNA template specification. A list of the raw materials as well as the chromatography resins and filters used in the manufacture of the linear DNA template is provided. All materials used are animal origin free and sourced from approved suppliers.

Specifications for the circular plasmid DNA as well as for the DNA linear template are provided. Process- and product-related impurities including host cell genomic DNA, RNA, proteins, endotoxins, bioburden and plasmid isoforms, for the plasmid DNA, are quantified routinely. The reference material for plasmid identity testing is not described. Results from three different batches are provided for the circular and linearized plasmid and the proposed specification limits seem to be justified by the yet limited available data. No descriptions of the analytical methods used for the control of the linear DNA template nor evidence regarding their qualification/validation have been yet provided. This information is, however, considered critical for quality of the final product. The Applicant is reminded that implementation of changes in the manufacture of the linear DNA template should be applied for in a variation application.

Control of critical steps and intermediates

Process parameters and tests that are used to control the process and drug substance quality are provided. The Applicant claims that due to rapid development of additional process knowledge, process parameters and ranges are expected to be updated in a subsequent submission to the MAA prior to its approval. This is found acceptable, but the Applicant is reminded that all process parameters and ranges should be sufficiently validated. All changes in future submissions prior to MAA or CMA approval should be clearly stated. Some clarifications about the list of critical process parameters (CPPs), in-process tests for control (IPT-C), and hold times are already requested.

The in-process test methods are defined either as in-process testing for control (IPT-C) or in-process testing for monitoring (IPT-M). The sole IPT-C is determination of RNA concentration in the ultrafiltration/diafiltration (UFDF) pool (pre- or post-dilution) by UV spectroscopy. This method is performed as described for the corresponding DS specification test. Three IPT-Ms are listed; determination of RNA concentration in the proteinase K pool by UV Spectroscopy (same as above), bioburden and bacterial endotoxin testing. All three methods are applied to test the proteinase K pool (post-hold), the UFDF pool (post-hold), and the UFDF end of diafiltration 2 retenate (pre-recovery) samples. Bioburden and bacterial endotoxin testing are compendial methods.

Process validation

The process validation is ongoing at Wyeth BioPharma, Andover. For the process validation studies a total of five validation batches will be included, all these batches have been manufactured representing the commercial batch size of 37.6 L. Results are available for three out of the five consecutive batches. The results from batches PPQ4 and PPQ5 are still pending.

No validation data are available to confirm consistent removal of impurities, which is not acceptable. In addition, residual DNA template is present at higher level in PPQ3 batch (211 ng DNA / mg RNA) than in PPQ1 and PPQ2 batches (10 and 23 ng/mg) which does not confirm the robustness of DNase I digestion.

The final filtration refiltration was validated at lab scale using a commercial scale filtration pool and will be confirmed at commercial scale. This is acceptable.

Several validation studies are still pending and will be updated once the data has been generated. ATP and CTP volumes added at the beginning of IVT were increased from the third PPQ batch and onwards. The results for PPQ4 and PPQ5 batches are therefore necessary to confirm the consistency of the process after this change. Therefore, a time-plan for the submission of these additional process validation data should be provided before marketing authorization approval.

<u>Hold times</u>

It is stated that in-process pool hold times are not required for routine processing, but strategic holds in the process ≥24 hours to aid in manufacturing scheduling were validated. The small scale in-process hold studies are intended to support biochemical stability at commercial scale. The hold times for the Proteinase K pool, UFDF pool and DS before freezing as listed in S.2.2 are all acceptably validated for hold times ≤72 hours.

<u>Filter Qualification and Validation</u>

The final filtration refiltration was validated at lab scale using a commercial scale filtration pool, and will be confirmed at commercial scale, which is pending. This is acceptable.

Shipping Performance Qualification

The shipping qualification strategy are described in detail and considered both thermal and mechanical aspects of shipping. The shipping procedures and configuration for transport of frozen DS to the DP manufacturing sites were validated to maintain product temperature in the acceptable range for durations up to 106 hours.

UFDF membrane lifetime

The strategy for UFDF membrane lifetime validation is to perform concurrent validation of the membranes at commercial scale. Parameters related to performance and cleaning of membranes will be evaluated as listed in Table S.2.5-9. This strategy is found appropriate since control of process parameters and IPC-tests are in place for every batch.

Manufacturing process development

Data for this section is pending.

Development history and Comparability

Process development changes were adequately summarised. Two DS processes have been used during the development history; Process 1 and 2. Details about process differences, justifications for making changes, and results from a comparability study is provided. The major changes between DS Process 1 and 2 are; increased process scale, DNA template changed from a PCR template to linearized plasmid DNA, magnetic bead purification replaced with proteinase K digestion and UFDF steps.

No comparability study was provided for non-clinical versus clinical batches, but the batch analysis results are provided.

The comparability study was performed between process 1 GMP batches and process 2 batches manufactured at Andover and will be completed when all PPQ data will be available.

In the comparability study a decrease in RNA integrity was observed for the Process 2 batches compared to Process 1 batches (78.1-82.8% compared to 59.7%). After adjustment of process parameters for CTP and ATP volumes batch 20Y513C501 (PPQ3) was manufactured with RNA integrity level of 75%, more consistent with the Process 1 batches. No analysis of the capillary electropherogram was provided. It is therefore not possible to conclude if the differences in RNA integrity are quantitative or qualitative. Additional batch data are needed to confirm that the optimized Process 2 allows to reach RNA integrity levels consistent with the Process 1 batches. **(Part of MO).**

Regarding the 5’ cap end of the DS, LC- UV/MS characterisation confirmed that the 5'-capped and uncapped structures are the same in Process 1 and 2, but that there is a slight shift towards higher 5'-capping levels in Process 2. It is noted that the capped-intact RNA was not measured, but only deducted from the results of 5’-cap and RNA integrity. Therefore, this argument cannot be used to fully confirm the comparability of Process 2 versus Process 1.

Furthermore, the poly(A)tail of the 3’ end was characterised by LC-UV/MS. The expected short (A30) and long (L70) segments of the poly(A) tail were observed after RNase T1 cleavage. While the results for the A30 segment were demonstrated to be comparable between Process 1 and Process 2 batches, significant differences were identified for the L70 segment. As expected, poly(A) tail heterogeneity was observed both for Process 1 and Process 2 batches, due to transcriptional slippage. Longer poly(A) tails were observed for the Process 2 batch, while the most abundant L70 segments of the Process 1 batch were demonstrated to contain an additional cytidine residue. Differences in the poly(A)tail pattern were observed when comparing the Process 1 and Process 2 DS batches. The differences in the extent of cytidine monophosphate incorporation and transcriptional slippage needs to be further investigated and

the possible impact on efficacy and safety should be discussed. The only Process 2 DS included in the comparison was manufactured prior to the adjustment of CTP and ATP volumes. Results obtained on the PPQ batches manufactured after adjustment (PPQ 3, 4 and 5) also needs to be presented.

The overall primary sequence of BNT162b2 drug substance was demonstrated to be comparable by LC/MS/MS -oligonucleotide mapping. Circular dichroism (CD) spectroscopy confirmed that the higher-order structure of Process 1 and Process 2 DS batches were comparable.

To demonstrate functionality, the protein size after in-vitro expression of BNT162b2 drug substance was determined using Western blot. The expressed protein sizes were demonstrated to be comparable between Process 1 and Process 2 batches. However, the method is only briefly described, and the relevance of the results is therefore difficult to assess.

Critical Quality Attributes (CQAs)

A summary of the quality attributes with the rationale for the criticality assignment is provided. The rationale for classification into CQA or QA is presented for each attribute and appears reasonable. The identified CQAs are; RNA integrity, 5'-cap, Poly(A) tail, residual DNA template and double stranded RNA (dsRNA). To be noted, for poly(A) tails, both percentage of Poly(A) positive mRNA molecules as well as the length of the Poly(A) tails are considered CQAs. A related concern is raised in S.4.

Process Development and Characterization

Data for this section is pending.

Process characterisation studies based on Cause and Effect Matrices (C&E) assessment, Failure Modes and Effects Analysis (FMEA), design of experiments (DOE), using scale-down models of individual unit operations, were/will be performed. To be noted, the overall control strategy including the approaches taken to identify critical process parameters (CPPs) are presented but some parameter and ranges may be updated after PPQ and additional characterization studies are completed. As for assessment of overall control strategy, a complete set of data and information is needed and therefore the final evaluation of the control strategy cannot be made at this point.

It should also be noted that future changes to any of the process parameters listed in S.2.2, regardless of the classification of CPP or non-CPP, should be applied for as variation applications.

Initially, addition volumes for ATP and CTP were identified as non-CPPs as both were supplied in theoretical excess. Following the Pfizer GMP campaigns and additional smalls scale studies it was shown that these volumes could be limiting, and the ranges were widened at the higher end. The approach to only change the higher end of the ranges need to be further justified and clarified. It is noted that after the adjustment of these volumes the percentage of RNA integrity was increased to levels more consistent with the Process 1 batches.

In the In vitro transcription (IVT) step T7 RNA polymerase and pyrophosphatase are added to start the reaction. The ribonucleotide building blocks are assembled by the T7 polymerase. T7 polymerase is magnesium dependent, but the magnesium can be chelated by pyrophosphate released by the addition of each ribonucleotide to the growing chain. Pyrophosphatase is used to maintain sufficient levels of free magnesium by breaking down the pyrophosphate. It is claimed that the added volumes of these two enzymes have been identified as non-CPPs as they are most likely to impact yield only. This conclusion is not agreed upon, the added volumes of the enzymes should be classified as CPPs.

Risk Assessment of Process Related Impurities

Data for this section is pending.

A safety risk assessment for potential process-related impurities included in the drug substance process relative to patient safety is provided in this section. The potential impurities include small molecules, enzymes and the NTP/Capping Structure. The sources of the impurities are sufficiently addressed.

The safety risk assessment strategy involves comparison of the theoretical worst-case concentration of impurities, assuming no removal, to calculated safety concern thresholds. If the worst-case level of an impurity exceeds the pre-determined safety limits, any available commercial scale data for the specific impurity will be provided in the relevant section and at a minimum will be monitored as part of process validation to demonstrate consistent removal to acceptable levels.

The worst-case levels of NTPs, 5' cap, small molecule process related impurities, RNase inhibitor, DNase I and pyrophosphatase from the BNT162b2 drug substance manufacturing process were calculated to be significantly below the pre-determined safety limits. This is found acceptable. The T7 RNA polymerase and proteinase K levels were further investigated and it was demonstrated that the detected concentrations in the clinical, initial emergency supply and PPQ BNT162b2 DS batches were well below the safety concern threshold. The Applicant states that data will be provided for additional batches once testing is complete. This is found acceptable. However, the Applicant should provide data on the T7 RNA polymerase and proteinase K levels in additional commercial scale DS batches, once testing is complete. In addition, the Applicant should briefly describe the methods applied to determine the concentrations of these two enzymes in the BNT162b2 DS samples.

Characterisation

Elucidation of structure and other characteristics

Analytical characterisation was performed on BNT162b2 drug substance batch 20Y513C101, which was manufactured by DS Process 2 at commercial scale. This is found acceptable.

The physico-chemical characterisation involved primary structure, 5' cap structure, poly(A)tail and higher order structure evaluation. Primary structure was confirmed by oligonucleotide mapping and the orthogonal method, RNA sequencing using the Illumina MiSeq Next Generation Sequencing (NGS) technology. The results confirm the RNA sequence. The 5'-cap and 3' poly A tail were confirmed by two separate LC-UV/MS-methods. It was demonstrated that the predominant form of the 5' terminus is the full-length nucleotide sequence with the 5'-Cap, but that there are also other minor species including phosphorylated, truncated and extended species. Analysis of the 3' poly A-tail demonstrated that BNT162b2 DS contains the expected tail, but that there is some heterogeneity due to transcriptional slippage. Un-capped RNA and/or truncated/extended forms are possible at minor to trace levels but a precise quantification of each uncapped or incompletely capped specie was not provided. It is also not specified if and how these species contribute to the potency of the BNT162b2 DS. The higher order structure of BNT162b2 mRNA DS was characterized in solution using circular dichroism (CD) spectroscopy. Overall, state-of-the-art methods were applied for physico-chemical characterisation and the results confirmed the expected sequence and quality attributes.

A severe deficiency of the characterisation section is that no biological characterisation is presented and that the mode of action is not described. This is not found acceptable and the dossier should be updated with relevant information. Even though full biological characterisation is not possible to perform on DS, the strategy to determine potency and relevant functional assay(s) should be described in section 3.2.S.3. Results obtained on DP could be included, to demonstrated functionality. Furthermore, it is observed that in the Development History and Comparability section (3.2.S.2.6), the expressed protein size is evaluated by in vitro expression followed by Western blot. Results obtained

by this method could be regarded as biological characterisation and should be included in section 3.2.S.3. The method needs further description and the results should be sufficiently characterized.

Impurities

Process-related and product-related impurities as well as potential contaminants are described in this section. Five batches were evaluated for impurities, i.e. clinical, initial emergency supply and PPQ batches. It is noted that this section is incomplete and will be updated after PPQ completion.

The sole product-related impurity addressed is double-stranded RNA, derived from the in-vitro transcription reaction. Results from the five DS batches demonstrate that the level of double stranded RNA is low, acceptable and consistent.

In addition to double stranded RNA, there are more product-related impurities, i.e. truncated RNA, also referred to as fragmented species. Truncated RNA is reflected in the DS specification in terms of RNA integrity. However, the characterisation of BNT162b2 DS is currently not found acceptable in relation to the CQA RNA integrity. Significant differences between batches manufactured by Process 1 and 2 are observed for this specific attribute. Even though two methods, namely agarose gel electrophoresis and capillary gel electrophoresis, have been applied to determine RNA integrity of BNT162b2 DS, no characterisation data on RNA integrity and truncated forms is presented and the potential safety risks associated with truncated RNA isoforms are not addressed. This is especially important considering that the current DS and DP acceptance criteria allows for up to 50% fragmented species. Therefore, the dossier should be updated with additional characterisation data and discussion on mRNA integrity, **this is considered a major objection.**

Residual DNA template is a process-related impurity derived from the linearised DNA template added to the in-vitro transcription reaction. Residual DNA template is controlled by qPCR as defined in the DS specification, and the levels for all five batches are demonstrated to be well below the acceptance criteria. However, a drift towards higher level was observed for the third PPQ batch and therefore additional batch data are needed to conclude on the consistent removal of this impurity. Additional process-related impurities, including nucleoside triphosphates (NTPs) and capping structure, small molecules, and enzymes, are evaluated and assessed in Section 3.2.S.2.6 Risk Assessment of Potential Process Related Impurities. Taking section 3.2.S.2.6 into account, the process-related impurities are sufficiently described. Some uncertainty remains regarding the approach to determine the levels of T7 RNA polymerase and proteinase K.

The potential contaminants described in this section are endotoxin and bioburden. Acceptable results are shown for the Proteinase K pool, UF retentate pre recovery, UF-product pool and the drug substance.

Specification, analytical procedures, reference standards, batch analysis, and container closure

Specifications

Table S. 4-1. Specifications

Quality Attribute	Analytical Procedure	Acceptance Criteria
Composition and Strength		
Clarity	Appearance (Clarity) [a]	≤ 6 NTU
Coloration	Appearance (Coloration) [a]	Not more intensely coloured than level 7 of the brown (B) colour standard

Quality Attribute	Analytical Procedure	Acceptance Criteria
pH	Potentiometry [a]	7.0 ± 0.5
Content (RNA Concentration)	UV Spectroscopy	2.25 ± 0.25 mg/mL
Identity		
Identity of Encoded RNA Sequence	RT-PCR[b]	Identity confirmed
Purity		
RNA Integrity	Capillary Gel Electrophoresis	≥ 50% intact RNA
5'- Cap	RP-HPLC	≥ 50%
Poly(A) Tail	ddPCR	≥ 70%
Process Related Impurities		
Residual DNA Template	qPCR[b]	≤ 330 ng DNA/mg RNA
Product Related Impurities		
dsRNA	Immunoblot[b]	≤ 1000 pg dsRNA/µg RNA
Safety		
Bacterial Endotoxin	Endotoxin (LAL) [a]	≤ 12.5 EU/mL
Bioburden	Bioburden [a]	≤ 1 CFU/ 10 mL

a. Compendial

b. Assay not performed on stability.

Abbreviations: NTU = Nephelometric Turbidity Units; B = brown; RT-PCR = reverse transcription polymerase chain reaction; ddPCR = droplet digital PCR; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus amebocyte lysate; EU = endotoxin unit; CFU = colony forming unit

The proposed specification for drug substance is at large found acceptable with respect to the analyses chosen for routine release testing. The CQAs RNA integrity, 5'-cap, Poly(A) tail, residual DNA template and double stranded RNA (dsRNA) are all included in the release specification. However, the length of the poly(A) tails in BNT162b2 DS is important for RNA stability and translational efficiency and therefore should be included in DS release testing. It is also noted that no method references are included, this needs to be updated.

Potency testing is not included in the control of DS but instead is performed at the level of DP release. Considering the nature of this product, the approach is endorsed.

Analytical procedures and reference standards

Analytical procedures

All analytical methods used for testing of the drug substance are described in the dossier.

The following tests are performed in accordance with Ph Eur; clarity (Ph Eur 2.2.1), colour (Ph Eur 2.2.2), pH (Ph Eur 2.2.3), bacterial endotoxins (Ph Eur 2.6.14) and bioburden (Ph Eur 2.6.12).

A general comment which applies to all non-compendial analytical methods is that rather brief details are given. Some of the analytical methods are not presented in sufficient detail and often method descriptions are based on "examples" of procedures, controls and standards as well as on "typical" system operating parameters. This hampers a full understanding the operation or, sometimes, the scientific basis of the assay. Furthermore, since several of these assays are none standard and complex, this interferes with assessment of suitability. The lack of sufficient information on critical

reagents, standards or equipment hinders regulatory control of critical aspects of the assays. Several concerns are raised for specific assays requesting additional information on critical procedures, reagents, standards and equipment.

It is claimed that the analytical methods were validated against the parameters presented in ICH Q2(R1). However, the validation summaries presented are far too brief to be able to conclude on suitability of the in-house analytical methods. The quality of BNT162b drug substance cannot be properly assessed, if the reliability of the analytical methods cannot be guaranteed.

Capillary gel electrophoresis (CGE) is used to determine the percent integrity of RNA in both drug substance (DS) and drug product (DP). The test sample is subjected to a denaturant containing formamide that unfolds the RNA and dissociates non-covalent complexes. When subjected to an electric field, the denatured RNA species migrate through the gel matrix, as a function of length and size, toward the anode. An intercalating dye binds to RNA and associated fragments during migration allowing for fluorescence detection. The intact RNA is separated from any fragmented species allowing for the quantitation of RNA integrity by determining the relative percent time corrected area for the intact (main) peak.

Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) is used to measure the relative amount of 5'- capped RNA species. Test samples are digested using RNase H followed by affinity purification and (RP-HPLC) with UV detection. After an annealing process to a biotinylated probe complementary to the last 26 bases of the 5' end of the RNA, samples are digested with RNase H, followed by streptavidin-matrix based affinity purification of the resultant duplexes from the much larger mRNA remnants. The short oligonucleotide capped, and uncapped species are eluted from the streptavidin-matrix, and relative quantification of the 5'-cap is accomplished by RP-HPLC analysis of the ensemble of RNA capped and un-capped molecules. The relative amount of capped species is determined by dividing the capped species signal by the total species signal.

The in-house analytical methods for CGE and RP-HPLC are at large well described and includes details on typical test conditions, operating parameters, representative electropherograms and chromatograms as well as information on system suitability testing.

An RT-PCR method is used to determine the identity of the encoded RNA sequence, a quantitative polymerase chain reaction (qPCR) analytical procedure is used to quantify the residual DNA template and an immunoblot analytical procedure is used to detect double stranded RNA (dsRNA) in BNT162b2 drug substance. All these assays are deemed suitable for their intended purpose and, in general, although brief, the descriptions provided are considered relevant. Several concerns regarding additional details on method description, controls and in some cases further clarifications of criteria established to support method suitability are raised.

The ddPCR technology is proposed for the quantification of the poly(A) tail in the messenger ribonucleic acid (mRNA). The technical procedure is considered, in general, sufficiently described but the suitability of this method for the intended purpose needs additional clarifications. The rationale by which the method determines the percent poly(A) relative to the theoretical input (which is not clearly described) should be further addressed.

Release and stability testing can be performed at several testing sites. However, the method transfer plan or activities was not submitted in the RR. It should be noted that, if method transfer was / will be performed, the following information are requested. For the non-compendial tests, it should be confirmed that the validation acceptance criteria for the receiving sites will be the same as for the transferring site (which will be assessed during the RR). For the analytical methods where comparative analysis will be proposed, it should be confirmed that the acceptance criteria will be the same as for the intermediate precision validated at the transferring site (and assessed during RR).

Reference standard

The current reference standard is referred to as the Clinical Reference Material (CRM). It is stated that the CRM will be used for clinical supplies, process validation and initial commercial supplies. The CRM is prepared from the GMP BNT162b2 DS batch 20Y513C201. Release data is presented in the dossier. The intended storage condition is -20 ±5 °C, but an alternative storage condition of -60 to -90 °C is also evaluated. A stability protocol is provided. There are several concerns regarding the reference standard, including the suitability of the batch chosen as CRM, if additional standards have been used during early development and issues related to the formal stability protocol. It should also be clarified for what release and stability testing methods the reference standard is used and will be used in future.

