Title: BNT162b2 (V9) Immunogenicity and Evaluation of Protection against SARS-CoV-2 Challenge in Rhesus Macaques

Study Number: COVID Rh2020-01 (NIRC study #: 8725-2005)
(SNPRC Study #: Covid-1778)

Parent Compound Number(s): PF-07302048

Alternative Compound Identifiers: N/A

Pfizer Vaccine Research and Development
401 N. Middletown Rd.
Pearl River, NY

Title: BNT162b2 (V9) Immunogenicity and Evaluation of Protection against SARS-CoV-2 Challenge in Rhesus Macaques

PRINCIPAL INVESTIGATOR: (b) (6)

CONTRIBUTING SCIENTIST(S): (b) (6) ;

(b) (6)

PREPARED BY:

(b) (6)

APPROVED BY:

(b) (6)

Title: BNT162b2 (V9) Immunogenicity and Evaluation of Protection against SARS-CoV-2 Challenge in Rhesus Macaques

SYNOPSIS

Prime-boost vaccination of rhesus macaques with BNT162b2 (V9) elicited SARS-CoV-2 neutralizing geometric mean titers 10.2 to 18.0 times that of a SARS-CoV-2 convalescent human serum panel. BNT162b2 generated strong Th1 type CD4+ and IFNγ+ CD8+ T cell responses in rhesus macaques. The BNT162b2 vaccine candidate protected the lungs of immunized rhesus macaques from infectious SARS-CoV-2 challenge, with no evidence of vaccine-elicited disease enhancement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Title: BNT162b2 (V9) Immunogenicity and Evaluation of Protection against SARS-CoV-2 Challenge in Rhesus Macaques

Study Number: COVID Rh2020-01

(Associated Study Numbers: NIRC study #: 8725-2005;
SNPRC Study #: Covid- 1777 and Covid-1778)

Functional Area: Vaccine Research and Development

Test Facility: Pfizer Vaccine Research, 401 North Middletown Road,
Pearl River, NY 10965

Immunizations In-Life Test Facility: New Iberia Research Center (NIRC),
4401 W. Admiral Doyle Drive,
New Iberia, LA 70560

Challenge In-Life Test Facility: Southwest National Primate Center (SNPRC),
8715 W. Military Dr.

San Antonio, TX 78227-5302

Neutralization Assay Test Facility: University of Texas Medical Branch (UTMB)
(b) (4)
(b) (4)
Galveston, TX 77555

Study/Testing Initiation Date: 07Apr2020

Study/Testing Completion Date: 01Nov2020

1. OBJECTIVES

The purpose of this study was to evaluate BNT162b2 (V9)-elicited immune responses and the ability of the vaccine to protect against SARS-CoV-2 challenge in rhesus macaques (*Macaca mulatta*).

2. INTRODUCTION

The coronavirus disease 2019 (COVID-19) vaccine (BioNTech code number BNT162, Pfizer code number PF-07302048) is an investigational vaccine intended to prevent COVID-19, which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The vaccine candidate BNT162b2, otherwise known as BNT162b2 (V9), is a m1Ψ nucleoside modified mRNA (modRNA) expressing full-length S with two proline mutations (P2) to lock the transmembrane protein in an antigenically optimal prefusion conformation.[1,2] The vaccine is formulated in lipid nanoparticles (LNPs).

BNT162b2 was assessed for immunogenicity and for protection against an infectious SARS-CoV-2 challenge in rhesus macaques. SARS-CoV-2 infection in humans manifests as both asymptomatic infection and as the disease COVID-19, with diverse signs, symptoms, and levels of severity. Based on published reports, SARS-CoV-2 challenged rhesus macaques develop an acute, transient infection in the upper and lower respiratory tract and have evidence of viral replication in the gastrointestinal tract, similar to humans.[3,4] The human and rhesus ACE-2 receptor have 100% amino acid identity at the critical binding residues, which may account for the fidelity of this SARS-CoV-2 animal model.[5]

3. MATERIALS AND METHODS

3.1. Immunogenicity Study Design

The study was performed in 2–4 year old, male rhesus macaques (*Macaca mulatta*) designed with 3 groups as shown in Table 1. Animals were vaccinated with 30 µg or 100 µg of BNT162b2 (n=6 per group) or with saline control (n=6) on days 0 and 21, administered in a 0.5 mL dose volume by the intramuscular (IM) route. Serum and peripheral blood mononuclear cells (PBMCs) were collected at the indicated times post immunization.

Immunizations were performed at the University of Louisiana at Lafayette-New Iberia Research Center (NIRC), which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC, Animal Assurance #: 000452). The work was in accordance with USDA Animal Welfare Act and Regulations and the NIH Guidelines for Research Involving Recombinant DNA Molecules, and Biosafety in Microbiological and Biomedical Laboratories. All procedures performed were in accordance with regulations and established guidelines and were reviewed and approved by an Institutional Animal Care and Use Committee or through an ethical review process.

