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24 May 2023

s 9(2)(a)

[redacted]
Ref: H2023024241

Tēnā koe [redacted]

Response to your request for official information

Thank you for your request under the Official Information Act 1982 (the Act) to Manatū Hauora (the Ministry of Health) on 26 April 2023. You requested:

> *I would like the full disclosure of the above report that can be located at this link, which is the Australian version.*
> *https://www.tga.gov.au/sites/default/files/foi-2389-06.pdf*
>
> *As this copy is in the public arena, there is no reason that the New Zealand version is not available. The copy on the MoH website at this link only contains 5 pages and is unacceptable when the Australian version has released 55 pages.*
> *https://www.health.govt.nz/system/files/documents/information-release/h202106950-_response.pdf*
>
> *In order to give Informed Consent New Zealanders must be informed that this mRNA vaccine in fact bio-distributes systemically and does not stay at the injection site as the Government and other officials are telling us. The high concentrations are in our spleens, liver, adrenal glands and ovaries. It also gets into our bone marrow (highly concerning) and brains???? This is misinformation (stays at the injection site) and comes close to propaganda from the government.*
>
> *I want to know this bio-distribution information that the MoH holds before I consent to the booster, not after the event.*
>
> *Please release this report as there is no longer any need for it to be kept secret when we have access to the Australia version.*

Please find the Pfizer non-clinical evaluation report attached to this letter as Appendix 1. Some information has been withheld under the following sections of the Act, which is noted in the document itself:

- Section 9(2)(a), to protect the privacy of natural persons,
- Section 9(2)(b)(ii), where its release would likely unreasonably prejudice the commercial position of the person who supplied the information; and
- Section 9(2)(g)(ii), to protect Ministers, members of organisations, officers, and employees from improper pressure or harassment.

Where information is withheld under section 9 of the Act, I have considered the countervailing public interest in releasing information and consider that it does not outweigh the need to withhold at this time

I trust this information fulfils your request. Under section 28(3) of the Act, you have the right to ask the Ombudsman to review any decisions made under this request. The Ombudsman may be contacted by email at: info@ombudsman.parliament.nz or by calling 0800 802 602.

Please note that this response, with your personal details removed, may be published on the Manatū Hauora website at: www.health.govt.nz/about-ministry/information-releases/responses-official-information-act-requests.

Nāku noa, nā

Chris James
Group Manager
Medsafe

Medicine Evaluation; Non-Clinical Studies

FINAL

1 PRODUCT DETAILS

File number:	TT50-10853
Product name:	Comirnaty (COVID-19 mRNA vaccine) (Pfizer-BioNTech) 0.5 mg/mL concentrate for injection (TT50-10853).
Dose form:	Concentrate for injection
Drug substance and strength:	BNT162b2 [mRNA], 0.5 mg/mL (as 225 µg/0.45 mL) Each 0.3 mL dose of the diluted vaccine delivers 30 µg drug substance.

Evaluator: s 9(2)(a) [redacted]

By email to s 9(2)(g)(ii) [redacted]**, Acting Manager Product Regulation, Medsafe 28 Jan 2021 12:27 am**

Peer reviewer: s 9(2)(a) [redacted]

By email to s 9(2)(g)(ii) [redacted]**, Acting Manager Product Regulation, Medsafe 28 Jan 2021 10:32 am**

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Limited glossary / abbreviations

Abbreviation	Expansion
ACE2	Angiotensin Converting Enzyme 2 Receptor for
ADE	Antigen Dependent Enhancement
ALC-0159	2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide
ALC-0315	((4-hydroxybutyl) azanediyl) bis (hexane-6,1-diyl) bis(2-hexyldecanoate)
Bw;bwt	Bodyweight
DART	Developmental and Reproductive Toxicity
EM	Electron Microscopy
GMT	Geometric Mean Titre
HEK	Human Embryonic Kidney Cells
HCS	Human Convalescent Serum
IM	Intra muscular injection
IFN	Interferon
IL	Interleukin
LNP	Lipid Nano-Particles (specifically LNP8 unless otherwise specified)
MACS	Magnetic Antigen Cell Separation
MOE	Margin of Exposure
mRNA	messenger Ribonucleic acid
modRNA	nucleoside modified mRNA
OP	OroPharyngeal
P2	two proline mutations
pVN_{50}	A measure of the serum antibody Titre (The reciprocal of the serum dilution resulting in a 50% neutralization of a pseudo-virus). A higher value indicates a greater response/titre
pVNT	Pseudo Virus Neutralisation Titre
q.s.	Quatum satis
QSAR	Quantative Strutural Activity Relationship
RNA	Ribonucleic acid
RBD	Receptor Binding Domain
S protein	SARS-CoV-2 spike glycoprotein
TGA	Therapeutic Goods Administration (Australia)
Th1	T helper type 1 cells
Th2	T helper type 2 cells
TNF	Tumour Necrosis Factor
TTC	Threshold of Toxicological Concern
V8&9	Viral variants of SAR-CoV-2
VAERD	vaccine-associated enhanced respiratory disease

Non-Clinical Assessment

1 INTRODUCTION

This new medicine application is for a new biological entity, BNT162b2 [mRNA], hereafter referred to as BNT162b2 (BioNTech code number BNT162, Pfizer code number PF-07302048), developed by Pfizer and BioNTech. The drug product (COMIRNATY) is an RNA-based vaccine indicated for the active immunisation of individuals aged 16 (originally 18 in the TGA application but amended by the applicant) years and over against COVID-19 disease caused by the SARS-CoV-2 virus.

The vaccine will be administered intramuscularly (IM) in the upper arm (deltoid muscle) as a series of two 30 µg doses of the diluted vaccine solution (0.3 mL each) according to the following schedule: a single 0.3 mL dose followed by a second 0.3 mL dose 21 days later (prime/boost regimen).

The drug substance is a nucleoside-modified mRNA that encodes a prefusion stabilised full-length variant of the SARS-CoV-2 spike (S) glycoprotein and is manufactured by a cell-free *in vitro* transcription process. The final clinical variant and related developmental variant RNAs were encapsulated lipid nanoparticles (LNPs), which facilitate entry of the RNA into host cells. The RNA is translated in the host cells to the S protein, which induces a protective immune response in the vaccinated individual. The vaccine is formulated as a preservative-free concentrated suspension for injection, presented in a multi-dose vial. The product is supplied frozen (-80°C to -60°C) and must be thawed and diluted with sterile sodium chloride (0.9%) solution prior to administration.

The Interim TGA evaluation report (Nonclinical Evaluation Report (**Interim**), reference PM-2020-05461-1-2, 4 December 2020) has been provided to Medsafe by the TGA. The applicant has declared that the nonclinical data provided to the TGA is identical to that provided to Medsafe. The TGA Interim evaluation report has been used as the primary basis for this report. Studies evaluated previously by the TGA are not re-evaluated in the current report other than to verify the conclusions of the TGA against the submitted studies. Where the TGA has evaluated an interim report for a study that has subsequently been updated with the final data, or where a study has been submitted to MedSafe after the TGA has finalised its interim report, that study has been evaluated in detail, (see Section 1.1 and Table1).

The LNP component of the Pfizer vaccine formulation contains two novel excipient lipids, ALC-0159 (2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide) and ALC-0315(4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate). These lipids are a key aspect of the formulation contributing both to the particle size of LNPs and the stability of the mRNA in the formulation.

1.1 Data Update Status

The data available for this review includes study reports or components of study results that were not available for the TGA at the time of their interim report. The data noted as pending in the TGA report and the current status is as follows:

- The histopathological evaluation of the lungs of monkeys from the immunogenicity and protection study (Study No. VR-VTR-10671)
    - Provided as a component of the 10 December 2020 update to MedSafe and evaluated in the Detailed Evaluation Section of this report.
- The final report of the repeat dose rat study with the BNT162b2 (V9) including histopathology, immunology and recovery data (Study No.20GR142)
    - The full report has been made available and the additional data assessed in the Detailed Evaluation Section of this report.
- A developmental and reproductive toxicity (DART) study with BNT162b2 (V9), (Study No 20256434)
    - Supplied as a component of the 11 January 2021 update to MedSafe and evaluated in the Detailed Evaluation Section of this report.

The full list of studies provided for this Medsafe review, whether they have been assessed by the TGA and their status as interim or final is provided in Table 1.

Table 1. Data Available for this Review and that reviewed in the TGA Interim Report

Study No	Submission Date	TGA Review	Status	Comment
Primary Pharmacology				
R-20-0112	20/10/2020	Y	Final	
R-20-0211	20/10/2020	Y	Final	
VR-VTR-10671	20/10/2020	Y	Interim	Lung histopathology data not available for the TGA interim review.
VR-VTR-10671	10/12/2020	N	Final	Report includes lung histopathology data. Evaluated in section 4.1.1.1 of the Detailed Evaluations Section of this report,
VR-VTR-10741	11/01/2021	N	Final	Structural and biophysical characteristics of the Spike protein. Evaluated in section 4.1.1.1 of the Detailed Evaluations Section of this report.
R-20-0054	10/12/2020	N	Final	Immunogenicity of LNP V8. Evaluated in section 4.1.1.1 of the Detailed Evaluations Section of this report.
R-20-0085	10/12/2020	Y	Final	
R-20-0357	10/12/2020	N	Final	Cytokine/chemokine release from human mononuclear cells. Evaluated in section 4.3.9 of the Detailed Evaluations Section of this report.
R-20-0360	10/12/2020	N	Final	In vitro expression of spike protein by V8 & V9. Evaluated in section 4.1.1.1 of the Detailed Evaluations Section of this report.
Pharmacokinetics				
072424	20/10/2020	Y	Final	
R-20-0072	20/10/2020	Y	Final	
01049-20008	20/10/2020	Y	Final	

01049-20009	20/10/2020	Y	Final	
01049-20010	20/10/2020	Y	Final	
01049-20020	20/10/2020	Y	Final	
01049-20021	20/10/2020	Y	Final	
01049-20022	20/10/2020	Y	Final	
043725	20/10/20	Y	Final	
185350	10/12/2020	N	Final	Distribution of ALC-0315, 0159. Evaluated in section 4.2.2 of the Detailed Evaluations Section of this report.
Toxicology				
20GR142	20/09/2020	Y	Interim	Awaiting recovery phase & immunogenicity
20GR142	11/01/2021	N	Final	Report includes recovery data and immunogenicity data. Evaluated in section 4.3.2 of the Detailed Evaluations Section of this report.
38166	20/09/2020	Y	Final	
20256434	11/01/2021	N	Final	Development and reproduction toxicity report not available at the time of the TGA interim review. Evaluated in section 4.3.6 of the Detailed Evaluations Section of this report.

There are no outstanding nonclinical studies identified as of the date of the current report.

2 FORMULATION

The formulation for the BNT162b2 vaccine is provided in Table 2. The vaccine is supplied as a concentrate in 5 dose vials which are then diluted prior to administration with 1.8 ml of normal saline for injection to give a total volume of 2.25 ml containing 1 µg of the active mRNA per µl so that a 0.3 ml injection contains 30 µg.

With the exception of the two novel lipids, ALC-0315 & ALC-0159, and the mRNA active, all other ingredients are common parenteral excipients and all except sucrose are naturally present in physiological fluids. From a safety/toxicology perspective the principal components of interest are the two novel lipid excipients and the mRNA active (& the S protein it expresses *in vivo*).

Table 2. BNT162b2 vaccine Formulation

Ingredient	Function	Qty/vial (mg)	Conc. mg/ml	Qty/dose, µg
s 9(2)(b)(ii)	[redacted]			

s 9(2)(b)(ii)

3 NON-CLINICAL OVERVIEW AND CONCLUSIONS

References to study numbers include "-TGA" where the summaries in this section have been developed from the study reports in the December 2020 TGA interim evaluation and "-New" or "amended" where provision of the study, or a component of a study report, post-dates the TGA report and have been evaluated *de novo* in this evaluation.

3.1 OVERVIEW OF NONCLINICAL TESTING STRATEGY

BNT162b2 is an investigational vaccine (now in clinical use) intended to prevent COVID-19, which is caused by SARS-CoV-2. The active part of BNT162b2 is a nucleoside-modified mRNA (modRNA) expressing full-length viral spike protein (S) with two proline mutations (P2) to lock the transmembrane protein in an antigenically optimal prefusion conformation. The active vaccine ingredient is encapsulated in a lipid layer composed of four lipids, two novel lipids (ALC-0315, ALC-0159) and two conventional ones, (DSPC, and cholesterol). Other excipients in the formulation include sucrose, NaCl, KCL, Na_2HPO_4, and KH_2PO_4.

The data provided in the Application includes studies on a number of viral variants that were investigated during the development of the vaccine, Table 3. Variants V8 and V9 code for the identical full-length SARS-CoV-2 spike protein (identical amino acid sequences) and differ only in their codon optimisation sequences. s 9(2)(b)(ii) Due to the identical S protein amino acid sequences, studies on V8 are relevant for the evaluation of V9 unless otherwise stated in this report. Only BNT162b2 (V9) has been evaluated in clinical trials and is the subject of the current application. Other variants included in studies submitted by the applicant were not sufficiently similar to V9 to provide information that could be reliably extrapolated to the V9 variant and have not been considered in the evaluation.

Table 3. Variants of BNT162 mRNA vaccine candidates

BNT162 vaccine candidate	RNA type	Encoded antigen
BNT162a1	Non-modified uridine mRNA	SARS-CoV-2 receptor binding domain, a secreted variant
BNT162b1	Nucleoside modified mRNA	SARS-CoV-2 receptor binding domain, a secreted variant
BNT162b2 (V8 and V9)	**Nucleoside modified mRNA**	**Full length SARS-CoV-2 spike protein bearing mutations preserving neutralisation-sensitive sites**
BNT162b3	Nucleoside modified mRNA	SARS-CoV-2 receptor binding domain, a membrane-bound variant
BNT162c1	Self-amplifying mRNA	SARS-CoV-2 receptor binding domain, a secreted variant
BNT162c2	Self-amplifying mRNA	Full length SARS-CoV-2 S protein bearing mutations preserving neutralisation-sensitive sites

Table 4. Nomenclature of the Principal Vaccine Candidates

Product Code	RNA Platform	Antigen Variant	Description/Translated Protein	Variant Code	GLP Tox Data	Clinical Candidate
BNT162b2	modRNA	V8[a]	P2 S	RBP020.1	Yes	No
BNT162b2	**modRNA**	**V9[a]**	**P2 S**	**RBP020.2**	**Yes**	**Yes**

a. The V8 and V9 variants of the P2 S antigen have the same amino acid sequence. Different codon optimizations were used for their ribonucleotide sequences.

Bold: BNT162b2 (V9) vaccine candidate submitted for licensure.

In summary the Nonclinical testing strategy was as follows:

- Primary pharmacology, distribution, metabolism, and safety of BNT162b2 vaccine
  - In *in vitro* and *in vivo* (rat) studies,
  - IM administration to reflect clinical use,
  - Rats chosen as a commonly used toxicology/immunogenicity model and because they mount an antigen-specific immune response to vaccination with BNT162b2,
  - Biodistribution of LNPs & Pharmacokinetics of novel excipients in mice and rats using LNP-Luciferase modRNA,
  - Biodistribution of LNPs & Pharmacokinetics of novel excipients in mice using radiolabelled LNPs,
  - Metabolism of ALC-0315 and ALC-0159 in mouse, rat, nonhuman primates, and human blood, liver microsomes, S9 fractions, and hepatocytes and *in vivo* in rat plasma, urine, faeces, and liver samples from the Pharmacokinetic study.
- Immunogenicity & Protection
  - in mice (immunogenicity only), rats (immunogenicity only), and nonhuman primates (immunogenicity and protection).
  - Serum antibody responses (S1 and RBD-binding IgG responses) assessed in mice and rats.
  - Functional antibody responses

        - tested by a SARS-CoV-2 pseudo-type neutralization assay (pVNT).
        - In nonhuman primates, S1-binding IgG responses were tested in a direct Luminex-based immunoassay (dLIA) and functional antibody responses were assessed in an authentic SARS-CoV-2 neutralization assay.
        - S-specific T cell responses in mouse and nonhuman primates using IFNγ ELISpot & intracellular splenocyte cytokine staining flow cytometry-based Th1/Th2 profile analysis.
    - A SARS-CoV-2 challenge study in BNT162b2 (V9)-immunized nonhuman primates – assessment of protection against infection and lack of disease enhancement
- Toxicology
    - BNT162b2 (V8) and (V9) in repeat-dose toxicity studies in rats,
    - Development and reproduction study of V9 in rats.

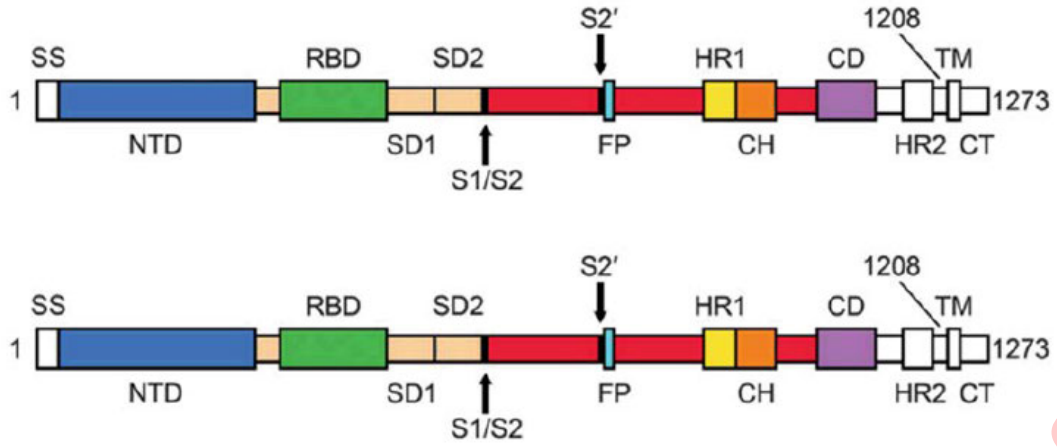
3.2 PHARMACOLOGY

The function and immunological significance of the S protein is described in the nonclinical overview as follows:

> "SARS-CoV-2 S is a large, trimeric glycoprotein that exists predominantly in a prefusion conformation on the virion. It is cleaved by furin into an N-terminal S1 and a C-terminal S2 fragment. S attaches to the host cell receptor ACE2 (Angiotensin Converting Enzyme 2 Receptor) by its receptor binding domain (RBD), which is contained in the S1 furin cleavage fragment. Spontaneously and during cell entry, the S1 fragment dissociates, and the S2 fragment undergoes a fold-back rearrangement to the post-fusion conformation in a process that facilitates fusion of viral and host cell membranes. S is the main target of virus neutralizing antibodies. Most of the antibodies with SARS-CoV-2 neutralizing activity are directed against the RBD."