In future, a two-tiered system for future commercial reference material will be implemented. A PRM and an initial WRM will be established in 2021 for the drug substance reference material. The PRM will be the standard against which WRMs are qualified and the PRM will be intended to last the lifetime of the commercial product. The Applicant claims that further information on the selection, preparation, qualification and stability of the PRM and WRM will be provided in the future.

The use of a two-tiered system is encouraged. It is understood that the PRM and WRM is not yet established. The Applicant is reminded that the implementation of the two-tiered system should be applied for in a Type II variation application. Alternatively, information on the preparation, qualification and stability evaluation of the PRM and WRMs should be included in a PACMP.

Batch analysis

Batch results are presented for DS batches used for nonclinical toxicology, clinical trials, process performance qualification (PPQ), emergency supply, and stability.

In general, the results obtained using the commercial process (DS Process 2) demonstrate batch to batch consistency with a few exceptions. The results for RNA integrity are higher for batch PPQ3 (20Y513C501) as a volume adjustments was made for ATP and CTP volumes before manufacturing of this batch. Batch results should be presented for the two newly manufactured batches PPQ4 and PPQ5 verify that the commercial manufacturing process consistently results in RNA integrity levels similar to levels achieved in process 1 batches.

Justification of specification

The rationale used to establish the acceptance criteria is described in detail and based a limited data set representative of BNT162b2 DS manufactured at the intended commercial scale and process. It is endorsed that the specification for BNT162b2 DS will be reassessed when more batches have been manufactured. However, from the available data, it appears that RNA integrity, dsRNA, Poly(A) tail and 5'-cap acceptance criteria are too wide and need to be tightened yet to better reflect data obtained from available lots used in clinical studies (and considered clinically qualified) and data from lots used for PPQ.

Container closure

The drug substance is stored in 12 L or 16.6 L single-use, flexible, disposable bags of ethylene vinyl acetate (EVA). Compliance with Ph. Eur. 3.1.7 *Ethylene-Vinyl Acetate Copolymer for Containers and Tubing for Parenteral Nutrition Preparations* is claimed. Schematic drawings of the bags are provided in the dossier but no specification or certificate of analysis for the container or the EVAM contact layer are included.

The information regarding container closure system is in general acceptable. However, the Applicant should verify compliance with Ph. Eur. 3.1.7 with a certificate of analysis for one representative batch of the EVAM contact layer.

A controlled extraction study has been performed on the EVA container film; all the compounds were observed below the Safety Concern Threshold of 1.5 µg/day TDI. Considering that the intended storage of the DS is -20 °C, a temperature which has a lower risk of leachables, it is reasonable that no specific leachable compounds have been selected for further studies. Nevertheless, a leachable study will be initiated to detect semi quantitate unexpected leachable compounds equal to or greater than 1.5 µg/day TDI. This approach can be accepted.

Stability

The initial proposed commercial shelf life of the drug substance is 6 months when stored at the intended storage condition of -20 ± 5°C in EVA bags. The initial shelf life is based on the currently available data from stability studies utilizing material from three clinical DS batches manufactured using Process 1 and two clinical DS batches (up to 3 months data presented) and three process validation batches manufactured by Process 2 (up to 1 month data presented).

It is claimed that the results of the comparability studies support that stability data generated using drug substance manufactured using Process 1 can be considered predictive of the drug substance manufactured by Process 2. This conclusion is not fully agreed with as detailed above in section S.2.6.

Based on the currently very limited stability data presented for process 2 batches (only 1-month data available for one batch) no conclusion can be drawn in relation to the proposed shelf life for the DS. Therefore, in order to support shelf life setting for drug substance updated reports from the ongoing stability studies on the primary batches (including data from the ongoing process validation batches) should be provided.

It is stated that sponsor will extend the assigned shelf life without notification providing the real time stability data at the intended storage condition is acceptable and within commercial specifications. This kind of extensions can be accepted for clinical trials but not after marketing authorization approval. This statement should be removed from the dossier.

2.1.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

The BNT162b2 drug product is supplied as a preservative-free, multi-dose concentrate to be diluted for intramuscular injection, intended for 5 doses. The drug product is a sterile dispersion of RNA-containing lipid nanoparticles (LNPs) in aqueous cryoprotectant buffer.

Each vial, containing 0.45 mL of the drug product at pH 7.4 is designed to deliver a total of 5 doses after dilution by addition of 1.8 mL of sterile 0.9% sodium chloride solution for a total volume of 2.25 mL, with each dose containing 30 µg of RNA in 0.3 mL. There is no manufacturing overage. The justification for the overfill is discussed, but the final volume exceeding the nominal volume is questioned.

The drug product is supplied in a 2 mL glass vial sealed with a bromobutyl rubber stopper and an aluminum seal with flip-off plastic cap.

The composition of the drug product, including amounts per vial and function and quality standard applicable to each component, are given in Table P.1-1.

All ingredients, including process aids used in the manufacture, should be specified in the composition together with a footnote that they are processing aid removed during manufacturing. Therefore, ethanol and citrate buffer and the excipients present in the DS (HEPES and EDTA) should be added to the composition.

Table P.1-1. Composition of BNT162b2 drug product, multi-dose vial (225 µg/vial).

Name of Ingredients	Reference to Standard	Function	Concentration (mg/mL)	Amount per vial	Amount per dose
BNT162b2 drug substance	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[a]	1.4 mg	0.2 mg
Sucrose	Ph. Eur.	Cryoprotectant	103[a]	46 mg	6 mg
Sodium chloride	Ph. Eur.	Buffer component	6	2.7 mg	0.36 mg
Potassium chloride	Ph. Eur.	Buffer component	0.15	0.07 mg	0.01 mg
Dibasic sodium phosphate, dihydrate[b]	Ph. Eur.	Buffer component	1.08	0.49 mg	0.07 mg
Monobasic potassium phosphate[c]	Ph. Eur.	Buffer component	0.15	0.07 mg	0.01 mg
Water for Injection	Ph. Eur.	Solvent/vehicle	q.s.	q.s.	q.s.

a. Values are rounded to maintain the same level of precision as the label claim, with trailing zeros not shown, where applicable. For example, 46 mg sucrose is rounded from 46.35 mg (103 mg/mL).
b. Dibasic sodium phosphate, dihydrate is named as disodium phosphate dihydrate in the Ph. Eur.
c. Monobasic potassium phosphate is named as potassium dihydrogen phosphate in the Ph. Eur.
Abbreviations:
ALC-0315 = ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)
ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide
DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine
q.s. = quantum satis (as much as may suffice)

All excipients except the functional lipids ALC-0315 and ALC-0159 and the structural lipid DSPC comply to Ph. Eur. grade. The functional lipid excipients ALC-0315 and ALC-0159 are classified as novel excipients. Both structural lipids DSPC and cholesterol are used in several already approved drug products. DSPC is used in several products approved in the EU (Marqibo, Doxil, Ambisome, Onpattro), though not by the same route of administration. Further justification that DSPC is not a novel excipient is requested.

The vial, stopper and seal components are compliant with the appropriate Ph. Eur. monographs for primary containers and closures.

Pharmaceutical development

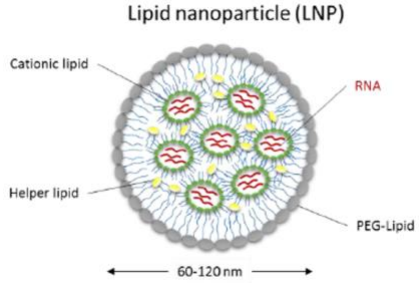
Formulation development

The section on formulation development describes and justifies the chosen formulation and is sufficiently comprehensive.

The formulation development studies of the RNA containing lipid nanoparticles have been thoroughly described. The development of a robust LNP formulation platform was performed at Acuitas Therapeutics. Studies are comprehensively described and were performed with available drug substance, representative of the mRNA platform and included in the drug product.

The LNPs consists of four lipids, each has a functional or structural purpose. The ionizable cationic lipid ALC-0315 interact electrostatically with negatively charged nucleic acids and encapsulate the mRNA. The PEGylated lipid ALC-0159 is preferably inserted at the LNP surface as a steric barrier to interactions with surfaces or other LNPs to avoid aggregation during storage. The phospholipid DSPC and cholesterol are structural lipids providing a stable bilayer and enabling mobility of the lipid components in the LNP structure.

The formed RNA-containing LNPs are solid particles relatively homogeneous in size, largely spherical in shape and has a nearly neutral surface. Furthermore, the accumulated batch-data to date show a consistent manufacturing of lipid nanoparticles both with respect to size and polydispersity.



Critical quality attributes related to LNP formation and payload delivery are primarily LNP size, encapsulation efficiency, and in vivo potency (RNA integrity). Additionally, surface area is considered critical to avoid aggregation both during storage and with serum components in vivo. The ratio cationic lipid to RNA (N/P) is also critical for formation of LNP. An access of cationic lipid is required and a ratio of about 6 is found reasonable.

The DP is stored frozen at the recommended storage temperature of -90 to -60°C. Stability studies are ongoing for the determination of DP shelf-life.

The same DP formulation composition has been used throughout the nonclinical and clinical studies and will also be used for the manufacturing of the pending full scale commercial PPQ-batches.

There are no formula overages in the drug product, only an overfill which has been acceptably justified ensuring that five doses can be removed from the multi-dose vial and delivered.

Screening studies were performed to confirm that the ALC-0315/ALC-0159/DSPC/CHOL at molar ratio 47.5/10/40.7/1.8 with a ratio of cationic lipid to RNA (N/P ratio) of 6.3 provide LNP with acceptable quality and stability. Physicochemical and biological properties were studied (density, viscosity, DSC characteristics). Moreover, size distribution and particle shape were studied showing a narrow distribution with a hydrodynamic radius and an almost spherical shape in the entire size distribution. The zeta potential was narrow and monomodal. The pegylated surface of the LNPs was studied showing consistence with the proposed LNP architecture: presence at the surface of PEG and hydrophilic head of ALC-0315. While the effort made by the applicant to provide sufficient development data in a very brief time is acknowledged, and taking into account that some additional heightened characterization information will be added, the formulation development lacks some characterisation studies showing the homogeneity of the suspension during storage at long-term or accelerated conditions, after freeze/thaw, or after dilution with 0.9% NaCl.

Manufacturing process development

The development history of the drug product is sufficiently described.

The initial LNP and drug product formulation processes were developed at Acuitas Therapeutics, followed by scale-up and manufacture at Polymun Scientific for clinical trial material and emergency

supply. The process has been transferred to Pfizer commercial facilities in Kalamazoo, MI, USA, and Puurs, Belgium, for manufacture of later clinical materials (Puurs), emergency supply and commercial supply.

The DP analytical comparability evaluation employed release testing and extended characterization methods. It is agreed that comparability has been reasonable demonstrated between the clinical supply lots manufactured with the "classical" LNP process and the representative emergency supply lot manufactured with the "upscale" LNP process with only small differences noted.

It is stated in the dossier that the applicant has a plan for a comprehensive demonstration of comparability among clinical supplies and the commercial product including an assessment of the starting drug substance batches, raw materials (e.g. ALC-0315, DSPC and cholesterol) from different vendors, process designs and comprehensive characterization of the resulting product quality. Data for this section is pending and will be updated once the data has been generated, analyzed, and verified. Four commercial PPQ-batches will be manufactured in November and December 2020. The results for the comparability of the commercial PPQ-batches versus the clinical supply batches of DP is pending and will be provided for assessment during the procedure.

In summary, no final conclusion on comparability can be drawn until all comparability data among clinical supplies and the commercial product (PPQ-batches) will be provided for assessment.

Critical Quality Attributes include appearance, visible particulates, subvisible particles, pH, osmolality, extractable volume, lipid identities and contents, RNA identity and content, LPN size and polydispersity, RNA encapsulation, RNA integrity, 5'-cap, poly(A) tail, in vitro expression, endotoxins, sterility, container closure integrity. Even though the risk assessment was not explained in detail, no issue is raised on that point since the DP specification contains the expected parameters.

The development of the manufacturing process is extensively described, and critical process parameters are defined. Process characterisation studies based on Cause and Effect Matrices (C&E) assessment, Failure Modes and Effects Analysis (FMEA), design of experiments (DOE), using scale-down models of individual unit operations, were / will be performed. It is noted that some results of process characterisation studies are pending. The overall documentation related to criticality assignment and NOR/PAR establishment will be assessed when completed. In addition, it is highlighted that for the process characterisation studies already presented, the level of information was not sufficient to allow assessment. Therefore, the PARs are not considered acceptable at this stage.

The lipid nanoparticle (LNP) formation is one critical manufacturing step. The process development is described and physicochemical properties (e.g. LNP size, polydispersity, RNA encapsulation, lipid to RNA ratio (N/P) as well as LNP topology by X-ray scattering) has been evaluated during upscale. The provided results are comparable. The tested parameters are considered relevant, covering the critical attributes size, shape, encapsulation and lipid to RNA molar ration.

The in-process hold times, dilution and mixing of DS parameters, and lipid weight and organic phase mixing parameters will be studied during PPQ. For buffer exchange and concentration step, residual ethanol and citrate should be studied during PPQ and process validation. Process characterisation studies were satisfactorily provided for DS thawing, sterile filtration, aseptic filling, stoppering, sealing and capping, and freezing steps. However, PPQ data will be needed to verify the filling weight of BNT162b2 filled at the commercial filling lines. Moreover, no development data showing homogeneity of LNP or RNA concentration in the vials during filling process was provided. Drug product robustness to freezing and warming during storage was studied and confirmed that BNT162b1 quality was not impacted by different thawing processes, but this will have to be confirmed for BNT162b2 DP.

Overall control strategy was presented but some parameter and ranges may be updated after PPQ and additional characterization studies completed. As for assessment of overall control strategy, a complete set of data and information is needed, this document will be assessed when finalised.

The analytical testing strategy of drug product has changed throughout the development and these changes have been described. Bridging studies have been performed for analytical tests that have been changed or replaced (subvisible particles, identity of encoded RNA sequence and RNA integrity). This is found acceptable.

Container closure system

The development of the container closure system is sufficiently presented. The primary packaging is composed of glass vial and rubber stopper and are compliant with the compendial requirements of Ph. Eur.

Controlled extraction studies have been performed on the bromobutyl rubber stopper. Leachables studies are planned to be set up the applicant should commit to provide the updated results from the leachables study for assessment.

Microbiological attributes:

Sterility and endotoxin testing is performed at DP release. A rapid sterility test may be utilized. CCI will be verified by dye ingress testing or head-space analysis. These tests were demonstrated to be able to detect CCI failure.

Compatibility

The drug product is frozen, and after thawing, the solution/suspension must be diluted with sterile 0.9% sodium chloride solution. The studies described have been performed to assess physicochemical stability of the DP after dilution with 0.9% sodium chloride solution in the original glass vial as well with commonly used commercially available administration components and using worst-case conditions for dosage and administration in the clinical setting. The thawed hold time (in-use period) of undiluted DP are ongoing as part of the stability program in section P.8.

Results presented support physicochemical stability of DP diluted in 0.9% sodium chloride solution for up to 24 hours at ambient or refrigerated temperatures and compatibility with dosing components (syringes and needles) for up to 6 hours. Furthermore, a microbiological in-use hold time study was performed by a challenge test including five compendial micro-organisms. No significant growth (>0.5log10 from the start-point) was observed for any of the microorganisms within 12 hours of inoculation with storage at 20-25°C of diluted DP in 0.9% sodium chloride solution. However, while the representativity of 0.05 mg/mL concentration against the 0.1 mg/mL concentration is accepted, there was no confirmation that the analytical methods are valid at this dilution, and the in-use specifications should be the same as the shelf-life specifications. It is noted, however, that this section may be updated as additional studies are completed

Compatibility of drug product is at large acceptably demonstrated by the dilution and administration simulation studies performed.

Manufacture of the product and process controls

Table P.3-1 lists the sites that have responsibilities in the production of BNT162b2 drug product and their specified functions.

Table P.3-1. Sites and responsibilities for BNT162b2 drug product manufacture

Site	Responsibility
Pfizer Manufacturing Belgium NV Rijksweg 12 Puurs, 2870 Belgium	LNP fabrication and bulk drug product formulation Fill and finish Primary packaging Secondary packaging Release and stability testing (Composition, Adventitious Agents) Batch release by Qualified Person in EEA [European Economic Area]
Wyeth BioPharma Division of Wyeth Pharmaceuticals LLC[a] 1 Burtt Road Andover, MA 01810 United States	Release and stability testing (Composition and Strength, Identity, Potency, Purity, Adventitious Agents)
Pfizer Inc. 875 Chesterfield Parkway West Chesterfield, MO 63017 United States	Release and stability testing (Composition and Strength, Identity, Potency, Purity, Adventitious Agents)
Pfizer Ireland Pharmaceuticals Grange Castle Business Park Clondalkin, Dublin 22 Ireland	Release and stability testing (Identity, Composition)
Hospira Zagreb Ltd.[b] Prudnička cesta 60 10291 Prigorje Brdovečko Croatia	Release testing (Sterility)
SGS Lab Simon SA Vieux Chemin du Poète 10 Wavre, 1301 Belgium	Release testing (Sterility)
BioNTech Manufacturing GmbH Kupferbergterrasse 17-19 55116 Mainz Germany	Batch release by Qualified Person in EEA [European Economic Area]

a. The legal entity name change from Wyeth BioPharma Division of Wyeth Pharmaceuticals was changed at the acquisition by Pfizer in 2009, since then the Wyeth Pharmaceuticals manufacturing site in Andover, Massachusetts belongs to Pfizer's production sites and is embedded in Pfizer´s GMP system. Pfizer will be utilized throughout the CTD.
b. Hospira is a wholly owned subsidiary of Pfizer Inc.

The DP is manufactured tested and batch released by Pfizer Manufacturing Belgium NV, Puurs, Belgium. Batch release can also be done at BioNTech Manufacturing GmbH, Mainz, Germany. Several testing sites are listed, in addition to Pfizer, Puurs, Belgium. Some clarifications are requested for GMP activities of sites located in Europe (**MO**). Moreover, as Mutual Recognition Agreement is not in force for human vaccines, the provided documentation for sites located in the USA is not considered sufficient (**MO**).

The manufacturing process includes lipid nanoparticle (LNP) fabrication and bulk drug product formulation followed by fill and finish. The target drug product batch size is 139 L (approximately 309,000 vials). The batch formula is provided but lacks process aids.

LNP fabrication and bulk drug product formulation

The frozen drug substance (mRNA) is thawed and diluted in water for injection to a target concentration of 2.0 mg/mL. The lipids are diluted in ethanol. To form the LNPs the aqueous phase with mRNA and the organic phase with the lipids are fed into one or more parallel T-mixers with pre-set flow rates to get 3:1 volume ratio. The LNP bulk is then first buffer exchanged with citrate buffer to

remove ethanol from the suspension then with phosphate-buffered saline (PBS) at pH 7.4, suitable for intramuscular administration. Sucrose is added as cryoprotectant, the concentration is adjusted, and the solution mixed until homogenous. Hold times during the bulk drug product formulation process are established.

Sterile filtration and aseptic filling

The bulk drug product is sterile filtered into a holding vessel using two sequential redundant sterilizing grade filters. Integrity of these filters are controlled by pre- and post-use integrity testing. A sample is taken for bioburden prior to filtration. The holding vessel is aseptically connected to the filling line and then sterile filtered bulk drug product is aseptically filled into sterile vials and capped. Vials are 100% inspected for defects either through automated visual inspection or manual visual inspection. Inspected vials are individually labelled and packed. All hold times following sterile filtration will be within the validated media fill times, ensuring acceptable microbial control during the drug product manufacturing process.

Controls of critical steps and intermediates

Critical manufacturing steps are discussed, and relevant in-process controls are applied.

Residual ethanol is not controlled in-process or in the final drug product specification. Data provided demonstrates that ethanol is sufficiently removed in the final drug product. Absence of test is therefore considered acceptable.

The lipid nanoparticle (LNP) formation is one critical manufacturing step and some additional information is requested regarding this step such as that a drawing of the T-mixer should be provided as well as the number T-mixers defined.

Process validation and/or evaluation

No full commercial scale batches are included in section 3.2.P.3.5 and the applicant states that "Data for this section is pending and will be updated once the data has been generated, analysed, and verified."

However, it is stated in the dossier that four commercial PPQ-batches will be manufactured in November and December 2020. These batches will be executed according to defined protocols and evaluated with predetermined acceptance criteria. Furthermore, these batches will be used both to demonstrate the comparability of the commercial PPQ-batches versus the clinical supply batches as well as for process validation of the manufacturing process of the drug product. In addition, validation data on process hold-times, shipping validation and verification of in-process test methods are incomplete. **Since all these validation data are pending, no final conclusion on process validation in section 3.2.P.3.5 can be drawn until these data are provided for assessment.**

Media fills have been performed to validate the aseptic filling process and were run in accordance to guidelines. Results have been provided from three consecutive simulation studies and gave satisfactory results without any contaminated units. Results for the media fill cover the maximum process time for the manufacturing of drug product (maximum filling time is 112 hours) and simulate worst-case manufacturing conditions. The media fill validation demonstrated that aseptic conditions are maintained during the filling process.

Acceptable information has been provided for filter validation on the 0.2 µm-filters used for sterile filtration, describing the material, pore size and surface area. All study results met the predetermined acceptance criteria and the studies for microbial retention, membrane compatibility, extractable substances and integrity test determination have shown that the 0.2 µm-filters are appropriate for

sterile filtration of the drug product. However, the applicant should clarify if the 0.2 µm-filter used for bioburden reduction is identical with the 0.2 µm-filters used for sterile filtration.