Table 1. Immunization Study Design

Gp#	No. of Rhesus Macaques	Animal IDs	Immunogen Description	Vaccine Encoded Antigen	Dose (µg)	Dose Vol / Route	Vax (Day)	Bleed (Week)
1	6	A16N100 A17N102 A17N037 A16N140 A16N020 A16N193	Saline (0.9% sodium chloride (Lot# 10-106-JT)	-	-	0.5 mL/IM	0, 21	Pre[a], 6hr, 24hr, 1, 2[a], 3, 4, 5, 6[a]
2	6	A17N143 A17N149 A17N138 A17N125 A17N107 A17N134	BNT162b2 (V9) (Lot # CoVVAC/270320)	Spike Protein P2 variant	30	0.5 mL/ IM	0, 21	Pre[a], 6hr, 24hr, 1, 2[a], 3, 4[a], 5, 6[a], 8

Table 1. Immunization Study Design

Gp#	No. of Rhesus Macaques	Animal IDs	Immunogen Description	Vaccine Encoded Antigen	Dose (µg)	Dose Vol / Route	Vax (Day)	Bleed (Week)
3	6	A17N109 A17N139 A17N167 A17N105 A17N113 A17N114	BNT162b2 V9 (Lot # CoVVAC/270320)	Spike Protein P2 variant	100	0.5 mL/ IM	0, 21	Pre[a], 6hr, 24hr, 1, 2[a], 3, 4[a], 5, 6[a], 8

a. PBMC collection timepoints

3.2. Test Article Information

The BNT162b2 (V9) drug product was provided by BioNTech (Mainz, Germany). Analytical testing of the drug product was performed at Pfizer VRD Early Bioprocess and Development in Pearl River, NY.

Table 2. Analytical Characterization of BNT162b2 (V9) Drug Product

Lot Number	Description of RNA	Cap (%)	PolyA (%)	Integrity (%)	Endotoxin (EU/mL)	Spike Protein Expression (%)[a]
CoVVAC/270320	BNT162b2 V9	(b) (4)				

3.3. General Formulation Instructions

All test articles were opened under aseptic conditions. The LNP formulations were handled with care to prevent potential RNase contamination. Prior to dose preparation, the frozen modRNA LNP vials were completely thawed at ambient temperature and diluted to the corresponding target concentrations at 60 and 200 µg/mL by using saline solution. The diluted modRNA LNP articles were well mixed by gentle swirling and/or inversion to ensure a homogeneous mixture.

3.3.1. RNase Reduction Measures

All preparation steps were performed under a laminar flow hood or PCR Dead Air Box. After disinfection with Terralin® liquid (alcohol-based disinfectant or similar surface disinfectant) all work surfaces, gloves, instruments and equipment were treated with RNase*Zap*™.

3.3.2. Source of Study Materials

Materials provided by BioNTech:

- BNT162 RNA LNP vial(s), 0.5 mg/mL RNA, 0.5 mL extractable volume, stored at -70 °C ± 10 °C

Materials provided by NIRC:

- 0.9% Sodium Chloride (referred to dilution buffer) ICU Medical, 1 L bag, 0.9% sodium chloride, Injection, USP, NDC 0990-7983-09, Lot 10-106-JT, Exp 01 Oct 2021

3.3.3. Vaccine Preparation

1. Test vials were removed from -70 °C ± 10 °C storage and warmed to room temperature (approximately 5-10 minutes) under a laminar flow hood.

2. While the test materials thawed, sterile, RNase free glass vials were prepared with the appropriate volume of dilution buffer (0.9% sterile sodium chloride/saline). A similar empty vial was prepared for the pooling of BNT162b2 (V9).

3. Vials containing test materials were gently inverted three to five times to ensure thorough mixing.

4. Flip caps and rubber stoppers were carefully removed on the BNT162b2 (V9) test item vials.

5. Using sterile, RNase-free pipet tips, the volume from each vial was pooled to provide a sufficient volume of homogeneous material.

6. After pooling, the appropriate volume of BNT162b2 (V9) was transferred into the 5 mL glass vials that contained the buffer that was added in step two. Exact volumes can be found in the dilution scheme below.

7. Vials were carefully closed and gently inverted to ensure a homogeneous mixture.

8. Syringes were aseptically filled and transported on ice to the animal facility. In the interest of animal welfare, the syringes were warmed to room temperature immediately prior to administration. All animals were injected within two hours of vaccine preparation.

Table 3. Dilution Scheme for BNT162b2 (V9) [Concentration = 0.5 mg/mL]

Group	BNT 162b2 (V9) Application Dose [µg/0.5mL]	Factor of Dilution	Dilution Step 1	
			Volume BNT162 Test Item (mL)	Volume Dilution Buffer (mL)
2	30	8.33	0.48	3.52
3	100	2.5	1.6	2.4

3.4. Pre-Screen

Rhesus macaques were selected based on pre-study physical exams and body weights were recorded. Selected macaques were identified by unique body tattoos prior to beginning any study related procedure.

3.5. Anesthesia

All vaccinations and peripheral blood draws were performed with the macaques appropriately sedated using Ketamine HCl (10 mg/kg), administered as an intramuscular (IM) injection.

3.6. Vaccine Administration

Vaccines were administered as a single 0.5 mL intramuscular injection in the left quadricep muscle. Sites were shaven and prepped per NIRC standard operating procedures (SOPs) prior to injection.

3.7. Daily Observations

Animals were observed daily for any abnormal clinical signs and/or signs of illness, behaviors departing from species specific behavior, or distress starting upon assignment to study. Any abnormal observations would have been reported to the Study Director and Study Veterinarian. Evaluation of vaccine administration sites were included in the daily observations for signs of redness, swelling, and/or localized reactions.

3.8. Sample Collection and Handling

3.8.1. Serum

Blood was collected into serum separator tubes with volumes determined based on body weight, according to Table 4. Samples were centrifuged at 3000 rpm/ 2095 RCF (x g) for 10 minutes, per NIRC SOPs for serum separation and harvest. Samples were barcoded, recorded and electronic files were sent with each shipment. Each serum sample was divided into 4 x 0.25 mL aliquots and any remaining volume was stored at approximately 1.0 mL per barcoded cryovial and stored at -70 °C until shipment. For SARS-CoV-2 neutralization assay testing at the UTMB BSL-3 facility, one of the four 0.25 mL aliquots were heat-inactivated (56 °C for 30 minutes in a water bath) and shipped directly to UTMB. All samples were handled in a manner to maintain sterility.

