24 November 2020

EMA/CHMP/BWP/633407/2020

Human Medicines Division

BWP Rolling Review report to ETF / CHMP

Rolling Review #2 (1st CMC wave) – Adopted

COVID-19 mRNA vaccine (nucleoside modified)

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

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

**Biologics Working Party (BWP)**

MEETING ON 24 November 2020

Chair: S. Ruiz

Vice Chair: N. Kruse

| **1. BACKGROUND INFORMATION**  |
| --- |
| **Name of the product** | COVID-19 mRNA Vaccine BioNTech |
| **Applicant** | BioNTech Manufacturing GmbH |
| **Active substance (INN / Common name)** | COVID19 mRNA (nucleoside modified), BNT162b2, 5’capped mRNA encoding full length SARS-CoV-2 Spike protein |
| **Therapeutic indication** | TBD |
| **Rapporteurs** | **Filip Josephson (SE), Jean-Michel RACE (FR)** |
| **BWP Rapporteurs** | **M. Welin (SE), A. Mambole-Dema (FR)** |
| **BWP Project Manager** | **T. van der Stappen** |
| **Time of procedure at next CHMP** | **RR#2 OP (CMC)** |

**Product classification**

|  |  |
| --- | --- |
| **Product category** | Product features |
|  | Other/Novel type |
| Choose an item. | Choose an item. |
|  | Choose an item. |

**Key words**

|  |  |  |  |
| --- | --- | --- | --- |
| Active substance | Finished product | Process  (AS/FP) | Control |
| **Characterisation** | Excipients | Process validation | Specifications |
| **Impurities (e.g. genotoxic)** | Comparability | Choose an item. | Choose an item. |

| **2. BWP recommendation to the CAT/CHMP**  |
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In conclusion, based on the review of the quality data provided as part of the RR#2 (1st CMC wave), the BWP concludes that there are Quality Major Objections identified. The Applicant will also need to sufficiently address the Quality related Other Concerns. Other Quality elements in further rolling review cycles are expected to define the overall quality profile of the vaccine.

Details of the Major Objections and Other Concerns are given in Annex I to this report.

| **3. BWP discussion**  |
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Only a Quality dossier has been submitted and assessed during rolling review cycle 2 (RR#2, 1st CMC wave). The Quality dossier is not yet complete.

On behalf of the Rapporteurs, A. Barbu and S. Nilsson highlighted the main elements and outcome of the assessment. Three MOs have been raised; on the GMP status for DS and DP manufacturing sites, comparability between clinical and commercial material, and omission of data on DP manufactured at the commercial site. Comments have been received from Peer Reviewer and several Member States. Overall, BWP agreed with the outcome of the Rapporteurs’ assessment. The following issues were discussed in more detail.

Based on the latest information on the GMP inspection strategy as clarified by C. Facchini (EMA), it was agreed to slightly reword MO1 on the GMP status.

It was agreed that further qualitative/quantitative information is needed about truncated mRNA species and translated protein as part of the product characterisation. Any implication on product functionality and safety/efficacy should be discussed and the specification limits proposed for the commercial product need further justification. It was noted that process adjustments already introduced could improve product quality in terms of the critical quality attribute %RNA integrity. The MO2 on comparability was amended accordingly.

It was acknowledged that information on the control strategy and novel excipients is currently outstanding but since the Applicant has already indicated this information will be provided in the next submission, the BWP agreed not to raise this as a MO.

There was general agreement that most of the (MO) issues raised could be solved post-approval in case of positive B/R but the BWP considered that the %RNA integrity issue in relation to the safety/efficacy needs to be addressed prior to opinion.

| **4. Quality overview**  |
| --- |

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.

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.

Hold times

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.