Control of excipients

ALC-0315 and ALC-0159 are novel excipients, not previously used in an approved drug product within EU. Additional information is provided separately in Section A.3.

DSPC is a non-compendial excipient sufficiently controlled by an in-house specification.

Cholesterol is sufficiently controlled according to the Ph. Eur. monograph with additional tests for residual solvents and microbial contamination.

The other excipients (sucrose, sodium chloride, potassium chloride, disodium phosphate dihydrate, potassium dihydrogen phosphate and water for injection) are controlled according to respective Ph. Eur. monograph. However, appropriate documentation for processing aids (ethanol and citrate buffer) and for drug substance buffer (HEPES and EDTA) should be provided.

Product specification, analytical procedures, batch analysis

The release and stability testing specifications for BNT162b2 drug product are provided in Table P.5-1.

Table P.5-1. BNT162b2 drug product specifications.

Quality Attribute	Analytical Procedure[a]	Acceptance Criteria
Composition and Strength		
Appearance	Appearance (Visual)	White to off-white suspension
Appearance (Visible Particulates)	Appearance (Particles) [b]	Essentially free from visible particulates
Subvisible Particles	Subvisible Particulate Matter [b, c]	Particles ≥10 µm: ≤6000 per container[b,c]
		Particles ≥ 25 µm: ≤600 per container[b,c]
pH	Potentiometry[b]	6.9 – 7.9
Osmolality	Osmometry [b, d, e]	425 - 625 mOsmol/kg
LNP Size	Dynamic Light Scattering (DLS)	40 to 180 nm
LNP Polydispersity	Dynamic Light Scattering (DLS)	≤ 0.3
RNA Encapsulation	Fluorescence assay	≥ 80%
RNA content	Fluorescence assay	0.50 ± 0.13 mg/mL
ALC-0315 content	HPLC-CAD	4.50 to 9.25 mg/mL
ALC-0159 content	HPLC-CAD	0.55 to 1.20 mg/mL
DSPC content	HPLC-CAD	0.90 to 2.05 mg/mL
Cholesterol content	HPLC-CAD	1.80 to 3.90 mg/mL
Container content for injections	Volume of injections in containers[e, f]	Not less than the sum of the nominal volumes of 5 doses
Identity		
Lipid identities	HPLC-CAD[e]	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)
Identity of encoded RNA sequence	RT-PCR[e]	Identity confirmed
Potency		
In Vitro Expression	Cell-based flow cytometry	≥ 30% Cells Positive
Purity		
RNA Integrity	Capillary Gel Electrophoresis	≥ 50% intact RNA
Adventitious Agents		
Bacterial Endotoxin	Endotoxin (LAL) [b]	≤ 12.5 EU/mL
Sterility	Sterility[b]	No Growth Detected
Container Closure Integrity	Dye incursion[g]	Pass

a. All assays performed on stability unless otherwise noted.
b. Compendial
c. USP<787> (obscuration method), and aligned with upcoming (Jan 2021) revision of Ph. Eur. 2.9.19
d. USP<785>; also in accordance with Ph Eur. 2.2.35, with minor difference in instrument calibration
e. Assay not performed on stability.
f. Procedure is aligned with Test for Extractable Volume of Parenteral Preparations.
g.Tested at release and on stability for stability batches only
Abbreviations: LNP = Lipid nanoparticles; CAD = charged aerosol detector; RT-PCR = reverse transcription polymerase chain reaction; FACS = fluorescence activated cell sorter; ddPCR = droplet digital PCR; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus amebocyte lysate; EU = endotoxin unit

Specification and justification of specifications

The specifications document for drug product in section 3.2.P.5.1 includes a comprehensive panel of relevant tests along with corresponding acceptance criteria.

With the exception of osmometry, volume of injections in containers, HPLC-CAD (lipid identities) and RT-PCR (identity of encoded RNA sequence), which are performed only at DP release, all other analytical procedures are conducted at release and stability studies for drug product. It is stated by the applicant that the acceptance criteria used for stability during shelf-life will be the same as the acceptance criteria used for lot release, but this remains to be confirmed.

Test method numbers are missing and should be given to all analytical procedures used in the specifications for release and end-of-shelf-life and should consequently be inserted in the drug product specifications document and to the descriptions and validations of analytical procedures.

LNP size for drug product is measured by dynamic light scattering (DLS) and the efficacy of the drug product depends on the size of the LNP. The proposed acceptance criteria of 40 to 180 nm seem wide compared to clinical batch data that is found in the range of 59-74 nm for the small scale clinical

batches ("classical LNP process) and 68-71 nm for the emergency supply ("upscale" LNP process). The acceptance criteria should therefore be tightened to be in line with what has been qualified in the clinical studies or clinically qualified by other means and set such that a clinically qualified level is assured throughout the shelf-life of the drug product.

Potency: In-vitro expression is a cell-based flow cytometry assay. The assay was implemented recently and the proposed acceptance criteria of ≥30% cells positive seem wide compared to the limited batch release data available to date, i.e. emergency supply lots that is in the range of 63-65%. In addition, some data are presented for the small-scale clinical batches used in comparability testing, where data are found in the range of 50-71% (Table 3.2.P.2.3-5 in the dossier). The proposed acceptance criteria need to be thoroughly justified and tightened in line with the levels qualified in clinical studies or clinically qualified by other means. This justification should include the applicant's total current knowledge of the drug product.

RNA encapsulation of drug product is measured by a fluorescence assay where free and total RNA are determined and the difference between the total and free RNA corresponds to RNA encapsulation. Encapsulation is used to ensure delivery of the RNA and improve the chances of transfection. The proposed acceptance criteria of ≥80% seem wide compared to clinical batch data that is found in the range of 92-94%. The proposed acceptance criteria for RNA encapsulation should therefore be tightened based on clinical qualification or clinically qualified by other means and set such that a clinically qualified level is assured throughout the shelf-life of the drug product.

The proposed acceptance criteria of ≥50% intact RNA for RNA integrity as measured by capillary gel electrophoresis seem wide compared to clinical batch data that is found in the range of 69-81%. The proposed acceptance criteria for RNA integrity should therefore be tightened based on clinical qualification or clinically qualified by other means and set such that a clinically qualified level is assured throughout the shelf-life of the drug product. Additionally, it should also be clarified if the emergency lots EE8492 and EE8493, both with results for RNA integrity of 55%, have actually been used in the clinical trials or not. In this context, it is also unclear whether there is a decrease in RNA integrity during the manufacturing of DP or not and a consequential need for a more stringent DS specification. The applicant should therefore discuss, and present comparative results for DS and DP, on RNA integrity. Sections S.4.1 and P.5.1 in the dossier should be aligned and updated accordingly. (**MO**)

The proposed acceptance criteria for LNP polydispersity as measured by DLS are wide and should be tightened in line with batch results for clinical batches, i.e. NMT 0.2 (0.22 observed on stability).

The proposed acceptance criteria for appearance, subvisible particles, pH, osmolality, volume of injection in containers, identity of encoded RNA sequence, RNA content, bacterial endotoxin, sterility and container closure integrity are all found acceptable.

Lipid content: Both safety and efficacy are dependent on the total amount of lipid relative to the RNA DS. A consistent molar ratio of lipid/RNA is expected in the DP vial, driven by the encapsulation process. Absolute lipid content may vary but composition (relative molar %) of the four lipids remains consistent. The acceptance criteria ranges have been calculated from worst-case low and high RNA content. No batches manufactured to date have exhibited results at or below the low RNA content estimate while the high RNA content level has been justified by development batches manufactured at worst-case high RNA contents. Although the absolute range of each lipid appears somewhat broad, the acceptance criteria are found acceptable. However, to further strengthen the control strategy given that a fixed molar ratio of cationic lipid and RNA is critical for LNP formation, acceptance criteria for the molar ratio N/P should be included in the specification unless further justified.

A separate test for in vitro release is not included in the specification. This is considered acceptable since test for potency is included by a cell-based method.

Analytical procedures

Some of the analytical procedures are common to both DS and DP. Several analytical procedures are specific to DP and are detailed and validation results are presented.

The compendial methods have been verified for use in accordance to the appropriate Ph. Eur. chapters.

It is claimed that all non-compendial methods were validated against the parameters given in ICH Q2. However, the validation summaries presented are far too brief to be able to conclude on the suitability of the analytical method. More comprehensive validation summaries of all non-compendial methods, for example in the form of short validation reports should be provided. The validation summaries should include all relevant calculations, acceptance criteria, description of and results obtained for individual samples. Chromatograms and dose response curves should be included, where applicable. The dossier should be updated accordingly.

Furthermore, in all of the in-house analytical methods used in the release of DP, method descriptions are based on "examples" of procedures, controls and standards as well as on "typical" system operating parameters. These terms raise uncertainties regarding the developmental stage, and the control of critical steps of these assays. The analytical methods used in the control of DP are expected to be finalized. The applicant is requested to confirm this and to update the relevant parts of the dossier with unequivocal method descriptions and additional details, if needed. The applicant should also confirm that any significant changes in analytical procedures will be applied for in a variation application.

In addition, it is stated in the dossier that a complete description of the rapid sterility test is pending. Therefore, method description and validation summary of the rapid sterility test should be provided during the procedure.

Potency: Cell based flow cytometry is used to confirm the in vitro expression of SARS-CoV-2 spike protein encoded by the RNA in BNT162b2 drug product (DP). Although the principle and method procedure are, at largely described, additional details are requested on critical reagents (such as antibodies), drug product control samples, equipment, assay suitability, gating strategy as well as further justification of the use of HEK293 cells in the assay.

Batch analysis

Batch analysis data have been provided including DP batches used in toxicology studies, clinical trials, emergency supply and stability. All these batches have been manufactured with the "classical" LNP process (nonclinical, clinical supply lots) or the "upscale" LNP process (emergency supply) and comparability has been reasonable demonstrated between the clinical supply lots and the emergency supply lot with only small differences noted. All DP batches manufactured and presented met the acceptance criteria in the DP specification. However, no DP batches at the intended full commercial scale have been manufactured to date.

Characterisation of impurities

The impurity profile of the DP is based on the impurity profile of the materials that are used for the manufacturing as well as the lipid impurities.

There are four process-related impurities identified for the DP; ethanol, citrate, HEPES and EDTA. Removal of ethanol will be demonstrated during process validation against the ICH Q3C limit (5000 ppm, class 3 solvents). EDTA, citrate and HEPES have been shown through safety risk assessment and theoretical worst-case calculations to be significantly below established safety limits. This is found acceptable.

The lipids are controlled via the acceptance criteria in their specifications. However, no information is provided on the lipid-related impurities originating from the degradation of the lipid nanoparticles and such data needs to be provided.

The applicant plans to update the dossier with further evaluations of lipid-related impurities and states that for section 3.2.P.5.5 "Data for this section is pending and will be updated once the data has been generated, analysed, and verified". Until these data are available for assessment, no final conclusions can be drawn on section 3.2.P.5.5.

A summary of risk assessment on elemental impurities in line with the ICH Q3D is missing. A summary of this risk assessment based on the general principles outlined in Section 5.1 of ICH Q3D should be submitted.

In summary, no final conclusion on the section 3.2.P.5.5 can be drawn until all data on the characterization of impurities will be provided for assessment.

Reference standard

The current reference standard for the BNT162b2 drug product is the clinical batch EE8493, stability data is being acquired. The applicant intends to establish a primary (PRS) and a working reference standard (WRS). A question is raised on the preparation, qualification and stability of PRS and WRS.

Stability of the product

The proposed initial shelf-life for drug product is 6 months when stored at the recommended storage condition of -90 to -60°C.

The applicant has provided stability results up to 4 months at -80 to -60°C of one clinical batch and up to 3 months of a non-clinical batch of drug product. Additionally, up to 3 months results at -80 to -60°C are also provided for supportive stability studies for two clinical lots of drug product.

The applicant has also initiated stability studies on two emergency supply lots (only release data exists to date) and has plans to initiate stability studies on the future PPQ-batches.

In addition, stability data has also been provided at accelerated (-40°C to +5°C) and stressed (+25°C to +30°C) storage conditions.

The stability studies are performed in accordance with ICH Q5C (Quality of biotechnological products: Stability testing of biotechnological/biological products) and the same or representative container-closure system are used in these stability studies as will be used for commercial batches.

Data is presented in P.2.5 for the container closure include extractables and leachabels, container integrity, and for functional tests for the bromobutyl stopper (penetrability, fragmentation, and self-sealing). A question is raised regarding the self-sealing test for the bromobutyl stopper after freezing and thawing.

All stability results for the clinical and non-clinical batches as well as for the supportive stability studies stored at -80 to -60°C complies with the clinical acceptance criteria in place at the time of testing. Overall, the presented stability data indicate no signs of degradation, significant trends or changes in terms of quality.

At accelerated conditions of +5°C-storage and up to 4 months testing of a clinical batch of drug product, LNP polydispersity and RNA integrity were out of specification at the 3 and 4 month-points.

As discussed, and concluded in section 3.2.P.2.3, it is agreed that comparability has been reasonable demonstrated between the clinical supply lots manufactured with the "classical" LNP process and the

representative emergency supply lot manufactured with the "upscale" LNP process. However, the applicant has a plan for a comprehensive demonstration of comparability among clinical supplies and the full commercial scale product but data for this section is pending. Four commercial PPQ-batches will be manufactured in November and December 2020. In summary, no final conclusion on comparability can be drawn until all comparability data among clinical supplies and the commercial product (PPQ-batches) of drug product will be provided for assessment. In addition, the claimed shelf-life is not yet acceptable since the batches are not representative of commercial supply (manufacturer, scale, drug substance process), the batches used represent less than 1% of the commercial scale, and only very limited data is available.

Photostability testing as well as temperature cycling studies are planned, and results are pending to date. While normally this data should be provided before the end of the RR procedure, it is acknowledged that the outer container (carton box) will provide protection from light; this information should be clearly stated in the SmPC/PIL.

Furthermore, it should be confirmed that future extensions of the assigned DP shelf life will be applied for in formal variation applications. The following statement should be removed for Module 3.2.P.8.1 of the dossier; "The sponsor will extend the assigned shelf life without notification providing the real time stability data at the intended storage condition is acceptable and within commercial specifications."

Post-approval stability protocol and stability commitment

A minimum of one batch of drug product will be added to the on-going post-approval stability program annually. The annual post-approval stability protocol has been provided and found acceptable although this protocol is part of GMP and therefore not assessed in this report. However, the applicant should confirm that they commit to continue all the ongoing stability studies at long-term conditions until completion.

Concluding remarks on the proposed shelf-life and storage conditions

The proposed initial shelf-life for the drug product is 6 months at the recommended storage temperature of -90 to -60°C. In order to support the suggested shelf-life for drug product, updated reports from the ongoing stability studies should be provided.

Post approval change management protocol(s)

Not applicable.

Adventitious agents

Adventitious agents safety evaluation has been provided for the DS manufacturing site [Andover] and for the DP manufacturing site [Puurs]. Information regarding the DP manufacturing site [BNT &Rentschler] is pending.

Proteinase K used in DS manufacturing and LB broth used in the establishment of the pST4-1525 MCB and WCB are the only materials of animal origin used in the manufacturing of BNT162b2. The applicant has identified contamination of the product by Transmissible Spongiform Encephalopathy (TSE) agents as the main theoretical risk associated with these ingredients, deemed minimal.

No information is provided regarding viral safety of these materials. Considering the stringent conditions routinely used in the heparin production, the risk for viral contamination is considered negligible for this material. Additional clarifications are requested for pyrophosphatase, T7 polymerase and RNase inhibitor, spermidine, DNase I and excipients ALC-0315, ALC-0159, DSPC and Cholesterol.

No information is included in A.2 on the control of other non-viral adventitious agents and only sterility testing performed at the level of DP is named. However, sufficient details on the aseptic validation filling and media fills have been provided in P.3 Manufacture. Furthermore, adequate testing for bioburden and endotoxin is performed at different stages of the manufacturing process, as described in section S.2.4. Therefore, based on the information existing in other parts of the dossier and pending new information regarding the BNT & Rentschler manufacturing site as well as new information requested on the control of materials, the overall risk for contamination is considered minimal at this point and no additional concerns are raised.

GMO

N/A

Novel excipients

Two novel excipients are included in the drug product, the cationic lipid ALC-0315 the PEGylated lipid ALC-0159. No final conclusion can be drawn until all data are provided. Some questions with regards to batch size and validation of analytical methods are raised at this point. Additional information on chemical synthesis, quality control of starting material, specification limits and retest period will be provided for assessment during the procedure.

2.1.4. Discussion and conclusions on chemical, pharmaceutical and biological aspects

Drug substance

Where data is submitted, the dossier is overall of acceptable quality. However, a substantial amount of information is pending, due to the very short time frame of product development and will be submitted in the subsequent submission(s). Information on the manufacturing process and process controls for the manufacturing site Andover is provided, while the corresponding information for site BNT Mainz & Rentschler is pending.

Based on the significant differences observed between batches manufactured by DS Process 1 and 2 for the CQA mRNA integrity, a MO is raised regarding comparability, characterisation and clinical qualification of the proposed acceptance criteria of ≥50% intact RNA. Whilst some testing results of biological activity/functionality has been submitted in support of comparability and potency testing is part of the DP release specifications, biological characterisation of the active substance is limited, and additional data and discussion is requested to address functionality.

The reference standard was poorly characterised, and the final two-tiered system is not yet in place.

The proposed initial shelf-life for the drug substance is 6 months at the recommended storage temperature of -20°C. In order to support the proposed shelf-life for drug product, updated reports from the ongoing stability studies should be provided.

Drug product

The drug product is a preservative-free, multi-dose concentrate to be diluted for intramuscular injection, intended for 5 doses. The drug product is a sterile dispersion of RNA-containing lipid nanoparticles (LNPs) in aqueous cryoprotectant buffer.

The formulation development studies of the RNA containing lipid nanoparticles have been thoroughly described including studies that were performed with available drug substance, representative of the mRNA platform and included in the drug product.

The development of the manufacturing process is extensively described, and critical process parameters are defined.

The manufacturing process includes lipid nanoparticle fabrication and bulk drug product formulation followed by fill and finish, and the process has at large been acceptably described.

However, no drug product batches at the intended full commercial scale have been manufactured to date. It is described in the dossier that four commercial PPQ-batches will be manufactured in November and December 2020. These batches will be used both to demonstrate the comparability of the commercial PPQ-batches versus the clinical supply batches as well as for process validation of the manufacturing process of the drug product. **Therefore, no final conclusion on drug product comparability, process validation, and shelf life can be drawn until additional data will be provided for assessment.**

The specifications document for drug product includes a comprehensive panel of relevant tests along with corresponding acceptance criteria. Several questions are raised concerning tightening of acceptance criteria for LNP size, polydispersity, potency, RNA integrity and RNA encapsulation to be in line with what has been qualified in the clinical studies or clinically qualified by other means.

The proposed initial shelf-life for the drug product is 6 months at the recommended storage temperature of -90 to -60°C. In order to support the suggested shelf-life for drug product, updated reports from the ongoing stability studies should be provided.

Conclusion

Three major objections are identified that precludes a marketing authorization: The first MO relates to the GMP status of the DS and DP manufacturing sites. Comparability between clinical and commercial material has not yet been demonstrated, which is addressed in MO 2. In particular, significant differences between batches manufactured by DS Process 1 and 2 are observed for the CQA mRNA integrity. Characterisation of truncated forms, more comprehensive comparability data, results on additional batches and impact on safety and efficacy is requested. The third MO concerns omission of data on DP manufactured at the commercial site. Batch results at release, data on comparability of commercial batches with clinical batches and additional stability data is required.

In addition, several deficiencies have been noted which should be appropriately addressed by the applicant before a positive CHMP opinion can be granted.

2.2. Non-clinical aspects

2.2.1. Pharmacology

The pharmacology dossier is based on initial studies of the functionality of the BNT162b2 (V9) RNA-based product and the encoded SARS-CoV-2 P2 S protein as well as on supporting studies of SARS-CoV-2 P2 S protein structure. This is followed by characterization of the humoral and cellular immune response in mouse and nonhuman primate upon immunization with BNT162b2 (V9) and ends up with a SARS-CoV-2 challenge study of BNT162b2 (V9) immunized nonhuman primates. No secondary pharmacodynamic, safety pharmacology or pharmacodynamic drug interaction studies with BNT162b2 have been conducted due to the nature of the RNA-based vaccine product, which is according to applicable guidelines.

Mechanism of action

The SARS-Cov-2 virus infect the body by the use of the Spike protein (S) to attach to specific cell surface receptors, especially, as recently suggested, the angiotensin converting enzyme 2 (ACE2). In addition to the initial attachment to a host cell, the S protein is also responsible for viral envelope fusion with the host cell membrane resulting in genome release. Due to its indispensable role, the S protein is a major target of virus neutralizing antibodies and has become a key antigen for vaccine development. By immunization with BNT162b2, encoding for the S protein, the intention is to trigger a strong and relatively long-lasting production of high affinity virus neutralizing antibodies, which can act through blocking the S-protein and it's receptor-binding domain (RBD) interaction with host cell receptors but also by opsonization mediated virus clearance. In addition, the immunization with BNT162b2 is also intended to elicit a concomitant T cell response of the Th1 type, supporting the B cells responsible for the production of S-specific antibodies and cytotoxic T cells that kill virus infected cells.