Following uptake of BNT162b2 LNP particles into host cells the P2-S is expressed from the RNA and is surface membrane bound or secreted from the cell, eliciting a humoral neutralizing antibody response and Th1-type CD4+ and CD8+ cellular response which is then able to block virus infection and kill virus infected cells, respectively.

Figure 1. Schematic of the Organization of the SARS-CoV-2 S Glycoprotein



The S1 furin cleavage fragment includes the signal sequence (SS), the N terminal domain (NTD), the receptor binding domain (RBD, which binds the human cellular receptor, ACE-2), subdomain 1 (SD1), and subdomain 2 (SD2). The furin cleavage site (S1/S2) separates S1 from the S2 fragment, which contains the S2 protease cleavage site (S2′) followed by a fusion peptide (FP), heptad repeats (HR1 and HR2), a central helix (CH) domain, the connector domain (CD), the transmembrane domain (TM) and a cytoplasmic tail (CT).
Source: modified from Wrapp et al, 2020.

3.2.1 S Protein Expression

To demonstrate S protein expression HEK293 (Human Embryonic Kidney Cells) cells were transfected with the naked BNT162b2 RNA (V8 & V9) using a commercial transfection kit (**Study No R-20-0360- New**) or as both the naked and LNP encapsulated BNT162b2 (V9) (**Study R-20-0211-TGA**). The expressed S protein co-localised with an endoplasmic reticulum (ER) marker, suggesting the S protein is synthesised and processed within the ER for surface expression or secretion.

SARS-CoV-2 P2 S expressed from DNA that encodes the same amino acid sequence as BNT162b2 RNA was investigated for structural and binding characteristics (**Study VR-VTR-10741-New**). The encoded P2 S antigen was found to accurately reflect the ACE2 RBD and other epitopes targeted by SARS-CoV-2 neutralizing antibodies.

3.2.2 Immunogenicity

Dedicated Immunogenicity studies have been conducted in mice (**R-20-0085-TGA, R-20-0112-TGA**, **R-20-0054-New**) and in nonhuman primates (VR--**VTR-10671-TGA and amended**). Additional immunogenicity investigation was included in the rat repeat dose toxicity and reproduction and development studies (**20GR142-TGA and amended, 38166-TGA, 2056434-New**).

BNT162b2 (V9) was demonstrated to induce both humoral and cellular immunity in mice and nonhuman primates and demonstrated a strong humoral response in rats.

In mice BNT162b2 (both V8 & V9) was highly immunogenic eliciting strongly antigen-binding IgG providing a high titre of neutralizing antibody and a Th1-phenotype CD4+ response as well as an IFNγ+, IL-2+ CD8+ T-cell response after a single dose (**R-20-0054-New, and R-20-0085-TGA, R-20-0112-TGA)**. Total IgG ELISA demonstrated a vaccine induced strong, dose-dependent IgG response to S1 and RBD. The vaccine also elicited high neutralizing titres in a

pseudo-type neutralization assay. Isolated splenocytes, collected 28 days after immunization, stimulated with an S protein specific overlapping peptide pool produced robust CD4+ and CD8+ T-cell IFNγ responses and a Th1-dominant profile was demonstrated in quantification of cytokines (IL-2 and IFNγ) in the corresponding culture supernatants.

In repeat dose rat studies (**20GR142-TGA and amended, 38166-TGA, 2056434-New**) SARS-CoV-2 neutralizing antibody responses were seen in both sexes at the end of the dosing (Day 17) and recovery phases (Day 21) but not prior to vaccine administration or in controls. In a developmental and reproduction study in rats where BNT162b2 (V9) was administered 21 and 14 days prior to being mated with untreated males, and on Gestation Days (GD) 9 and 20, BNT162b2 elicited SARS-CoV-2 neutralizing antibody responses in the majority of females just prior to mating, at the end of gestation, and at the end of lactation as well as in most offspring (foetuses on GD21 and pups on PND21) but not prior to vaccine administration or in controls. Cellular immunity was not examined in the rat.

Following immunisation of Rhesus macaques with BNT162b2 (V9) IM with 30 µg or 100 µg on Days 0 and 21, S1-binding IgG and SARS-CoV-2 neutralizing titres (NT_{50}) were found from 14 days after the first dose and substantial increases were observed after the second dose. Seven days after the second dose (at 30 µg) the neutralising geometric mean titre (GMT) in the macaques was 8-fold higher than that found in the sera from 38 convalescing human patients (HCS). Although both, S1-binding IgG levels and neutralizing titres, declined through to the last measured time point (Day 56) it remained above the neutralizing GMT and the S1-binding geometric mean concentration (GMC) of the HCS. Strong S-specific Th1-dominant IFNγ+ T-cell responses were observed in all immunized macaques, consistent with findings in mice. Intracellular cytokine staining analysis demonstrated a dose-dependent increase in S-specific CD4+ T cell responses with a strong Th1-bias evidenced by high frequency of IFNγ+, IL-2+, or TNF-α+ cells.

Notably, CD8+ T-cell responses were also detectable in BNT162b2-immunized animals.

3.2.3 Protection

Protection was assessed only in the Rhesus Monkey (**VR-VTR-10671-TGA and amended**). Although immunogenicity could be demonstrated in the mouse model, the SARS-CoV-2 S protein does not effectively bind to ACE2 of wild type mice to initiate replication [1,2].

In rhesus monkeys administered BNT162b2 V9 LNP at 30 or 100 µg in 0.5 ml of normal saline, IM, on days 0 and 21 and challenged with SARS-CoV-2, 55 days after the second dose, no significant clinical signs of illness were observed (including in control). Viral RNA detection in bronchoalveolar lavage (BAL) fluid showed a highly statistically significantly ($p<0.0014$) lower level in immunised animals. The combination of an absence of clinical signs in control animals but substantially lower viral load in BAL fluid in immunised animals indicates the rhesus monkey is a useful model of infection and immunisation but not of COVID-19 disease. Radiographic evidence (X-ray and CT) of pulmonary abnormality was

[1] Fontela, C, et al. **Animal models for COVID-19**. Nature. Vol 586 22 October 2020
[2] Wan, Y., Shang, J., Graham, R., Baric, R. S. & Li, F. Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. J. Virol. 94, e00127-20 (2020).

observed in challenged controls but not in challenged BNT162b2- immunised animals and no radiographic evidence of vaccine elicited enhanced disease was observed. The main Histopathology finding in the lung was inflammation which was similar between control and BNT162b2-immunized animals, with no evidence of enhanced respiratory disease. The discordance between the higher lung inflammation in controls observed by radiography and the absence of this difference histologically is likely due to the transient nature of lung inflammation in the Rhesus Monkey and the time gap between the Day 1 to 3 peak of viral shedding and acute inflammation observed by radiography and tissue sampling for histology at Days 7 to 8 after SARS-CoV-2 challenge.

Both antibody-mediated and T cell-mediated immunity are known to be important contributors for effective protection against SARS-CoV-2[3] and, if neutralising antibody-mediated protection is incomplete, cytotoxic CD8+ T cells are crucial for viral clearance[4].

A study of recovered COVID 19 patients found that all had S protein-specific CD4+ T cells in the circulation and 70% had S protein-specific CD8+ T cells[5]. Studies in mice reinforce a protective role of T cells in host defence against SARS-CoV[6]. In this respect the findings of the pharmacology studies that BNT162b2 (V9) induces humoral, cellular, and functional immune responses in mice and monkeys are consistent with prospective clinical efficacy.

3.2.3.1 Potential for Antibody Dependent Enhancement (ADE) or Vaccine-associated Enhanced Respiratory Disease (VAERD)

ADE can occur where by two mechanisms i) enhanced antibody-mediated virus uptake into Fc gamma receptor IIa (FcγRIIa)-expressing phagocytic cells leading to increased viral infection and replication, or ii) by excessive antibody Fc-mediated effector functions or immune complex formation causing enhanced inflammation and immunopathology[7]. Both ADE pathways can occur when non-neutralizing antibodies or antibodies at sub-neutralizing levels bind to viral antigens without blocking or clearing infection. In this respect BNT162b2 (V9) provided protection against SARS-CoV-2 challenge in monkeys with no viral RNA detected in respiratory tract swabs and BAL and no radiographic (X-ray and CT) lesions in immunised, challenged animals, while viral replication and lung radiographic abnormalities were evident in un-immunised, challenged controls. There was no evidence of vaccine-elicited disease enhancement as detected from radiographic analysis. No clinical

[3] Tay M.Z., Poh C.M., Rénia L. et al. (2020) The trinity of COVID-19: immunity, inflammation and intervention. Nat. Rev. Immunol 20:363–374
[4] Arunachalam P.S., Charles T.P., Joag V. et al. (2020) T cell-inducing vaccine durably prevents mucosal SHIV infection even with lower neutralising antibody titres. Nat. Med 26:932–940
[5] Grifoni A., Weiskopf D., Ramirez S.I. et al. (2020) Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell 181:1489–1501.e15
[6] Zhao J., Zhao J. & Perlman S. (2010) T cell responses are required for protection from clinical disease and for virus clearance in severe acute respiratory syndrome coronavirus-infected mice. J. Virol. 84: 9318–9325
[7] Lee, W., Wheatley, A., Kent, S., and DeKosky, B. (2020) **Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies Nature**. Microbiology, VOL 5, OCTOBER 2020, 1185–1191

signs were observed in any challenge groups, which is consistent with other studies in SARS-CoV-2-infected non-human primates. The preclinical studies do not indicate a risk of ADE.

Nonclinical studies with several potential mRNA-LNP vaccines undergoing clinical trials (influenza, rabies and Zika virus infections)[8,9] have yielded similar findings to BNT162b2 (V9) and support the conclusion of an absence of ADE risk. Additionally, the Th1-biased response in mice and monkeys suggests a low risk of ADE and VAERD.

3.3 PHARMACOKINETICS

Because of the mechanism of action of most vaccines, pharmacokinetic studies are not normally a critical or significant aspect of a vaccines' efficacy and/or safety assessment and are not routinely required under relevant guidelines. For the BNT162b2 vaccine however uptake of the LNP carrier for the BNT162b2 mRNA by target cells is critical to its efficacy. Additionally, the LNP component of the vaccine contains 2 new excipients that require pharmacokinetic characterisation. The LNPs used in the studies summarised below have been confirmed by the sponsor to be identical to that in the proposed vaccine.

Pharmacokinetics of ALC-0159 & ALC-0315

The proposed vaccine lipid nano-particle contains 2 novel excipients designated ALC-0159 & ALC-0315. The plasma kinetics, distribution to the liver, and elimination of these excipients were studied in rats after IV administration of LNPs containing a luciferase encoding mRNA (**study 07424-TGA**). Plasma levels of ALC-0159 & ALC-0315 declined rapidly with initial $t_{1/2}$ of 1.72 and 1.62 h respectively but the terminal $t_{1/2}$ was considerable slower at 72.7 and 139 hrs respectively. The liver was a primary site for distribution from the blood (20% & 60% of the dose for ALC-0159 & ALC-0315 respectively), although data for other tissues was not obtained. Elimination of the parent (unmetabolized) lipid was entirely in the liver (47% & 1% of the dose for ALC-0159 & ALC-0315 respectively) with no unchanged lipid found in the urine.

The metabolism of ALC-0315 and ALC-0159 *in vitro* was evaluated in blood, liver microsomes, S9 fractions, and hepatocytes from mice, rats, monkeys, and humans, and the *in vivo* metabolism was examined in plasma, urine, faeces, and liver from the iv rat PK study summarised above (**043725-TGA & 01049-20008,20009,20010, 20020, 20021, 20022 - TGA)**. Metabolism for both ALC-0159 and ALC-0315 appears to occur relatively slowly across most species *in vitro* and *in vivo*. Across all species studied both ALC-0159 and ALC-0315 are metabolized by hydrolytic metabolism of the amide or ester functionalities, respectively.

Nanoparticulate LNPs

Systemically administered nano-particulates, including LNPs, are rapidly removed by normal immune surveillance mechanisms by cells of the reticulo-endothelial system (RES). In the

[8] Sahin U., Karikó K. & Türeci Ö. (2014) mRNA-based therapeutics - developing a new class of drugs. Nat Rev Drug Discov 13(10):759-80.
[9] Pardi N., Parkhouse K., Kirkpatrick E. et al. (2018) Nucleoside-modified mRNA immunization elicits influenza virus hemagglutinin stalk-specific antibodies. Nat Comm 9(1) (08):3361

absence of specific surface derivatisation of nano-particulates to prevent or diminish protein binding, and therefore immune cell recognition of the particulates, the circulating half-life is very short and of the order of a few tens of minutes. In the current context prolonged circulation is not advantageous and rapid uptake and expression of the mRNA is the desired outcome.

The distribution of LNPs following IM administration to mice or rats was investigated in two studies. In the first of these studies (**R-20-0072 – TGA**), conducted in females only, the LNP contained a mRNA expressing luciferase to provide a marker for distribution analysis using whole body bioluminescence after luciferin administration. Expression of luciferase was largely confined to the injection site with some distribution to the liver. The luciferase signal declined to background levels in the liver after 48 hrs and at the injection site after 216 hrs, reflecting the gradual degradation of the mRNA and expressed protein.

In the second study in rats (Study **185350 – New**), conducted in both sexes, the LNP again contained a mRNA expressing luciferase but also included trace levels of a radiolabelled, nonexchangeable, non-metabolisable lipid (designated as [3H]-CHE) to provide a more sensitive marker for tissue distribution. Once intracellular, the [3H]-CHE does not recirculate and therefore allows assessment of distribution of the particles. The content and concentration of total radioactivity in blood, plasma and a comprehensive range of tissues were determined in 3 animals per sex at 0.25 1, 2, 4, 8, 24 and 48 hours post-dose. The injection site again accounted for the greatest proportion of the dose but low levels of radiolabel were found in most tissues. Levels at the injection site were highly variable due to technical challenges in accurately visualising and excising the site. Over 48 hrs the radiolabel distributed primarily to the liver, adrenal glands, spleen and ovaries. Total recovery of radiolabel outside the injection site was greatest in the liver at 21.5% of dose, with $\leq$ 1.1% in the spleen and $\leq$ 0.1% in the adrenal glands and ovaries. Although levels in tissues other than the liver were low as a percentage of total dose this was partly a reflection of relative organ sizes. The concentration in the ovaries was 12 µg equiv. lipid/g in ovaries compared to 30 µg equiv. lipid/g for the liver, potentially giving rise to concern regarding reproductive effects (subsequently discounted by the submitted developmental and reproduction study). There were no substantive differences in the pattern of distribution between sexes.

BNT162b2 mRNA

Study (**R-20-0072 – TGA**) discussed above investigate the tissue distribution of an mRNA expressing luciferase administered in LNPs as a readily detectable surrogate for administered BNT162b2 mRNA in the same[10] LNPs (designated LNP8) used in the final formulation. At 6 h, 24 h, 48 h, 72 h, 6 d, and 9 d after IM injection to mice, the *in vivo* luciferase expression was measured by administration of luciferin and whole body imaging to detect expression and therefore uptake of luciferin mRNA. As imaging is conducted on

[10] The TGA has requested that the sponsor confirm this point due to some ambiguity in the study report – ie the relationship between the LNP used in the study to that in the final product formulation is not explicitly stated.

intact animals, signal strength is dependent on tissue depth and tissues with low levels of expression are unlikely to be identified. Luciferin expression was found at the injection site and as expected, in the liver at 6 hours after administration, was only detectable at the injection site by 24 hrs and was undetectable by 9 days after administration. Uptake by, and expression in, RES tissues other than the liver, such as lymph nodes and spleen, is highly likely based on the pharmacokinetics of other nano-particulates and observed for the radiolabelled LNPs discussed above) but any expression present in this study was below the limit of detection of the imaging system. Disappearance of detectable luciferase detection by 9 days reflects the anticipated progressive degradation of mRNA and the expressed luciferase.

3.4 TOXICOLOGY

3.4.1 Single Dose Toxicity

No separate single dose toxicity studies were conducted.

3.4.2 Repeat Dose Toxicity

Two 17 day, repeat dose, GLP, toxicity studies have been conducted in the same strain of rats (Crl:WI(Han)), one included the V8 variant (**Study 38166-TGA**) and the other included the V9 variant (**Study 20GR142-TGA and amended**). Both studies included other variants not relevant to the proposed vaccine product that are not discussed here. The V8 study utilised a dose of 100 µg RNA. The V9 study used a dose of 30 µg of RNA. Both studies administered the dose IM on days 1, 8 and 15 with 10 animals per group sacrificed on day 15 and 5 per group sacrificed after a recovery period of 3 weeks. The 30 µg/animal dose for the V9 strain represents an MOE (Margin of Exposure) per dose of approximately 240-300-fold over the proposed clinical dose. Findings in both studies were closely comparable qualitatively. The principal findings were those expected from a vaccine producing a robust antigen-specific immune response. Immune-response related findings for both variants included: erythema and oedema of the injection site with an elevated body temperature (on most occasions the treated group mean was in the order of 1^0C above the concurrent control), transient reductions in food consumption and body weight gain after each injection, enlarged spleen and draining lymph nodes, elevated acute phase proteins (alpha-1 acid glycoprotein, alpha-2-macroglobulin, fibrinogen), elevated WBC, reduced albumin:globulin ratio, and increased haematopoiesis in spleen and bone marrow. There was no indication of systemic toxicity. GGT (a marker primarily for bile duct damage) was elevated in the study on the V8 variant but there were no increases in other markers of hepatic damage and no histopathology evidence of effects on the biliary system (or other relevant tissues). As GGT was not increased for the V9 variant under the same protocol, and given the very close similarity between the two variants, the elevation of GGT for the V8 variant is likely to be incidental. The only finding not directly related to elicitation of an immune response was vacuolation of hepatocytes, without association with histological evidence of hepatic tissue damage or elevation of serum hepatic enzymes, seen with both variants. The applicant postulates this is due to the PEG-lipid component of the LNPs and cites a review of PEGylated biopharmaceuticals which found vacuolation of various tissues

as a common finding. Although the postulated mechanism is plausible the relationship is uncertain as hepatocytes were not a common target identified for PEGylated biopharmaceuticals. The effect was fully reversible over the 3-week recovery phase and given the high MOE achieved and the use of 3 doses given in weekly intervals to rats compared to 2 doses over 3 weeks proposed clinically, the observed hepatocyte vacuolation is unlikely to present a safety issue for the proposed product. All changes in clinical pathology parameters and acute phase proteins were reversed at the end of the recovery period. At the end of the recovery period inflammation at the injection site and at the iliac (draining) lymph node showed evidence of progressive but incomplete recovery.