Filter Qualification and Validation

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****VD:{all specifications Table 3.2.S.4.1-1}VS:{http://gdms.pfizer.com/gdms/drl/objectId/090177e19508a2ba }DC:{all specifications - quality attributes, analytcial procedure and acceptance criteria. Table 4-1. PPQ drug substance specification.}VT:{2}DL:{T}VO:{Denton, Mary}DV:{Nagarajan,Srinivasan Raj (SSNAGA) |15-Oct-20 17:50:19}VC:{}DI:{201015175016}** | **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 |
| 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-PCRb | 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 | qPCRb | ≤ 330 ng DNA/mg RNA |
| **Product Related Impurities** |
| dsRNA | Immunoblotb | ≤ 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 attributes 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.

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 mLVD:{product description}VS:{DMID D2000091-01 http://gdms.pfizer.com/gdms/drl/objectId/090177e194b8d442 }DC:{DMID describes 0.45 mL target fill at 0.5 mg/mL and in-vial dilution to total volume of 2.25 mL. Simple math derives the volume of 1.8 mL to be added (2.25 - 0.45 mL). DMID describes total content of 225 ug/vial. Simple math derives concentration of 100 ug/mL (225 ug/2.25 mL) and therefore equivalent concentration of 30 ug/0.3 mL.}VT:{2}DL:{D}VO:{Webb, Chandra}DV:{Thomas, Jade (THOMJ109) |13-Oct-20 2:22:39 PM}VC:{DV - Jade, 13-Oct-2020}DI:{20828202813}. 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).***



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***



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.***



*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.

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.

| **ANNEXES**  |
| --- |
| ***Annex I – List of Questions*** |

**Major Objections**

***GMP***

1. GMP status for DS and DP manufacturing sites is currently not acceptably demonstrated:
	1. 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.
	2. 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***

1. 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.
	1. 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.
	2. 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.
	3. 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.
	4. 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.
	5. 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.
2. 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)

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

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

1. 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.
2. 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:
	1. It should be indicated that the incubation time during GTP/N1-methylpseudo UTP bolus feeds is a global time for the 11 feeds
	2. 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.
	3. 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.
	4. 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)

1. 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.
2. 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.
3. 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*

1. 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.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
7. The manufacturing process to obtain the linear DNA template should be completed with the following information:
	1. The quantity of linear DNA template obtained in each batch should be stated
	2. The chemical agent used for chemical lysis of the cells should be mentioned and its clearance should be demonstrated to be sufficient.
	3. The mention “or equivalent” for the restriction enzyme should be deleted.
	4. The Applicant should confirm that implementation of changes in the manufacture of the linear DNA template will be applied for in a variation application.
8. 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.
9. 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.
10. The reference material for plasmid identity testing should be described.
11. 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)

1. 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)

1. Several validation studies and full PPQ data are still pending for the manufacturing process at Wyeth BioPharma, Andover. Therefore, additional information is needed:
	1. 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.
	2. A time-plan for the submission additional process validation data should be provided before marketing authorization approval.
2. 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)

1. 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.
2. 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.
	1. It needs to be further justified why these parameters are not classified as CPPs.
	2. 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.
	3. 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).
3. 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.
4. 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.
5. 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.
6. 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)

1. 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.
	1. A brief method description including conditions for protein expression, gel separation, and western blot assay should be provided.
	2. The expected protein size should be stated and supported by theoretical calculations.
	3. 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.
	4. The Applicant should provide data on protein expression in terms of percentage of successfully transduced HEK293 cells using the lipofectamine transfection system.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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)

1. 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)

1. 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.
2. Regarding the RT-PCR method for determination of DS and DP identity:
	1. Information regarding the positive control used in the should be provided.
	2. 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.
	3. 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.
3. Regarding the ddPCR-based method for determination of poly(A) tails in the mRNA DS:
	1. Information regarding the internal control used in the should be provided.
	2. 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.
	3. 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.
4. 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.
5. With respect to the immunoblot analytical method used for determination of dsRNA in BNT162b2 drug substance:
	1. 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.
	2. 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.
6. 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)

1. 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.
2. 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)