The structural elements of the vector backbones of the BNT162b2 are optimized for prolonged and strong translation of the antigen-encoding RNA. The potency of the RNA vaccine is further optimized by encapsulation of the RNA into lipid nano particles (LNPs), which protects the RNA from degradation by RNAses and enable transfection of host cells after intramuscular (i.m.) delivery. BNT162b2 is nucleoside-modified by a substitution of 1-methyl-pseudouridine for uridine and thus its inherent adjuvant activity mediated by binding to innate immune sensors such as toll-like receptors (TLRs) 7 and 8, is dampened, but not abrogated.

The S protein is a trimeric class I fusion protein that exists in a metastable prefusion conformation before engaging with a target cell. BNT162b2 encodes a P2 mutant (P2 S) variant of S where two consecutive proline mutations have been introduced in order to lock the RBD in the prefusion conformation.

The RNA is formulated with functional and structural lipids forming lipid nano particles (LNPs), which protect the RNA from degradation and enable transfection of the RNA into host cells after IM injection. The composition of the LNPs may also affect the distribution of injected BNT162b2. In addition, it cannot be excluded the LNP composition contributes to the overall immunogenicity.

Primary pharmacodynamic studies in vitro

To confirm the functionality of the BNT162b2 (V9) RNA-based product, protein expression, transfection frequency from BNT162b2 and cell surface expression of the SARS-CoV-2 P2 S protein antigen was assessed. Regarding the results obtained from the Western Blot, a semi quantitative analysis of the results should be provided to improve the readability of the protein expression and in the analysis of the blot, some missing scientific information and explanations should be added by the applicant **(OC)**. BNT162b2 (V9) transfection of HEK293T cells indicated SARS-CoV-2 P2 S was correctly expressed on the cell surface, as indicated by flow cytometry staining of non-permeabilized cells with an anti-S1 monoclonal antibody. In addition, the cellular localization of expressed S1 protein was investigated. The S protein co-localized with an ER marker, as detected by immunofluorescence experiments in HEK293T cells expressing BNT162b2-RNA, suggesting the S protein is processed within the ER.

In a set of supportive studies, it was investigated whether BNT162b2 RNA encodes for an amino acid sequence that authentically express the ACE2 binding site. No study report for this data set could be found and should be provided (**OC**). Recombinant P2 S was expressed from DNA encoding for the same amino acid sequence as BNT162b2 RNA encodes for. Flow cytometry staining with spike protein (S) binding agents, as human ACE2 and monoclonal antibodies known to bind to authentic S-protein all indicated an authentically presented P2 S protein and ACE2 binding site. Low nano molar affinity of P2 S binding to ACE2 PD and B38 mAb was demonstrated with the use of biolayer Interferometry.

To further structurally characterize the P2 spike protein, a cryo-electron microscopy (cryoEM) investigation of purified P2 S, expressed from DNA, was conducted. The cryoEM revealed, according to the Applicant, a particle population closely resembling the prefusion conformation of SARS-CoV-2 spike protein. By fitting a previously published atomic model on to a processed and refined cryoEM dataset, a rebuilt model was obtained showing good agreement with reported structures of prefusion full-length wild type S and its ectodomain with P2 mutations. In the prefusion state the RBD undergo hinge-like conformational movements and can either be in an "up" position (open for receptor binding) or in a "down" position (closed for receptor binding). Three-dimensional classification of the dataset showed a class of particles that was in the conformation one RBD 'up' and two RBD 'down". This partly open conformation represented 20.4% of the trimeric molecules. The remainder were in the all RBD 'down' conformation. Although potent neutralizing epitopes have been described when the RBD is in the "heads down" closed conformation, the "heads up" receptor accessible conformation exposes a potentially greater breadth of neutralizing antibody targets. It is concluded that antibodies to both the up and down conformations will potentially be formed upon immunization with the P2 S encoding BNT162b2. Regarding the Structural and Biophysical Characterization, the applicant is asked to provide a) A schematic description of the V8 and V9 variants, so as to identify the exact position of optimized codons in the sequence, as well as the position of added cytosines nucleotides. The exact position of these optimized codons inside the modRNA sequence should be provided. b) The exactly detailed mRNA structure of BNT162b2, including coding and non-coding sequences. c) A comparison in the V8 and V9 codon sequences, highlighting their differences and mΨU residues. d) An estimation of mΨU content in both V8 and V9 sequences and discuss on the potential difference in immunogenicity between these two sequences. E) A comparison on the protein expression obtain from both variants (V8 and V9) to ensure that the expected protein is expressed in non-clinical models **(OC)**.

Primary pharmacodynamic studies in vivo

The humoral and cellular immune response following IM administration of BNT162b2 (V9) was investigated in mice and nonhuman primates.

Balb/c, females were immunized IM on day 0 with 0.2, 1 or 5 µg RNA/animal of BNT162b2 (V9), or with buffer alone (n=8). Blood samples were collected on Days 7, 14, 21 and 28 after immunization. The IgG antibody response to SARS-CoV2- RBD or S1 was analyzed by ELISA. Immunization with BNT162b2 induced IgGs that bound to S1 and RBD, as detected by ELISA, and on day 28 after immunization showed a binding affinity of KD 12 nM or 0.99 nM (geometric mean) respectively, as detected by surface plasmon resonance.

To further characterize the antibody response to BNT162b2 and its potential capacity to reduce SARS-Cov-2 infections, a pseudo virustype neutralization assay (pVNT) was used as a surrogate of virus neutralization since studies with authentic SARS-CoV-2 requires a BSL3 containment. The pVNT was based on a recombinant replication-deficient vesicular stomatitis virus (VSV) vector that had been pseudotyped with SARS-CoV-2 S protein according to published protocols. A dose-dependent increases in SARS-CoV-2-S VSV pseudovirus neutralizing antibodies were observed in sera from BNT162b2-immunized mice. On day 14, the difference of the group treated with 5 µg RNA compared to the buffer control was statistically significant (p = 0.0010). On days 21 and 28, the differences of the groups treated with 1 µg and 5 µg BNT162b2 compared to the buffer control were statistically significant. The relevance of the pseudovirus assay for authentic SARS-Cov-2 was not discussed. Concerning study R-20-0085 on the immunogenicity of the LNP formulated modRNA encoding the viral S protein (V9), the applicant is asked to a) justify the absence of IgG2A and IgG1 characterization for RBD; b) justify why the results were not expressed in titers that would also allowed comparisons across experiments. Indeed, comparison with pVNT experiments expressing results in titers could help to determine the levels of neutralizing and non-neutralizing antibodies present in the sera **(OC)**. Immunization of mice

with BNT162b2 also induced IFN-γ secreting cells of both the CD4+ and CD8+ T-cell subsets. This was shown by ELISPOT after ex vivo re-stimulation of splenocytes with an S-protein overlapping peptide pool Day 28 after immunization. Cytokine profiling was also carried out by Multiplex analysis of cytokine release from the Day 28 Splenocytes. High levels of the Th1 cytokines IFNγ and IL-2 but minute amounts of the Th2 cytokines IL-4, IL-5 and IL-13 were detected after re-stimulation with S but not RBD overlapping peptide mix. In addition, an elevated secretion of TNFα, GM-CSF, IL-1β, IL-12p70 and IL-18 was recorded after re-stimulation. In order to characterize the immunophenotype of B-and T-cells appearing in lymph nodes from mice immunized with BNT162b2 (V9), B- and T-cell subsets in draining lymph node cells were quantified by flow cytometry 12 days after immunization. Higher numbers of B cells were observed in the samples from mice that received BNT162b2 compared to controls. That included plasma cells, class switched IgG1- and IgG2a-positive B cells, and germinal centre B cells. T-cell counts were elevated, particularly numbers of T follicular helper (Tfh) cells, including subsets with ICOS upregulation, which play an essential role in the formation of germinal centres (Hutloff 2015).

In the nonhuman primate (rhesus macaques) studies, BNT162b2 (V9) was shown to be immunogenic after intramuscular administration. The serum concentrations of both S1-binding and the SARS-CoV-2 neutralizing antibody titers were at least an order of magnitude higher after BNT162b2 immunization of rhesus macaques than for the panel of SARS-CoV-2 convalescent human sera. Regarding Study VR-VTR-10671 : BNT162b2 (V9) Immunogenicity and Evaluation of Protection against SARS-CoV-2 Challenge in Rhesus Macaques, the applicant needs to a) precise for the Luminex data how the reference curve for has been constructed, what does represent the arbitrary U/ml used and how it is referring to the serum dilution factor; b) define the criteria for choosing a 10-30% infection rate of Vero cells; c) Methods to quantify antibody production in the different experiments differ and consequently cross-comparison between experiments is hardly impossible. Indeed, it is important to distinguish neutralizing antibodies from non-neutralizing antibodies. In this study, total antibody response is measured using a luminex assay and results expressed on U/ml and for the neutralization assay results are expressed in VNT 50. The applicant needs to provide an estimation of the non-neutralizing antibodies in the whole antibody response. d) It is important to notice that on figure 6 of study report, neither panel A nor panel B highlight the consumption of IgG S1 binding antibodies after challenge nor the increase due to B memories response following the challenge: this would need to be further justified by the Applicant **(OC)**.
Antigen specific S-reactive T-cell response after BNT162b2 immunization of the macaques was measured by ELISPOT and ICS. While S-specific T cells were low to undetectable in naïve animals, strong IFNγ but minimal IL-4 ELISpot responses were detected after the second 30 or 100 µg dose of the BNT162b2. Intra cellular staining (ICS) confirmed that BNT162b2 immunization elicited strong S-specific IFNγ producing T cell responses, including a higher frequency of CD4+ T cells that produced IFNγ, IL-2, or TNF-α but a lower frequency of CD4+ cells that produce IL-4. An S-specific IFNγ producing CD8+ T cell response was also recorded.

A challenge study in rhesus macaques served as nonclinical proof of concept (PoC). Rhesus macaques share a 100% homology with the human ACE2 sequence that interacts with the RBD of the S protein. BNT162b2 (V9) immunized macaques were challenged with SARS-CoV-2 intra nasally and intra tracheally 55 days after the second immunization with BNT162b2. Rhesus macaques were immunized on days 0 and 21. Some other covid-19 vaccine candidates have different prime-boost intervals, such as 4 weeks for both ChAdOx1 (Graham et al., 2020) and mRNA-1273 (Corbett et al., 2020). Considering that the time between the first and second vaccine dose may have a significant impact on the immunological response, the applicant is asked to provide the rationale for the chosen prime-boost interval (21 days) (**OC**). At the time of challenge, SARS-CoV-2 neutralizing titers ranged from 260 to 1,004 in the BNT162b2 (V9)-immunized animals. Neutralizing titers were undetectable in animals from

the control-immunized and sentinel groups. The presence of SARS-CoV-2 RNA was monitored by nasal and oropharyngeal (OP) swabs and bronchoalveolar lavage (BAL). Viral RNA was detected in BAL fluid from 2 of the 3 control-immunized macaques on Day 3 after challenge and from 1 of 3, on Day 6. At no time point sampled was viral RNA detected in BAL fluid from the BNT162b2 (V9)-immunized and SARS-CoV-2 challenged macaques. The difference in viral RNA detection in BAL fluid between BNT162b2-immunized and control-immunized rhesus macaques after challenge is statistically significant (p=0.0014). From control-immunized macaques, viral RNA was detected in nasal swabs obtained on Days 1, 3, and 6 after SARS-CoV-2 challenge; from BNT162b2 (V9)-immunized macaques, viral RNA was detected only in nasal swabs obtained on Day 1 after challenge and not in swabs obtained on Day 3 or subsequently. The pattern of viral RNA detection from OP swabs was similar to that for nasal swabs. No signs of viral RNA detected vaccine-elicited disease enhancement were observed. The viral RNA levels between control-immunized and BNT162b2-immunized animals after challenge were compared by a non-parametric analysis (Friedman's test), and the p-values are 0.0014 for BAL fluid, 0.2622 for nasal swabs, and 0.0007 for OP swabs. The data from the individual animals should be provided for the RT-qPCR test for presence of SARS-CoV-2 RNA (**OC**).

Despite the presence of viral RNA in BAL fluid from challenged control animals, none of the challenged animals, immunized or control, showed clinical signs of illness (weight change, body temperature change, blood oxygen saturation and heart rate). The Applicant concluded, the absence of clinical signs in any of the challenged animals, immunized or control, despite the presence of viral RNA in BAL fluid from challenged control animals, indicates that the 2-4 year old male rhesus monkey challenge model appears to be an infection model, but not a clinical disease model. However, a further investigation by lung radiograph and computerized tomography (CT) was conducted. Radiographic evidence of pulmonary abnormality was observed in challenged controls but not in unchallenged sentinels nor in challenged BNT162b2-immunized animals except for a CT-score signal in 1 of 6 pre infection and 2 out of six at Day 10/EOP in BNT162b immunized animals. The CT score signal was at the same level as the control at Day 10/EOP and is of unclear significance due to the presence in one animal before challenge. No radiographic evidence of vaccine-elicited enhanced disease was observed. Histopathological examination of lung tissues is ongoing and will be submitted as an addendum (**OC**). Overall, the challenge study is suboptimal as it comes with a number of uncertainties. The limitations can be listed regarding the model: absence of sars-cov2-clinical signs in control and challenged NHP, use of juveniles NHP, lack of females NHP, one out of three age-matched saline control-immunized (n=3) male rhesus macaques not responding to challenge (no viral RNA neither in the BAL and nasal swab), low numbers of animals with a low statistical significance, questionable selection of titer of the viral challenge (1.05. 10^6 PFU). Moreover, some important data are missing to date like the absence of cytokines measurement in the NHP BAL. The applicant is asked to discuss all these limitations and should provide further scientific information on the NHP model relevance. Although the model is considered adequate to demonstrate immunogenicity, and viral clearance, it is considered insufficient to demonstrate efficacy against the disease (OC).

Secondary pharmacodynamic studies

No secondary pharmacodynamics studies were conducted with BNT162b2. This is accepted.

Safety pharmacology programme

No safety pharmacology studies were conducted with BNT162b2. The Applicant refers to that they are not considered necessary according to the WHO guideline (WHO, 2005). In addition, no findings on vital organ functions have been recorded in the repeat dose toxicology studies. Thus, the absence of safety pharmacology studies is accepted.

Pharmacodynamic drug interactions

No pharmacodynamics drug interaction studies were conducted with BNT162b2. This is accepted.

2.2.2. Pharmacokinetics

The applicant has determined the pharmacokinetics of the two novel LNP excipients ALC-0315 (aminolipid) and ALC-0159 (PEG-lipid) in plasma and liver as well as their elimination and metabolism in rats. Furthermore, the applicant has studied the biodistribution of the two novel lipids (in rats) and a LNP-formulated surrogate luciferase RNA in mice. No traditional pharmacokinetic or biodistribution studies have been performed with the vaccine candidate BNT162b2.

No validated methods of analysis to support the non-clinical PK/biodistribution studies have been submitted. However, the applicant claims to have used a qualified LC-MS/MS method to support quantitation of the two novel LNP excipients without providing such data **(OC).**

PK studies with the two novel LNP-excipients ALC-0315 and ALC-0159: Wistar Han rats were IV bolus injected with LNP formulated luciferase-encoding RNA at 1 mg/kg and ALC-0315 and ALC-0159 concentrations at 15,3 mg/kg and 1,96 mg/kg respectively. ALC-0315 and ALC-0159 levels in plasma, liver, urine and faeces were analysed by LC-MS/MS at different time-points up to 2-weeks. No other organs besides the liver were investigated and therefore distribution to other organs cannot be excluded. The clinical administration route is IM the PK study was performed with a different administration route (IV) **(OC).**

ALC-0315 and ALC-0159 were rapidly cleared from plasma during the first 24 hours with an initial t½ of 1.62 and 1.72 h, respectively. 24 hours post-dosing, less than 1% of the maximum plasma concentrations remained. A slower clearance rate was observed after 24 hours with ALC-0315 and ALC-0159 terminal elimination t½ of 139 and 72.7 h, respectively.

Following plasma clearance, the liver appears to be to major organ to which ALC-0315 and ALC-0159 distribute. The applicant has estimated the percent of dose distributed to the liver to be ~60% for ALC-0315 and ~20% for ALC-0159. The observed liver distribution is consistent with the observations from the biodistribution study and the repeat-dose toxicology, both using IM administration.

For ALC-0315 (aminolipid), the maximum detected concentration in the liver (294 µg/g liver) was reached 3 hours after IV injection. ALC-0315 was eliminated slowly from the liver and after 2-weeks the concentration of ALC-0315 was still ~25% of the maximum concentration indicating that ALC-0315 would be eliminated from rat liver in approximately 6-weeks time. For ALC-0159 (PEG-lipid), the maximum detected concentration in the liver (15.2 µg/g liver) was reached 30 minutes following IV injection. ALC-0159, was eliminated from the liver faster than ALC-0315 and after 2-weeks the concentration of ALC-0159 was only ~0,04% of the maximum detected concentration. The applicant is asked to comment on the differences in the kinetics of the two novel excipients as well as on the relatively long liver clearance of ALC-0315 **(OC).**

While there was no detectable excretion of either lipid in the urine, the percent of dose excreted unchanged in faeces was ~1% for ALC-0315 and ~50% for ALC-0159.

Biodistribution of a LNP-formulated luciferase surrogate reporter: To determine the biodistribution of the LNP-formulated modRNA, the applicant did not study distribution of the modRNA used in the vaccine candidate BNT162b2, but instead, in a non-GLP study, determined the biodistribution of a surrogate luciferase modRNA formulated with a LNP with identical lipid composition used in BNT162b2. Since several LNP formulations were tested in the study it is not completely clear which of the LNP formulation is used in the clinical version of BNT162b2 **(OC).**

The study used three female BALB-c mice per group and luciferase protein expression was determined by in vivo bioluminescence readouts using an In Vivo Imaging System (IVIS) following injection of the luciferase substrate luciferine. The readouts were performed at 6h, 24h, 48h, 72h, 6d and 9d post IM injection (intended clinical route) in the right and left hind leg with each 1 µg (total of 2µg) of LNP-formulated luciferase RNA. The biodistribution method has not been validated or qualified and no discussion on its sensitivity has been included **(OC).**

In vivo luciferase expression was detected at different timepoints at the injection sites and in the liver region indicating drainage to the liver. As expected with an mRNA product, the luciferase expression was transient and decreased over time. Luciferase signals at the injection sites, most likely reflecting distribution to the lymph nodes draining the injection sites, peaked 6h post injection with signals of approximately 10 000 times of buffer control animals. The signal decreased slowly during the first 72 hours and after 6 and 9 days the signals were further weakened to approximately levels of 18 and 7 times the signals obtained from animals injected with buffer control.

The signals from the liver region peaked 6h post injection and decreased to background levels 48h after injection. The liver expression is also supportive of the data from the rat PK study and the findings in the rat repeat-dose toxicological study showing reversible liver vacuolation and increased gGGT levels.

Immunogenicity of a LNP formulated luciferase modRNA: Activation of the innate immune system following IM injection of a LNP-formulated luciferase reporter RNA into mice was assessed in a Luminex-based multiplex assay were serum samples (day -1 (pre), 6 h and day 9) were tested for levels of the following chemokines and cytokines: MCP-1, MIP-1β, TNF-α, IFN-α, IFN- γ, IL-2, Il-6, IL-10, IL1-β, IP-10. The applicant tested 3 different LNPs, all formulated together with luciferase RNA. The results suggest that the LNP formulation used in BNT162b2 (LNP8) slightly increased levels of MCP-1, IL-6, and IP-10 at 6h post immunisation. All chemokine/cytokine levels dropped to background levels at day 9. The applicant is asked to clarify issues regarding the data and discuss the possible clinical relevance of the transiently increased IL-6 levels **(OC)**. In addition to innate immune activation, LNP formulated luciferase modRNA was able to induce IFN-γ T-cell responses (when challenged with MHC I-specific luciferase peptide pools) measured in splenocytes isolated from the mice at day 9.The LNP formulated luciferase modRNA did not induce the formation of luciferase-specific IgGs as measured by ELISA.

Metabolism of the two novel LNP-excipients ALC-0315 and ALC-0159: Metabolism studies were conducted to evaluate the two novel lipids in the LNP, ALC-0315 (aminolipid) and ALC-0159 (PEG-lipid). No metabolic studies were performed with the modRNA or the other two lipids of the LNP. Overall, it seems as both ALC-0159 and ALC-0315 are metabolised by hydrolytic metabolism of the amide or ester functionalities, respectively, and this hydrolytic metabolism is observed across the species evaluated.

The metabolism of the novel excipients, ALC-0159 and ALC-0315, were examined *in vitro* using blood, liver S9 fractions and hepatocytes, all from mouse, rat, monkey and human. The *in vivo* metabolism was examined in rat plasma, urine, faeces, and liver from a rat pharmacokinetics study where a luciferase-encoding modRNA formulated in an LNP was used.

Metabolism of ALC-0315 appears to occur via two sequential ester hydrolysis reactions, first yielding the monoester metabolite followed by the doubly de-esterified metabolite. The monoester metabolite was observed in vitro in rat blood, monkey S9 fraction, and in vivo in rat plasma and rat liver. The doubly de-esterified metabolite was observed in vitro in mouse and rat blood; monkey liver S9 fraction; and in vivo in rat plasma, urine, faeces and liver. Subsequent metabolism of the doubly de-esterified metabolite resulted in a glucuronide metabolite which was observed in urine only from the

rat pharmacokinetics study. Additionally, 6-hexyldecanoic acid, the acid product of both hydrolysis reactions of ALC-0315, was identified in vitro in mouse and rat blood; mouse, rat, monkey and human hepatocytes; mouse, rat and human liver S9 fractions; and in vivo in rat plasma.