3.4.3 Genotoxicity and carcinogenicity

Genotoxicity and carcinogenicity studies have not been conducted on either the formulation or its key novel constituents. This is consistent with vaccine international guidance documents (ICH S1A, WHO 2005) and with the duration of use and the low dose levels of the vaccine and its components. The BNT162b2 vaccine however contains 2 novel excipients (designated ALC-0159 and ALC-0315) and warrant further comment.

The two novel excipients are not listed on the TGA's ingredient database and are therefore novel both structurally and pharmaceutically. These excipients are however lipids and neither they nor the mRNA would be expected to have genotoxic or carcinogenic potential based on a consideration of their structure. *In silico* QSAR analysis did not identify a genotoxic risk potential. The total dose of the novel lipids from a single injection of the BNT162b2 vaccine is 430 µg/dose for ALC-0315 and 53.4 µg/dose for ALC-0159. For ALC-0159 the dose is less than the TTC (Threshold of Toxicological Concern), 120 µg/day - which is considered an acceptable daily exposure for a mutagenic carcinogen for durations of up to 1 month (ICH M7 [R1], 2017) - but for ALC-0315 the dose is greater than the lifetime TTC of 120 µg/day. However, the carcinogenic risk is dependent on both dose and duration of exposure (ICH M7 [R1], 2017). There is a linear relationship between the amount of daily intake of a mutagenic impurity corresponding to a 10^{-5} cancer risk and the number of treatment days. The daily dose corresponding to 10^{-5} cancer risk (lifetime acceptable intake) of a mutagenic carcinogen which is administered 2 days is 19162.5 µg (1.5 µg x 365 days x 70 years/2 days), significantly greater than the daily dose of ALC-0315 administered in BNT162b2. The issue is further discussed in response to a TGA question to the applicant in Section 3.7.

The BNT162b2 vaccine is unlikely to present a genotoxic or carcinogenic risk in clinical use.

3.4.4 Long term studies

No long-term studies have been conducted which is consistent with WHO guidance and normal practice for vaccine development.

3.4.5 Reproduction and Developmental Toxicity

A developmental and reproduction toxicity study was conducted in rats with BNT162b2 V9 administered IM at the proposed human clinical dose of 30 µg given 21 and 14 days prior to mating with untreated males and on Gestation Days 9 and 20, for a total of 4 dosing days (**Study 20256434-New**). Half the females were sacrificed on GD 21 for examination of

developmental effects and the remainder were permitted to litter and rear their pups until weaning on post-natal day 21. There were no treatment related effects on any reproduction or developmental parameter (ovarian, uterine, or litter parameters, including embryo-foetal survival, growth, or external, visceral, or skeletal malformations, anomalies, or variations, postnatal development, including postnatal growth, physical development [pinna unfolding and eye opening], neurodevelopment [pre-weaning auditory and visual function tests], macroscopic observations, and survival). Treatment related effects were confined to findings consistent with the administration of a vaccine and the consequent immune response (injection site oedema, transient body weight and food consumption effects after each dose). Treated parental females developed a SARS-CoV-2 neutralizing antibody response which was also detected in all foetuses from the caesarean section and pups from the littering groups. The results raise no potential safety issues for the use of this vaccine in pregnant human patients. The doses administered in this study provide an MOE compared to the proposed single clinical dose of 30 µg, of 250-fold. The overall MOE is considerably larger due to both the shorter interval between doses and the greater number of doses (4 in rats versus 2 as the proposed clinical dose).

3.4.6 Local Tolerance

No specific local tolerance studies have been conducted however repeat dose toxicology studies and immunogenicity studies utilising the proposed clinical IM route of administration have demonstrated the anticipated local erythema and oedema associated with inflammation from a vaccine induced immune response. The incidence and severity of the reactions were higher after the second or third injections compared with the first. The majority of animals had very slight oedema or rarely slight erythema after the first dose. After the second or third dose, the severity of oedema and erythema increased up to moderate or rarely, severe grades. These observations resolved prior to the next injection or for recovery animals resolved during the 3-week recovery phase. The inflammation at the injection site was characterized by infiltrates of macrophages, granulocytes, and lymphocytes into the muscle, and variably into the dermis and subcutis. Some mostly mild myofiber degeneration, occasional muscle necrosis, and mostly mild fibrosis were observed. Inflammation was minimal to mild at the end of the recovery period indicating progressive resolution.

3.4.7 Immunotoxicity

The dose dependence of cytokine and s 9(2)(b)(ii) release was investigated in human peripheral blood mononuclear cells (PBMCs - a mixture of monocytes and lymphocytes [T cells, B cells, and natural killer cells]) from 3 donors, after transfecting the cells with BNT162b2 V9 (**Study R-20-0357-New**). Cytokine (TNF-α, IL-6, IFN-γ, and IL-1β) release was low and close to background levels across a wide dose range of BNT162b2. Higher levels of chemokine release compared to cytokines were observed but in all 3 donors for all the chemokines measured (IP-10, MIP-1β, and MCP-1), tested doses up to 0.2 µg BNT162b2 per well showed low or background chemokine levels. These findings are concordant with that from the 17- day rat study with various BNT162 mRNA variants (including BNT162b2 V8 but not V9) which found no increase in cytokine levels across 3 doses given once weekly.

3.5 Overview and conclusions.

To a large extent the expanding clinical experience with the proposed vaccine has overtaken the nonclinical data and provides a more robust basis for assessment of most potential clinical risks. Areas where this is not the case include reproductive and developmental toxicity and the potential for genotoxicity and carcinogenicity.

The preclinical studies have not identified any significant potential safety risks (local or systemic) for the proposed vaccine when used as indicated by the applicant. A reproduction and development study identified no effects on female reproduction (including mating performance), or foetal or pup development.

There were no observations suggestive of immunotoxicity across toxicology, immunology or protection studies in mice, rats or nonhuman primates. Immunogenicity and protection studies indicated that the proposed vaccine produces cellular and humoral immunity and, in monkeys, was protective against SARS-CoV-2 challenge.

Consistent with international guidance genotoxicity and carcinogenicity studies have not been conducted on either the formulation or its key novel constituents (ALC-0159, ALC-0315). There is no basis for postulating a genotoxic risk for mRNA or the expressed protein. Although ALC-0315 has a long half-life and the single dose exposure exceeds the TTC for a genotoxic impurity, a QSAR (Quantitate Structure Activity Relationship) analysis did not identify a genotoxic risk, the repeat dose studies achieved a MOE of approximately 300, and dose frequency is limited to 2 doses. Overall, the BNT162b2 vaccine is unlikely to present a genotoxic or carcinogenic risk in clinical use.

3.6 Comment on the nonclinical overview and summaries.

The data submitted was generally of good quality, utilised appropriate methods and were well documented. At least partially because of the urgency of development and the novelty of the vaccine mechanism and components many studies were not conducted under GLP certification, most were investigational/research in nature but were documented to an acceptable standard. Pivotal toxicology studies were GLP certified and followed appropriate international guidelines as did the overall nonclinical data generation. Appropriate animal models were used for toxicology, immunogenicity and protection studies. The applicant has adequately addressed the considerable number of questions put to them by the TGA and EMA, which cover areas of potential weakness or incompleteness in the nonclinical data.

Overall, the nonclinical data has addressed adequately the key human safety issues not addressable from the currently available and accumulating clinical experience with the proposed vaccine.

3.7 Pfizer Responses to EMA and TGA Questions

Pfizer have provided copies of their responses to questions from EMA and TGA regarding their data submissions. Those questions, and responses are presented below (Q. Agency Question, A. Pfizer Answer, C. This evaluators comments). As many questions related to a central theme the Pfizer response is consolidated and summarised where practicable.

Many of the issues raised, particularly around immunogenicity and immunotoxicity, are largely rendered redundant by clinical trials and rapidly accumulating clinical experience but are included here for reference.

Details for the citations in questions and responses can be found in the original documentation from the applicant.

EMA:

BNT162b2 – EMEA/H/C/005735. Responses to List of Questions (15 December 2020)

Questions related to Pharmacokinetics of Novel lipids

Q. **Discuss the long half-life of ALC-0315 in the body**

Q. **To discuss the non-clinical toxicologic effects of ALC-0315 in non-clinical studies and the clinical relevance of the persistence of this lipid (4-5 months) in man.**

Q. **To discuss the impact of a potential increase in the number of boosts on the effect of the persistence of lipids in humans.**

A. Pfizer have provided separate answers to each of these questions that cover a common range of facts:

- There is no further data on the pharmacokinetics of these lipids, however,
- there were no nonclinical safety issues in the repeat dose toxicity studies (Study 38166 and Study 20GR142) at doses (on a mg/kg basis) much greater than that administered to humans (on the order of 300-1000x) and administered at shorter intervals (3 doses a week apart vs 2 doses 3 weeks apart) where the vaccine was tolerated without evidence of systemic toxicity.
- Vacuolation in the liver related to lipid distribution was observed at the end of the dosing phase but was resolved at the end of the 3-week recovery period. There was no evidence of liver injury or of systemic toxicity.
- Given the above it is unlikely that the administration of a booster dose will lead to significant accumulation of any of the vaccine components that would adversely impact the current therapeutic window (assuming additional dose or doses occur at an interval that is greater than the half-life).

C. The responses are reasonable. The MOE on a mg/kg bw basis is very substantial and further increased by administration of 3 doses to rats a week apart in comparison to the clinical schedule of 2 doses at least 3 weeks apart. Effects in rats attributable to the lipid component of the LNP were limited to vacuolation of hepatocytes, fully reversible over 3 weeks and not associated with other histopathology or clinical chemistry findings indicative of an adverse effect. The long half-life in and of itself does not present a potential risk where a compound is not being administered repeatedly over a prolonged period. From a toxicological perspective the AUC is the key metric for internal (systemic) dose rather than half-life. As each individual dose is small and only 2 doses are likely to be given over a period of a year or so, the long

half-life does not raise safety issues and the very high MOE achieved in the rat studies provides evidence of at most low systemic toxicity at doses very greatly exceeding those proposed for clinical use.

Questions Related to Reproduction and Development

Q. **The impact on the post treatment contraception duration**

Q. **Regarding the new study 185350 submitted, the Applicant is asked to discuss: - The concentrations of [3H]-08-A01-C01 in the ovaries over 48 hours, the precise location of the labelled lipid in the ovaries and the clinical relevance of this finding. Another discussion regarding the toxicity of this findings on fertility is also expected.**

A. The Pfizer responses were:

- In the developmental and reproductive toxicity (DART Study 20256434) study in rats there were no vaccine-related effects on female fertility or pregnancy, or embryo-fetal or offspring development. These results support no requirements for contraception. The draft DART study report was submitted to EMA on 15 Dec 2020.
- No work has been carried out to understand the precise cellular location of the lipids (or any other components of the LNP) in the ovaries. Given the nature of the radiolabel detection methodologies, such precise cellular localization of the radiolabel is not possible. Only a small amount of the total dose that was administered to rats was present in the ovaries (0.1% of the dose). Most of the LNP remained at the injection site. In the repeat-dose toxicity studies (Study 38166 and Study 20GR142), there was no evidence of vaccine-related macroscopic or microscopic findings in the ovaries.

C. The responses are reasonable. There is no evidence across the entire database of adverse effects attributable to the lipid component of the LNP that would indicate a potential risk in clinical use generally or in pregnancy specifically. The proposed data sheet notes that there is limited experience in pregnant women and provides appropriately cautious advice that “Administration of COMIRNATY in pregnancy should only be considered when the potential benefits outweigh any potential risks for the mother and fetus.” Although not directly addressable from the nonclinical data, there may be a basis in the current circumstances for concluding the risk of vaccination during pregnancy is outweighed by the potential benefits of immunity particularly for specific subpopulations at heightened risk. This is a clinical consideration but there is no nonclinical data that would preclude or limit this consideration.

Questions related to anti RNA and PEG antibodies

Q. **Content of vacuoles, found in portal regions of the liver - Did the Applicant perform analysis in order to detect anti-RNA antibodies, anti-PEG antibodies and auto-antibodies in order to identify autoimmune disease among participants and during non-clinical studies?**

Q. **The Applicant is asked to discuss the potential immunogenic effect of the PEG contained in ALC-0159.**

Q. **The Applicant is asked to discuss a potential decrease in vaccine efficacy due to the anti-peg antibodies that could be observed.**

A. The Pfizer responses made the following points (summarised here);

- Analysis for anti-RNA, anti-PEG, or auto antibodies was not included in the nonclinical or clinical studies.
- An effect of anti-PEG antibodies would have been observed after dosing and up until the mRNA has been delivered into the cytoplasm. In nonclinical studies, BNT162b2 elicited a robust immune response, which had higher titres after repeated dose administration (up to 3 total doses). If Anti-PEG antibodies were present, the animals still mounted an immune response to the BNT162b2 antigen.
- In the C4591001 clinical study, no reduced immunogenicity has been observed in humans following the second booster dose of BNT162b2 based on Clinical evidence of potential autoimmune-related adverse events will be closely monitored during the long-term follow up out to 24 months for adverse events in the pivotal trial (C4591001).
- Polyethylene glycol (PEG) is a component of many different cosmetics and therapeutics developed for human use. Thus, exposure to PEG is not uncommon (Webster et al, 2007)[11].
- Although there is potential for PEG antibodies to form in response to administration of BNT162b2, there was no evidence that if these types of anti-PEG antibodies did form in the nonclinical studies, that they had any impact on the immune response through administration of multiple doses of the vaccine.

C. The clinical efficacy data provides the principal response to these questions. The animal data however is consistent with an absence of anti-PEG antibody related reduction in immunogenic efficacy but does not preclude formation of such antibodies. Liposome PEGylation is a common mechanism for reducing immune recognition of nano particulates, and therefore prolonging circulation of therapeutic liposome preparations. As the applicant notes exposure to PEG is not novel. The very high MOEs in the animal studies compared to human clinical doses suggest the readily reversible hepatocyte vacuolation effects in the animal studies are not likely to be manifest at clinical doses. Ultimately the clinical data are the appropriate source for answers to these issues. Whether an animal study on the formation of anti-RNA or anti-PEG antibodies is warranted is dependent on a clinical judgement of the adequacy of the clinical data to address the concern.

Other Questions

[11] Webster R, Didier E, Harris P, et al. PEGylated proteins: evaluation of their safety in the absence of definitive metabolism studies. Drug Metab Dispos 2007;35(1):9-16.

Q. **The findings observed in the liver of one animal (prominent lobular architecture) in link with all hepatotoxicity findings observed in toxicology studies.**

A. These findings were observed in a non-GLP distribution study, using a surrogate with the identical LNP of the BNT162b2. In general, the macroscopic finding of prominent lobular architecture is a nonspecific term reflecting zonal changes in the liver which may be associated with an assortment of microscopic findings, or sometimes no findings at all. However, in absence of microscopic analysis, no clear conclusion can be made. In the repeat-dose toxicity studies with BNT162b2 (Study 38166 and Study 20GR142), there was mostly minimal portal hepatocyte vacuolation, which was interpreted to be the result of LNP lipid distribution into the hepatocytes and was without evidence of liver injury.

C. This is an odd question given the absence of such findings in the repeat dose toxicology studies. The finding of 1 animal across multiple studies with an altered macroscopic appearance of the liver is clearly incidental to treatment and raises no toxicological or safety concerns. The Pfizer response is reasonable. The issue of vacuolation is addressed in response to a question from the TGA below.

Q. **Major GLP concern. The exclusion of the serology phase from the GLP compliance is not indicated in the study director statement and nothing in the final report shows that the study director is aware that the antibodies titration is out of GLP compliance. The impact of such non-compliance about the value of the antibodies titration should have been stated by the study director.**

A. Serology analysis for 20GR142 was conducted under Good Clinical Laboratory Practice (GCLP). Although the GLP compliance statement does not directly state that the serology was not performed under GLP conditions, the serology study report (Study 20GR142, Appendix B) states it was performed under GCLP conditions and the study director was aware of this. The conduct of the serology analysis under GCLPs had no impact on the integrity or data interpretation of the study.

C. The EMA question is somewhat overstated. GLP *certification* is related to documentation and internal Quality Assurance processes, not to experimental or analytical competence. Competence is assessed through *accreditation*. Most laboratories will undergo both processes. While GLP certification is an important quality control mechanism high quality studies can be conducted in non-GLP laboratories and poor studies are not infrequently generated by GLP certified facilities. Provided a study is reported at a level of detail consistent with GLP requirements and appropriate QA and QC procedures are documented for test facilities, the absence of GLP certification does not negate or diminish the reliability of such analyses. The applicants' response is appropriate and reasonable.

Q. **hPBMC study. The Applicant is asked to discuss the robustness and the results of this study with regard to:**

- the low number of donors,

- the single time point used for the cytokine measurement,

The impact of the direct correlation between dose and cytokine secretion in donor 1 should be discussed and analysed in term of inflammation and immunotoxicity. The Applicant is asked to discuss the mechanism underlying chemokines elevations in the three donors as well as clinical impact of high levels of chemokine secretion compared to cytokines (IP-10, MIP-1b, MCP-1).

A. The Sponsor performed this exploratory study to assess a first innate cytokine secretion profile *in vitro* in hPBMC following uptake of BNT162b2 LNPs. For this purpose, a low number of donors was appropriate. In summary, the data in R-20-0357 showed low inflammatory response. Given expected variability in responses among human donors, any specific interpretation from a single donor within this n=3 data set would not be appropriate. The kinetics of the innate response was not performed with hPBMCs; however, based on data in BNT162b2-immunized nonhuman primates, the innate immune response is transient, with a modest response between 6h and 24h post immunization that returns to baseline at the next measured timepoint of 7 days. The non-clinical data support a modest innate immune response, which is expected based on use of modified RNA. In response to the question of the cell types involved in LNP uptake, expression of the protein encoded by the RNA, we have used the LNP-formulation from BNT162 encapsulating a modRNA encoding for the green fluorescent protein (GFP) to understand which cells were transfected and express the modRNA encoded protein. We have detected a dose-dependent expression, especially in monocytes and dendritic cells (DCs), but only weak expression signals in B and T cells. This, in part, may explain the mechanism of higher chemokine levels compared to cytokines as in this regard, monocyte chemoattractant protein (MCP-1) or macrophage inflammatory protein (MIP) are especially expressed by antigen presenting cells after infection correlated with a Th1 response (Trumpheller et al, 1997, Ruiz Silva et al, 2016)[12,13].