Table 4. Blood Volume Collection Guidelines

Body Weight Range (kg)	Collection Volume (mL)
<4.5	5.0
4.6-5.5	8.5
5.6-7.2	12.0
>7.3	17.0

kg, kilogram; mL, milliliter

3.8.2. PBMCs

Whole blood was collected from each animal at specified time points in EDTA vacutainer tubes. PBMCs were retained at room temperature then processed per NIRC SOP 8725-06.07. After processing, cells were frozen at 5 x 10^6 cells/mL cell concentration in liquid nitrogen. No less than 5 x 10^6 cells/mL or more than 1 x 10^7 cells/mL were frozen per vial. Plasma from individual animals was aliquoted and stored at -70 °C.

3.9. Shipping and Storage Conditions

Test materials were shipped from Pfizer (Pearl River) to NIRC in a manner to maintain frozen conditions during transport. Test materials were inventoried and stored at -70 °C upon arrival. Serum samples were shipped over night on dry ice with Temptale included. PBMC samples for each animal were split into two boxes, send and retains. Cryoshippers were used to transport PBMCs to the Pearl River Pfizer facility.

3.10. Immunological Assays

3.10.1. SARS-CoV-2 S1-Binding IgG Luminex Assay

A direct binding Luminex immunoassay (dLIA) was used to quantify S1-binding serum IgG levels (VR-MQR-10211). A recombinant SARS-CoV-2 S1 with a C-terminal Avitag™ (Acro Biosystems) was bound to streptavidin-coated Luminex microspheres. Bound nonhuman primate S1-binding IgG was detected with a R-Phycoerythrin-conjugated goat anti-human polyclonal secondary antibody (Jackson Labs). Data were captured as median fluorescent intensities (MFIs) using a Luminex reader and converted to U/mL antibody concentrations using a reference standard curve with arbitrary assigned concentrations of 100 U/mL and accounting for the serum dilution factor. Assay results were reported in U/mL of IgG.

3.10.2. SARS-CoV-2 Neutralization Assay

The SARS-CoV-2 neutralization assay used a previously described strain of SARS-CoV-2 (USA_WA1/2020) that had been rescued by reverse genetics and engineered by the insertion of an mNeonGreen (mNG) gene into open reading frame 7 of the viral genome.[6] This reporter virus generates similar plaque morphologies and indistinguishable growth curves from wild type virus. Viral master stocks were grown in Vero 76 cells as previously described.[7] When testing human convalescent serum specimens, the fluorescent neutralization assay produced comparable results as the conventional plaque reduction

neutralization assay. Serial dilutions of heat-inactivated sera were incubated with the reporter virus (2 x 10^4 PFU per well) to yield approximately a 10-30% infection rate of the Vero CCL81 monolayer for 1 hour at 37 °C before inoculating Vero CCL81 cell monolayers (targeted to have 8,000 to 15,000 cells in the central field of each well at the time of seeding, one day before infection) in 96-well plates to allow accurate quantification of infected cells. Cell counts were enumerated by nuclear stain (Hoechst 33342) and fluorescent virally infected foci were detected 16-24 hours after inoculation with a Cytation 7 Cell Imaging Multi-Mode Reader (Biotek) with Gen5 Image Prime version 3.09. Titers were calculated in GraphPad Prism version 8.4.2 by generating a 4- parameter (4PL) logistical fit of the percent neutralization at each serial serum dilution. The 50% neutralization titer (VNT50) was reported as the interpolated reciprocal of the dilution yielding a 50% reduction in fluorescent viral foci.

3.10.3. IFNγ and IL-4 ELISpot Assays

PBMCs were tested with commercially available nonhuman primate IFNγ and IL-4 ELISpot assay kits (Mabtech, Sweden). Cryopreserved rhesus macaque PBMCs were thawed in pre-warmed AIM-V media (Thermo Fisher Scientific, US) with Benzonase (EMD Millipore, US), washed once and the concentration was adjusted to 2.5 x 10^6 cells/mL in AIM-V. Pre-coated PVDF 96-well microplates were washed three times with PBS and blocked with AIM-V. PBMCs were added at 1.0 x 10^5 cells/well for IFNγ and 2.5 x 10^5 cells/well for IL-4. PBMCs were stimulated with a peptide pool spanning the entire S protein (15 mers, 11 amino acid overlap, JPT, Germany) at 1 μg/mL for 24 hours for IFNγ and 48 hours for IL-4 at 37 °C in 5% CO_2. Tests were performed in triplicate wells and medium-DMSO, a CMV peptide pool (JPT, Germany) and PHA (Sigma, USA) were included as controls. Cells were removed and plates washed six times with PBS. Biotinylated detection antibody diluted to a concentration of 1 μg/mL in PBS/0.5% BSA was added to the plates and incubated for two hours at room temperature. Plates were washed six times with PBS and Streptavidin-HRP diluted at 1:1000 in PBS/0.5% BSA was added and incubated for one hour at room temperature. Plates were washed six times with PBS and AEC substrate (BD, US) was added and incubated for 10 minutes for IFN-γ and 30 minutes for IL-4 at room temperature until red spots were developed. The plates were washed with distilled water, dried for 1-2 hours at room and scanned and counted using a CTL ImmunoSpot S6 Universal Analyzer (CTL, US). Results shown are background (Media-DMSO) subtracted and normalized to SFC/10^6 PBMCs.