ALC-0315 was stable over 120 min (>93% remaining) in liver microsomes and S9 fractions and over 240 min (>93% remaining) in hepatocytes in all species and test systems.

The primary route of metabolism for ALC-0159 appears to involve amide bond hydrolysis yielding *N*,*N*-ditetradecylamine. This metabolite was identified in mouse and rat blood as well as hepatocytes and liver S9 from mouse, rat, monkey and human. Theoretical metabolites were arrived at via examination of the excipient molecules and consideration of commonly observed biotransformations (hydroxylation, *N*-dealkylation, hydrolysis, glucuronidation, sulfation, oxidation and combinations thereof). Given that the acetamines have been reported to be carcinogenic in animals, including liver tumors, potentially based on genotoxic mechanism, the applicant is asked to provide a discussion on the distribution and metabolism of the ALC-0159 focusing on the acetamide moiety **(OC** raised in toxicology section).

ALC-0159 was stable over 120 min (>82% remaining) in liver microsomes and S9 fractions and over 240 min (>87% remaining) in hepatocytes in all species and test systems.

Excretion of the two novel LNP-excipients ALC-0315 and ALC-0159: Excretion of the two novel lipids in the LNP, ALC-0315 (aminolipid) and ALC-0159 (PEG-lipid) was studied in the rat PK study. No excretion studies were performed with the modRNA or the other two lipids of the LNP which is considered acceptable.

While there was no detectable excretion of either lipid in the urine, the percent of dose excreted unchanged in faeces was ~1% for ALC-0315 and ~50% for ALC-0159. Since almost no unchanged ALC-3015 was detected in urine or faeces, metabolism may play a bigger role in the elimination of ALC-0315 than ALC-0159.

2.2.3. Toxicology

The toxicological dossier for BNT162b2 is based on a total of three pivotal toxicological experimental studies; two repeat-dose toxicity rat studies (one full study submitted, one intermittent study submitted) and one DART/EFD rat study (not yet submitted beyond a study plan). The test substance is BNT162b2 (variant 8 and the clinically relevant variant 9), a modified RNA in a lipid nanoparticle (LNP) formulation. The differences between the variants are due to codon optimization. The LNP contains four excipients whereof two are novel (ALC-0315 and ALC-0159).

General toxicity: The two general/repeat-dose toxicity studies involved i.m. exposure of Han Wistar rats to BNT162b2 for a total of 17d (three exposures; 1ggr/w) followed by three weeks of recovery. One study used variant 8 of BNT162b2 (dose 100ug) and one study used variant 9 (30ug). Overall, the study designs only included a single experimental group each with a variant of BNT162b2, with no dose-response assessment or specific experimental groups for the LNP alone or its novel excipients. This somewhat limits the risk assessment but is acceptable. No test substance-linked mortality or clinical signs were observed (except a slight increase [<1C] in body temperature). No ophthalmological and auditory effects were found. The animal model of choice, the rat, has not been assessed in the pharmacological dossier but a limited absorption/distribution study has been conducted in pharmacokinetics dossier. Immunogenicity was assessed in the toxicology studies.

Body weight and food intake: Exposure generated a slight reduction of absolute BW within 24h after 1st exposure (-6.8% to -11.3%; BNT162b2 V8) alternatively a weak body weight increase reduction [BNT162b2 v9]. No changes in food intake were observed.

Gross pathology and organ weights: At 100ug BNT162b2 V8 and 30ug BNT162b2 V9, the tissue at the injection site was thickened/enlarged with oedema and erythema at the end of exposure in a reversible manner. The spleen was enlarged (reversible) with up to 60% for both vaccine variants and doses. There was also an enlargement of the lymph nodes at 100ug. Overall, there were signs of a significant immune response which is likely linked to – and expected to a certain degree for - the test substance. There was a trend of slightly enlarged liver in females at 100ug (BNT162b2 V8).

Histopathology: At 100ug BNT162b2 V8, there were observations of various inflammatory signs at the injection site (e.g. fibrosis, myofiber degeneration, oedema, subcutis hyperplasia). Also, there was inflammation of the perineural tissue of the sciatic nerve and surrounding bone in most rats at d17. The bone marrow demonstrated increased cellularity and the lymph nodes showed plasmacytosis, inflammation and increased cellularity. The spleen demonstrated increased haematopoiesis in half the animals at d17. The liver showed hepatocellular and periportal vacuolation at d17 (partially or fully reversed during recovery) which may be related to hepatic clearance of the PEGylated lipid in the LNP. Only some organ and tissue samples from the main organs were used for BNT162b2 V8 histopathology (adrenal gland, brain, epididymis, heart, kidney, liver, lungs, lymph nodes, ovary, pituitary gland, prostate, spleen, testicle, thymus, thyroid). Other tissues/structures (nasal body cavity, clitorial gland, dorsal root ganglion, larynx, mandibular lymph node, tibial nerve, preputial gland, ureter, Zymbal's gland) were preserved for additional histopathology if needed. The interim data for 30ug BNT162b2 V9 did not include histopathology data and no specification of what tissues are to be examined/stored.

Immunogenicity: Treatment of rats with 100ug BNT162b2 V8 generated SARS-CoV-2 S-binding IgG antibodies against the S1 fragment and the RBD (based on ELISA and pseudovirus neutralization test on blood samples).

Haematology: At 30ug BNT162b2 V9 and 100ug BNT162b2 V8, there was a moderate to strong reduction of reticulocytes (48-74%, not specified for V9) coupled to lowered red cell mass parameters (RBC, HGB, and HCT). There was a very strong increase (>100%) in large unclassified cells [LUC; 295.5-319.5% for V8, not specified for V9], neutrophils [606-680% for V8, not specified for V9], eosinophils [419-509% for V8, not specified for V9], basophils [105-147% for V8, not specified for V9] and fibrinogen [160-205% for V8, not specified for V9]. The changes were reversible (assessed for V8). No effects on coagulation were observed for a V8 and a slight increase in mainly males with V9.

Clinical pathology: A very strong but reversible increase (>100%) in pro-inflammatory acute phase proteins in the blood (A1AGP, A2M) was seen with both 30ug BNT162b2 V9 and 100ug BNT162b2 V8. Also, indicative of pro-inflammation, a slight to moderate reduced albumin/globulin ratio was seen for both variants. V8 (100ug) exposure generated increased levels of gGT (>200%) and increased gGT enzyme activity and increased AST levels (+ ~19%). V9 (30ug) exposure led to slight to moderate increases in ALT and ALP levels, possible indicative of liver effects. There were no changes in cytokine levels (IFNg, TNFa, Il-1b, Il6, Il-10) after 100ug V8 exposure. For 100ug V8, there were no changes measured in urine whereas there was a slight-moderate reduction in pH for 30ug V9.

Genotoxicity: No genotoxicity has been provided. The components of the vaccine formulation are lipids and RNA that are not expected to have genotoxic potential. That being said, the novel lipids possess an acetamide moiety which is classified as possible human carcinogen (IARC Group 2B) with debated genotoxic mechanism, which should be discussed further (**OC**).

DART: No results have yet been submitted nor has a discussion been provided on choice of animal model (rat) or experimental design (**OC**).

Local tolerance: No dedicated local tolerance studies available but assessment included in repeat-dose toxicity studies. At 100ug BNT162b2 V8, there was mostly light to moderate oedemas but in some cases severe oedema. The severity increased with the 2nd and 3rd injections. The interim data for 30ug BNT162b2 V9 exposure indicated similar effects.

2.2.4. Ecotoxicity/environmental risk assessment

As the active substance is a vaccine product (which additionally is based on naturally degradable mRNA and lipids), no ERA is considered necessary.

2.2.5. Discussion on non-clinical aspects

Pharmacology: The proposed medical product is composed of a modRNA formulated with functional and structural lipids forming lipid nano particles (LNPs), the latter having the purpose to protect the modRNA from degradation and enable transfection of the modRNA into host cells after IM injection. The composition of the LNPs is likely to affect the distribution of injected BNT162b2. In addition, it cannot be excluded the LNP composition contributes to the overall immunogenicity (see also toxicology below). Applicant should provide a more detailed clarification of the mode of action of BNT162b2, e.g. which cells types will take up the LNP, translate the modRNA and express the S-protein on the surface. Moreover, which cell types/organs will be targeted by the immune defence system, when the vaccine is in action. Further information on the potential activity/mode of action of the two novel excipients should be provided (**OC**).

Regarding the structural and biophysical characterization of the modRNA, some information is missing (e.g. a schematic description and comparison of both vaccine variants including the position of added cytosines nucleotides and optimized codons, coding and non-coding sequences, mΨU content, an assessment of sequence structure relation to immunogenicity)(see **OC for details**). Regarding the results obtained from the Western Blot in the in-vitro studies, a semi quantitative analysis of the results should be provided and in the analysis of the blot, some missing scientific information and explanations should be added by the applicant (**OC**). It can be noted that an overview of the structural and biophysical characterization of P2 S as a vaccine antigen has been provided. While it is not considered to be of critical importance for the assessment in this procedure, it still provides a scientific understanding supporting the nonclinical key studies of humoral and cellular immune response, including SARS-CoV-2 neutralizing IgG, as well as SARS-CoV-2 challenge nonclinical PoC.

In-vivo pharmacodynamics: The humoral and cellular immune response following IM administration of BNT162b2 (V9) was investigated in mice and nonhuman primates but a more in depth discussion on the suitability of these pharmacological animal models has not been provided (e.g. susceptibility for SARS-CoV-2 infection; potential bias for Th1- or Th2-skewed responses has been well characterized for certain mice strains) and the relevance of the immunogenicity data for the clinic (e.g. only single immunisation in mice). Also, no or limited attention to the induction of long-term memory responses nor immunogenicity and protection in aged animals has been paid (**OC**). That being said, the induction of virus neutralizing antibodies in both mice (VSV-SARS-CoV-2 S) and primates (SARS-CoV-2) indicated that BNT162b2 immunization has the potential to induce neutralizing antibodies also in humans. Thus, vaccination with modRNA is expected to induce robust neutralising antibodies and a concomitant T cell response to achieve protective immunity. Nevertheless, no further discussion was provided regarding the possibility of autoimmune responses induced by the ModRNA. The Applicant is invited to further discuss the risk that the mRNA vaccine can trigger potential autoimmune responses and how they plan to possibly evaluate their occurrence (**OC**).

In mice, the immune response was assessed by single immunization only. Taking the phenotyping of B and T cells in aggregate, the data indicates a concurrent induction of SARS-CoV-2 S-specific neutralizing antibody titers and a Th1-driven T-cell response by immunization with BNT162b2 (this was also seen in nonhuman primates).

There are some issues with study R-20-0085 regarding the immunogenicity of the LNP formulated modRNA encoding the viral S protein (V9; e.g. regarding the absence of IgG2A and IgG1 characterization of RBD, experimental design that would allow an effective titer comparaison between experiment) (see **OC for details**). There are also some issues with the study on multiplex analysis of cytokine release from murine Splenocytes Day 28 after Immunization with BNT162b2 (the use of "5" as compared to "1 ug" BNT162b2 for the Luminex analysis in the Pharmacology written summary, page 18 (last paragraph) as compared to in the report R-20-0085) (**OC**). Moreover, it is noted that high levels of the Th1 cytokines IFNγ and IL-2 in multiplex immunoassays were detected after re-stimulation with the S but not RBD overlapping peptide mix, although RBD is part of the S protein. This should also be clarified (**OC**).

Concerning the nonhuman primate (rhesus macaques) studies, the applicant considers the human convalescent serum panel as an assessable benchmark to judge the quality of the immune response to the vaccine. The reasoning behind this can be followed. The assumption that the immune response to SARS-CoV-2 infection provides some measure of protection from disease upon subsequent exposure to the virus, appears plausible. There were a number of specific questions regarding the NHP proof of concept study (study VR-VTR-1067) which could be considered demonstrate immunogenicity and viral clearance in NHP but insufficient to fully demonstrate efficacy against the disease (issues to consider were e.g. on the report itself, the animal model relevance, technical aspects, endpoints, immunological aspects) (**OC**).

The applicant needs to a) precise for the Luminex data how the reference curve for has been constructed, what does represent the arbitrary U/ml used and how it is referring to the serum dilution factor; b) define the criteria for choosing a 10-30% infection rate of Vero cells; c) Methods to quantify antibody production in the different experiments differ and consequently cross-comparison between experiments is hardly impossible. Indeed, it is important to distinguish neutralizing antibodies from non-neutralizing antibodies. In this study, total antibody response is measured using a luminex assay and results expressed on U/ml and for the neutralization assay results are expressed in VNT 50. The applicant needs to provide an estimation of the non-neutralizing antibodies in the whole antibody response (**OC**). d) It is important to notice that on figure 6 of study report, neither panel A nor panel B highlight the consumption of IgG S1 binding antibodies after challenge nor the increase due to B memory response following the challenge: this would need to be further justified by the Applicant **(OC)**. The report VR-MQR-10211, on S1-binding rhesus macaque serum IgG levels detected by a direct binding Luminex immunoassay, was not provided. This should be submitted (**OC**)

Concerning the characterization of the T cell responses, the Applicant suggests the S-specific IFNγ producing T cell responses, including a high frequency of CD4+ T cells that produced IFNγ, IL-2, or TNF-α but a low frequency of CD4+ cells that produce IL-4, indicates a Th1-biased response occurred after the BNT162b2 (V9) immunization. This reasoning appears plausible, however, there was no reference to what to expect from a typical Th2 biased response to enable a comparison of the current data. Nevertheless, the role of such a Th1 biased response was put in the context of antigen-specific T-cell responses playing an important role in generation of antigen-specific antibody response as well as in elimination of infected cells to mediate protection against disease. However, the potential importance of T-cell effector cells for a putative protection against SARS-Cov-2 infection after BNT162b2 (V9) immunization was not further investigated or discussed.

When immunized macaques were challenged with SARS-CoV-2, a clear and statistically significant effect was observed on reduced presence of viral RNA in bronchoalveolar lavage (BAL), nasal and oropharyngeal (OP) swabs. A clear effect was also recorded by blinded X ray scoring of the lungs. A protective effect is also evident in the CT score Day 3 after challenge, however at Day 10/EOP, there was a CT signal in 2 out of six BNT162b immunized monkeys at the same level as observed in the control group. That signal is of unclear significance since also in 1 out of 6 pre infection BNT162b immunized animals a similar CT-score signal was observed. The size of the study prevents any firm conclusion on these observations. A histopathological examination of lung tissues is ongoing and a submission ASAP as an addendum is awaited (**OC**). Furthermore, the data from the individual monkeys should be provided for the RT-qPCR test for presence of SARS-CoV-2 RNA (**OC**).

In the NHP pharmacology study (Study VR-VTR-10671), rhesus macaques were immunized on days 0 and 21. Some other covid-19 vaccine candidates have different prime-boost intervals, such as 4 weeks for both ChAdOx1 (Graham et al., 2020) and mRNA-1273 (Corbett et al., 2020). Considering that the time between the first and second vaccine dose may have a significant impact on the immunological response, the applicant is asked to provide the rationale for the chosen prime-boost interval (21 days). (Graham et al., 2020: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7385486/ Corbett et al., 2020: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7449230/) **(OC).** The Spike protein of SARS-CoV-2 undergo mutations, and it thus critically important to investigate the biological significance of these variants in relation to the development of Spike-based covid-19 vaccine candidates. For example, Korber et al. present evidence that there are now more SARS-CoV-2 viruses circulating in the human population globally that have the G614 form of the Spike protein versus the D614 form that was originally identified from the first human cases in Wuhan, China. Further, Li et al., states that as of May 6, 2020, 329 naturally occurring variants in Spike protein have been reported in the public domain. The applicant is asked to discuss how the chosen Spike antigen variant in BNT162b2 relates to the Spike variants currently on the dominant SARS-CoV-2 viruses circulating in the human population. **(**Korber et al., 2020: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7332439/ Li et al., 2020: https://doi.org/10.1016/j.cell.2020.07.012) **(OC).** The rhesus macaques were challenged with the SARS-CoV-2 USA-WA1/2020 isolate. To our knowledge, this strain does not contain the D614G mutation. This mutation is reported to rapidly accumulate in the circulating SARS-CoV-2 strains and may increase the infectivity several-fold compared to the original Wuhan-1 strain. The applicant is asked to discuss the relevance of the NHP challenge study results in relation to the strain used for challenge and the strains circulating in the human population **(OC)**.

In conclusion of the preclinical pharmacology, the presented data, including immunogenicity, triggering of neutralizing IgGs and Th1 response and reduced presence of viral RNA in challenged animals as well as radiological lung parameters (to be confirmed by histopathology), provide some support for the vaccination approach. It can be noted that in the primary proof-of-concept study, the use of juvenile rhesus monkeys with no or only mild clinical symptoms for the preclinical efficacy testing has limitations in its value as a disease animal model for human Covid-19 (which is a clearly age stratified disease, mostly affecting the elderly). In addition, the low number of animals of the male sex only that were studied only for a short time period weakens the conclusiveness of the study. However, due to species differences in the immune system between animal model species and humans, the final call on whether this candidate vaccine will work sufficiently well in humans will entirely rely on the clinical outcome.

Pharmacokinetic (regarding the two novel LNP excipients): The applicant is requested to provide qualification data for LC-MS/MS method used to quantify the two novel LNP lipids in the non-clinical PK study **(OC).** The Applicant is also asked to justify the choice of an IV study instead of an IM study,

which would have a more clinical relevance. The difference observed in terms of PK absorption should be discussed **(OC).**

It is worth to notice that the lipid displaying a persistent kinetic over time in liver is ALC-0159, ie the one that does not contain any PEG, although PEG is known to be used to increase half-life of many recombinants. The Applicant will have to justify this observation, as well as to discuss the difference of kinetics profile between the two lipids. The Applicant is also requested to estimate the delay of the clearance of the ALC-0315 from the liver, as this could have an impact on the safety profile **(OC).**

Biodistribution: As expected for an RNA, the expression of the surrogate luciferase reporter RNA was transient and decreased over time. It is acknowledged that the biodistribution of the mRNA mostly will be dependent on the composition of LNP and the applicant has provided data that differences in LNP formulation affects the biodistribution of the luciferase modRNA and luciferase protein expression. The Applicant mentions that the LNP-formulated luciferase-encoding modRNA tested in this study have the exact same lipid composition than BNT162b2. It is however not clear to understand which of the three tested LNP formulation is present in the drug product, BNT162b2 variant V9. The Applicant should comment **(OC).**

RNA stability and kinetics are not expected to be the same for all RNAs and are influenced by the nucleosides of the RNA and although expression of the full-length spike (S) protein is expected to follow similar kinetics of that of the luciferase with a transient expression fading over time, it cannot be excluded that differences in stability/persistence of the signal could differ between the luciferase protein and the spike (S) protein. It can be noted that there is no information on the similarities of the mRNA modifications of the non-coding regions between the luciferase modRNA used in the study and the modRNA used in BNT162b2. The applicant is asked to provide more information on the luciferase reporter RNA, in particular, whether the untranslated sequences are similar to that of the BNT162b2 modRNA and therefore at least the stability of the mRNAs are somewhat comparable. **(OC)**

The biodistribution of the vaccine has been evaluated in mice, using 2 µg mRNA (encoding for luciferase). In humans and in the repeat-dose study in rat using the V9 version, 30 µg (per administration) was used. It is not clear if this difference in RNA concentration results differences in the amount of LNP used. The applicant is therefore asked to clarify if there were differences in the amount of LNP used in the biodistribution study and the repeat-dose study /clinical trials and if so, discuss how this could affect the distribution and safety evaluation observed in the clinic compared to non-clinical data **(OC)**.

The bioluminescence method used to determine the surrogate luciferase modRNA biodistribution has not been validated or qualified and no discussion on its sensitivity has been included. Only three females were investigated which is considered a low number. Moreover, only one dose level (given as single injection) was tested (compared to two injections given clinically). The sensitivity of the method and dose proportionality effects have therefore not been determined.

The applicant has only discussed distribution at the injection site and to the liver. To the untrained eye it is not clear that the bioluminescence signal is solely liver specific. Although the signal appears to be in the liver region, from the data submitted it cannot be excluded that the bioluminescence signal could include distribution to other organs located in proximity of the liver.

Several literature reports indicate that LNP-formulated RNAs can distribute rather non-specifically to several organs such as spleen, heart, kidney, lung and brain. The observed extra-hepatic distribution of modified RNAs have been detected at much lower levels compared to the liver when measured with techniques detecting nucleic acids (for example branched DNA analysis). This raises the concern if the sensitivity of the bioluminescence method is sufficient to detect potentially weaker biodistribution to sites besides the liver and injection sites?

The vaccine is intended to be given to patients twice with a booster dose after 22 days. The booster dose scheme could increase the risk for inflammatory reactions at sites of expression and therefore a wide biodistribution profile might not be optimal from a safety perspective. The applicant is therefore asked to provide more information regarding the biodistribution assay and should discuss the sensitivity of the biodistribution method. The choice of using a non-validated/non-qualified bioluminescence method to determine the biodistribution of a surrogate luciferase protein instead of choosing to detect the actual modRNA used in the vaccine candidate BNT162b2 should be justified **(OC).** Moreover, the applicant is asked to consider the possibility of a wider biodistribution pattern than observed and discuss the possible safety consequences of a wider biodistribution profile of BNT162b2. **(OC)**

Results from the Luminex-based multiplex assay revealed that immunisation with LNP formulated luciferase modRNA transiently increased levels of MCP-1, IL-6, IP-10, at 6 hours post-immunization. These results in mice suggest that the LNP can activate the innate immune system of mice, by the synthesis of pro-inflammatory cytokines. As the effect was transient, this effect could be considered as an adjuvant-like effect. Overall, on the basis of above, the LNP formulation is expected to have not only a role to protect modRNA from nucleases degradation, and facilitating cellular transfection, but also adjuvant like effects.