C. Given the completion of phase III clinical trials, the progressive roll out of the vaccine internationally, and the evidence from animal studies that cytokines are not excessively elevated this question is largely redundant. The response is reasonable.

Q. **The Applicant is asked to discuss the propensity for C activation in non-clinical studies as well as in patients' blood samples prior to and/or after treatment with comirnaty.**

A. There were no findings suggestive of complement activation in the GLP toxicity studies in rats. Rats are known to demonstrate clinical manifestations of

[12] Ruiz Silva M, van der Ende-Metselaar H, Mulder H, et al. Mechanism and role of MCP-1 upregulation upon chikungunya virus infection in human peripheral blood mononuclear cells. Sci Rep 2016;6:32288.
[13] Trumpfheller C, Tenner-Racz K, Racz P, et al. Expression of macrophage inflammatory protein (MIP)-1alpha, MIP-1beta, and RANTES genes in lymph nodes from HIV+ individuals: correlation with a Th1-type cytokine response. Clin Exp Immunol 1998;112(1):92-99.

complement activation-related pseudoallergy (Dezsi et al, 2019)[14], none of which were noted in either of the two toxicity studies performed for BNT162b2. For this reason, no further investigations of systemic complement activation after intramuscular administration of BNT162b2 were conducted. Patient samples were not monitored for complement activation. Close monitoring and long-term follow up out to 24 months for adverse events, including CARPA-like events, will be performed in the pivotal trial (C4591001).

C. The response is reasonable.

Q. **Increases in eosinophils and basophils in toxicity pivotal studies. The observed increases in neutrophils, monocytes, eosinophils and basophils observed in the study 20GR142 should be discussed in regard of the clinical observations. The underlying mechanism as well as the clinical relevance regarding immunotoxicity/antigenicity should be discussed. Large unstained cells should also be characterized.**

A. Increases in neutrophils, monocytes, eosinophils and basophils observed in the Study 20GR142 were related to the inflammatory/immune response to BNT162b2 administration. Similar findings were identified in Study 38166 in animals administered 100 µg BNT162b2. The increases in eosinophils and basophils are a minor component of the inflammatory leukogram, which is dominated by increases in neutrophils. Large unstained cell (LUC) increases of this low magnitude are commonly observed with inflammation/immune responses and are attributed to increased monocytes and/or reactive lymphocytes. Characterization of LUC by peripheral blood smear review is not indicated in such circumstances because the identification of occasional large mononuclear cells or reactive lymphocytes provides no additional information.

C. The applicants' response is reasonable and correct, the observations are expected where inflammation is demonstrated to be present as is the case in rats dosed IM with an immunogenic (or irritant or infectious) preparation.

TGA

The TGA forwarded 2 rounds of questions to Pfizer. As the questions and responses were extensive and multiple questions related to individual issues these are consolidated and abbreviated where possible below. Some of the questions post-date the TGA Interim report and refer to studies not covered in that report (but are evaluated in the current report).

Q. **In the repeat dose study in rats with different variants of BNT162, the dosing interval was one week, whereas the immune response peaks 2-3 weeks after dosing, and the clinical dosing interval is 3 weeks. In addition, the novel lipid excipients have long elimination half-lives. Repeat dose toxicity studies with a**

[14] Dézsi L, Mészáros T, Őrfi E, et al. Complement Activation-Related Pathophysiological Changes in Anesthetized Rats: Activator-Dependent Variations of Symptoms and Mediators of Pseudoallergy. Molecules 2019;24(18):3283.

dosing interval of 2 or 3 weeks and 4 or more doses would be more appropriate for investigating the potential toxicity of the vaccine. What was the rationale for the dosing interval and number of doses used in the study?

A. Per the WHO guideline for nonclinical development of infectious disease vaccines, the dosing interval used in the toxicity studies may be more frequent compared with the clinical dosing regimen (WHO, 2005). Also, the guideline indicates that the number of doses administered in nonclinical toxicity studies should be equal to or more than the number of doses that are planned in the clinic. The repeat-dose toxicity studies with BNT162b2 included administration of 3 total doses, one more than is administered in the clinic, consistent with standard industry practice for infectious disease vaccine evaluation. As platform data was available, a shortened administration paradigm was used in the repeat dose toxicity studies in order to assess the toxicity of the vaccine with a shorter study timeline allowing more rapid transition into clinical trials. There was a robust immune response elicited by BNT162b2 by the end of the dosing and recovery phases of the study, thus supporting that toxicity related to the immune response was evaluated. At the time of the study initiation, the half-life of the novel excipients was not known. However, the amount of lipid would presumably be significantly less at the end of recovery phase and all findings observed during the dosing phase were either resolved or partially resolved by the end of the 3-week recovery period. In addition, the doses used in the toxicity studies are 300-1000 fold higher than the clinical dose when normalized for body weight.

C. The response is reasonable.

mRNA Kinetics

Q. **In the biodistribution study (Study no. R-20-0072), no tissues/organs were collected and processed to analyse the distribution of the mRNA/expressed protein. Sensitivity of the body imaging method to detect expressed protein is most likely low. A more sensitive method could have been used. Please justify.**

There are no distribution and degradation data on the S antigen-encoding mRNA. Please justify.

A. The S antigen encoding modRNA distribution will mirror that of the lipid nanoparticle (LNP) (studied in R-20-0072 and 185350). Following uptake into cells and the release of the modRNA from the LNP, the modRNA will be degraded by RNAase, a conserved clearance mechanism across species. Luciferase expression after IM administration of a modRNA encoding the luciferase protein was observed up to 9 days at the injection site, which is a sum of both the protein expression rate (translation of the modRNA) and half-life of the target protein. During this time, the luciferase signal at the injection site was reduced ~2000-fold (from approximately 1×10^9 to 5×10^5) indicating that the protein and therefore the modRNA has been eliminated.

The qualitative distribution data produced in study R-20-0072 are also supported by a radiolabelled biodistribution study carried out with a luciferase modRNA in an identical LNP to BNT162b2 (Study 185350) that showed 18% of the administered dose was present in the liver. Beyond the liver and the injection site, all other tissues examined had % of dose values of approximately 1% or lower.

C. The additional study has been evaluated in the current report and supports the overall conclusions from study R-20-0072. The response is reasonable.

Hepatocyte Vacuolation

Q. **In the toxicity study with BNT162b2 (V8) and other mRNA variants (Study. 38166), hepatocyte vacuolation was observed in all treated groups. The sponsor indicated that "vacuolation of liver hepatocytes has been associated with hepatic clearance of the PEGylated lipid in the LNPs (Ivens et al, 2015)". The sponsor's statement is inconsistent with the findings reported in the cited article. As noted in the article, pegylated substances are taken up by macrophages, resulting in vacuolation of Kupffer cells in liver, but not vacuolation of hepatocytes. Please explain the hepatocyte vacuolation findings in rats.**

A. IM LNP-luciferase mRNA biodistribution studies demonstrated liver distribution and IV and IM ADME studies demonstrated that both ALC-0159 and ALC-0315 distribute to the liver and the microscopic appearance and distribution of the vacuoles are consistent with lipid. Because hepatocyte vacuolation, in addition to phagocytic cell vacuolation, had been reported with PEGylated drugs, the preliminary thought was that the vacuoles reflected distribution or clearance of the ALC-0159 PEG-lipid, or the PEG component of the ALC-0159 PEG-lipid, to the liver. Because ALC-0315 is the predominant lipid in the LNP and has slower clearance than the PEG-lipid ALC-0159, the hepatic vacuoles more likely reflect ALC-0315 and not ALC-0159 distribution to the liver.

C. The observation of vacuolation was not associated with adverse clinical chemistry or histopathology findings and were fully reversible 3 weeks following the last dose. Given the very large MOE achieved in these studies the findings do not raise safety issues and the applicants response is reasonable.

Questions related to LNP Toxicity & Characterisation in NonClinical Studies

Q. **The results of the biodistribution study with luciferase-encoding mRNA (Study R-20-0072) were expected to be representative of BNT162b2, as the LNP formulated luciferase-encoding mRNA had the same lipid composition but the study description stated LNP8 had a particle size of 171 nm, much bigger than the particle size of the LNP in the clinical candidate (60 – 80 nm).**

Please also provide detailed information on LNP used in BNT162b2 formulations in all nonclinical studies (pharmacology, pharmacokinetics and toxicity studies).

Several manufacturing process changes for BNT162b2 mRNA were introduced during development. The proposed commercial scale manufacturing process includes use of linearised plasmid DNA template for mRNA production, whereas in early development phases PCR-amplification of DNA template was used. It is not clear which manufacturing process was used for BNT162b2 mRNA used in nonclinical studies. Please clarify.

A. The particle size of LNP8 in the study R-20-0072 was 71 nm & the report has been corrected and re-submitted (ie 171 was a typo). We confirm that the LNP formulation used in BNT162b2 was termed "LNP8". LNP8 has the identical lipid composition as that used in BNT162b2. Pharmacological and toxicology study reports include detailed certificates of analysis. For other studies, key details are summarized in the table provided, Table 5. For RBP020.1 LNP and RBP020.2 LNP, both RNAs were produced using the manufacturing process starting with (linear) PCR product. This PCR product was used as a template for mRNA production.

C. The response is reasonable

Table 1. LNP Characterisation for Nonclinical Studies

Study	LNP identity (batch)	Size [nm]	Encapsulation efficacy [%]	RNA [mg/mL]	Lipid			
					ALC-0315 [mg/mL]	ALC-0159 [mg/mL]	DSPC [mg/mL]	Cholesterol [mg/mL]
R-20-0072	LNP8 (FM-1074-D)	71	90	1	s 9(2)(b)(ii)			
PF-07302048_06Jul20_072424	FM-1261A	94	93	0.99				
185350	NC-0552-1	89	94	1.0	-[a]	-[a]	-[a]	-[a]
R-20-0085	BNT162b2 (V9) (RBP020.2 LNP)	76	94	0.508	s 9(2)(b)(ii)			
R-20-0112								
VR-VTR-10671								
20GR142								
20256434								
LPT 38166	BNT162b2 (V8) (RBP020.1 LNP)	58	91	0.554	s 9(2)(b)(ii)			

a. Not presented in the certificate of analysis.

Q. **In the rat distribution study of LNP (Study 185350), total radioactivity recovery was very low. This was possibly because the draining lymph nodes to the injection site were not collected and faeces, urine, carcass and cage-wash samples were not analysed. Please provide these data or justify otherwise.**

A. Study 185350 was a mRNA & LNP distribution rather than an excretion balance study carried out with a non-diffusible lipid marker, rather than individually labelled

components. Because of this aim, no excreta were measured and the sponsor did not digest and count the remains of the carcass, making the calculation of a balance impossible. The tissues collected for this study were a standard panel of tissues that did not include the draining lymph nodes; therefore, we cannot provide data on the portion of the dose in the draining lymph nodes. However, it seems likely, especially as we did detect radioactivity in the mandibular and mesenteric lymph nodes, that a portion of the dose would be found in these lymph nodes.

C. The response is reasonable.

Q. **No genotoxicity study was conducted with the novel excipients (ALC-0159 and ALC-0315) in the LNP formulation. Please justify or provide genotoxicity studies with the two excipients.**

ALC-0159 and ALC-0315 in the LNP formulation are novel excipients. The toxicity of the novel excipients was not studied alone. While the safety of the novel excipients was assessed in one species (i.e. rats) in the repeat dose studies with the vaccine formulation, the safety was not assessed in a second species. Please justify.

A. The toxicity of the lipids have been assessed as part of the 2 repeat-dose toxicity and one DART study using doses that provide a very high MOE over human clinical use and in accordance with WHO guidance, a single species is sufficient for the assessment of vaccine toxicity. Exposure to the novel lipids in the BNT162b2 LNP, ALC-0315 and ALC-0159, (which are not mutagenic based on in silico QSAR analysis) is low. For ALC-0159 the dose is 53.4 µg (administered twice in BNT162b2) which is less than the TTC, 120 µg/day - which is considered an acceptable daily exposure for a mutagenic carcinogen for durations of up to 1 month (ICH M7 [R1], 2017) - and for ALC-0315 the dose is 430 µg administered twice in BNT162b2, which is greater than the lifetime TTC of 120 µg/day. However, the carcinogenic risk is based on both dose and duration of exposure (ICH M7 [R1], 2017). There is a linear relationship between the amount of daily intake of a mutagenic impurity corresponding to a 10^{-5} cancer risk and the number of treatment days. The daily dose corresponding to 10^{-5} cancer risk (lifetime acceptable intake) of a mutagenic carcinogen which is administered 2 days is 19162.5 µg (1.5 µg x 365 days x 70 years/2 days), significantly greater than the daily dose of ALC-0315 administered in BNT162b2. In addition, the primary metabolites of ALC-0159 and ALC-0315 have been identified in vitro in a range of species, including human, and in the rat pharmacokinetic study (Study PF-07302048_05Aug20_043725) which show all metabolites seen in human are in the rat as well as in a range of other species used in the pharmacological testing.

ALC-0159 and ALC-0315 or their metabolites are expected to remain sequestered within the lysosomal compartment, not able to enter the nucleus and thus will not be able to interact directly with DNA or chromosomes. It has been well established that the active lipids in this class remain associated with the LNP and lysosomal pathway after intracellular delivery in vitro and in vivo (Marcusson et al, 1998; Song

et al, 2002; Gilleron et al, 2013; Sahay et al, 2013; Maier et al, 2013; Sabnis et al, 2018), with the cationic lipid and/or LNP lipid markers being excluded from the nucleus and remaining peri-nuclear (Marcusson et al, 1998; Song et al, 2002; Maier et al, 2013; Sabnis,et al, 2018). As the lysosomal compartment matures, the lipids are metabolized and excreted (Maier et al, 2013). Additionally, much of the PEG-lipid is lost from the LNP before entry into target cells (Mui et al, 2013).

Finally, ALC-0315 and ALC-0159 are highly similar in structure and function to the ionizable cationic lipid DLin-MC3-DMA and the PEGylated lipid PEG-2000-C-DMG in the siRNA drug Patisiran (ONPATTRO®; Akinc et al, 2019), which received marketing authorization in the US and Europe in 2018. DLin-MC3-DMA and PEG-2000-C-DMG were evaluated individually and in combination as the drug substance in an extensive genetic toxicology test battery (Onpattro FDA multidiscipline review, 2018). Both the drug substance and the individually tested lipids were negative in all genetic toxicology tests. Taken together, this assessment supports that exposure to ALC-0159 and ALC-0315 as components of BNT162b2 does not present a genotoxic risk.

C. The novel lipid excipients ALC-0159 and ALC-0315 and their metabolites are, as presented by Pfizer, highly unlikely to be genotoxic and exposures from clinical use are very low. The use of TTC values for genotoxic impurities is highly conservative and the arguments presented by the applicant are sound.

Questions Regarding immune response

Q. **Potent type I interferon responses were found to be associated not only with inflammation but also potentially with autoimmunity.[1] IFN-γ has also been found to play a role in autoimmunity.[2,3] IFN-γ was increased in animals immunised with BNT162b2. Please comment on the clinical relevance of these findings.**

A. The spike antigen-specific IFN-γ response measured in mouse, nonhuman primate, and humans represents a coordinated immune response against a viral intrusion (Vabret et al, 2020). IFN-γ is a key cytokine for several anti-viral responses (McNab et al, 2015; Lee and Ashkar, 2018). It acts in synergy with type I interferons to inhibit replication of SARS coronaviruses (Sainz et al, 2004). The strongly TH1-biased CD4+ T-cell response and robust production of IFNγ from CD8+ T cells, with both anti-viral and immune-augmenting properties, is a pattern favored for vaccine safety and efficacy (Lambert et al, 2020). No evidence of immunopathology has been observed in nonclinical studies and there has been no evidence of systemic immunotoxicity in the ongoing clinical trials.

C. The response is reasonable.

Q. **Rhesus macaques do not show clinical signs and generally develop only mild lung pathology from SARS-CoV-2 infection. In the immunogenicity and protection study (Study no. VR-VTR-10671), almost similar lung inflammation was observed in both**

challenged control and immunised animals in the protection study. Please comment.

A. Lung histology was performed at Days 7 to 8 after SARS-CoV-2 challenge, several days past the Day 1 to 3 peak of viral shedding and acute inflammation observed by radiography (e.g. pulmonary CT scan), respectively (Munster et al, 2020). Similar kinetics have been observed in the literature and highlights the transient nature of the infection and inflammation in the rhesus challenge model. The use of younger animals in this study likely contributes to the limited nature of pulmonary inflammation (Singh et al, 2020; Yu et al, 2020; Hewitt et al, 2020). Lastly, SARS-CoV-2 pulmonary inflammation in monkeys tends to be multifocal (Rockx et al, 2020) & it is possible that the selected tissue trimmed for, did not overlap affected areas.

C. The response is reasonable.

Q. **In the immunogenicity study in monkeys (Study no. VR-VTR-10671), S protein binding and virus neutralising antibodies, and to a lesser extent, S specific CD4+ T cells, declined relatively quickly over 5 weeks after the second dose. Are there data on antibodies and T cells beyond 5 weeks? Please comment on the clinical relevance of these findings.**

In the same monkey study, BNT162b2 administration induced a high frequency of specific CD4+ T cells producing IFN-γ, IL-2, or TNF-α and a low frequency of CD4+ cells that produce IL-4, indicating a TH1-biased response, but one monkey had a higher TH2 response than TH1. In the mouse immunogenicity study (Study no. R-20-0085), two higher doses (1 and 5 µg) induced higher IgG2a than IgG1, but the low dose (0.5 µg) induced higher IgG1 than IgG2a. In addition, the vaccine increased TH1 type cytokines (IFNγ, IL-2, TNFα) release and very low levels of TH2 cytokines (IL-4, IL-5 and IL-13) when stimulated with a S peptide pool, but high levels of IL-4, IL-5 and IL-13 were noted in one out of 8 animals in the 1 µg dose group. Were the high IL-4, IL-5 and IL-13 levels observed in the same animal? Please comment on these findings in relation to the potential of the vaccine for antibody-dependent disease enhancement (ADE) and vaccine-associated enhanced respiratory disease (VAERD).