1. 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)

1. 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.
2. 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)

1. 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.
2. 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.
3. 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.
4. 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.
5. 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)

1. The following additional information should be included in Module 3.2.S-6 of the dossier.
	1. A certificate of analysis of one representative batch of the EVAM contact layer demonstrating compliance with Ph. Eur. 3.1.7.
	2. A specification for the container closure system including dimensions (currently only schematic drawings are included).
2. 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)

1. 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.
2. 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)

1. 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.
2. 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)

1. 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.
2. 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.
3. Development data showing homogeneity of LNP or RNA concentration in the vials during filling process should be provided.
4. 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.
5. 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)

1. The batch formula should be completed with process aids.
2. The lipid nanoparticle (LNP) formation is one critical manufacturing step and some additional information is requested regarding this step.
	1. The range number of DS bags and DS batches to be thawed should be stated.
	2. 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.
	3. A drawing of the T-mixer including further details should be provided, e.g. geometry and dimensions.
3. 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: (1) The environment grades should be indicated for each step; (2) holding times will be assessed when complementary data will be available.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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)

1. 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).
2. 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.
3. 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.
4. 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.
5. 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.
6. 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).
7. 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)

1. 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.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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:
	1. 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.
	2. 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.
	3. Complete examples of results (including the three population: P1, P2 and P3) should be provided for NC, DPC and TS samples
	4. 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.
	5. 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.
	6. 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
	7. 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.
8. 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.
9. 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.
10. 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.
11. The method description and validation summary of the rapid sterility test should be provided during the procedure.
12. 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.
13. 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.
14. 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).
15. Detailed description of analytical methods should be provided in P.5.2; these details should be in line with the validation data:
	1. for all methods, a list of materials needed for analysis
	2. for the DLS method for particle size and polydispersity, further details of the instrument and the sample size
	3. for the fluorescence assay method: the surfactant and its concentration, sample and standard concentration and the range of the calibration curve.
	4. for the CAD method, the sample diluent.
	5. 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.
	6. for the RT-PCR method: criteria for the selection of primers used for the test.
16. 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.
17. 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)

1. 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.
2. 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)

1. 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.
2. 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.”
3. Results on photostability testing as well as temperature cycling studies are pending to date and needs to be provided for assessment.
4. The applicant should confirm that they commit to continue all the ongoing stability studies at long-term conditions until completion.
5. 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.
6. The applicant needs to clearly define in P.8 and in the future SmPC/PIL in line with available data and practical needs:
	1. the shelf-life under recommended, refrigerated, and ambient conditions
	2. the in-use shelf-life and storage conditions after dilution with saline and after first use
	3. 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

1. 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.
2. 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.

1. The commercial batch size should be provided.
2. 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.
3. The method description should include the GC chromatography parameters.
4. 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.
5. 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.

1. The synthesis scheme is illegible, a readable scheme should be provided.
2. The commercial batch size should be provided.
3. The method description should include the GC chromatography parameters.
4. 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.
5. A test for molecular weight and polydispersity should be included unless otherwise justified.
6. It should be confirmed that the packaging materials are conform to Ph Eur or EU regulation 10/2011 amended.

| ***Annex II – New Active Substance status*** |
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1. Recommendation

Based on the review of the data the BWP considers that the active substance BNT162b2, 5’capped mRNA encoding full length SARS-CoV-2 Spike protein contained in BioNTech’s COVID-19 mRNA (nucleoside modified) vaccine is to be qualified as a new active substance in itself.

1. Executive summary
	1. Problem statement

The applicant requested the active substance BNT162b2 contained in the above medicinal product to be considered a new active substance in itself.Scientific evaluation

* 1. Quality aspects

Description of the COVID-19 mRNA Vaccine

The active ingredient of the COVID-19 mRNA Vaccine is a modified messenger ribonucleic acid (mRNA) encoding for a mutated full-length variant of the SARS-CoV-2 S protein. The RNA is encapsulated into lipid nanoparticles, which protect the RNA from degradation and enable transfection of the RNA into host cells after intramuscular injection for vaccination.