3.10.4. Flow Cytometry Intracellular Cytokine Staining (ICS) Assay

PBMCs were thawed as above, rested for 3 to 4 hours, and were stimulated with a peptide pool spanning the entire S protein (15 mers, 11 amino acid overlap, JPT, Germany) at 1 μg/mL, Staphylococcus enterotoxin B (SEB; 2 μg/mL) as positive control, or 0.2 % DMSO as negative control, in AIM-V medium in 96-well plates. CD107a monoclonal antibody (mAb) (BioLegend; clone H4A3, APC), GolgiStop, and GolgiPlug were added to each well and cells were incubated at 37 °C for 12 to 16 h. Following incubation, cells were stained with Viability Dye eFluor 780 (eBioscience™) and Fc block added prior to surface staining with mAbs specific for CD4 (clone SK3, BV480), CD3 (clone SP34.2, Alexa 700), and CD8 (clone RPA-T8, BB700). Cells were then washed once with 2% FBS/PBS buffer, fixed and

permeabilized with BD CytoFix/CytoPerm solution, washed twice in BD Perm/Wash buffer and intracellular staining performed with the following mAbs: CD154 (BioLegend; clone 24-31, BV605), IFN-γ (clone B27, FITC), IL-2 (eBioscience™; clone MQ1-17H12, PE-Cy7), IL-4 (clone MP4-25D2, BV421), TNF-α (clone Mab11, BUV395), CD3 (clone SP34.2, Alexa 700) in perm/wash buffer for 30 min at RT. Cells were washed, resuspended in 2% FBS/PBS buffer and acquired on a LSR Fortessa. All mAbs are from BD Biosciences, except mentioned. Data analyzed by FlowJo (10.4.1). Cytokine-expressing cells were gated within the CD154+ CD4 T cells and CD69+ CD8 T cells. Results shown are background (medium-DMSO) subtracted.

3.11. SARS-CoV-2 Challenge of Rhesus Macaques

Infectious SARS-CoV-2 challenge was performed on the BNT162b2-immunized animals (100 µg dose level; n =6) and age-and sex-matched saline-immunized animals (n=3; Animal ID# A17N118, A17N157, A17N128) at the Southwest National Primate Research Center. Animal husbandry followed standards recommended by AAALAC International and the NIH Guide for the Care of Use of Laboratory Animals. This study was approved by the Texas Biomedical Research Institute Animal Care and Use Committee.

The SARS-CoV-2 inoculum was obtained from a stock of 2.1×10^6 PFU/mL previously prepared at Texas Biomedical Research Institute (San Antonio, TX), aliquoted into single use vials, and stored at -70 °C. The working virus stock was generated from two passages of the SARS-CoV-2 USA-WA1/2020 isolate (a 4th passage seed stock purchased from BEI Resources; NR-52281) in Vero 76 cells. The virus was confirmed to be SARS-CoV-2 by deep sequencing and identical to the published sequence (GenBank accession number MN985325.1). Approximately two weeks prior to challenge, animals were moved to the Southwest National Primate Research Center (SNPRC; San Antonio, TX) and into the ABSL-3 facility. BNT162b2-immunized (n=6) and age-matched saline control-immunized (n=3) male rhesus macaques (control) were challenged with 1.05×10^6 plaque forming units of SARS-CoV-2 USA-WA1/2020 isolate, split equally between the intranasal (IN) and intratracheal (IT) routes as previously described.[8] The challenge was performed 55 days after the second BNT162b2 immunization. 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). Animals were monitored regularly by a board-certified veterinary clinician for rectal body temperature, weight and physical examination. Specimen collection was performed under tiletamine zolazepam (Telazol) anesthesia as described.[8] Nasal and oropharyngeal swabs were collected from all macaques pre and at Days, 1, 3, and 6 (relative to the day of challenge), 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. BAL was performed by instilling four times 20 mL of saline. These washings were pooled, aliquoted and stored frozen at -70 °C. 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. See Appendix, Supportive Table 8.5 for a summary of individual animals.

3.12. Chest X-rays and Computed Tomography Scans

X-rays and computed tomography (CT) scans were performed under anesthesia as previously described.[9,8] For radiographic imaging, 3-view thoracic radiographs (ventrodorsal, right and left lateral) were obtained one week prior to challenge, and post-challenge on Days 1, 3, 6 and end of project (Day 7/8) or Day 10. High-resolution CT was performed one week prior to challenge and post-challenge on Day 3 post-challenge for BNT162b2-immunized and control animals and end of project (Day 7/8) or Day 10 for all groups. The animals were anesthetized using Telazol (2-6 mg/kg) and maintained by inhaled isoflurane delivered through a Hallowell 2002 ventilator anesthesia system (Hallowell, Pittsfield, MA). Animals were intubated to perform end inspiratory breath-hold using a remote breath-hold switch. Lung field CT images were acquired using Multiscan LFER150 PET/CT (MEDISO Inc., Budapest, Hungary) scanner. Image analysis was performed using 3D ROI tools available in Vivoquant (Invicro, Boston, MA). Images were interpreted by two board-certified veterinary radiologists blinded to treatment groups. Scores were assigned to a total of 7 lung regions on a severity scale of 0-3 per region, with a maximum severity score of 21. Pulmonary lesions that could not be unequivocally attributed to the viral challenge (such as atelectasis secondary to recumbency and anesthesia) received a score of "0".

3.13. Reverse-transcription Quantitative Polymerase Chain Reaction

To detect and quantify SARS-CoV-2 in rhesus macaques, viral RNA was extracted from nasal swabs, OP swabs, and BAL specimens as previously described[10,11,12] and tested by RT-qPCR as previously described.[8] Briefly, 10 µg yeast tRNA and 1×10^3 PFU of MS2 phage (Escherichia coli bacteriophage MS2, ATCC) were added to each thawed sample, and RNA extraction performed using the NucleoMag Pathogen kit (Macherey-Nagel). The SARS-CoV-2 RT-qPCR was performed on extracted RNA using a CDC-developed 2019-nCoV_N1 assay on a QuantStudio3 instrument (Applied Biosystems). The cut-off for positivity (limit of detection, LOD) was established at 10 gene equivalents (GE) per reaction (800 GE/mL). Samples were tested in duplicate. Any specimens that had, on repeated measurement, viral RNA levels on either side of the LLOD, were categorized as indeterminate and excluded from the graphs and the analysis.