A. Assessment of duration of immunity in rhesus macaques was limited to approximately 1 month after the Dose 2 as animals were subsequently used to assess protection against SARS-CoV-2 challenge. The SARS-CoV-2 neutralizing GMT in BNT162b2-immunized rhesus macaques continued to decay but remained above the GMT of a human convalescent serum panel. In the ongoing Phase 1 studies in the US and Germany (C4591001 and BNT162-01, respectively), persistence of antibody and T cell responses are being assessed up to 2 years following vaccination and will provide the most relevant and reliable measure of long-term memory.

The elevated levels of IL-4, IL-5 and IL-13 were observed in the same mouse. While 7 out of 8 mice indicated a TH1-biased response based on Luminex and IgG subtype ratio, 1 mouse out of 8 showed a stronger TH2 induction. Differences in total IgG and neutralizing antibodies were not observed; in addition, the IFNγ release analysis

based on ELISpot showed a strong cytokine release also for this mouse, still indicating a potent TH1 T-cell response. In the monkey study, one BNT162b2-immunized animal (100 µg dose) had an overall lower spike-specific T cell response. More equivalent levels of IFNγ and IL-4 secretion by ELISpot were observed in contrast to slightly higher IL-4 levels detected by intracellular cytokine staining (ICS) analysis. These results are not interpreted as a TH2-skewed T cell response, in which case much higher IL-4 levels would be expected relative to IFNγ by both ELISpot and ICS. In addition, in a more diverse HLA setting, human data from the BNT162-01 clinical trial has demonstrated a strong TH1-dominant CD4+ T cell and IFNγ+ CD8+ T cell response following two doses of 30 µg BNT162b2 (Sahin et al, 2020b) and BNT162b1 (Sahin et al, 2020a). After the challenge monkeys, no enhanced disease was observed in any BNT162b2 immunized monkey arguing against vaccine-induced ADE and VAERD.

C. The response is reasonable.

Other Issues

Q. **In the mouse immunogenicity study (no. R-20-0112), results of B cells in the spleen in Figure 11 b and c (page 58) are labelled as "cells per LN". Please clarify whether the results were for spleen or lymph node**.

A. the labelling of the y-axis in Fig. 11 b and c reflects the results observed in spleen.

Q. **In Study 20GR142 microscopic findings were noted in the draining lymph node. Please specify which lymph node.**

A. The iliac lymph node is the draining lymph node for a quadricep IM injection

Q. **A summary on P2S antigen expression in mammalian cells Expi293 and structural and biophysical characterisation was provided in the nonclinical pharmacology written summary, without providing the studies in the dossier. Please provide the complete study report(s).**

A. Study report has been provided and is reviewed in the current evaluation

4 DETAILED EVALUATION

4.1 PHARMACOLOGY

4.1.1 Primary Pharmacodynamics

4.1.1.1 Immunogenicity

s 9(2)(a) Author. s 9(2)(a) Responsible person (2020) **Covid-19: Immunogenicity Study of The LNP formulated ModRNA Encoding the Viral S Protein-V8.** Study Report. R-20-0054. 27 Nov 2020

Test Material:	BNT162b2 V8
Batch:	BNT162b2 /RBP020.1, LNP Polymun batch RBP020.2 LNP with the lot:CoVVAC/270320
Animals / Source	Female BALB/c mice, source not stated. 9 wks old
Study dates:	19 Mar 2020 – 16 Apr 2020
Laboratory:	BioNTech SE, An der Goldgrube 12, 55131 Mainz, Germany
GLP & QA:	Non-GLP, QA was certified
Test Guideline:	None stated

Conduct of the Study

This study examined the immunogenicity of a non-modified uridine-containing mRNA encoding the antigen variant 8 (V8) of the BNT162b2 mRNA. A closely similar study was conducted with the V9 mRNA variant in study R-20-0085 that was reviewed in the TGA interim report. That study utilised the same species of mice, the same doses and timing and the same analyses.

Four groups of 8 female BALB/c mice were immunized on day 0 with doses of 0.2 µg, 1 µg, or 5 µg of RNA per animal of the mRNA encapsulated in (LNPs), or with the buffer alone (control group), by intramuscular injection in a volume of 20 µL. Blood was collected on days 7, 14, 21, and 28 after immunization to analyse the antibody immune response by ELISA and pseudo-virus based neutralization test (pVNT). On day 28, spleens were collected for splenocyte isolation and analysis of T-cell responses using interferon γ (IFN-γ) -specific ELISpot assays. Luminex assays and intracellular cytokine staining (ICS) were performed to assess cytokine/ chemokine responses.

Results

IgGs against recombinant S1 protein or receptor binding domain (RBD) were detected by ELISA screening analysis in serum samples obtained on study days 7, 14, and 21. Treatment with BNT162b2 V8, but not the control buffer group, induced the formation of IgGs specific for S1 protein and the RBD, at all tested doses in a dose related manner.

Figure 2 .ELISA endpoint titration on day 28

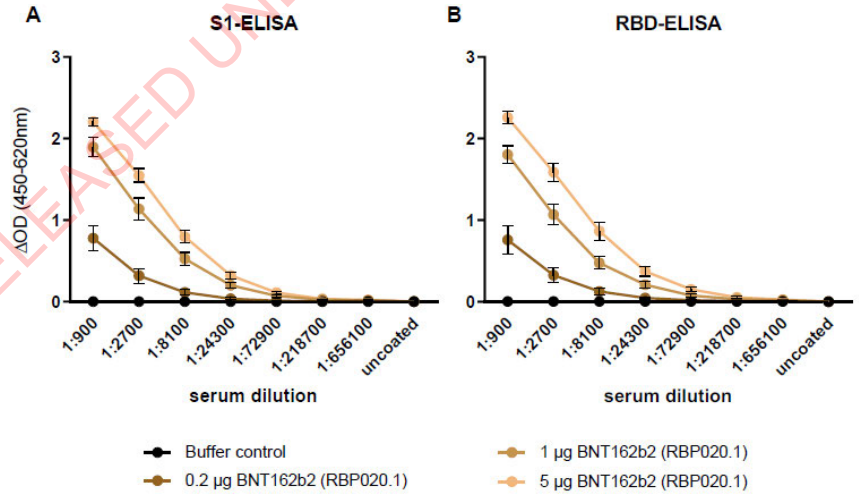


Figure 3. ELISA screening analysis on days 7, 14, and 21 against the recombinant S1 protein

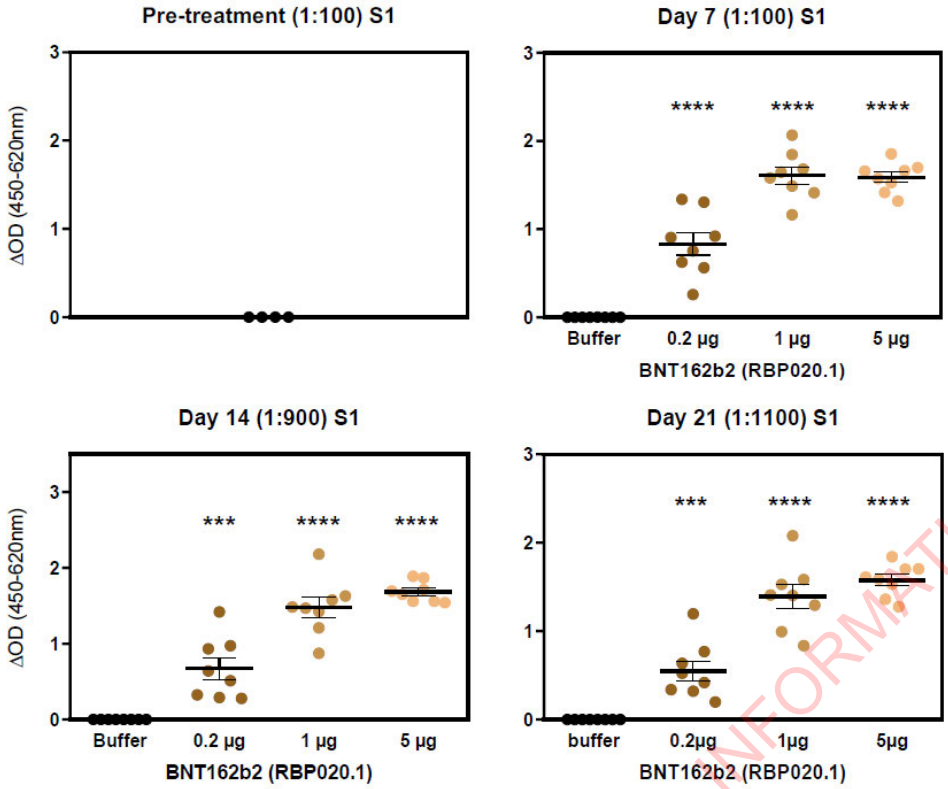
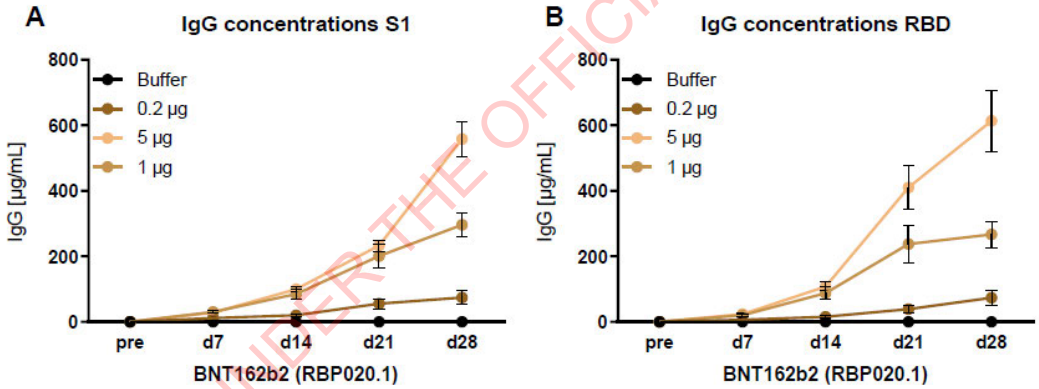


Figure 4.Kinetic of the antibody concentration against the viral antigen



BNT162b2 V8 induced a dose dependent formation of IgG1 and IgG2A specific for S1 protein, with all doses inducing a balanced ratio with slightly higher IgG2A concentrations compared to IgG1.

Virus-neutralizing antibodies in serum samples obtained on study days 14, 21, and 28 were detected by pVNT with doses of 1 µg and 5 µg inducing the formation of virus neutralizing antibodies with temporally increasing pVN_{50} titres, while no or only very low formation of neutralizing antibodies was observed for the group treated with the lowest dose of 0.2 µg.

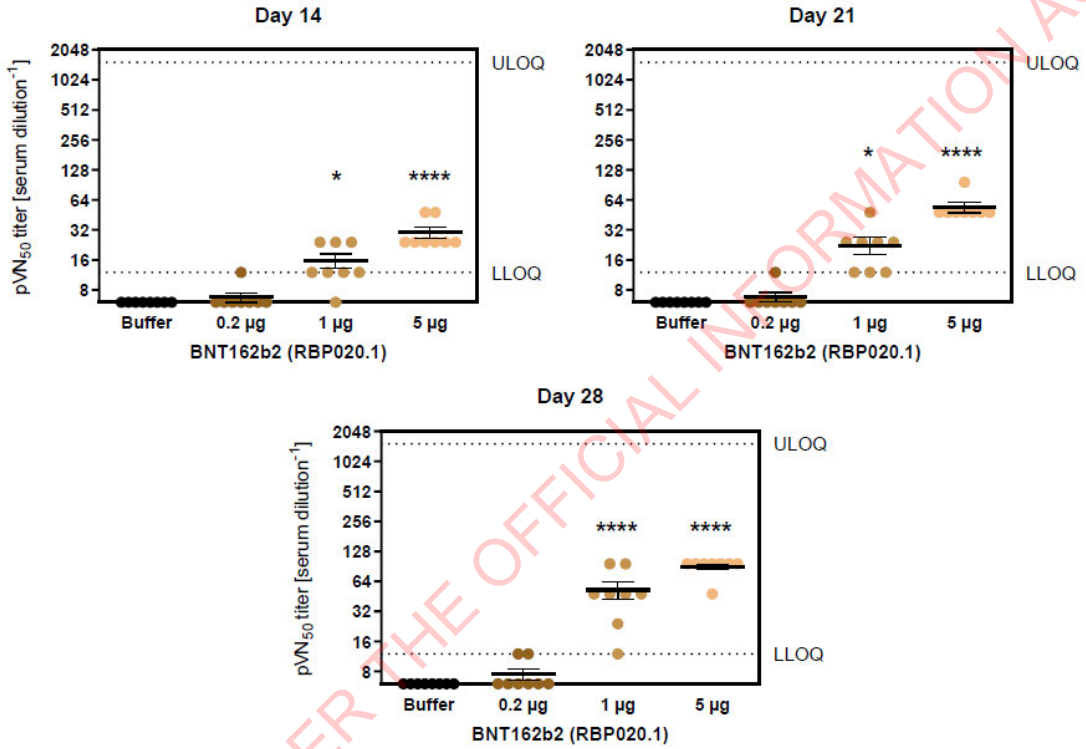
Antibody titres on Day 28 are provided in Table 6.

Table 6. Antibody tires on day 28

	BNT162b2 0.2µg	BNT162b2 1µg	BNT162b2 5µg
Anti-S1 protein total IgG [µg/mL]	74.0 ± 21.3	296.2 ± 37.2	558.4 ± 53.
Anti-RBD protein total IgG [µg/mL]	73.4 ± 23.1	266.9 ± 40.6	410.5 ± 66.3
pVN_{50} titer [reciprocal dilution]	7.5 ± 0.9	52.5 ± 10.6	90.0 ± 5.6

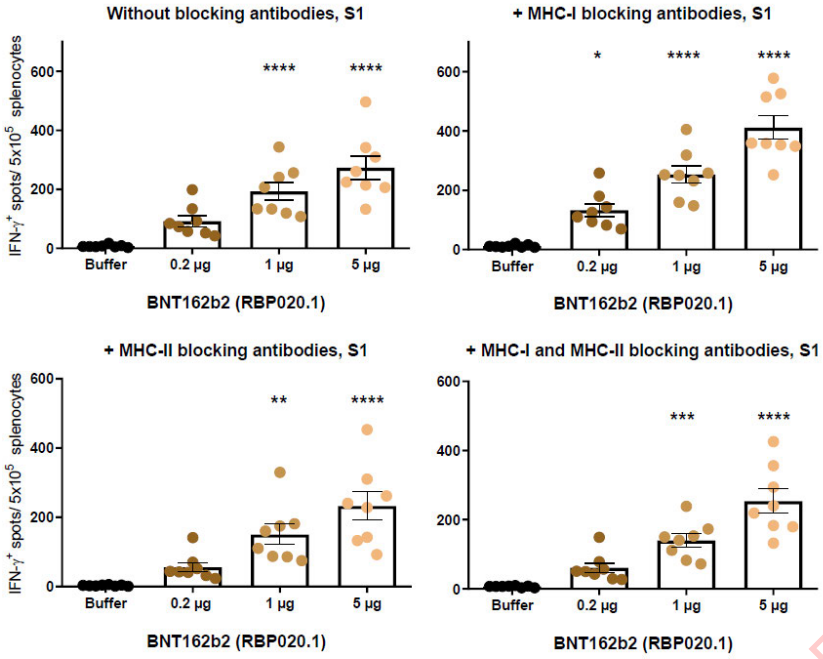
Antibody titres were slower to rise than for the V9 variant (study R-20-0085) but comparable by day 28 (see assessment in TGA report pg 13). The $pVNT_{50}$ values however were substantially (approx. 4 fold) lower than that for the V9 variant.

Figure 5.Titers of neutralizing antibodies on days 14, 21, and 28



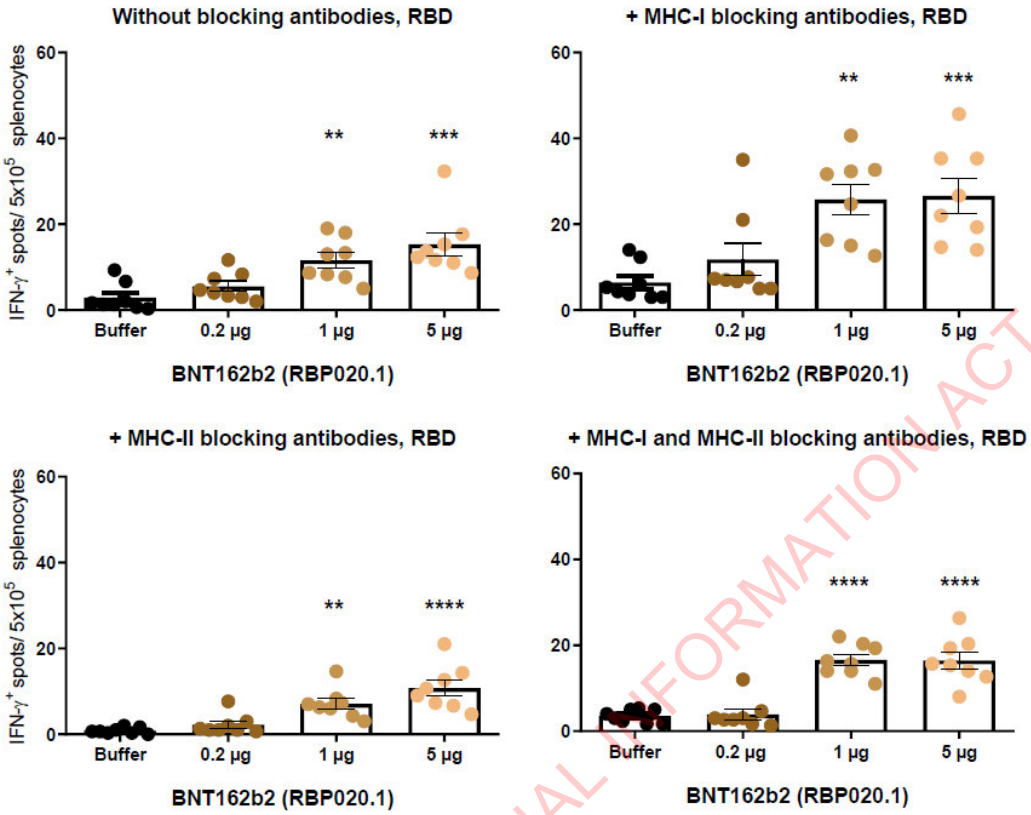
On day 28 following sacrifice splenocytes were isolated and stimulated with S1 and RBD peptide pools and IFNγ secretion was analysed. Stimulation of splenocytes with an S1-specific overlapping peptide pool without preincubation with MHC-I or MHC-II blocking antibodies induced IFN-γ responses in T cells of animals immunized with BNT162b2 V8 at all doses. An IFN-γ response to stimulation was also detected after pre-incubation with MHC-II blocking antibodies or with MHC-I and MHC-II blocking antibodies but only at 1 and 5 µL doses.