In view of potential acute immunotoxicity mediated by LNPs, does the Applicant possess data on other timepoints (earlier than 6h or beyond) regarding the cytokines measurements? **(OC)**

The Applicant is also asked to discuss the absence of an in vitro hPBMC stimulation assay in healthy donors to assess reactogenicity **(OC)**.

Extrapolating to clinics, the Applicant is requested to discuss the level of IL-6 cytokine induced by LNPs, considering that asymptomatic but infected subjects candidate to vaccination, could display higher IL-6 levels during early phase infection **(OC)**.

Toxicology: The first "V8"-repeat-dose toxicity rat study has some documentation issues that possible would have to be followed up regarding its GLP status (a GLP inspection has been initiated) and thereby increasing the uncertainty of the interpretation of the results **(OC)**. That being said, as the toxicological outcomes from the V8 and V9-studies are overall similar (and the two studies were conducted at different sites), the V8-results are considered to be useful for risk assessment. There is also some uncertainty regarding which production process (two possible) was used for the test substance in the V9 study (also dependent on the quality assessment which has yet to start).

Only the whole formulation (modified RNA in LNPs) were used, so there is no toxicological data on the LNP alone or its specific novel excipients. Overall, the V8 and V9 test substances invoked a strong but mostly reversible immune-linked response in rats after 17d exposure. It is unclear if or how much of that immune response is attributable to the LNP components (which are included in the formulation as excipients). There is some pharmacokinetic data that indicates that the LNP has the potential to induce a transient immune response, but it can also be noted that most exogenous biomolecules tend to generate some degree of transient immune response, so such observations would not be unexpected.

While no extensive pharmacological assessment has been conducted in rat (only in mouse and non-human primate, with no deeper discussion on the choice of animal models [**OC**]), immunogenicity tests on blood samples in the V8 repeat-dose toxicity study indicate that rats generate SARS-CoV-2 antibodies, partly supporting the choice of animal model. Other SARS-CoV-2 immune responses in rat remain unclear. The immune responses, especially at the injection sites (e.g. oedema, erythema), seem to increase with each injection in the studies (n=3). There was a marked increase in acute phase proteins, fibrinogen and reduced albumin-globulin ratio (but no increase in cytokines with V8, unclear for V9). There was also a general increase in immune cells (LUC, neutrophils, eosinophils, basophils)

and a decrease in red blood cell parameters (reticulocytes, RGB, HGB, HCT). The spleen was enlarged at both 30ug V9 and 100ug V9 and the lymph nodes were enlarged mostly at 100ug (V8) but also in a few animals at 30g (V9). While an immune response is expected from V8 and V9, the strong reaction of the injection site and immune system in rat is difficult to interpret/risk assess as the vaccine candidate(s) are derived from a novel vaccine platform. There is also the possibility, which is difficult to assess non-clinically or effectively in-silico, that the generated antibodies may react with endogenous proteins. An absence of dose-response designs in the studies increases the difficulty to interpret the effects.

As the pharmacokinetic distribution study in rat was limited (mainly giving data on liver), the distribution (and its effects) has to be inferred indirectly from the toxicological studies. There is some uncertainty in this regard as not all tissues have been investigated in the V8 study and histopathological details are unknown for the V9 study and it is recommended to study as many tissues as possible (the following tissues were not studied: nasal body cavity, clitorial gland, dorsal root ganglion, larynx, mandibular lymph node, tibial nerve, preputial gland, ureter, Zymbal's gland). While there was no severe pathogenesis in liver, there were some reversible functional hepatic and/or biliary effects with V8 and V9 (enlarged liver, vacuolation, strongly increased gGT levels at >200% and activity, minor-moderate increase in levels of ALT and ALP) which may be linked to the LNP. The gGT changes were not observed with 30ug V9, which may be due to variant differences and/or a lower dose. Considering that vaccines are expected to generate little or no toxicity (beyond local tolerance and immune response effects) and that BNT162b2 derives from a novel vaccine platform in the context of a pandemic, further discussion on these effects and a possible mention in the SmPC is required (see **OC for specific details**). DART data remains to be submitted and the choice of animal model and experimental design to be justified (**OC**).

With regard to the characterization of the novel LNP components, these are not considered primarily as adjuvant substances. While some degree of LNP-specific immune response cannot be ruled out (as demonstrated in the *in vivo* biodistribution study in Balb-C mice by pro-inflammatory cytokines induction [MCP-1, MIP-1β, TNF-α, IFN-γ, IL-6, IP-10] at 6 h post immunization – see study R-20-0072), no further experimental toxicological studies are considered necessary as the use of the whole formulation (RNA + LNP) in the repeat-dose toxicity and DART studies is sufficient to qualify the novel excipient lipids in combination that the overall effects are also being assessed in the clinical trials. That being said, as the lipids contain an acetamide moiety which has been linked to carcinogenicity in animals, including liver tumors, potentially related to genotoxicity, and liver distribution and functional effects have been observed in rat, an extended discussion of these lipids is requested (**OC**).

It is unclear at this stage of the rolling review how these effects are depicted in the SmPC.

2.2.6. Conclusion on non-clinical aspects

Based on the provided data so far there are no non-clinical major objections. The applicant will need to sufficiently address the other concerns raised to be granted MAA from a non-clinical perspective. Other non-clinical elements in further rolling review cycles are expected to define the safety profile of the vaccine.

2.3. Clinical aspects

N/A

2.4. Risk management plan

N/A

2.5. Pharmacovigilance system

N/A

3. SCIENTIFIC OVERVIEW AND DISCUSSION on responses to questions raised in previous cycle(s)

N/A

4. Benefit risk assessment

N/A

5. Rapporteur list of questions

5.1. Quality aspects

Major objections

GMP

1. GMP status for DS and DP manufacturing sites is currently not acceptably demonstrated:

a. A statement on GMP compliance issued by EU supervisory authority of the DS and DP manufacturing and testing sites Wyeth BioPharma Division, Andover, United States and Pfizer Inc, Chesterfield, United States should be available by adoption of the CHMP opinion.

b. The MIA for Pfizer Puurs is limited to the formulation and filling only. It should be clarified if authorisation will be extended to all operations listed in 3.2.P.3.1, including LNP manufacturing. Moreover, GMP certificate or a statement of GMP compliance issued by the Supervisory authority of BioNTech Manufacturing GmbH, Mainz, Germany should cover batch certification of the DP.

Drug substance and Drug product

2. Comparability between clinical and commercial material has not yet been demonstrated, which raises uncertainties about consistency of product quality and hence uncertainties as regards product safety and efficacy of the commercial product. Significant differences between batches manufactured by DS Process 1 and 2 are observed for the CQA mRNA integrity. In addition, the

characterisation of BNT162b2 DS is currently not found acceptable in relation to this quality attribute. This is especially important considering that the current DS and DP acceptance criteria allows for up to 50% fragmented species. Therefore, the dossier should be updated with additional characterisation data on mRNA integrity in sections 3.2.S.2.6 (comparability) and 3.2.S.3 of the dossier.

a. Truncated and modified RNA species should be regarded as product-related impurities. Even though two methods, namely agarose gel electrophoresis and capillary gel electrophoresis (CGE), have been applied to determine RNA integrity of BNT162b2 DS, no characterisation data on truncated forms is presented. Results obtained on RNA integrity by CGE and agarose gels should be included in the characterisation section (3.2.S.3). The truncated forms should be sufficiently characterised, i.e. they should be described, and it should be discussed if the fragmented species are expected to be similar between batches. In addition, the possibility of translated proteins other than the intended spike protein (S1S2), resulting from truncated and/or modified mRNA species should be addressed and relevant protein characterization data for predominant species should be provided, if available.

b. Upon changing to DS Process 2, a decrease in RNA integrity was observed (only numerical values provided). Concerning this difference in RNA integrity between Process 1 and Process 2 DS batches the Applicant is requested to provide capillary electropherograms together with an evaluation of any batch differences in peak patterns. The potential safety risks associated with truncated RNA isoforms should be thoroughly discussed with reference to the batches used, clinical experience and possibly literature data. The quantitative and qualitative differences observed between Process 1 and 2 should be discussed with respect to their impact on safety and efficacy.

c. For Process 2, the CTP and ATP volumes were adjusted before the manufacture of DS batch PPQ3 to align better with RNA integrity results from Process 1. Additional batch data (from PPQ4 and PPQ5) should be provided to confirm that the optimised Process 2 allows for reaching RNA integrity levels consistent with the Process 1 batches.

d. After contact with the applicant it was confirmed that DP batches manufactured from early Process 2 batches, with lower RNA integrity, have been recently introduced in clinical trials. However, as the cut-off date for the clinical Interim Analysis (IA) was changed, the IA doesn't include data from subjects dosed with Process 2 material, and the Company does not expect to have Process 2 included in the Final Analysis dataset. Therefore, the proposed acceptance criteria of ≥50% intact RNA for RNA integrity is considered too wide compared to clinical batch data, 69-81%. The proposed release and shelf-life acceptance criteria for the DP should therefore be tightened based on the clinical data included in the dossier or clinically qualified by other means.

e. Release data provided for some of the DP batches indicates a possible decrease in mRNA integrity during the manufacturing of DP. The applicant should therefore discuss possible root causes, and present comparative results for DS and DP, on RNA integrity. A consequential need for a more stringent DS specification should be considered. Sections S.4.1 and P.5.1 in the dossier should be aligned and updated accordingly.

3. Drug product batches manufactured at the commercial facility (whole manufacturing process at the commercial site Pfizer, Puurs, at commercial scale, drug substance from process 2) were not presented. Process validation (PPQ) for commercial scale batches are already initiated and validation data should be provided. Batch results for at least 2 commercial scale batches representative of the commercial process should be presented. Comparability of commercial batches with clinical batches should be demonstrated and the data should be provided. The

claimed shelf-life and storage condition are not yet acceptable since no stability data is available for batches from the commercial manufacturing site and scale and shelf-life is based on very small scale (development) batches (less than 1% of the commercial scale), not representative of the commercial batches (manufacturing site, scale, process for the drug substance). Additional stability data (6 months at long-term storage condition) should be presented.

Other concerns

Drug substance

The applicant plans to update a number of sections along the dossier and states the following: "Data for this section is pending and will be updated once the data has been generated, analysed, and verified". Until these data are available for assessment, no final conclusions can be drawn on the concerned sections.

General information (S.1)

4. The proposed mechanism of action should be presented in S.1 General Information.

Description of manufacturing process and process controls (S.2.2)

5. Information on the final batch volume should be provided. The Applicant should state either the total batch volume or the approximate number of DS containers generated from one batch. Section 3.2.S.2.2 should be updated accordingly.
6. It is noted that some parameters and ranges may be updated after PPQ and additional characterization studies are completed. These updates could have an impact on overall assessment of the manufacturing process description, leading to additional issues. However, the following issues have already been identified and should be addressed:
    a. It should be indicated that the incubation time during GTP/N1-methylpseudo UTP bolus feeds is a global time for the 11 feeds
    b. The strategy for UFDF membrane lifetime validation is to perform concurrent validation of the membranes at commercial scale. This is found acceptable, provided that the Applicant will include control of the feed flow rates, transmembrane pressure and membrane surface area in section 3.2.S.2.2. The dossier should be updated accordingly.
    c. The transfers of the UFDF pool into a single PE flexible container before and/or after 0.45/0.2 µm filtration should be clarified and should appear in the DS process flow diagram.
    d. The DS filling volume range in the EVA flexible containers should be defined in line with the volumes validated for shipping.

Control of materials (S.2.3)

7. Representative CoAs or full specifications should be provided for starting and non-compendial raw materials used in the manufacturing of BNT162b2 DS. It is expected that information regarding the

microbiological control is included. Additionally, all raw materials should be demonstrated to be free from contaminating RNases, unless otherwise justified.

8. It is noted that for starting and raw materials used at Andover, additional material testing will be performed and provided when available. Where relevant, the applicant should consider in house testing for the functional activity of starting and critical raw materials such as the enzymes used in the manufacturing process. The information should also be completed with the analytical methods.

9. As the 5'-cap structure is complex, its synthesis should be described. The impurities and by-products generated during its synthesis should be discussed.

Linear DNA template

10. Additional details on relevant characteristics and origin of the E. coli strain DH10B as well as source and an overall description of generation (flow chart of the successive steps) of the plasmid used as template for the production of Drug Substance should be provided.

11. Release testing of plasmid MCB and WCB should be completed with a percentage of the expected sequence rather than "comparable to the reference sequence". Moreover 100 % homology is requested for the coding sequence; for the other parts of the plasmid any mutation should be assessed.

12. The specification for the future WCBs should be completed with the percentage of viable cells with an appropriate acceptance limit. Moreover, an acceptance limit for viable cell concentration should be set, and a percentage of the expected sequence (% homology) for DNA sequencing as requested for plasmid MCB and current WCB should be proposed. Finally, the analytical methods should be indicated.

13. The cell bank stability protocol (including test parameters and corresponding acceptance criteria) should be provided. Otherwise, the performance of the WCB should be checked during the manufacture of each batch of plasmid DNA, for example by following the trends in bacterial growth and plasmid yield.

14. It is recommended that cell banks be stored in two or more separate locations to minimize the risks of their total loss as a result of a catastrophic event. It is indicated that Pfizer facility at 875 Chesterfield Parkway West, Chesterfield is the only proposed storage site for MCB and WCB. A clarification whether any risk amelioration strategies are in place to avoid the loss of cell banks should be requested.

15. Information should be provided regarding the reference material used in the restriction map analysis and DNA sequencing determination for MCB and WCB used for plasmid DNA template production.

16. The manufacturing process to obtain the linear DNA template should be completed with the following information:

    a. The quantity of linear DNA template obtained in each batch should be stated

    b. The chemical agent used for chemical lysis of the cells should be mentioned and its clearance should be demonstrated to be sufficient.

    c. The mention "or equivalent" for the restriction enzyme should be deleted.

    d. The Applicant should confirm that implementation of changes in the manufacture of the linear DNA template will be applied for in a variation application.

17. The specification for the linear DNA template should be revised with narrower limits for purity and process-related impurities taking into account the batch analysis results. A high level of DNA impurities could impact the activity of the T7 polymerase during the Transcription phase of the DS production.

18. Appropriate descriptions of all analytical methods used in the release control of the linear DNA template as well as summaries of the results obtained in the method validation/qualification studies should be provided.

19. The reference material for plasmid identity testing should be described. (Rapp Q10)

20. The stability of the linear DNA template and the stability of the filtered circular plasmid DNA intermediate should be addressed. A shelf life for the linearized DNA template should be established and a stability protocol covering the proposed storage period should be included. Relevant available data should be provided to support this proposal.

Control of critical steps and intermediates (S.2.4)

21. It is stated that OOS result for in-process controls would trigger an evaluation of the deviation to determine if the batch could be further manufactured. It should be confirmed that OOS results will lead to batch rejection.

Process validation and/or evaluation (S.2.5)

22. Several validation studies and full PPQ data are still pending for the manufacturing process at Wyeth BioPharma, Andover. Therefore, additional information is needed:

    a. Results for PPQ4 and PPQ5 batches should be provided to confirm the consistency of the DS manufacturing process after the change of ATP and CTP volumes in the IVT vessel at PPQ3 and onwards. The description of deviations and investigation conclusions should be provided, as well as the evaluation of removal of impurities for the five PPQ batches.

    b. A time-plan for the submission additional process validation data should be provided before marketing authorization approval.

23. Residual DNA template is present at higher level in PPQ3 batch (211 ng DNA / mg RNA) than in PPQ1 and PPQ2 batches (10 and 23 ng/mg); the robustness of DNase I digestion step should be further investigated.

Manufacturing process development (S.2.6)

24. It is noted that the ranges studied for addition volumes for CTP and ATP as stated in 3.2.S.2.6 are 81.0-143.8 and 90.0-135.1 mg/L respectively and that the acceptable ranges proposed are 85.4-143.8 and 85.4-135.1 mg/L. It seems as if the lower acceptable range of 85.4 mg/L proposed for ATP volume have not been studied, this needs to be clarified. In addition, it needs to be justified why the lower end of the ranges for both CTP and ATP volumes remained unchanged although the target ranges were increased (from 90 to135.1 and 107.9 mg/L respectively), to avoid that these nucleotides will be limiting in order to increase the percentage of the RNA integrity.
    These ranges need to be further justified and clarified and the dossier updated accordingly.

25. In the In vitro transcription (IVT) step, the magnesium dependent T7 RNA polymerase assembles ribonucleotide building blocks. Since magnesium can be chelated by pyrophosphate released by the addition of each ribonucleotide pyrophosphatase is important to maintain sufficient levels of free magnesium. The Applicant states that added volumes of these two enzymes have been identified as non-CPPs as they are most likely to impact yield only. This conclusion is not entirely agreed upon.

    a. It needs to be further justified why these parameters are not classified as CPPs.

    b. Regardless of the classification as non-CPPs or CPPs it is strongly recommended to include an appropriate control of the added volumes of the enzymes T7 polymerase and pyrophosphatase in sections 3.2.S.2.2 and 3.2.S.2.4 of the dossier.

    c. In addition, it needs to be clarified if the actual volumes loaded are calculated based on enzyme activity as stated in the certificates of the actual batch of the enzymes that are used. (See also question in section 3.2.S.2.3 above).

26. The Applicant should provide data on the T7 RNA polymerase and proteinase K levels in additional commercial scale DS batches, once testing is complete. In addition, the Applicant should briefly describe that the methods applied to determine the concentrations of these two enzymes in the BNT162b2 DS samples and confirm that these methods are fit for purpose.

27. Differences in the poly(A)tail pattern were observed when comparing the Process 1 and Process 2 DS batches. The differences in the extent of cytidine monophosphate incorporation and transcriptional slippage should be further investigated and the possible impact on efficacy and safety should be discussed. The only Process 2 DS included in the comparison was manufactured prior to the adjustment of CTP and ATP volumes. Results obtained on the PPQ batches, manufactured after adjustment (PPQ 3, 4 and 5) should also be presented and discussed.

28. The level of information in the dossier presenting the available process characterisation studies is not sufficient to allow assessment: the results of the studies should be presented, preferably summarised in figures or tables.

29. An overall control strategy was presented but some parameter and ranges may be updated after PPQ and additional characterization studies completed. As for assessment of overall control strategy, a complete set of data and information is needed, this document will be assessed when finalised. A time plan for the submission of the final data set of the control strategy should be provided.

Characterisation (S.3)

30. In the Development History and Comparability section (3.2.S.2.6), the expressed protein size is evaluated by in vitro expression followed by Western blot. Results obtained by this method could be regarded as biological characterisation and should be included in section 3.2.S.3. The method needs further description and the results should be sufficiently characterized.

    a. A brief method description including conditions for protein expression, gel separation, and western blot assay should be provided.

    b. The expected protein size should be stated and supported by theoretical calculations.

    c. The identities of the two distinct bands should be explained. If possible, the identities of the bands should be confirmed and characterized by LC-MS/MS.

d. The Applicant should provide data on protein expression in terms of percentage of successfully transduced HEK293 cells using the lipofectamine transfection system.

31. Even though biological characterisation might not be possible to perform on DS, the strategy to determine potency and relevant functional assay(s) should be described in section 3.2.S.3. Results obtained on DP could be included, to demonstrated functionality.

32. NGS technology has been used as an orthogonal method to confirm primary sequence but details are missing about the results of this analysis in terms of coverage of the target genome. A brief description of the NGS method, and the results obtained with it should be provided.

33. As regards 5' end of the RNA, relative abundance of each species (capped, non-capped and/or incompletely capped) is given as major (>50%) for the expected 5'-cap structure, minor (5 to 50%) and trace (<5%) for other species. However, a more precise quantification of each uncapped or incompletely capped species should be provided. Moreover, the potential contribution of uncapped or incompletely capped structures to the potency of the BNT162b2 DS should be discussed.

34. The Applicant should discuss the relationship between 5'-cap heterogeneity and dsRNA production. A risk assessment should be provided. This should be also taken into account in the justification of DS specification.

35. It should be addressed whether, under expected storage conditions, individual base modifications occur (e.g. depurination, oxidation). Based on this discussion it may be necessary to review the impurity methods and specifications for appropriateness to detect relevant degradation under long-term conditions.

Control of drug substance, Specifications (S.4.1)

36. The proposed commercial drug substance specifications, the method descriptions and the method validation summaries should be updated to include in-house method identification numbers for the non-compendial methods. The information is required in order to provide a clear link between the specification and the descriptions and validations of analytical procedures used for routine testing. Furthermore, for the compendial methods references to relevant parts of the Ph Eur should be included. Section 3.2.S.4.1, 3.2.S.4.2 and 3.2.S.4.3 of the dossier should be updated accordingly.

Control of drug substance, Analytical procedures (S.4.2)

37. In all the in-house analytical methods used in the release of DS, method descriptions are based on "examples" of procedures, controls and standards as well as on "typical" system operating parameters. These terms raise uncertainties regarding the developmental stage, and the control of critical steps of these assays. The analytical methods used in the control of DS are expected to be finalized. The applicant is requested to confirm this and to update the relevant parts of the dossier with unequivocal method descriptions, including relevant lists of materials and additional details, if needed. The applicant should also confirm that any significant changes in analytical procedures will be applied for in a variation application.