3.14. Macroscopic and Microscopic Pathology

Necropsy, tissue processing, and histology were performed by SNPRC. Histopathological assessments were performed at Days 7 or 8 following infectious SARS-CoV-2 challenge on the BNT162b2-immunized animals (100 µg dose level; n =6) and age- and sex-matched saline-immunized and SARS-CoV-2-challenged control animals that were included in the histopathology animal cohort (n=3; Table 5). Tissues collected and microscopically evaluated included lung (7 sections- 1 sample of each lobe on L & R), kidney, liver, spleen, skin, large and small intestine, heart [with coronary arteries], bone marrow, nasal septum, tongue, trachea, mediastinal lymph node, and mucocutaneous junctions. Tissues were fixed in 10% neutral buffered formalin and routinely processed into paraffin blocks, sectioned to 5 µm and stained with hematoxylin and eosin.

Microscopic evaluation was performed independently by a SNPRC and a Pfizer pathologist, both blinded to treatment group. Lungs were evaluated using a semi-quantitative scoring

system with inclusion of cell types and/or distribution as appropriate. An inflammation area score, based on the estimated area of the lung section with inflammation, was used to grade each lung lobe: 0=normal; 1=<10%; 2=11-30%; 3=30-60%; 4= 60-80%; 5=>80%. Samples were unblinded after agreement on diagnoses and severity grades. For each animal, the inflammation area score for each lung lobe was averaged to generate a single inflammation area score for that animal. That score was used to evaluate the severity of respiratory disease after SARS-CoV-2 challenge.

Table 5. Pathology Cohorts

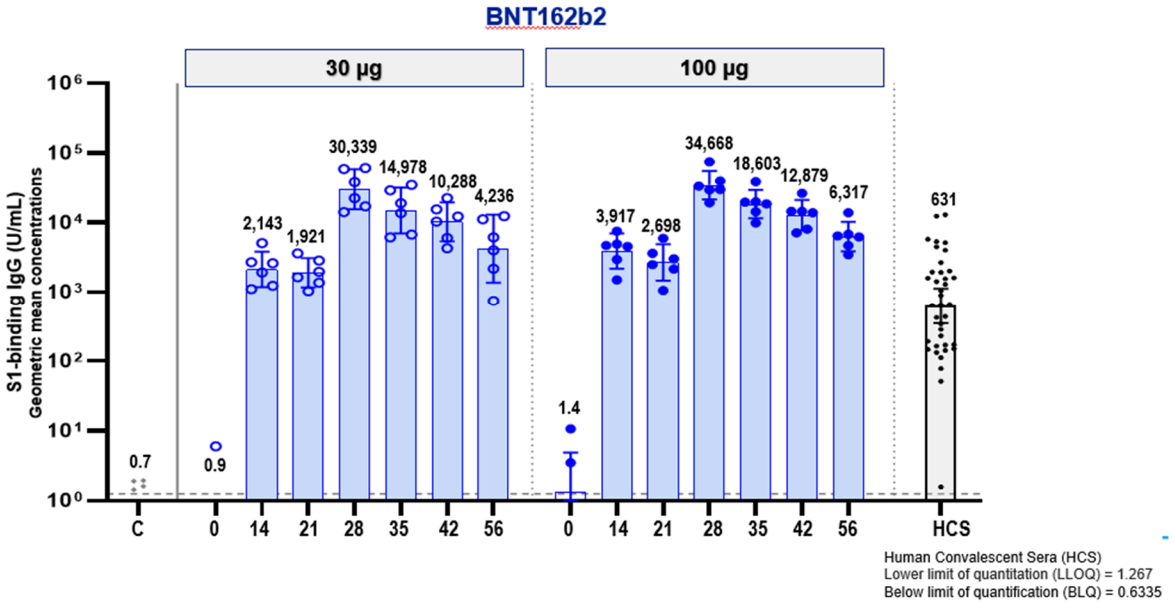
Group	Number of Animals (Animal ID)
Control[a]	3 (A16N193, A17N037, A17N102)
BNT162b2	6 (A17N109, A17N139, A17N167, A17N105, A17N113, A17N114)

a. Age- and sex- matched control (saline-immunized and challenged) animals

4. RESULTS AND DISCUSSION

To assess BNT162b2-mediated protection in non-human primates, groups of six male, 2-4 year old rhesus macaques were immunized IM with 30 or 100 µg of BNT162b2 or saline control on Days 0 and 21. S1-binding IgG was readily detectable by Day 14 after Dose 1, and levels increased further after Dose 2 (Figure 1). Seven days after Dose 2 (Day 28), the GMCs of S1-binding IgG were 30,339 units (U)/mL (30 µg dose level) and 34,668 U/mL (100 µg dose level). For comparison, the S1-binding IgG GMC of a panel of 38 SARS-CoV-2 convalescent human sera was 631 U/mL, substantially lower than the GMCs of the immunized rhesus macaques after one or two doses.