Figure 6. ELISpot analysis after stimulation with an S protein-specific peptide pool on day 28



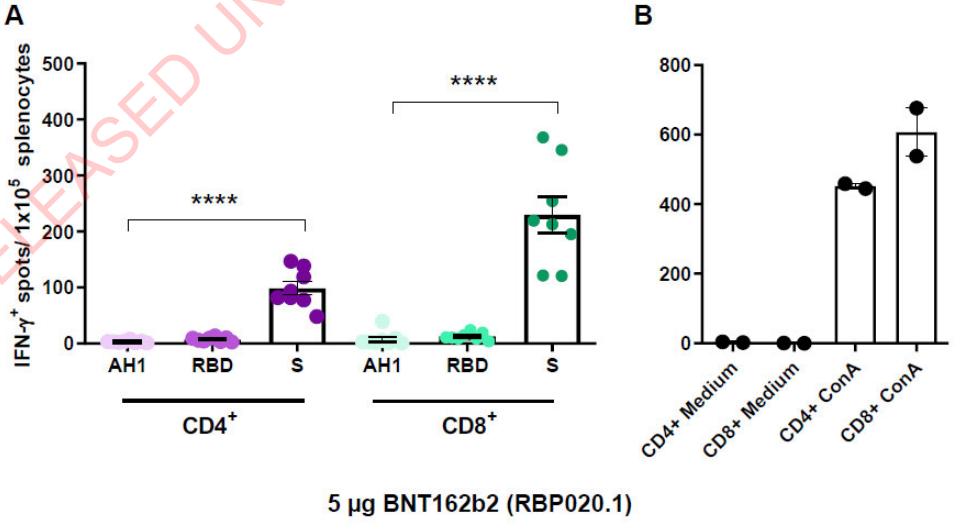
Stimulation of splenocytes with an RBD-specific overlapping peptide pool without preincubation with MHC-I or MHC-II blocking antibodies induced IFN-γ responses in T cells of animals immunized with BNT162b2 at all doses Cells from animals treated with 1 µg or 5 µg BNT162b2 which were stimulated after pre-incubation with MHC-I and/or MHC-II blocking antibodies also displayed significantly higher spot numbers than control splenocytes.

Figure 7. Cytokine analysis after stimulation with an RBD-specific peptide pool on day 28



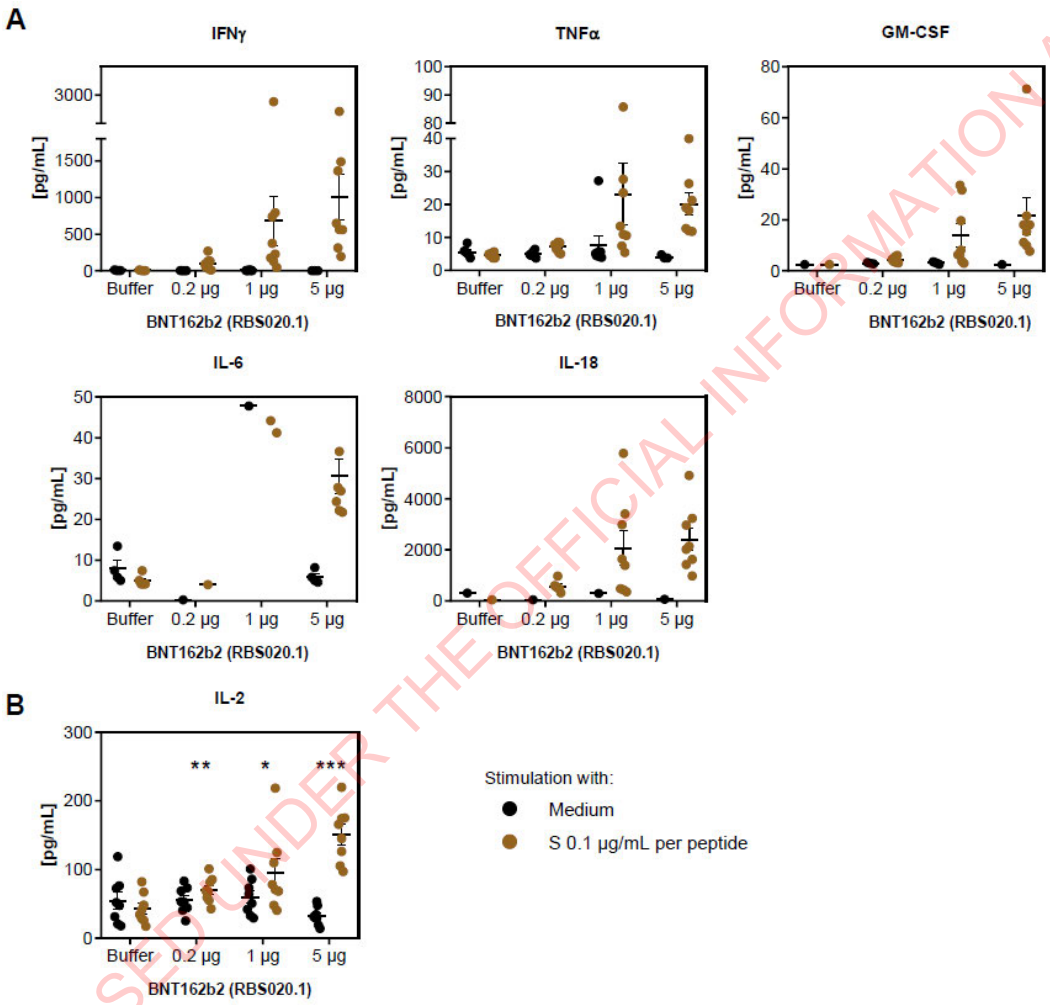
To identify the responding T-cell subtype, an additional analysis was performed after separation of CD4+ and CD8+ cells by MACS (magnetic antigen cell separation) isolation using splenocytes isolated from the group treated with 5 µg RNA. After stimulation with an S protein-specific peptide pool, but not after stimulation with an irrelevant peptide (derived from endogenous retroviral gene product envelope glycoprotein 70, AH-1) both CD4+ and CD8+ cells displayed IFN-γ responses.

Figure 8. ELISpot analysis using splenocytes on day 28 after MACS cell separation



For cytokine analysis, splenocytes from immunized animals were stimulated with either medium, PMA and ionomycin, or the S protein- or RBD-specific overlapping peptide mix. Immunization with BNT162b2 V8 induced an increased level of most TH1-specific analytes and IL-2. Stimulation of splenocytes with 0.1 µg/mL per peptide of the S-specific overlapping peptide induced a stronger increase in cytokine concentrations than 0.165 µg/mL per peptide of the RBD-specific overlapping peptide pool. For IFN-γ, TNF-α, GM-CSF, IL-18, and IL-2, a dose-dependent stimulation was observed.

Figure 9.Cytokine concentrations in supernatants of re-stimulated splenocytes 28 days after



The study authors concluded that "the vaccine candidate was immunogenic, inducing a high titre of IgGs against the antigen and a desired Th1-driven T-cell response. pVN antibody titres however were lower than expected for the V8 platform. Therefore, while the candidate would be suitable for clinical testing, a candidate which induces higher titres of functional antibodies might be desired."

Conclusions

BNT162b2 V8 given to mice as a single dose of 0.2 µg, 1 µg, or 5 µg, induced anti-S and anti-RBD IgG and neutralising antibodies against the antigen, with high binding affinity to S1 and

the RBD, as well as a Th1-biased T cell response including a cytotoxic T-cell response. The results of this study are concordant with, and support, those obtained for the V9 variant although the V8 variant produced a $pVNT_{50}$ titre 4 fold less than the V9 variant.

BNT162b2 (V9) Immunogenicity and Evaluation of Protection against SARS-CoV-2 Challenge in Rhesus Macaques. COVID Rh2020-01 (NIRC study #: 8725-2005), (SNPRC Study #: Covid-1778), Study No. VR-VTR-10671

Test Material:	BNT162b2 V9 LNP
Batch:	CoVVAC/270320
Animals / Source	rhesus macaques 2-4 years old
Study dates:	7/04/20 – 1/11/20
Laboratory:	Pfizer Vaccine Research and Development, 401 N. Middletown Rd. Pearl River, NY
GLP & QA:	No
Test Guideline:	Non-guideline

The interim study report, lacking the histology of lung tissue results, was evaluated in the TGA interim report and the conclusions provided below, other than for the histology findings are drawn from the TGA report after cross verification against the final study report.

Conduct of the Study

Male rhesus macaques in groups of 6 were administered normal saline (control) or BNT162b2 V9 LNP at 30 or 100 µg in 0.5 ml of normal saline, IM, on days 0 and 21 of the study. Blood samples were collected at pre-dose, and weekly after dosing to day 56. S-specific T-cell responses were analysed by ELISpot and ICS.

At 55 days after the second immunisation (day 76 of the study) the group immunised with two IM doses of 100 µg BNT162b2 (V9) and 3 control monkeys were challenged with 1.05×10^6 plaque forming units of SARS-CoV-2 (strain USA-WA1/2020), split equally between the intranasal (IN) and intratracheal (IT) routes. A separate sentinel group of age- and sex-matched animals (n=3) from the 30 µg BNT162b2-immunized group was mock challenged with cell culture medium (DMEM supplemented with 10% FCS).

Nasal and oropharyngeal swabs were collected from all macaques pre challenge and at Days, 1, 3, and 6 (relative to the day of challenge), and additionally from BNT162b2-immunized macaques on Day 7 or 8, and from control and sentinel macaques on Day 10. Bronchoalveolar lavage (BAL) was performed on macaques the week before challenge and on Days 3 and 6 post-challenge and on BNT162b2-immunized macaques on Day 7 or 8. Samples were tested for the presence of SARS-CoV-2 RNA by RT-qPCR using the CDC-developed 2019-nCoV_N1 assay.

Necropsy was performed on BNT162b2-immunized animals on Day 7 or 8. Control and sentinel animals were not necropsied to allow further use in a separate study.

Results

Summary from TGA assessment

Immunogenicity

- S1 specific IgGs were detected by day 14 after the first dose, the IgGs markedly increased after dose 2 in a dose dependent manner. Negligible S1 specific IgG was detected in the control group.
- The geometric mean concentration (**GMCs**) of S1-binding IgG (30,339 U/mL and 34,668 U/mL at 30 µg and 100 µg doses, respectively) 7 days after the 2nd immunisation dose were significantly higher than the S1-binding IgG GMC (631 U/mL) of human convalescent serum (**HCS**). IgG relatively rapidly decreased to ~4000 and ~6000 U/mL on day 56 (5 weeks after the 2nd immunisation dose).
- BNT162b2 (V9) administration stimulated 50% serum neutralising titres (VNT_{50}) as detected in monkey sera by day 14 after the first dose (measured as **geometric mean titre or GMT**). GMTs peaked 1 week after the second dose (GMT 962, 14 days after dose 2 of 30 µg and GMT 1689 after dose 2 of 100 µg) and lasted to day 56 with GMTs significantly higher than neutralisation GMTs of the HCS (GMT 94). By 5 weeks after the second dose, neutralising titres decreased by 3 to 5 fold from the peak.
- Strong IFN-γ and low IL-4 responses were detected in BNT162b2-immunised animals (ELISpot analysis) on day 28 and 42.
- BNT162b2 administration induced a high frequency of S-specific CD4+ T cells producing IFN-γ, IL-2, or TNF-α but a low frequency of CD4+ cells that produce IL-4, indicating a TH1-biased response except for one monkey, which has a slightly higher TH2 response than TH1. S-specific IFN-γ producing CD8+ T cell responses were also observed in immunised animals (flow cytometry ICS analysis). The vaccine also increased IFN-γ CD8+ T cells.
- The T-cell responses were similar between the two dose levels. S protein specific T cell responses decreased from day 28 to day 42 by <2 fold (not determined on day 56), although the decrease was relatively small or there was no significant change for CD8+ T cells in the high dose group (100 µg)

Protection

- At the time of challenge, SARS-CoV-2 neutralising titres ranged from 260 to 1,004 in the BNT162b2 (V9)-immunised animals. Neutralising titres were undetectable in animals from the control-immunised and sentinel groups.
- Viral RNA was detected in BAL fluid from 2/3 control-immunised animals on day 3 and from 1/2 on day 6 post challenge whereas no viral RNA was detected in BAL fluid from the BNT162b2 (V9)-immunised and SARS-CoV-2 challenged macaques (statistically significant).
- Viral RNA was detected in nasal swabs obtained from control-immunised animals on days 1 (viral RNA: ~ 10^4 log10 copies), 3 (~ 10^4 log10 copies), and 6 (~ 10^3 log10 copies) post challenge. High viral RNA copies were detected in nasal swabs obtained from BNT162b2 (V9)-immunised animals on Day 1 (viral RNA: day 1 ~ 10^5 log10), but

RNA copies were below the limit of detection from day 3 (less than ~ 10^2 log10 copies).

- Low levels of RNA were observed from OP swabs in immunised monkeys on days 1 and 3, compared to the control-immunised monkeys.
- No significant clinical signs of illness were observed in any group of animals including control.
- Radiographic evidence (X-ray and CT) of pulmonary abnormality was observed in challenged controls but not in challenged BNT162b2- immunised animals nor in unchallenged sentinels. No radiographic evidence of vaccine elicited enhanced disease was observed.

New Data

The final study report incorporating the histology of the lungs of the 100 µg vaccinated group was provided on 10/12/20.

The main Histopathology finding in the lung was inflammation. The lung inflammation area score was similar between saline-immunized and BNT162b2-immunized animals, and there was no evidence of enhanced respiratory disease. Inflammatory cell infiltrates included macrophages, neutrophils, lymphocytes, plasma cells, and some eosinophils. There were no other significant microscopic findings in other tissues. The discordance between the higher lung inflammation in controls observed by radiography and the absence of this difference histologically is likely due to the transient nature of lung inflammation in the Rhesus Monkey and the time gap between the Day 1 to 3 peak of viral shedding and acute inflammation observed by radiography and tissue sampling for histology at Days 7 to 8 after SARS-CoV-2 challenge.

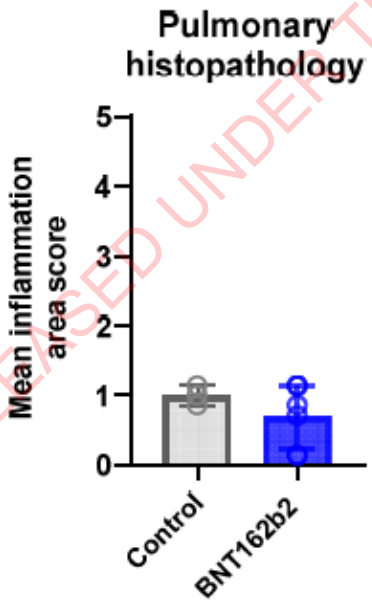


Figure 10. Lung inflammation area score on Day 7 or 8 after IN/IT SARS-CoV-2 challenge. Each data point (Control n=3, Test n=6) represents the mean lung inflammation area score of a single animal (mean score of the 7 lung lobes).

Conclusions

BNT162b2 (V9) is highly immunogenic in rhesus macaques and paralleled its immunogenicity in mice. Seven days after Dose 2 of 100 µg, the neutralizing Geometric Mean Titre reached 18-times that of a human SARS-CoV-2 convalescent serum panel and remained 3.3-times higher than this benchmark five weeks after the last immunization. The strongly Th1-biased CD4+ T cell response and IFNγ+ CD8+ T-cell response to BNT162b2 is a pattern consistent with vaccine safety and efficacy. BNT162b2 protected 2-4 year old rhesus macaques from infectious SARS-CoV-2 challenge, with reduced detection of viral RNA in immunized animals compared to those that received saline and with no radiological, microscopic, or clinical evidence of exacerbation. An absence of RT-qPCR detectable SARS-CoV-2 RNA in serial BAL samples obtained starting 3 days after challenge of BNT162b2-immunized rhesus macaques provides evidence of lower respiratory tract protection.

4.1.1.2 S Protein characterisation and Expression

s 9(2)(a) **Structural and Biophysical Characterization of SARS-CoV-2 Spike Glycoprotein (P2 S) as a Vaccine Antigen.** Study No. vr-vtr10741

Test Material:	SARS-CoV-2 P2 S Protein, AA sequence identical to that coded by BNT162b2 RNA
Batch:	NA
Animals / Source	NA
Study dates:	7 Apr 2020 – 19 Aug 2020
Laboratory:	Pfizer Discovery Sciences, Eastern Point Road, Groton, CT
GLP & QA:	No
Test Guideline:	Non-Guideline

Conduct of the Study

This study explores the structure and binding of SARS-CoV-2 P2 S expressed from DNA that encodes the same amino acid sequence as BNT162b2 RNA.

Antibody binding to transfected Expi293F cells was investigated using flow cytometry. Transfected Expi293F cells were incubated with (i) labelled anti-6xHis plus His-tagged human ACE2 peptidase domain (ACE2-PD); (ii) labelled anti-Rabbit IgG Fab plus either anti-SARS-CoV-2 Spike RBD (receptor Binding Domain), anti-SARS-Cov-2 Spike S1, or anti-SARS Spike S2; or (iii) labelled ant-Human IgG Fab plus either CR3022 therapeutic antibody, B38 neutralizing antibody, or H4 neutralizing antibody. For each condition, three replicates were measured with 3000 events collected per replicate.

The kinetics of binding of solubilized, purified P2 S to ACE2-PD and human neutralizing monoclonal antibody B38 was measured by biolayer interferometry.

For structural analysis P2 S protein was harvested from Expi293F transfected cells and purified by size-exclusion chromatography. A peak that consists of intact P2 S migrating at around 150 kDa, as well as dissociated S1 and S2 subunits (which co-migrate at just above 75 kDa), was used in the structural characterization. Spontaneous dissociation of the S1 and

S2 subunits occurs throughout the course of protein purification, starting at the point of detergent-mediated protein extraction, so that P2 S preparations also contain dissociated S1 and S2. The structure was examined using cryogenic electron microscopy.