38. Regarding the RT-PCR method for determination of DS and DP identity:

    a. Information regarding the positive control used in the should be provided.

b. The proposed assay acceptance criteria for the qualitative RT-PCR-based assay used for determination of DS identity requires a Ct value for the positive PCR control of NMT than 32 simultaneous with a Ct value for the negative controls of NLT 32. These criteria are not considered relevant to support method suitability. More stringent acceptance criteria should be established and supported by relevant data.

c. The mRNA extraction step needed for determination of the identity of BNT162b2 DP should be included in the description of the RT-PCR-based assay and this step should be appropriately described and addressed in the method validation procedure. This question relates to the DP part of the dossier.

39. Regarding the ddPCR-based method for determination of poly(A) tails in the mRNA DS:

    a. Information regarding the internal control used in the should be provided.

    b. From the limited description of the ddPCR-based assay for quantification of poly(A) tails it seems that the cDNA generated using a poly(T) primer is used both as a template for further amplification of the (poly(A) positive mRNA)-derived cDNA and also as the theoretical input based on which the final calculation of the Poly(A) tails is made. This strategy is not understood. The suitability of this approach and the rationale by which the method is able to determine the percent poly(A) tails in the mRNA DS relative to the input (which should be clearly defined) needs to be better described.

    c. With respect to the storage conditions of the cDNA prior ddPCR, storage at room temperature, however with no hold time defined, is mentioned in the method description, but a storage time of 3 days at –20°C is examined in the validation studies with respect to method robustness. These discrepancies should be clarified. Information on the qualified lot of linearized plasmid standard used in the qPCR-based method to quantify the residual DNA template in BNT162 b2 DS should be provided.

40. Information on the qualified lot of linearized plasmid standard used in the qPCR-based method to quantify the residual DNA template in BNT162 b2 DS should be provided.

41. With respect to the immunoblot analytical method used for determination of dsRNA in BNT162b2 drug substance:

    a. Additional information regarding the critical reagents (such as antibodies), standards and equipment used as well as representative dot blots and standard curves should be highlighted in the dossier. The robustness of the method should be appropriately demonstrated in the validation exercise, if different reagents, e.g. different clones or different vendors for the antibodies, are envisaged.

    b. An incubation time of >16h is defined for the primary antibody incubation step. An upper limit should be defined as well. Unless otherwise justified, all variable incubation times described in the method should be considered in the validation exercise, in order to demonstrate the robustness of the assay.

42. For the capillary gel electrophoresis method, it should be specified how the peaks are integrated to allow quantitation of the RNA integrity. An integrated electropherogram should be provided as an example.

Control of drug substance, Validation of analytical procedures (S.4.3)

43. The information in the dossier does not support that any of the in-house analytical procedures applied for drug substance has been properly validated in line with ICH Q2. The validation summaries provided are far too brief and important details are missing. The Applicant should submit more comprehensive validation summaries of all non-compendial methods, for example in the form of short validation reports. The validation summaries should include all relevant calculations, acceptance criteria, description of and results obtained for individual samples. Chromatograms and dose response curves should be included, where applicable. Module 3.2.S.4.3 of the dossier should be updated accordingly.

44. The method transfer plan or activities should be addressed. It should be noted that, if method transfer was / will be performed, the following information should be provided. For the non-compendial tests, it should be confirmed that the validation acceptance criteria for the receiving sites will be the same as for the transferring site (which will be assessed during the RR). For the analytical methods for which comparative analysis will be proposed, it should be confirmed that the acceptance criteria will be the same as for the intermediate precision validated at the transferring site (and assessed during RR).

Control of drug substance, Batch analyses (S.4.4)

45. Batch results should be presented for the two newly manufactured batches PPQ4 and PPQ5 to be able to assess process consistency. This is considered specifically important to verify that the volume adjustments made for ATP and CTP volumes before manufacturing of PPQ3 (20Y513C501) consistently provides reproducible results, in particular with RNA integrity levels similar to levels achieved in process 1 batches.

Control of drug substance, Justification of specifications (S.4.5)

46. The length of the poly(A) tails in BNT162b2 DS is important for RNA stability and translational efficiency and this test should therefore be included in DS release specification.

47. The proposed acceptance criteria for the percentage of 5'- Cap (≥50%), dsRNA (<1000 pg/µg mRNA) and Poly(A) tail (≥70%) are not considered justified and should be tightened to better reflect the data presented for the DS material used in the manufacturing of the clinical and PPQ batches. In addition, batch release results from two newly manufactured batches PPQ4 and PPQ5 should be included in the reassessment of the acceptance criteria.

Reference standards (S.5)

48. It should be clarified for what release and stability testing methods the reference standard is used and will be used in future. The function of the reference standard should be briefly stated for each assay, i.e. result evaluation/normalisation, sample compliance, assay control etc. The information could be provided preferentially in a tabulated form.

49. It is noted that the CRM is derived from a Process 2 DS batch that was established in September 2020. It should be explained if another reference standard was used to perform release tests on Process 1 DS batches. All initial reference materials should be listed.

50. The CRM is derived from an early Process 2 batch which has a slightly lower RNA integrity than the clinical batches and possibly also to future batches, due to target value optimisation. The Applicant should justify the suitability and address potential risks of using this material as a reference standard.

51. Neither the storage condition, nor the shelf-life is established for the CRM. The Applicant should explain if the reference standard is used in any of the methods included in the formal stability protocol. If this is the case, the Applicant should explain how compliance with the acceptance criteria can be guaranteed.

52. Since the Applicant intends to establish primary and working reference materials, information on the preparation, qualification and stability evaluation of the PRM and WRMs should be included in a PACMP. Otherwise it should be confirmed that a variation application will be submitted in connection with the introduction of these standards.

Container closure system (S.6)

53. The following additional information should be included in Module 3.2.S-6 of the dossier.

    a. A certificate of analysis of one representative batch of the EVAM contact layer demonstrating compliance with Ph. Eur. 3.1.7.

    b. A specification for the container closure system including dimensions (currently only schematic drawings are included).

54. A commitment to submit for assessment any unexpected leachable compound from EVA container closure system reproducibly observed above 1.5 µg/day TDI should be provided.

Stability (S.7)

55. Process 1 batch is not considered representative to process 2 batches. The only parameters studied for process 1 batch are RNA integrity and RNA content and the cGE method for RNA integrity was changed. Therefore, based on the currently very limited stability data presented for process 2 batches (only 1-month data available for one batch) no conclusion can be drawn in relation to the proposed shelf life for the DS. Thus, in order to support shelf life setting for drug substance updated reports from the ongoing stability studies on the primary batches (including data from the ongoing process validation batches) should be provided.

56. It should be confirmed that future extensions of the assigned DS shelf life will be applied for in formal variation applications. The following statement should be removed for Module 3.2.S.7.1 of the dossier; "*The sponsor will extend the assigned shelf life without notification providing the real time stability data at the intended storage condition is acceptable and within commercial specifications*."

Drug product

The applicant plans to update a number of sections along the dossier and states the following: "Data for this section is pending and will be updated once the data has been generated, analysed, and verified". Until these data are available for assessment, no final conclusions can be drawn on the concerned sections.

P.1 Description and composition of the drug product (P.1)

57. All ingredients, including process aids used in the manufacture, should be specified in the composition together with a footnote that they are removed during manufacturing. Therefore, ethanol and components of citrate buffer should be added to the composition. Moreover, HEPES and EDTA (excipients used in the drug substance buffer) should also be added to the composition table. Section P.1 should be updated accordingly. All these ingredients should be mentioned in the SmPC and PIL.

58. While the final volume of drug product after reconstitution (2.25 ml) exceeds the vial nominal capacity (2 ml), it is expected that during clinical trials it was demonstrated that the method of preparation is feasible and is robust in ensuring efficient mixing and uniformity of the solution. This issue should be addressed and if needed, appropriate instructions for use (IFU) should be given in the SmPC and PIL.

Pharmaceutical development (P.2)

59. Controlled extraction studies have been performed on the bromobutyl rubber stopper. Leachables studies are planned to be set up to support the proposed DP shelf-life of 24 months, the T0 will be provided later on during the procedure. The applicant should commit to provide the updated results from the leachables study for assessment.

60. It is noted that some additional heightened characterization information will be added in the formulation development file. However, the awaited data were not detailed. Formulation development should be completed with characterisation studies showing the homogeneity of the suspension during storage at long term or accelerated conditions, after freeze/thaw, or after dilution with 0.9% NaCl should be studied.

61. Development data showing homogeneity of LNP or RNA concentration in the vials during filling process should be provided.

62. Overall control strategy was presented but some parameter and ranges may be updated after PPQ and additional characterization studies completed. As for assessment of overall control strategy, a complete set of data and information is needed, this document will be assessed when finalised. A time-plan for the submission of the final data set of the control strategy should be provided.

63. The compatibility studies of the diluted suspension in the vial and in syringes were performed with DP diluted to 0.05 mg/mL while dilution for administration is intended to be 0.1 mg/mL: it should be confirmed that the analytical methods are valid at this dilution. Moreover, the specifications applied for RNA content and RNA integrity (+/- 20% of T0) are not acceptable; in use specifications should be the same as the shelf-life specifications. It is noted, however, that this section may be updated as additional studies are completed. The applicant still needs to define in P.8 and the SmPC/PIL the in-use shelf-life and storage conditions after dilution and first use, in line with available data.

Manufacture (P.3)

64. The batch formula should be completed with process aids.

65. The lipid nanoparticle (LNP) formation is one critical manufacturing step and some additional information is requested regarding this step.

a. The range number of DS bags and DS batches to be thawed should be stated.

b. According to pharmaceutical development (Section P.2.3.4) 2-8 parallel T-mixer may be used depending on the batch size and manufacturers equipment. In the description of manufacturing process (Section P.3.3) it is stated that "one or more" T-mixer(s) are used. The number of T-mixers should be defined in Section P.3.3 and the dossier should be updated accordingly.

c. A drawing of the T-mixer including further details should be provided, e.g. geometry and dimensions.

66. It is noted that some parameters and ranges may be updated after PPQ and additional characterization studies completed. These updates could have an impact on overall assessment of the manufacturing process description, leading to additional issues. From the first assessment, the manufacturing process description should already be completed with the following information:

    a. The environment grades should be indicated for each step;

    b. holding times will be assessed when complementary data will be available.

67. The applicant should clarify if the 0.2 µm-filter used for bioburden reduction is identical with the 0.2 µm-filters used for sterile filtration.

68. It is stated that OOS result for in-process controls would trigger an evaluation of the deviation to determine if the batch could be further manufactured. It should be confirmed that OOS results for acceptance criteria will lead to batch rejection.

69. The validation protocol should be completed with the minimum number of consecutive batches at commercial scale to be included in the PPQ validation process, which should not be less than 3 batches. DS thaw parameters should be studied. Each thawing method (controlled room temperature thaw or controlled thaw) should be validated on at least one batch. Moreover, the mixing speed during dilution of DS should be added in the list of studied parameters.

70. For PPQ, to validate the TFF efficiency, residual ethanol and citrate should be measured with appropriate limits. During aseptic filling, a homogeneity test of the filled vials should be added with appropriate sampling and acceptance criteria. Finally, some acceptance criteria are "report results" with limits to be developed after sufficient manufacturing experience. This is not endorsed and acceptance criteria should be fixed before PPQ validation.

71. Acceptance criteria for quality attributes that are requested to be narrowed in the DP specification should be narrowed as well in the process validation protocol.

72. It should be confirmed that the Kleenpak Capsule with Supor EKV Membrane will be the one used for routine DP manufacturing at Puurs. If other filters are used, the extractables / leachables should be studied before use.

Control of excipients (P.4)

73. It should be confirmed that cholesterol will be controlled in line with Ph. Eur. monograph Cholesterol for parenteral use (2397) for future batches and not Ph. Eur. monograph Cholesterol (0993).

74. Additional test for microbial contamination should be included for all compendial excipients, except for water for injection. Further, where relevant, a test for bacterial endotoxins should be added unless otherwise justified.

75. Appropriate documentation for the processing aid excipients ethanol and citrate buffer and the excipients for drug substance buffer HEPES and EDTA is missing and should be provided.

76. DSPC is used in several medicinal products approved in EU and administered intravenously. According to the guideline on excipients in the dossier (EMEA/CHMP/QWP/396951/2006), an excipient used by a new route of administration may be considered as a novel excipient. Therefore, further discussion should be provided to justify why DSPC administered intramuscularly is not considered as a novel excipient and how data from intravenous administration can support safety of the excipient for this drug product.

77. Specifications for DSPC should include a test for purity of stearic acid, identity of phosphorus, and the assay specification (90.0-110.0%) should be tightened in line with batch results from the supplier.

78. For cholesterol and DSPC, the analytical methods for residual solvents and microbial purity should be described in detail (e.g. detailed chromatographic conditions for GC, sample and standards preparation, detailed calculation formulae for the GC method and respectively the actual method of preparation and count for microbial purity).

79. Unless otherwise justified, controls for the absence of RNase should be included in the specification for excipients, especially Water for Injections.

Control of drug product (P.5)

80. In all of the in-house analytical methods used in the release of DP, method descriptions are based on "examples" of procedures, controls and standards as well as on "typical" system operating parameters. These terms raise uncertainties regarding the developmental stage, and the control of critical steps of these assays. The analytical methods used in the control of DP are expected to be finalized. The applicant is requested to confirm this and to update the relevant parts of the dossier with unequivocal method descriptions and additional details, if needed. The applicant should also confirm that any significant changes in analytical procedures will be applied for in a variation application.

81. The information in the dossier does not support that any of the in-house analytical procedures applied for DP has been properly validated in line with ICH Q2. The validation summaries provided are far too brief and important details are missing. The Applicant should submit more

comprehensive validation summaries of all non-compendial methods, for example in the form of short validation reports. The validation summaries should include all relevant calculations, acceptance criteria, description of and results obtained for individual samples. Chromatograms and dose response curves should be included, where applicable. Module 3.2.P.5.3 of the dossier should be updated accordingly.

82. With the exception of osmometry, volume of injections in containers, HPLC-CAD (lipid identities) and RT-PCR (identity of encoded RNA sequence), which are performed only at DP release, all other analytical procedures are conducted at release and stability studies for drug product. It is stated by the applicant in section 3.2.P.5.6 that the acceptance criteria used for stability during shelf life will be the same as the acceptance criteria used for lot release. This is found acceptable, however, the applicant should confirm that the same acceptance criteria are valid both at release and end-of-shelf-life for the drug product. The specifications document in 3.2.P.5.1 could preferably be updated to include a separate column for the end-of-shelf-life specifications.

83. Test method numbers are missing and should be given to all analytical procedures used in the specifications for release and end-of-shelf-life and should consequently be inserted in the drug product specifications document and to the descriptions and validations of analytical procedures. Sections 3.2.P.5.1, 3.2.P.5.2 and 3.2.P.5.3 should be updated accordingly.

84. LNP size for drug product is measured by dynamic light scattering (DLS) and the efficacy of the drug product depends on the size of the LNP. The proposed acceptance criteria of 40 to 180 nm seem wide compared to clinical batch data that is found in the range of 59-74 nm for the small scale clinical batches ("classical LNP process) and 68-71 nm for the emergency supply ("upscale" LNP process). The acceptance criteria should therefore be tightened to be in line with what has been qualified in the clinical studies or clinically qualified by other means and set such that a clinically qualified level is assured throughout the shelf-life of the drug product.

85. The mRNA extraction step needed for determination of the identity of BNT162b2 DP should be included in the description of the RT-PCR-based assay and this step should be appropriately addressed in the method validation procedure.

86. With respect to the cell-based flow cytometry method used to confirm the in vitro expression of SARS-CoV-2 spike protein encoded by the RNA in BNT162b2 DP:

    a. Information regarding critical reagents (such as antibodies), drug product control samples and equipment used should be provided in the dossier. The robustness of the method should be appropriately demonstrated in the validation exercise, if different reagents, e.g. different clones or different vendors for the antibodies or different instruments, are envisaged.

    b. It is stated that exact shapes and locations of gates are expected to be different between instruments and that gates will be shaped and sized to select for the relevant cell populations. The gating strategy should be established, clearly defined and a description of the rationale for establishing the gating strategy should be provided. Possible changes observed between different equipment should be appropriately cross-validated.

c. Complete examples of results (including the three population: P1, P2 and P3) should be provided for NC, DPC and TS samples

d. In the table defining assay acceptance criteria, a limit of >30% is established for results obtained using drug product control samples. In order to unequivocally demonstrate the suitability of this method, the lower limit strategy should be replaced by a target/interval value. A value of, or close to, 30% is considered too low for the demonstration of method suitability and should be updated based on relevant data.

e. The relevance of the results obtained in the in vitro expression test using a HEK293 cell line for the in vivo intended targeted cell population should be further discussed and, ideally, substantiated with characterization data, unless otherwise justified. Additionally, information on characterisation of the HEK293 cell line used, including specifications should be provided.

f. The cell culture and transfection steps included in the potency method should be appropriately considered in the method validation strategy. For example, substantial variation in the culture parameters (such as passage number and seeding densities) are allowed for HEK293 cells used in determining DP in vitro expression. Unless otherwise justified, these possible variations should be addressed in the validation exercise when investigating assay robustness

g. High variability is claimed in the comparability exercise in P.2.2; in method validation it is noted that variability (% RSD) decreases significantly with sample size (%RSD is 18% for 150ng (sample size per method) and 7.1% for 250ng). It should be discussed if the method is optimized for the intended use and this should be confirmed with comparability results with commercial scale batches.

87. In-vitro expression is a cell-based flow cytometry assay. The assay was implemented recently and the proposed acceptance criteria of ≥30% cells positive seem wide compared to the limited batch release data available to date, i.e. emergency supply lots that is in the range of 63-65%. In addition, some data are presented for the small-scale clinical batches used in comparability testing, where data are found in the range of 50-71% (Table 3.2.P.2.3-5 in the dossier). The proposed acceptance criteria need to be thoroughly justified and tightened in line with the levels qualified in clinical studies or clinically qualified by other means. This justification should include the applicant's total current knowledge of the drug product.

88. The proposed acceptance criteria of ≥80% for RNA encapsulation seem wide compared to clinical batch data that is found in the range of 92-94%. The proposed acceptance criteria for RNA encapsulation should therefore be tightened based on clinical qualification or clinically qualified by other means and set such that a clinically qualified level is assured throughout the shelf-life of the drug product.

89. The specification range of each lipid appears somewhat broad, but the acceptance criteria are found acceptable. However, to further strengthen the control strategy given that a fixed molar ratio of cationic lipid and RNA is critical for LNP formation, acceptance criteria for the molar ratio N/P should be included in the specification unless further justified.

90. The method description and validation summary of the rapid sterility test should be provided during the procedure.

91. A specification should be included for free lipids or the applicant should justify that the control strategy is sufficient in this regard. In addition, no information and discussion are provided on the lipid-related impurities originating from the degradation of the lipid nanoparticles and such data needs to be provided.

92. A risk assessment with respect to the potential presence of elemental impurities in the drug product based on the general principles outlined in Section 5.1 of ICH Q3D should be performed. A summary of this risk assessment should be submitted. The risk assessment should cover all relevant elements and sources in accordance with the guideline. The summary must enable a quantitative comparison of observed or predicted levels with the PDE:s given in the guideline. It should contain what is necessary to evaluate the appropriateness and completeness of the risk assessment, including any assumptions, calculations etc. made. The control strategy for elemental impurities should be justified based on the risk assessment.

93. The specification for LNP polydispersity index should be tightened in line with batch results for clinical batches, i.e. NMT 0.2 (0.22 observed on stability).

94. Detailed description of analytical methods should be provided in P.5.2; these details should be in line with the validation data:

    a. for all methods, a list of materials needed for analysis

    b. for the DLS method for particle size and polydispersity, further details of the instrument and the sample size

    c. for the fluorescence assay method: the surfactant and its concentration, sample and standard concentration and the range of the calibration curve.

    d. for the CAD method, the sample diluent.

    e. for the potency in vitro by cell based flow cytometry: the Drug Product Control (DPC) (e.g. qualification), for the flow cytometer acquisition: complete examples of results (including the three population: P1, P2 and P3) should be provided for NC, DPC and TS samples, and Assay and Sample acceptance criteria rationale should be explained and justified.

    f. for the RT-PCR method: criteria for the selection of primers used for the test.

95. Validation data for the CGE (RNA integrity) is referred to the drug substance section S.4.3. However, as the active substance is formulated (RNA is encapsulated in the LNP formula), the appropriate validation parameters for the drug product (specificity, accuracy, sensitivity, robustness) should be addressed.

96. Method transfer plan was not submitted in the RR but is requested to be discussed in the next submission. For the non-compendial tests, it should be confirmed that the validation acceptance criteria for the receiving sites will be the same as for the transferring site (which will be assessed during the RR). For the analytical methods where comparative analysis will be proposed, it should

be confirmed that the acceptance criteria will be the same as for the intermediate precision validated at the transferring site (and assessed during RR).

Reference standards or materials (P.6)

97. It should be clarified for what release and stability testing methods the reference standard (including the CRM) is used today and will be used in the future. The function of the reference standard should be briefly stated for each assay, i.e. results of evaluation/normalisation, sample compliance, assay control etc. This information could be provided preferentially in a tabulated form.

98. Since the Applicant intends to establish primary and working reference standards, information on the preparation, qualification and stability of the PRS and WRSs should be provided.

Stability (P.8)

99. The proposed initial shelf-life for the drug product is 6 months at the recommended storage temperature of -90 to -60°C. In order to support the suggested shelf-life for drug product updated reports from the ongoing stability studies should be provided.