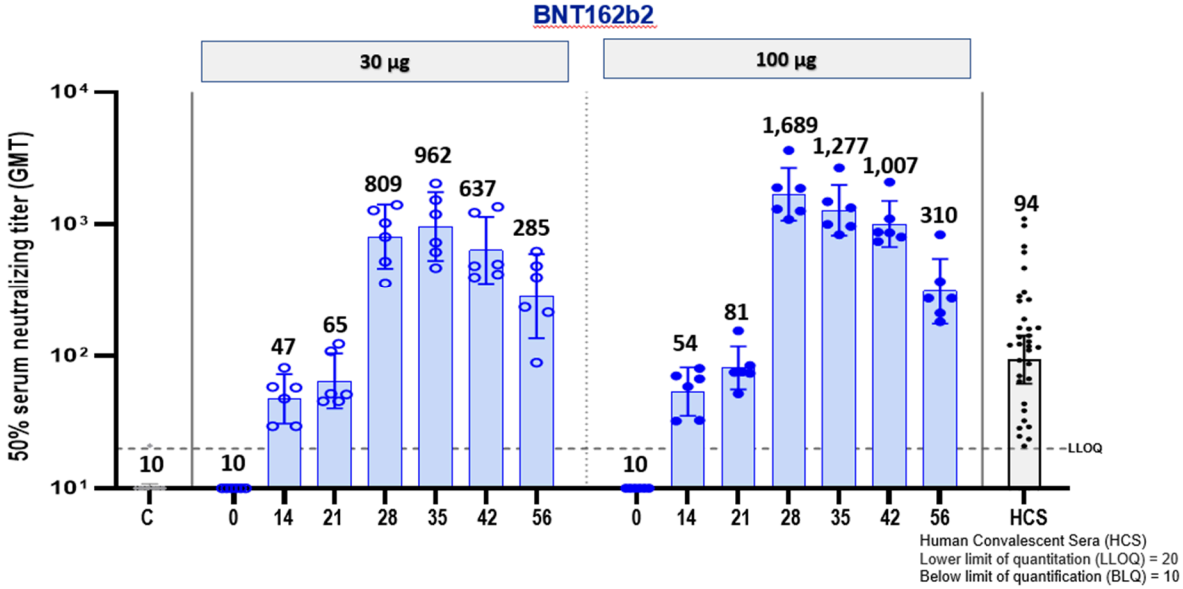
Figure 1. S1-binding IgG Concentrations Elicited by Immunization of Rhesus Macaques with BNT162b2 (V9)



Numbers on the x-axis indicate the day post first immunization. Heights of bars indicate GMCs, which are written above the bars; whiskers indicate 95% CIs; dots represent individual monkey IgG concentrations. Dotted line indicates the lower limit of quantification (LLOQ=1.267 U/ml). Values at or below LLOQ were set to ½ LLOQ. C – saline-immunization control; HCS – human convalescent serum panel.

Fifty percent virus neutralization GMTs, measured by an authentic SARS-CoV-2 neutralization assay,[6] were detectable in rhesus macaque sera by Day 14 after Dose 1 and peaked at a GMT of 962 (Day 35, 14 days after Dose 2 of 30 µg) or 1,689 (Day 28, 7 days after Dose 2 of 100 µg; Figure 2). Robust GMTs of 285 for 30 µg and 310 for 100 µg dose levels persisted to at least Day 56. For comparison, the neutralization GMT of the human convalescent serum panel was 94. A summary of the S1-binding IgG GMCs and SARS-CoV-2 neutralization GMTs are described in Appendix, Supportive Table 8.1.

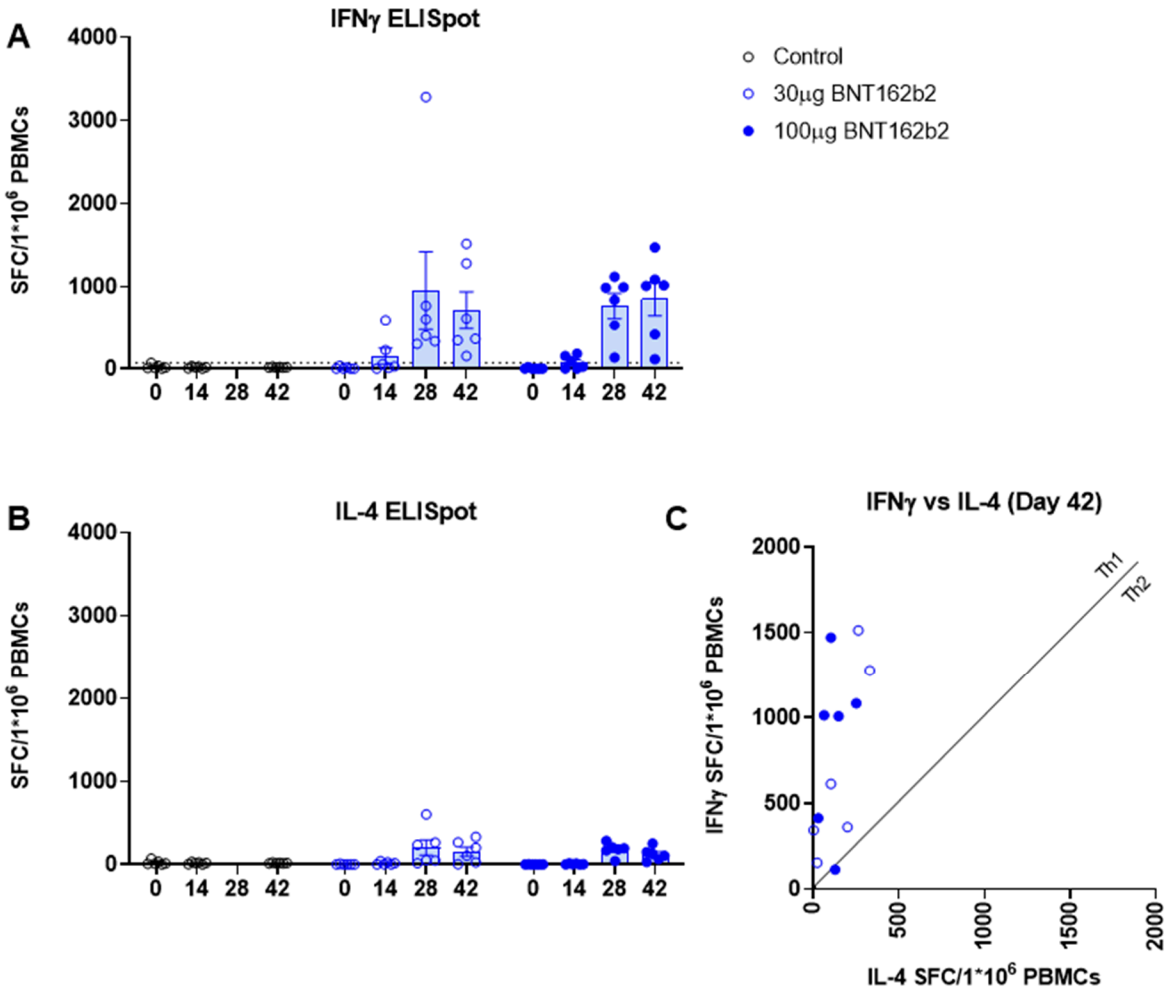
Figure 2. 50% Serum Neutralizing Titers Elicited by Immunization of Rhesus Macaques with BNT162b2 (V9)