Results

Binding of cell surface expressed P2 S to human ACE2 receptor and a panel of human neutralizing mAbs was confirmed in cells using flow cytometry. Protein expressed from DNA with the BNT162b2-encoded P2 S amino acid sequence was confirmed to be in the prefusion conformation by cryo-EM. This analysis showed that the antigenically important RBD can assume the 'up' conformation, with the receptor binding site, rich in neutralizing epitopes, accessible in a proportion of the molecules. Alternative states observed reflect a dynamic equilibrium between RBD 'up' and 'down' positions. Binding of expressed and purified P2 S to ACE2 and a neutralizing monoclonal antibody further demonstrates its conformational and antigenic integrity.

Conclusions

The encoded P2 S antigen of BNT162b2 RNA authentically presents the ACE2 binding site and other epitopes targeted by SARS-CoV-2 neutralizing antibodies.

s 9(2)(a) (2020) **In Vitro Expression of mRNA Constructs Encoding the SARS-COV-2 Spike Protein Variants V8 and V9.** Report No R-20-0360. 27 Nov 2020

Test material:	various V8 and V9 constructs in different RNA platforms
Batch:	Various
Animals / Source	HEK293T (Human embryonic kidney cells)
Study dates:	1 Apr 2020 – 9 Apr 2020
Laboratory:	BioNTech RNA Pharmaceuticals GmbH, An der Goldgrube 12, 55131 Mainz, Germany
GLP & QA:	Non-GLP, QA is certified
Test Guideline:	Non-Guideline

Conduct of the Study

This study is was conducted to investigate transfection and expression of various constructs of V8 and V9 in HEK293T cells. The study is similar to the later study Number R-20-0211-TGA but investigates multiple naked mRNA variants whereas the later study investigated only the variant V9, as both the naked mRNA and as the final drug product BNT162b2-LNP. Study R-20-211 is the definitive study and this study, R-20-0360-New, provides only background or supportive information.

Antigen expression from various V8 and V9 constructs in different RNA platforms were investigated by Fluorescence activated Cell Sorting (FACS). HEK293T cells in 12 well plates were transfected with the respective RNAs using a commercial transfection agent (RiboJuice) over an 18 hour incubation period. Following staining with the viability dye, eFluor, cells were stained with 50 µL rabbit anti-S1 antibody. Cells were then stained with a secondary anti-rabbit antibody conjugated to allophycocyanin before FACS analysis using flow cytometry.

A modRNA encoding green fluorescent protein (GFP) was used as a positive control.

Table 7. Overview of RNA platforms, constructs, and transfection amounts

Construct	RNA concentration (µg/well)
s 9(2)(b)(ii)	s 9(2)(b)(ii)
modRNA V8	1
s 9(2)(b)(ii)	s 9(2)(b)(ii)
modRNA V9	1
s 9(2)(b)(ii)	s 9(2)(b)(ii)
modRNA GFP	1

modRNA = nucleoside-modified mRNA, s 9(2)(b)(ii)
V = variant

Results

s 9(2)(b)(ii)

There were no major differences in cell viability observed between the groups of transfected cells and non-transfected cells.

Figure 11. s 9(2)(b)(ii)

s 9(2)(b)(ii)

Conclusions

s 9(2)(b)(ii)

4.1.2 Secondary & Safety Pharmacodynamics

Studies of secondary or safety pharmacology have not been conducted which is normal for a vaccine and consistent with WHO guidance.

4.1.3 Pharmacodynamic Drug Interactions

Studies of this type have not been conducted which is normal for a vaccine and consistent with WHO guidance.

4.2 PHARMACOKINETICS

4.2.1 Absorption

The vaccine is administered by IM injection. Absorption studies have not been conducted but distribution studies provide an indication of the relative proportion of mRNA expressed at the injection site and peripheral tissues.

4.2.2 Distribution

A Tissue Distribution Study of a [3H]-Labelled Lipid Nanoparticle-mRNA Formulation Containing ALC-0315 and ALC-0159 Following Intramuscular Administration in Wistar Han Rats. Study No. 185350, ALC-NC-0552

Test Material:	[3H]-08-A01-C01 An aqueous dispersion of lipid nanoparticles (08-A01-C01) which includes ALC-0315, ALC-0159, distearoylphosphatidylcholine, and cholesterol and a luciferase encoding mRNA
Batch:	NC-0552-1
Animals / Source	Han Wistar rats from s 9(2)(b)(ii)
Study dates:	16 Jul 2020 – 24 Sep 2020
Laboratory:	s 9(2)(b)(ii)
GLP & QA:	Not GLP but consistent with OECD GLP requirements
Test Guideline:	

Conduct of the Study

The test item, 08-A01-C01, is an aqueous dispersion of lipid nanoparticles (LNP), comprised of a proprietary mixture of lipid components (including ALC-0315, ALC-0159, distearoylphosphatidylcholine, and cholesterol) and mRNA encoding luciferase as a model protein. LNPs included trace amounts of radiolabelled [Cholesteryl-1,2-^{3}H(N)]-Cholesteryl Hexadecyl Ether ([3H]-CHE), a nonexchangeable, non-metabolisable lipid marker used to monitor the disposition of the lipid nanoparticles. Once intracellular, the [3H]-CHE does not recirculate and therefore allows assessment of distribution of the particles.

Wistar rats in groups of 21 per sex were administered the radiolabelled LNPs as a single IM dose equivalent to 50 µg mRNA/animal (1.29 mg/animal of total lipid dose). The content and concentration of total radioactivity in blood, plasma and tissues were determined in 3 animals per sex at 0.25 1, 2, 4, 8, 24 and 48 hours post-dose. The concentration of total radioactivity was measured by liquid scintillation counting (LSC).

Radiolabel was analysed in the following tissues: Adipose tissue, Ovaries, Adrenal glands, Pancreas, Bladder, Pituitary gland, Bone (femur), Prostate, Bone marrow (femur), Salivary glands, Brain, Skin, Eyes, Muscle, Heart, Small intestine, Injection site, Spinal cord, Kidneys,

Spleen, Large intestine, Stomach, Liver, Testes, Lung, Thymus, Lymph node (mandibular), Thyroid, Lymph node (mesenteric), and Uterus.

Results

Initially, 21 male rats were dosed at 100 µg mRNA/animal. Adverse clinical signs including body weight loss, piloerection, ungroomed, brown staining on muzzle, hunched posture and irregular respiration, were observed after approximately 24 hours post-dose with one animal humanely killed. A subsequent review of this initial data showed concentrations were well detected in tissues and therefore the target dose was consequently lowered to 50 µg mRNA/animal for the remainder of the study to avoid clinical signs of toxicty. All following comments relate to the 50 µg groups.

There were no adverse effects observed in males, and in females clinical signs of toxicity were limited to a single animal with decreased activity, and irregular respiration that was additionally hunched and piloerect at 48 hrs post dose.

A high inter-animal variability in concentration and % of injected dose values was found for the injection site, likely due to difficulty in identifying the total area that the injected bolus dose migrated to, as this was not visible. When dosing the male 50 µg mRNA group, the injection site was circled using a marker pen to help aid dissection of the injection area. The overall injection site concentrations and % dose values were higher in males than in females likely due to more consistent identification and collection in males, since concentrations in other tissues were broadly similar between the sexes.

Following a single intramuscular administration of [3H]-08-A01-C01, the greatest mean tissue concentration and, in most instances, % of injected dose was found remaining in the injection site at each time point in both sexes. The injection site mean concentration and equivalent % dose values are presented in the table below. The highest mean recovery of total radioactivity at the injection site occurred at 1 hour after dosing.

Table 8. Recovery of radiolabel at injection site

Timepoint (h)	Injection site (µg equiv lipid/g)		Injection site (% dose)	
	Male	Female	Male	Female
0.25	219.940	36.566	32.887	6.815
1	587.670	199.950	68.829	36.411
2	529.210	93.144	39.053	24.094
4	619.850	56.227	47.710	9.056
8	299.590	125.930	18.731	24.993
24	267.170	122.540	31.957	26.295
48	268.770	61.088	32.823	16.426

Low levels of radioactivity were detected in most tissues from 0.25 h with highest levels in plasma between 1-4 hours peaking at 4 hrs in males and 1 hr in females then steadily but slowly decreasing. The majority of total radioactivity was associated with the plasma fraction.

Table 9. Radiolabel levels in blood and plasma

Timepoint (h)	Blood (µg equiv lipid/g)		Plasma (µg equiv lipid/mL)		Blood:plasma ratio	
	Male	Female	Male	Female	Male	Female
0.25	3.003	0.936	6.035	1.894	0.48	1.15
1	2.809	5.928	5.379	10.884	0.49	0.54
2	4.028	6.773	8.714	9.091	0.46	0.64
4	3.400	2.698	8.755	4.251	0.42	0.60
8	2.000	0.628	3.573	1.147	0.56	0.55
24	1.274	0.544	2.621	0.945	0.49	0.57
48	0.535	0.305	1.085	0.524	0.50	0.58

Over 48 hrs radiolabel distributed to most tissues but only low levels were found in tissues other than the liver spleen, adrenal glands and ovaries. The majority of recovered radiolabel was found in the liver as would be expected for a particulate, and the size of the organ, and to a lesser extent the spleen. Tissue concentrations in the adrenals and ovaries were comparable to the liver and spleen however total recovery from these organs was low due to their comparative size. Levels in the liver and spleen peaked around 8 hours and at around 48 hours for the ovaries and adrenals.

Table 10.Tissue radiolabel levels as µg equiv lipid/g

Timepoint (h)	Values expressed as µg equiv lipid/g)						
	Liver		Spleen		Adrenal glands		Ovaries
	Male	Female	Male	Female	Male	Female	Female
0.25	1.151	0.323	0.354	°0.313	0.302	°0.240	°0.104
1	4.006	5.244	2.140	2.801	0.580	2.388	1.339
2	9.574	12.370	5.255	10.213	1.206	4.232	1.638
4	18.525	14.569	8.945	11.646	2.569	3.206	2.341
8	27.916	25.172	24.434	19.747	6.387	7.218	3.088
24	23.360	15.119	22.819	17.341	19.948	7.595	5.240
48	18.164	30.411	19.550	27.155	21.476	14.942	12.261

°=Mean includes results calculated from data less than 30 cpm above background

Table 11.Organ radiolabel as % of dose (radiolabel per organ)

Timepoint (h)	Liver		Spleen		Adrenal glands		Ovaries
	Male	Female	Male	Female	Male	Female	Female
0.25	0.995	0.209	0.014	°0.011	0.001	°0.001	°0.001
1	2.834	2.907	0.087	0.098	0.002	0.012	0.009
2	7.629	7.030	0.232	0.418	0.005	0.015	0.008
4	15.027	8.699	0.351	0.419	0.012	0.018	0.016
8	21.519	14.580	1.118	0.845	0.026	0.043	0.025
24	19.901	10.977	0.957	0.685	0.083	0.049	0.037
48	13.953	18.357	0.914	1.146	0.104	0.108	0.095

°=Mean includes results calculated from data less than 30 cpm above background

Conclusions

The distribution of radiolabelled LNPs (containing luciferase mRNA) was investigated in rats over a 48 hr period following IM administration of a single dose. Concentration was greatest at the injection site at all time points, with levels peaking in the plasma by 1-4 hours post-dose and distribution mainly into liver, adrenal glands, spleen and ovaries over 48 hours. Total recovery of radioactivity outside of the injection site was greatest in the liver, with much lower total recovery in spleen, and very little recovery in adrenals glands and ovaries. The mean plasma, blood and tissue concentrations and tissue distribution patterns were

broadly similar between the sexes and LNPs did not associate with red blood cells. These findings are consistent with observations in study R-20-0072-TGA which used whole body imaging to detect expression of luciferase, but the methods in this study provide considerably greater sensitivity to detect low level distribution to smaller organs/tissues.

4.2.3 Metabolism

Metabolism of ALC-0159 and ALC -0315 was investigated in a series of studies evaluated in the TGA interim report. (**043725-TGA & 01049-20008, 20009, 20010, 20020, 20021, 20022 – TGA).**

4.2.4 Pharmacokinetic Drug Interactions (nonclinical)

Not studied

4.3 TOXICOLOGY

4.3.1 Single-Dose Toxicity (in order by species, by route)

No single-dose studies were conducted.

4.3.2 Repeat-Dose Toxicity

17-Day Intramuscular Toxicity Study of Bnt162b2 (V9) And Bnt162b3c In Wistar Han Rats With A 3-Week Recovery. Testing Facility Study Number: 20GR142. **Full/Final Report**

Test Material:	BNT162b2 (V9): 0.5 mg/mL RNA encoding the full SARS-CoV-2 Spike (S) P2 variant protein. BNT162b3c: 0.5 mg/mL RNA encoding membrane-anchored, trimerised variant of the RBD of the SARS-CoV-2 S protein
Batch:	COVVAC/270320 (BNT162b2 [V9]), BCV/040620 (BNT162b3c)
Route and frequency	IM, weekly on days 1, 8 and 15
Animals / Source	Han Wistar rats from s 9(2)(b)(ii)
Study dates:	24 Jun 2020 – 13 Aug 2020
Laboratory:	Pfizer Worldwide Research & Development, Drug Safety Research & Development, Eastern Point Road, Groton, CT 06340 USA
GLP & QA:	Yes
Test Guideline:	Not Stated

Conduct of the Study

This study examined the repeat dose toxicity of two candidate COVID-19 vaccines, the commercialised version denoted as BNT162b2 (V9) and the BNT162b3c variant which has not been progressed to a commercial vaccine. Both vaccine candidates were formulated in a lipid nanoparticle (LNP)-RNA platform and both express the SARS-CoV-2 spike protein or its derivatives. As the LNP component of the vaccine is common to both variants the data for both are presented here.

The objective of this study was to determine the toxicity and development of a specific immune response to the antigen in each of the vaccine candidates following intramuscular (IM) administration of 30 µg of RNA once weekly to 10 rats per sex per group for a total of 3 doses (given on days 1, 8 and 15) to rats. The reversibility of effects was evaluated in

satellite groups of 5 rats per sex per group following a 3-week recovery phase. Animals were sacrificed on day 17. Immunogenicity tests for detection of neutralizing antibody titres to wild type live Sars-CoV-2 virus were conducted.

The actual achieved dose was approximately 120 µg/kg bw per dose in males and 150 µg/kg bw per dose for females yielding a margin of exposure of approximately 240 and 300-fold per dose over that proposed for clinical use.

Information available to Medsafe for this study that was not available to the TGA, and not evaluated in the TGA interim report provided to Medsafe consist of the immunogenicity, histopathology and recovery data and the associated integrated discussion of these results in the study report.

Results

Findings were largely comparable for the two variants. Minor differences are noted in the discussion below but are not material in the consideration of safety. There were no treatment related effects on: mortality, ophthalmology, or urinalysis. Other than effects related to expected and intended immune responses there were no treatment related effects on organ weights or gross pathology.

Body weight gains were lower in both treated male groups but were fully reversed over the recovery period where body weight gains were elevated over control. There was no effect on body weight gains in females. There were no, or only minor, effects on food consumption in either sex. In treated males statistically significant but small decreases in food consumption occurred in the 4-day block after each dose was administered (D1-4, & 8-11) but consumption was comparable to control in the inter-dosing periods (D4-8, 11-15) and overall only in the males administered BNT162b3c a slight and statistically significantly lower food consumption was noted over the entire 15 days of the treatment phase. The pattern of food consumption effects in males only is consistent with a general malaise, correlating with the greater increase in body temperature in males than in females after each dose, reducing appetite rather than a systemic toxicity effect.

Table 12. Principal immune response independent findings

	Male			**Female**		
Parameter	Control	BNT162b2 (V9)	BNT162b3c	Control	BNT162b2 (V9)	BNT162b3c
BW gain D 1-15 (g)	46.67	35.35**	29.83**	19.5	22.49	21.25
BW gain recovery (g)	40.18	61.09	58.32	13.78	24.10	12.54
Food consump. 1-15 (g)	295.87	285.59	269.31*	225.80	212.29	215.59

* P<0.05, ** P<0.01

Non-immune response related histopathology findings were limited to minimal portal hepatocyte vacuolation which was not associated with hepatic tissue damage or toxicologically significant elevations of liver enzymes (AST, ALT, ALP, GGT). Although some clinical chemistry parameters were increased, with occasional statistical significance achieved, they were within the normal range and not of sufficient magnitude to support a conclusion of an adverse effect.

The histological alterations in the liver were completely resolved by the end of the 3-week recovery phase. The study authors postulate that the hepatic vacuolation may be related to hepatic clearance of the PEGylated lipid component of the LNP, citing Ivens et al, 2015[15] in support. Although this mechanism is plausible the cited paper indicates a range of tissues may be affected and the liver, although affected for some compounds reviewed, is not consistently the target. The paper however supports the general conclusion that PEGylated lipids may cause cell vacuolation in various tissues including the liver. Given the absence of findings supportive of liver damage, the complete resolution over the 3 week recovery period, and the high margin of exposure compared to clinical dosing, the observation in the liver does not raise a safety concern regardless of the specific mechanism involved.

Immune Response Related Findings

Local reactions following administration consisted of moderate oedema with a very slight erythema at 24 hours. The oedema was generally observed up to 72 hours post dose, and both the oedema and erythema fully resolved prior to subsequent dose administration on Days 8 and 15.

Administrations of BNT162b2 or BNT162b3c on test days 1, 8, and 15 led to slightly, but statistically significantly increased body temperatures compared to the control animals after each dose by ~ 1°C in males and ~ 0.3-0.9°C in females. Body temperatures did not exceed 39 ^{0}C in treated groups after any dose.

Table 13 Principal immune related findings

	Male			**Female**		
Parameter	Control	BNT162b2 (V9)	BNT162b3c	Control	BNT162b2 (V9)	BNT162b3c
Body Temp D8	37.07	38.05**	38.33**	37.81	38.47**	38.73**
Body temp D15	37.34	38.37**	38.43**	38.02	38.15	38.35
WBC (10^3 /µL)	3.84	8.83**	8.60**	2.16	5.70**	6.37**
Monocytes (10^3/µL)	0.071	0.234**	0.254**	0.056	0.154**	0.176**
Neutrophils (10^3/µL)	0.674	0.449**	4.351**	0.409	2.469**	2.879**
Large Unstained Cells (10^3/µL)	0.026	0.209**	0.323.**	0..10	0.132**	0.190**
Fibrinogen (mg/dL)	253.1	596.7**	606.1**	217.2	596.7**	606.1**
Albumin/globulin	1.88	1.65**	1.65**	1.96	1.61**	1.66**
alpha-1 acid glycoprotein D4 µg/ml	174.36	1642.3**	2351.8**	239.8	1906.3**	1677.1**
alpha-2-macroglobulin D4 µg/ml	113.4	2318.1**	3911.6**	212.1	703.8**	887.1**
Spleen bw relative wgt	0.2008	0.2842**	0.3051**	0.2202	0.3492**	0.3231**

* P<0.05, ** P<0.01

At the end of the treatment period all clinical pathology findings (performed days 4 and 17) were generally similar between rats administered BNT162b2 (V9) or BNT162b3c, and consistent with expected immune responses to vaccines or secondary to inflammation. The main findings were present in both sexes on Days 4 and/or 17 and included higher acute

[15] Ivens IA, Achanzar W, Baumann A, et al. PEGylated biopharmaceuticals: current experience and considerations for nonclinical development. Toxicol Pathol 2015 Oct;43(7):959-83.

phase proteins (alpha-1 acid glycoprotein, alpha-2-macroglobulin and fibrinogen) and higher white blood cell count consisting of elevated levels of neutrophils, monocytes and large unstained cells (which typically represent large mononuclear cells) and lower albumin:globulin ratio due to slightly elevated globulin and slightly lower albumin.