100. It should be confirmed that future extensions of the assigned DP shelf life will be applied for in formal variation applications. The following statement should be removed for Module 3.2.P.8.1 of the dossier; "The sponsor will extend the assigned shelf life without notification providing the real time stability data at the intended storage condition is acceptable and within commercial specifications."

101. Results on photostability testing as well as temperature cycling studies are pending to date and needs to be provided for assessment.

102. The applicant should confirm that they commit to continue all the ongoing stability studies at long-term conditions until completion.

103. It should be confirmed that the specifications for the bromobutyl stopper include the tests in the Ph Eur 3.2.9, including the self-sealing test, and that the self-sealing test is still acceptable after the stopper exposure to freezing (down to -90°C) and thawing, since the vial is a multi-dose container intended for 5 doses.

104. The applicant needs to clearly define in P.8 and in the future SmPC/PIL in line with available data and practical needs:

    a. the shelf-life under recommended, refrigerated, and ambient conditions

    b. the in-use shelf-life and storage conditions after dilution with saline and after first use

    c. a storage condition to keep the vial in outer carton and protect from light, before and after dilution (since multi-dose container).

Appendices (3.2.A)

Viral safety

105. Regarding the Pyrophosphatase, T7 polymerase and RNase inhibitor, spermidine and DNase I provide a certificate stating that no product of biological origin has been used during the manufacture (production and purification) or provide adequate virological documentation, with regard to viruses and unconventional transmissible agents (NCTA or prions, compliance with EMEA/410/01 Rev.3 requirements) where applicable, for each of the components concerned.

106. Regarding the four lipid excipients: ALC-0315, ALC-0159, DSPC and Cholesterol provide a certificate stating that no product of biological origin has been used during the manufacture (production and purification) or provide adequate virological documentation, with regard to viruses and unconventional transmissible agents (NCTA or prions, compliance with EMEA/410/01 Rev.3 requirements) where applicable, for each of the components concerned.

Novel excipient – ALC-0315

Based on the limited information no final conclusion can be drawn on chemical synthesis, quality control of starting material, specification limits for impurities and retest period.

107. The commercial batch size should be provided.

108. The specification limit for assay (85-115%) is considered wide and should, if possible, be tightened. The specification limit should be re-evaluated as more batch data are available and then specification limits for impurities are set, i.e. the mass balance should be taken into account.

109. The method description should include the GC chromatography parameters.

110. A brief summary of validation of the GC method is provided. Extended information in form of a short validation report including relevant data, chromatograms and calculations should be submitted.

111. It should be confirmed that the packaging materials are conform to Ph Eur or EU regulation 10/2011 amended.

Novel excipient – ALC-0159

Based on the limited information no final conclusion can be drawn on chemical synthesis, quality control of starting material, specification limits for assay impurities and retest period.

112. The synthesis scheme is illegible, a readable scheme should be provided.

113. The commercial batch size should be provided.

114. The method description should include the GC chromatography parameters.

115. A brief summary of validation of the GC method is provided. Extended information in form of a short validation report including relevant data, chromatograms and calculations should be submitted.

116. A test for molecular weight and polydispersity should be included unless otherwise justified.

117. It should be confirmed that the packaging materials are conform to Ph Eur or EU regulation 10/2011 amended.

5.2. Non-clinical aspects

Major objections

None

Other concerns

Pharmacology

1. Applicant should provide a more detailed clarification of the mode of action of BNT162b2, e.g. which cells types will take up the LNP, translate the modRNA and express the S-protein on the surface. Moreover, which cell types/organs will be targeted by the immune defence system, when the vaccine is in action. Further information on the potential activity/mode of action of the two novel excipients should be provided. *([confidential information deleted])*

2. In study 20-0211, regarding the results obtained from the Western Blot, a semi quantitative analysis of the results to improve the readability of the protein expression should be provided and in the analysis of the blot, some missing scientific information and explanations should be added by the applicant *([confidential information deleted])*:
   a. The presence of the two bands for BNT162b2 ARN (at 100 KDa and 190 KDa respectively)
   b. The 76.5 kDa bands are not observed in both BNT162b2 nor in S1 control lanes, and the full S protein at 141.14 kDa is also not observed in BNT162b2 lane.
   c. The lack of an important expression for the S1 protein ctrl at 76.5 Kda

3. Regarding the structural and biophysical characterization, the applicant is asked to provide *([confidential information deleted])*:
   a. A schematic description of both variants, (V8 and V9) so as to identify the exact position of optimized codons in the sequence and including coding and non-coding sequences...
   b. A comparison between the V8 and V9 codon sequences, highlighting their differences on mΨU and cytosine residues. The exact position of these optimized codons inside the modRNA sequence should be provided
   c. An estimation of mΨU relative content in both V8 and V9 sequences and a discussion on the potential difference in immunogenicity between these two variants. Changes in cytosine and mΨU content can significantly change the modRNAs immunogenicity.
   d. A comparison on the protein expression obtain from both variants (V8 and V9) to ensure that the expected protein is expressed in non-clinical models.

4. The modRNA contains a substitution of 1-methyl-pseudouridine for uridine. This substitution decreases recognition of the vaccine RNA by innate immune sensors, such as toll-like receptors

(TLRs) 7 and 8, resulting in decreased innate immune activation and increased protein translation. Vaccination with modRNA is expected to induce robust neutralising antibodies and a concomitant T cell response to achieve protective immunity. Nevertheless, no further discussion was provided regarding the risk of autoimmune responses induced by the modRNA. The Applicant is invited to further discuss the possibility that the mRNA vaccine can trigger potential autoimmune responses and how do it plan to possibly evaluate their occurrence *([confidential information deleted])*.

5. The applicant is requested to provide a more extended discussion on the choice and relevance of the pharmacological animal models (also with regard to the choice of the rat as a toxicological animal model) and chosen endpoint in the pharmacological-immunological assessment (e.g. lack of assessment of long-term memory responses, no assessment of old age-dependent effects) *([confidential information deleted]).*

6. Concerning study R-20-0085 on the immunogenicity in mice of the LNP formulated modRNA encoding the viral S protein (V9):
    a. The applicant is asked to justify the absence of IgG2A and IgG1 characterization for RBD *([confidential information deleted])*;
    b. The applicant is asked to justify why the results were not expressed in titers that would also allowed comparisons across experiments. Indeed, comparison with pVNT experiments expressing results in titers could help to determine the levels of neutralizing and non-neutralizing antibodies present in the sera *([confidential information deleted])*.
    c. In the study report R-20-0085 section 4.5.3.1 a discrepancy was found between text (1, 5 or 10 ug/animal) and table of treatment schedule (0.2, 1 and 5 ug); the Applicant should clarify which is the correct piece of information *([confidential information deleted])*.
    d. Concerning the *Multiplex analysis of cytokine release from murine Splenocytes Day 28 after Immunization with BNT162b2,* it is referred to *immunization with "5"* as compared to *"1 ug" BNT162b2* for the Luminex analysis in the Pharmacology written summary, page 18 (last paragraph) as compared to in the report R-20-0085, respectively, this discrepancy could be clarified. Moreover, it is noted that high levels of the Th1 cytokines IFNγ and IL-2 in multiplex immunoassays were detected after re-stimulation with the S but not RBD overlapping peptide mix, although RBD is part of the S protein. This could be further clarified or commented *([confidential information deleted])*.

7. Regarding Study VR-VTR-10671: BNT162b2 (V9) Immunogenicity and Evaluation of Protection against SARS-CoV-2 Challenge in Rhesus Macaques *([confidential information deleted])*:
    a. The applicant needs to precise for the Luminex data how the reference curve has been constructed, what does represent the arbitrary U/ml used and how it is referring to the serum dilution factor;
    b. The applicant is asked to define the criteria for choosing a 10-30% infection rate of Vero cells
    c. Methods to quantify antibody production in the different experiments differ and consequently cross-comparison between experiments is difficult. Indeed, it is important to distinguish neutralizing antibodies from non-neutralizing antibodies. In this study, total antibody response is measured using a luminex assay and results expressed on U/ml and for the neutralization assay results are expressed in VNT 50. The applicant needs to provide an estimation of the non-neutralizing antibodies in the whole antibody response.
    d. It is important to notice that on figure 6 of study report, neither panel A nor panel B highlight the consumption of IgG S1 binding antibodies after challenge nor the increase due to B memories response following the challenge: this would need to be further discussed by the Applicant

8. The report VR-MQR-10211, on S1-binding rhesus macaque serum IgG levels detected by a direct binding Luminex immunoassay, was not provided. This should be submitted *([confidential information deleted])*.

9. The data from the individual animals should be provided for the *RT-qPCR test for presence of SARS-CoV-2 RNA after SARS-CoV-2 Challenge in BNT162b2 (V9) immunized nonhuman primates ([confidential information deleted])*.

10. In the NHP pharmacology and in the toxicology studies the control group is immunized with PBS and not with a mRNA in LNP expressing a non-correlated antigen. The Applicant is invited to further discuss the potential effect of the formulated mRNA on the immune response and toxicity *([confidential information deleted])*.

11. The Spike protein of SARS-CoV-2 undergo mutations, and it thus critically important to investigate the biological significance of these variants in relation to the development of Spike-based covid-19 vaccine candidates. For example, Korber et al. 2020 present evidence that there are now more SARS-CoV-2 viruses circulating in the human population globally that have the G614 form of the Spike protein versus the D614 form that was originally identified from the first human cases in Wuhan, China. Further, Li et al., states that as of May 6, 2020, 329 naturally occurring variants in Spike protein have been reported in the public domain. The applicant is asked to discuss how the chosen Spike antigen variant in BNT162b2 relates to the Spike variants currently on the dominant SARS-CoV-2 viruses circulating in the human population *([confidential information deleted])*. References: Korber et al., 2020: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7332439/ Li et al., 2020: https://doi.org/10.1016/j.cell.2020.07.012

12. The rhesus macaques were challenged with the SARS-CoV-2 USA-WA1/2020 isolate. To our knowledge, this strain does not contain the D614G mutation. This mutation is reported to rapidly accumulate in the circulating SARS-CoV-2 strains and may increase the infectivity several-fold compared to the original Wuhan-1 strain. The applicant is asked to discuss the relevance of the NHP challenge study results in relation to the strain used for challenge and the strains circulating in the human population *([confidential information deleted])*.

13. Overall, the challenge study appears questionable in its design and hardly supports the robustness of the immunological response. The above limitations can be listed regarding the model:

    a) Absence of clinical signs in control and challenged NHP,
    b) Use of juveniles NHP,
    c) Lack of females NHP,
    d) One out of three age-matched saline control-immunized (n=3) male rhesus macaques not responding to challenge (no viral RNA neither in the BAL and nasal swab),
    e) Low numbers of animals with a low statistical significance
    f) Questionable selection of titer of the viral challenge (1.05. 10^6 PFU)
    g) In the NHP pharmacology study (Study VR-VTR-10671), rhesus macaques were immunized on days 0 and 21. Some other covid-19 vaccine candidates have different prime-boost intervals, such as 4 weeks for both ChAdOx1 (Graham et al., 2020) and mRNA-1273 (Corbett et al., 2020). Considering that the time between the first and second vaccine dose may have a significant impact on the immunological response, the applicant is asked to provide the rationale for the chosen prime-boost interval (21 days) *([confidential information deleted])*. References: Graham et al., 2020:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7385486/ Corbett et al., 2020: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7449230/

Moreover, some important data are missing to date:

h) Lung histopathology and immunochemistry, mentioned by the Applicant as ongoing, should be provided.
i) Absence of cytokines measurement in the NHP BAL

The applicant is asked to discuss all these limitations and should provide further scientific information on the NHP model relevance. Although the model is considered adequate to demonstrate immunogenicity, and viral clearance, it is considered insufficient to demonstrate efficacy against the disease *([confidential information deleted])*.

Pharmacokinetics

14. Quantification of ALC-0315 and ALC-0159 in plasma, liver homogenates, urine, and faeces homogenates was conducted by LC-MS/MS in an in vivo PK study (PF-07302048_06Jul20_072424). No validation data for the LC-MS/MS method in the non-GLP IV PK study in rats (Study PF-07302048_06Jul20_072424) had been presented. The Applicant is requested to provide qualification data for this method *([confidential information deleted])*.

15. The Applicant is asked to justify the choice of an IV study instead of an IM study in the non-GLP IV PK study in rats (Study PF-07302048_06Jul20_072424), which would have a more clinical relevance. The difference observed in terms of PK absorption should be discussed *([confidential information deleted]).*

16. It's worth to notice that the lipid displaying a persistent kinetic over time in liver is ALC-0159, ie the one that does not contain any PEG, although PEG is known to be used to increase half-life of many recombinants. The Applicant will have to justify this observation, as well as to discuss the difference of kinetics profile between the two lipids. The Applicant is also requested to estimate the delay of the clearance of the ALC-0315 from the liver, as this could have an impact on the safety profile *([confidential information deleted])*

17. The Applicant mentions that the LNP-formulated luciferase-encoding modRNA tested in this study have the exact same lipid composition than BNT162b2. It is however not clear to understand which of the three tested LNP formulation is present in the drug product, BNT162b2 variant V9. The Applicant should comment. *([confidential information deleted])*

18. There are uncertainties regarding the biodistribution study performed with the surrogate luciferase reporter RNA. The applicant is therefore asked to provide more information regarding the biodistribution assay:

    a) The applicant is asked to justify and discuss the choice of using a non-validated/non-qualified bioluminescence method to determine the biodistribution of a reporter luciferase protein instead of detecting the actual BNT162b2 modRNA. The justification should include a discussion on the sensitivity of the method. *([confidential information deleted])*

    b) It can be noted that there is no information on the similarities of the mRNA modifications of the non-coding regions between the luciferase modRNA used in the

study and the modRNA used in BNT162b2. The applicant is asked to provide more information on the luciferase reporter RNA, and in particular, whether the untranslated sequences are similar to that of the BNT162b2 modRNA and therefore at least the stability of the mRNAs are somewhat comparable. *([confidential information deleted])*

c) The biodistribution of the vaccine has been evaluated in mice, using 2 µg mRNA (encoding for luciferase). In humans and in the repeat-dose study in rat using the V9 version, 30 µg (per administration) was used. It is not clear if this difference in RNA concentration results differences in the amount of LNP used. The applicant is therefore asked to clarify if there were differences in the amount of LNP used in the biodistribution study and the repeat-dose study /clinical trials and if so, discuss how this could affect the distribution and safety evaluation observed in the clinic compared to non-clinical data. *([confidential information deleted])*

d) The applicant is asked to consider the possibility of a broader biodistribution pattern than observed and discuss the possible safety consequences of a wider biodistribution profile of BNT162b2. *([confidential information deleted])*

19. Luminex-based multiplex assay:

a) In view of potential acute immunotoxicity mediated by LNPs, does the Applicant possess data on other timepoints (earlier than 6h or beyond) regarding the cytokines measurements? *([confidential information deleted])*

b) The Applicant is asked to discuss the absence of an in vitro hPBMC stimulation assay on healthy donors to assess reactogenicity. *([confidential information deleted])*

c) Extrapolating to clinics, the Applicant is requested to discuss the level of IL-6 cytokines induced by LNPs considering that asymptomatic but infected subjects candidate to vaccination, could display higher IL-6 levels during early phase infection. *([confidential information deleted])*

Toxicology

20. Further discussion is requested on how the immunological response to the vaccine observed in rats, the species used in the toxicological studies, compares to that observed in Rhesus monkeys, the species used in the virus challenge study and, if possible, humans *([confidential information deleted])*.

21. The qualitative and quantitative composition of the lipids constituting the LNP are not specified in the final study report #38166. It is thus not possible to check the composition of LNP; this point appears crucial as it is expected that the toxicity associated with modRNA formulated in LNP formulations is expected to be driven primarily by the LNP composition: This will have to be specified *([confidential information deleted])*.

22. Both the "V8" and "V9" repeat-dose toxicity rat studies indicate functional hepatic and/or biliary effects (enlarged liver, vacuolation, increased gGT, ALT and ALP) which may or may not be linked to the LNP and which requires further discussion *([confidential information deleted])*.

a. The applicant is requested to provide a discussion on the clinical relevance of these findings, and the need for a notation in the SmPC *([confidential information deleted])*.

b. The discussion should include the mechanism underlying the elevated plasma activity of liver/biliary enzymes and its potential relation to LNP lipids *([confidential information deleted])*.

c. The discussion should also include the findings of vacuolation of hepatocytes (minimal to mild) that was present in the portal regions of liver for all BNT162b2 (V8)-dosed animals (19 of 20 animals) at the end of the dosing phase *([confidential information deleted])*:

d. Confirm that the same LNPs composition was used amongst all treated groups (from a qualitative and quantitative point of view);

e. Explain and discuss the difference in vacuolation occurrence between sexes for all treated groups as well as the absence of vacuoles in the V9 study;

f. Justify the occurrence of vacuolation in hepatocytes, while this effect is usually seen with phagocytes and not hepatocytes;

g. Discuss the short delay of occurrence as well as the mechanism underlying these vacuoles at the time of sacrifice (i.e. only 3 IM weekly injections) (i.*e. Development of anti-PEG antibodies? adaptive response with or without functional change? imaging to determine if vacuoles contain PEG?).* Of note, the accumulation in the liver was mainly observed with ALS-0315 that does not contain PEG, in contrast of ALC-0159 which does contain PEG 2000. Discuss their potential toxicity from a non-clinical and clinical point of view.

h. In light of the *in vivo* PK study, showing persistence of ALC-315 inside liver (slow elimination kinetics inside liver) and considering the presence of PEG in the formulation of ALC-0159, the Applicant is requested to document more in depth the role/implication of ALC-0159 and ALC-315 in the occurrence of vacuolation of peri-portal hepatocytes *([confidential information deleted])*.

23. The Applicant will also have to detail and further document the elevated serum levels of the cytokines IFN-gamma, TNF-alpha, IL-1beta, IL-6, and IL-10 that were noted in the control group of study #38166 *([confidential information deleted])*.

24. Complement (C) activation-related pseudoallergy (CARPA) can be a serious side effect of liposomal drugs, biologicals, and many other modern therapeutic and diagnostic agents. The Applicant is asked to discuss the absence of quantitative and targeted assays of C3c and C4 proteins *([confidential information deleted])*.

25. The applicant is requested to provide an extended discussion on the distribution and metabolism of the novel excipient lipids (ALC-0315 and ALC-0159), their potential genotoxicity of the acetamide moiety in the lipids (which is classified as possible human carcinogen (IARC Group 2B) with debated genotoxic mechanism) in the context of the rat liver observations *([confidential information deleted])***.**

26. Some toxicological studies remain to be submitted: the full report for the #20GR142 study and the interim and full report for the DART study.

    a. The Applicant is also asked to provide at the next NC roll of submission, a detailed timeline for availability of preliminary data *([confidential information deleted])*.

    b. With regard to the DART study, a justification of the study design is requested to determine the value of this study for evaluation of the developmental risk in humans.

Primarily the choice of the rat as relevant animal species (rodent placental antibody transfer during the latter part of gestation is not considered similar to human antibody transfer during the third trimester of gestation) and the design of the dose regimen (whether this will lead to sufficient antibody transfer during lactation, which is equivalent to the third trimester exposure in humans) will need attention *([confidential information deleted])*.

27. In terms of GLP compliance, concerns have been raised by the assessors during the review of the non-clinical report amendment of the study #38166 *([confidential information deleted])*.

    About the test items and formulations:

    Page 26: The test item EE4 (G7) is designed as: LNP formulated modRNA encoding the RBD subunit of SARS-CoV-2 S protein ("BNT162b - 2"): what is the difference with b1 designed by the same terminology. b2 is not supported to code the full-length spike S glycoprotein?

    BNT162b - 2 is also associated with another name in the pathology report page 1563 ("LNP modRNA Sp2"). Is it the same test item? The applicant is ask to clarify the different terminologies used for the test item EE4.

    Information about stabilities of the test items during 6 hours at room temperature is not documented, whereas the test items were administrated 6 hours after thawing at room temperature. Could you provide evidence that the test items are stable for 6 hours at RT.

    Calculation of dose concentrations to be administrated to animals are difficult to be understood for Group7 (BNT162b2 as test item), if 200µl per animal is administrated, the total concentration seems to be 110µg and not 100ug as stated. The applicant should clarify.

    About the management of the study:

    A mistake in the conclusion page 61 concerning the sex of animal No.179 having eschar has been observed. It seems to be a male, and not a female as stated. And this presence of eschar was not found in the table for individual clinical signs page 152 for this female No.179. Same comment for male No.162 (reddened skin reported page 62) not found page 138. The applicant should explain these discrepancies and correct these issues.

    The final report had been amended justified by changes qualified as minor or/and corrections following sponsor comments. Some corrections could be considered as not minor but major, because they put into relief real mistakes in the issuance of the final report: "eschar formation was incorrectly described with occurrence on test days 14 and 15 instead of on test day 14 only" ; "on haematology and coagulation the finding of an increased number of eosinophils in groups 4, 5 and 7 was missing" ; "on clinical chemistry the directions of changes for albumin and globulin levels were incorrectly stated as an increase in albumin and a decrease in globulin plasma levels instead of a decrease in albumin and an increase in globulin plasma levels"; "the incorrect test item 'BNT162b1' instead of 'BNT162a1' was stated for Group 3".

5.3. Clinical aspects

N/A

5.4. Risk management plan

N/A

5.5. Pharmacovigilance system

N/A

5.6. New active substance status

N/A

6. Recommended conditions for future marketing authorisation and product information in case of a positive benefit risk assessment

N/A

7. Appendices (as appropriate)

N/A