Numbers on the x-axis indicate the day post first immunization. Heights of bars indicate GMTs, which are written above the bars; whiskers indicate 95% confidence intervals; dots represent individual monkey titers. LLOQ – 20. Titers at or below LLOQ were set to ½ LLOQ. Abbreviations as in Figure 1.

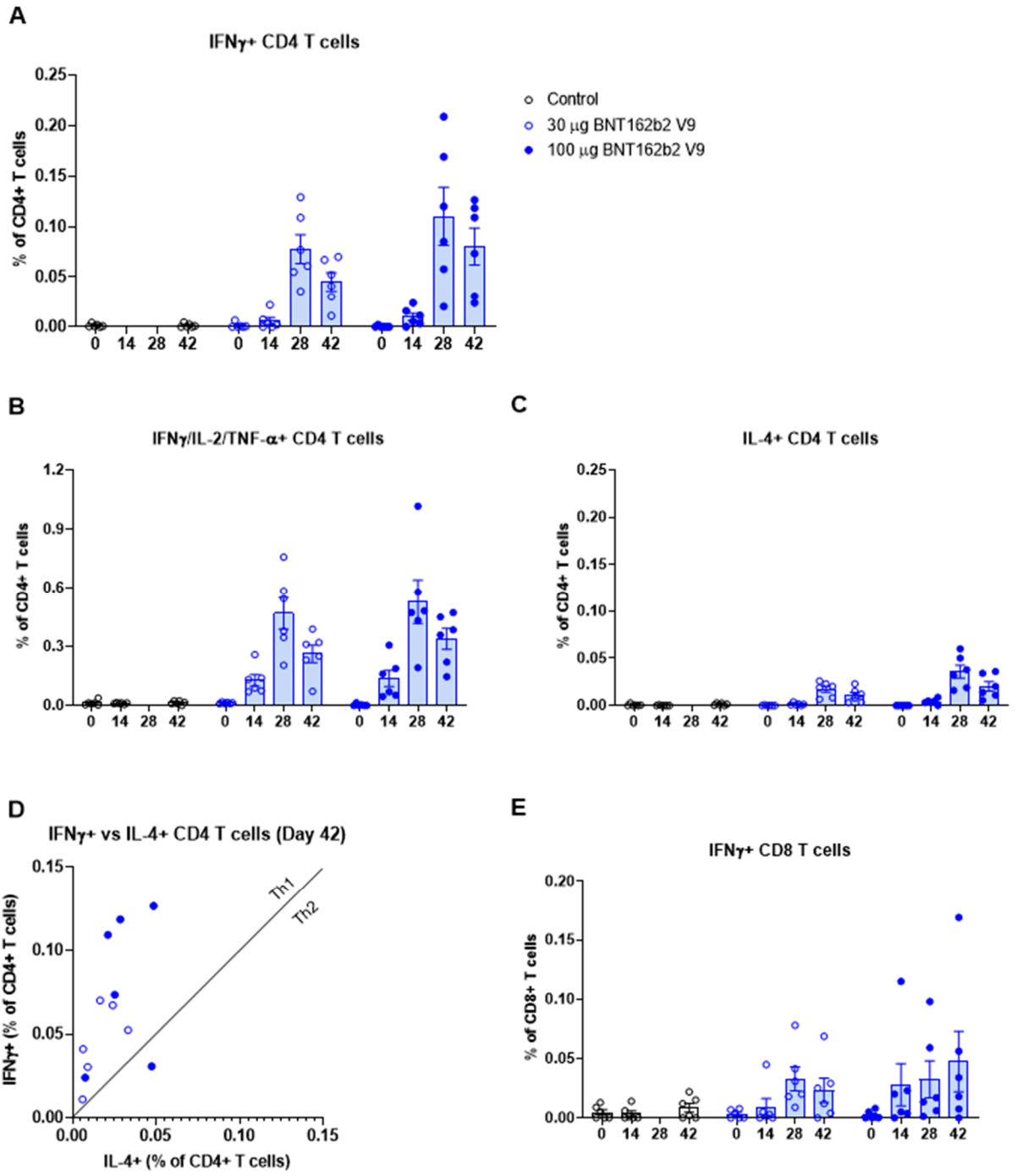
S-specific T-cell responses were analyzed by ELISpot and intracellular cytokine staining (ICS). Peripheral blood mononuclear cells (PBMCs) were collected before immunization and at the times indicated after Doses 1 and 2. In BNT162b2-immunized animals, strong IFNγ but minimal IL-4 responses were detected by ELISpot after Dose 2 (day 28 and 42) (Figure 3). ICS analysis confirmed that BNT162b2 elicited strong S-specific IFNγ producing T cell responses, including a high frequency of $CD4^+$ T cells that produced IFNγ, IL-2, or TNF-α but a low frequency of $CD4^+$ cells that produced IL-4, indicating a Th1-biased response (Figure 4A to Figure 4B). BNT162b2 also elicited S-specific $IFN\gamma^+$–producing $CD8^+$ T cells (Figure 4E).