Hyper-segmented neutrophils present on peripheral blood smears were considered by the study authors to be secondary to the robust increases in neutrophil counts and likely related to mobilization of bone marrow storage neutrophils and prolonged neutrophil lifespan in circulation (Ulich et al, 1988)[16] which is reasonable and consistent with the intended action of a vaccine.

Haematological changes correlated with minimally increased cellularity of hematopoietic cells (primarily myeloid) in the bone marrow and the spleen, minimal to moderate mixed cell inflammation at the injection site and increased cellularity in germinal centres of lymphoid organs observed histologically.

Additional findings included transiently lower reticulocyte counts on Day 4, and higher reticulocytes counts on Day 17 in females, with minor lower haematocrit on Days 4 and 17.

Lower reticulocytes were interpreted by the study authors to be a transient effect of innate immune responses (Abreu et al, 2018[17]; Brooks et al, 2017[18]; Kim et al, 2014[19]; Wrighting & Andrews,2006[20]) consistent with the literature cited.

Treatment related gross pathology observations consisted of large draining lymph nodes and dark/pale and/or firm injection sites in animals administered BNT162b2 (V9) or BNT162b3c, and large spleen and inguinal lymph nodes in animals administered BNT162b3c. Spleen weights (relative to bw or brain weight) were increased in both sexes for both treatments.

Treatment related histopathology findings consisted of the mixed cell inflammation and oedema of the injection site, increased cellularity with plasma cells and germinal centres in draining and inguinal lymph nodes, hepatocellular vacuolation, increased cellularity and hematopoietic cells with germinal centres in the spleen, and increased cellularity and hematopoietic cells in the bone marrow of both males and females administered BNT162b2 (V9) or BNT162b3c.

[16] Ulich TR, del Castillo J, Souza L. Kinetics and mechanisms of recombinant human granulocyte-colony stimulating factor-induced neutrophilia. Am J Pathol 1988;133(3):630- 38.
[17] Abreu R, Quinn F, Giri PK. Role of the hepcidin-ferroportin axis in pathogen-mediated intracellular iron sequestration in human phagocytic cells. Blood Adv 2018;2(10): 1089-100.
[18] Brooks MB, Turk JR, Guerrero A, et al. Non-Lethal Endotoxin Injection: A Rat Model of Hypercoagulability. PLoS One 2017;2(1),e0169976.
[19] Kim A, Fung E, Parikh SG, et al. A mouse model of anaemia of inflammation: complex pathogenesis with partial dependence on hepcidin. Blood 2014;123(8) 1129-136.
[20] Wrighting DM and Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. Blood 2006;108(9):3204-09.

SARS-CoV-2 neutralizing antibody responses were seen in both sexes at the end of the dosing (Day 17) and recovery phases (Day 21) but not prior to vaccine administration or in controls.

Recovery Phase Findings

No treatment related organ weight changes persisted at the end of the recovery phase and macroscopic findings were limited to large draining lymph nodes in 1 male administered BNT162b2 (V9) and 1 female administered BNT162b3c and large inguinal lymph nodes in 1 female administered BNT162b3c, indicating a partial recovery of these findings. There were no macroscopic findings at the injection sites or in the spleen indicating complete recovery of macroscopic effects at these sites.

Vacuolation of hepatocytes and increased cellularity of hematopoietic cells in the spleen and bone marrow were not observed at the end of recovery phase in BNT162b2 (V9) or BNT162b3c administered males and females.

Residual inflammation at the injection site was characterized by mostly lymphocytes and plasma cells with few neutrophils (indicating partial recovery) and no oedema (full recovery). However, increased cellularity of the germinal centres in the spleen partially recovered, as the incidence and/or severity of these findings were lower in recovery phase animals as compared with dosing phase animals in both males and females administered BNT162b2 (V9) or BNT162b3c. At the end of recovery phase, mature plasma cells had replaced the plasma-blasts identified in the inguinal and draining lymph nodes in the dosing phase animals. In recovery phase animals, infiltration of macrophages was observed in the draining lymph nodes (minimal to mild) in both sexes administered BNT162b2 (V9) or BNT162b3c and in the inguinal lymph nodes (minimal) in both sexes administered BNT162b2 (V9). This finding was considered indicative of a reparative process (consequence of phagocytosis), which can be seen following inflammatory reactions at the injection sites.

The overall picture in recovery animals was of a declining immune response to vaccine administration with partial or complete resolution of immune related effects.

Conclusions

Male and female rats were administered 3 doses (days 1, 8 and 15) of BNT162b2 (V9) at 120 µg/kg bw per dose in males and 150 µg/kg bw per dose for females yielding a margin of exposure of approximately 240 and 300 fold per dose over that proposed for clinical use. Treatment related effects were consistent with a vaccine induced immune response together with hepatocyte vacuolation likely due to the PEGylated lipid components of the LNP. In recovery phase animals retained for 3 weeks after the last dose the overall picture was of a declining immune response to vaccine administration with partial or complete resolution of immune related effects. There were no findings suggestive of potential safety issues in human subjects receiving clinical doses of the vaccine.

4.3.3 Genotoxicity

No genotoxicity studies were conducted. *In silico* QSAR studies on the novel lipid excipients ALC-0315 and ALC-0159 did not identify any genotoxic potential.

4.3.4 Carcinogenicity

No carcinogenicity studies were conducted.

4.3.5 Long-term studies

No-long term studies were conducted.

4.3.6 Reproductive and Developmental Toxicity

A Combined Fertility and Developmental Study (Including Teratogenicity and Postnatal Investigations) of BNT162b1, BNT162b2 and BNT162b3 by Intramuscular Administration in the Wistar Rat. Study No. 20256434,

Test Material:	BNT162b2
Batch:	CoVVAC/270320
Animals / Source	Wistar rats CRL:WI(Han) from s 9(2)(b)(ii) [redacted]
Study dates:	29 Jun 2020 – 12 Oct 2020
Laboratory:	s 9(2)(b)(ii) [redacted]
GLP & QA:	Yes
Test Guideline:	ICH S5(R3), & FDA, 2006 Guidance on Developmental Toxicity Studies in Vaccines for Infectious Disease Indications;

Conduct of the study

BNT162b2 (the final proposed vaccine) was administered by IM injection to 44 female Han Wistar rats at the human clinical dose (30 µg RNA/dosing day) 21 and 14 days prior to being mated with untreated males, and on Gestation Days (GD) 9 and 20, for a total of 4 doses. Half the females were sacrificed on day 21 and subject to caesarean section for assessment of embryofoetal development. The remaining females were allowed to litter and rear their young up to weaning on post-natal day 21. Pups were culled to 8 (4 of each sex where possible) on PND 4.

Based on an approximate body weight of 240 g at gestation day 1, the administered dose was approximately equivalent to 125 µg/kg bw giving a margin of exposure for each dose singly of approximately 250 with respect to the proposed clinical dose of 30 µg per person (125 µg/kg bw ÷ 30 µg/60 kg bw).

Dosing was not conducted during the mating phase and males were not dosed. The control group were administered saline by the same route and regimen.

Although the study included groups treated with two other LNP-formulated RNA vaccine candidates (BNT162b1 and BNT162b3) that did not proceed into Phase 2/3 clinical trials, results for those preparations are of limited relevance and are not discussed here, although the findings were generally similar across all candidate preparations.

Study parameters for all Parental females included: survival, clinical signs, body weights, body weight gains, food consumption, oestrous cycles, mating performance, fertility and macroscopic observations. Caesarean section Females were also examined for ovarian and

uterine contents, gravid uterine weights and foetuses were evaluated for viability, sex, body weights, and external, visceral, and skeletal morphology. Females permitted to litter were assessed for parturition, lactation, and maternal behaviour. Delivered pups were assessed for survival, clinical signs, body weights, physical development (pinna unfolding and eye opening), preweaning auditory and visual function tests to screen for normal neurodevelopment, and macroscopic observations.

Neutralizing antibody titres against SARS-CoV-2 live virus were measured in blood samples collected from all females before administration of the first dose (baseline) on the first day of cohabitation, on GD21 for the caesarean subgroup and their viable foetuses, and on post-natal day 21 for the littering group females and their pups.

Results

There were no unscheduled deaths, and no effects on: oestrous cycles, pre-coital interval, mating, fertility and pregnancy index, or on any ovarian, uterine, or litter parameters, including F1 pre and postnatal survival, growth, external, visceral, and skeletal morphology, or effects on pre-weaning physical and functional development of the F1 pups. There were no treatment related macroscopic effects in parental females at necropsy.

Clinical signs and macroscopic findings localized to the injection site as well as transient, non-adverse body weight and food consumption effects after each dose administration were observed in the treated group, consistent with administration of a vaccine and an inflammatory/immune response as expected.

There were no treatment related external, visceral or skeletal malformations, variations or anomalies in the BNT162b2 group (or other vaccine candidate groups). Isolated malformations observed were consistent with the background of findings for this strain of rat and, from 132 pups examined, consisted of single pups, from different litters with gastroschisis, a small mouth and agnathia (associated short and fused mandibles), a single pup with a right-sided aortic arch.

Parameter	**Control**	**BNT162b2**
Gestation bw Change (g) Days 9-12 Days 18-21 Days 0-21	 13.55 34.10 124.7	 5.70*** 24.82*** 108.9***
Lactation bw change D 1-21	33.07	35.94
Pre-coital interval days	3.0	2.8
Copulation Index %	100	100
Pregnancy rate %	98	95
Females with live/viable pups	43	42
Gestation index %	100	100
Gestation length (d)	22.1	22.0
Gravid uterus weight (g)	86.3	87.7
Live birth index (%)	98	99.3

Viability index (%)	99.0	98.9
Foetal weight (g)	4.89	4.90
Weaning index [PND 4-21] (%)	99.4	100
Live foetuses per dam	13.2	13.1
No of corpora Lutea	14.7	15.5
Pre-implantation loss	0.6	1.5*
Post-implantation loss	0.9	0.9

* p<0.05, *** P<0.001

BNT162b2 elicited SARS-CoV-2 neutralizing antibody responses in the majority of females just prior to mating, at the end of gestation, and at the end of lactation as well as in most offspring (fetuses on GD21 and pups on PND21) but not prior to vaccine administration or in controls.

Conclusions

IM administration of 30 µg of BNT162b2 to female rats 21 and 14 days prior to being mating with untreated males, and on Gestation Days (GD) 9 and 20, for a total of 4 doses did not affect reproductive factors in parental females or developmental parameters in pups. The results raise no potential safety issues for the use of this vaccine in pregnant human patients. The doses administered in this study provide a MOE compared to the proposed single clinical dose of 30 µg, of 250-fold.

BNT162b2 elicited SARS-CoV-2 neutralizing antibody responses in the majority of females just prior to mating (M0), at the end of gestation (GD21), and at the end of lactation (PND 21), and SARS-CoV-2 neutralizing titres were detected in most offspring.

4.3.7 Local Tolerance

No specific local tolerance studies have been conducted.

4.3.8 Antigenicity

Immunogenicity was evaluated as part of the primary pharmacology studies.

4.3.9 Immunotoxicity

s 9(2)(a) (2020) **In-Vitro Study of Cytokine/Chemokine Secretion in Human Peripheral Blood Mononuclear Cells Supporting BNT162b2 Preclinical Immunotoxicity.** Study Report R-20-0357. 27 Nov 2020

Test Material:	BNT162b2 (V9)
Batch:	RNA-RF200321-06, CoVVAC/270320
Animals / Source	Human peripheral blood mononuclear cells
Study dates:	2 Nov 2020 – 6 Nov 2020
Laboratory:	BioNTech RNA Pharmaceuticals GmbH, An der Goldgrube 12, 55131 Mainz, Germany
GLP & QA:	No, (research and development study)
Test Guideline:	Non-guideline

Conduct of the Study

The objective of this study was to assess dose dependence of cytokine and chemokine release in human peripheral blood mononuclear cells (PBMCs - a mixture of monocytes and lymphocytes [T cells, B cells, and natural killer cells]) from 3 donors, after transfecting the cells with BNT162b2 V9. The in-vitro human PBMC stimulation assay allows assessment of potential immunotoxicity effects and better understanding of the safety of BNT162b2 clinical drug candidate in human cells.

Doses of 0.01 to 3 µg LNP BNT162b2 per well (corresponding to 0.05 to 15 µg/mL BNT162b2) were used to test chemokine and cytokine release by PBMCs. 500,000 cells in a volume of 180 µL medium per well were first were first plated in a 96 well plate, BNT162b2 was added and the cells incubated for 24 hrs. Supernatants of transfected cells were collected after 24 h and cytokine and chemokine secretion were determined by Meso Scale Discovery (MSD) multiplexed ELISA.

Table 14. Dose of BNT162b2 per well or per mL

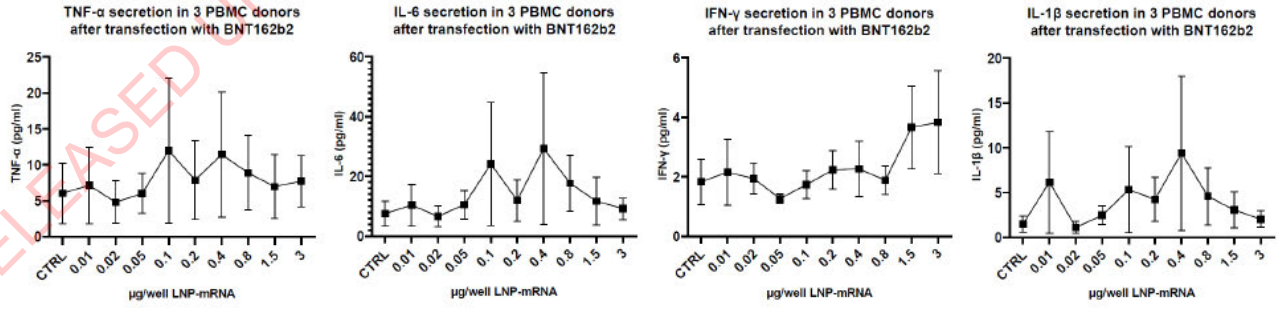
Dose in 96-well plate, human PBMCs	
µg LNP-mRNA/well	µg LNP-mRNA/mL
3	15
1.5	8
0.8	4
0.4	2
0.2	1
0.1	0.5
0.05	0.2
0.02	0.1
0.01	0.05

LNP = lipid nanoparticle, PBMCs = peripheral blood mononuclear cells

Results

In this study, secretion of the proinflammatory cytokines TNF-cr, IL-6, IFN-y, and IL-1P was low and close to background levels across the applied dose range: 0.01 µg to 3 µg BNT162b2 per well in human PBMCs from 3 donors.

Figure 12. dependence of cytokine secretion after transfection



Secretion of the chemokines IP-10, MIP-18, and MCP-1 showed low and close to background levels for doses up to 0.2 Ug BNT162b2 per well and overall low to-medium levels when doses from 0.4 to 3 µg BNT162b2 per well were applied to human PBMCs from 3 donors.

Figure 13. Dose dependence of cytokine secretion after transfection of PBMCs from 3 donors with BNT162b2 (mean ± SEM).

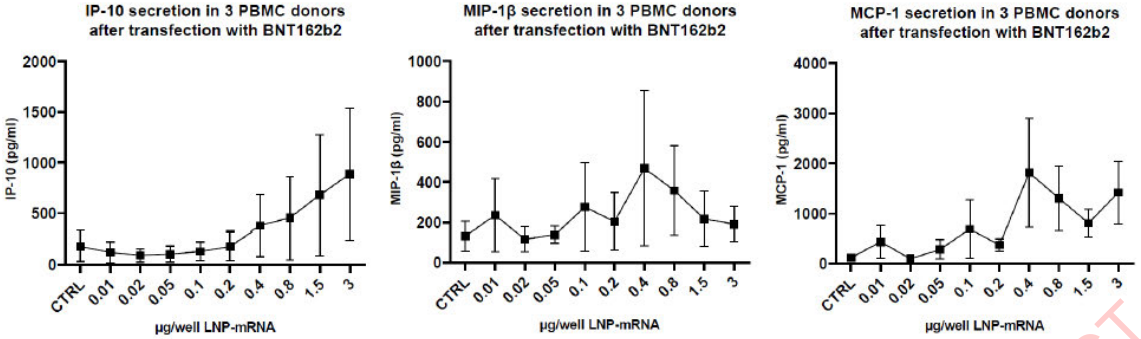


Figure 14. Maximal cytokine/chemokine secretion after transfection of PBMCs with 0.01 to 3 µg BNT162b2 per well

Cytokine/ chemokine	Secretion from donor 1 PBMCs (pg/mL)	Secretion from donor 2 PBMCs (pg/mL)	Secretion from donor 3 PBMCs (pg/mL)
TNF-α	7	32	5
IL-6	5	80	19
IFN-γ	7	4	2
IL-1β	4	27	3
IP-10	2,158	494	29
MIP-1β	196	1,241	183
MCP-1	2,474	3,984	1,070

Conclusions

Cytokine (TNF-α, IL-6, IFN-γ, and IL-1β) release from BNT162b2 transfect PBMCs from 3 human donors was low and close to background levels across a wide dose range of BNT162b2 (0.4 to 3 µg per well). Higher levels of chemokine release compared to cytokines were observed but in all 3 donors for all the chemokines measured (IP-10, MIP-1β, and MCP-1), tested doses up to 0.2 µg BNT162b2 per well showed low or background chemokine levels. These findings are concordant with that from the 17-day rat study with various BNT162 mRNA variants (including BNT162b2 V8 but not V9) which found no increase in cytokine levels across 3 doses given once weekly.