The mode of action relies on intracellular translation of the mRNA to generate the target protein. The antigen is incorporated into the cellular membrane or secreted into the extracellular environment to induce an adaptive immune response. The primary read-out are antibody titers for the SARS-CoV-2 S protein that are expressed on the viral membrane. The expressed SARS-CoV-2 S protein is expected to mediate an immune response to the virus in several ways: induction of neutralizing antibodies and T cell activation.

Structure of the active ingredient of the COVID-19 mRNA Vaccine

The mRNA structure is determined by the respective nucleotide sequence of the linear DNA used as template for in vitro RNA transcription and the 5'-cap analog. Of note, BNT162b2 uses N1-methylpseudouridine instead of uridine. In addition to the codon-optimized sequence encoding the target protein, the RNA displays common structural elements which have been optimized for maximal efficacy (5'-cap, 5'-UTR, sec, 3'-UTR, poly(A)‐tail).

New active substance status

The modified mRNA in the COVID-19 mRNA Vaccine is a chemical active substance that has not been previously authorised in medicinal products in the European Union. From a chemical structure point of view, the modified mRNA is not related or active metabolite or pro-drug to any other authorised substances. COVID-19 mRNA Vaccine is therefore classified as a New Active Substance and considered to be new in itself.

Justification for omission of a dedicated database search

A dedicated database research in specific databases has not been performed. This is justified as follows:

 The Coronavirus Disease 2019 (COVID-19) outbreak started in China in December 2019, and since then has spread to many countries in the world. COVID-19 is caused by a novel virus (SARS-CoV-2). The SARS-CoV-2 spike protein (S protein), which is encoded by the mRNA vaccine, is novel too. It was first described in early 2020. Based on research and review of all CHMP opinions and EC decisions from December 2019 onwards, one can conclude that the amino acid sequence of the target protein encoded by the mRNA does not share the same therapeutic moiety as active substance(s) previously authorised in medicinal product(s) for human use.

 The active ingredient of COVID-19 mRNA Vaccine is mRNA. To date, there is not a single mRNA product authorised in medicinal product(s) for human use that encodes for a specific target protein. The only RNA products approved in the EU are siRNAs that work by silencing the mRNA of proteins involved in causing a disease:

1) Onpattro (EPAR 2018) is a chemically-synthesized, double-stranded oligonucleotide. The sense strand and the antisense strand of the siRNA contain each only 21 nucleotides (EPAR). Onpattro is designed to interfere with protein production of an abnormal form of the protein transthyretin (TTR).

2) Givlaari (EPAR 2020) is a chemically-synthesized, double-stranded oligonucleotide. The siRNA is comprised of a combination of 2’ F and 2’ O-methyl nucleotides, conjugated to a triantennary N-acetyl galactosamine (GalNAc) ligand to facilitate delivery of the siRNA to the liver. Givlaari is designed to interfere with RNA production of the enzyme delta-aminolevulinate synthase 1 (ALAS1).

In summary, one can exclude that additional searches in chemical and drug databases such as ADISINSIGHT, EMBASE, IMSRESEARCH, etc. will reveal any mRNA product encoding for SARS-CoV-2 S protein.

1. Conclusions on quality aspects

Based on the review of data, BNT162b2 (5’capped mRNA encoding full length SRAS-CoV-2 Spike protein) is a biological substance not previously authorised in a medicinal product for human use in the European Union. Thus, BNT162b2 falls into first indent of “Annex 1 Definition of a new active substance” of the Notice to Applicants 2A chapter 1.

1. Overall conclusions

Based on the review of data, BNT162b2 (5’capped mRNA encoding full length SRAS-CoV-2 Spike protein) is a biological substance not previously authorised in a medicinal product for human use in the European Union.

1. List of questions

Not applicable.