Figure 3. IFNγ and IL-4 ELISpot Results in BNT162b2- and Control-Immunized Animals



Groups of six 2-4 year old rhesus macaques were immunized on days 0 and 21 with 30 or 100 µg BNT162b2 or saline (Control). Numbers on the x-axis indicate the day post first immunization (a Day 28 sample was not available from the Control group). Height of bars indicates the mean, whiskers indicate the standard error of mean (SEM), every symbol represents one animal. Dotted lines mark the lower limit of detection. (A) IFNγ ELISpot analysis. (B) IL-4 ELISpot analysis. (C) Correlation of frequency of IFNγ or IL-4 producing cells at Day 42 (21 days post dose 2).

Figure 4. S-specific CD4 and CD8 T-cell Responses in BNT162b2- and Control-Immunized Animals as Measured by ICS Assay



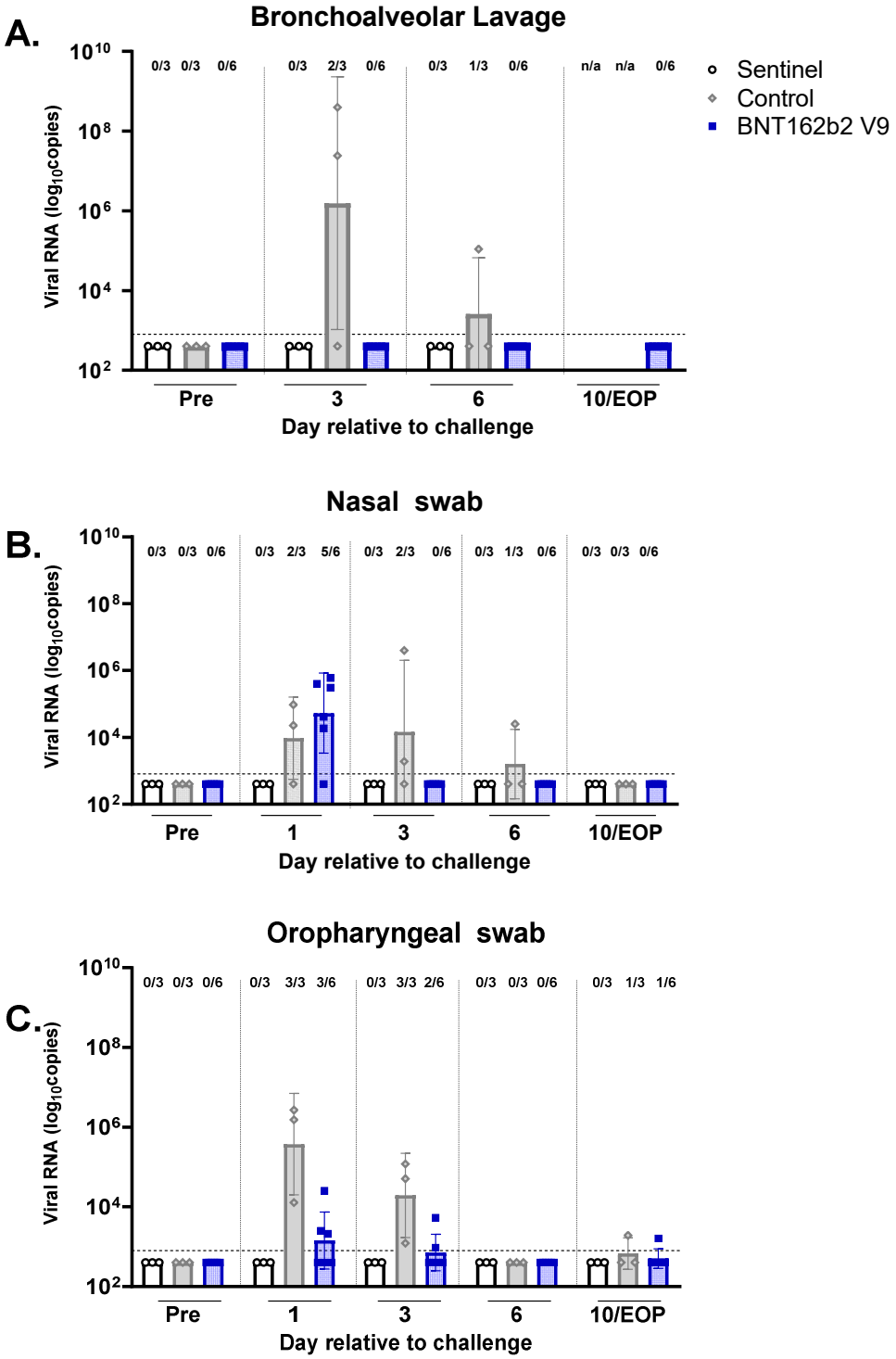
Numbers on the x-axis indicate the day post first immunization. Height of bars indicates the mean, whiskers indicate the standard error of mean (SEM), every symbol represents one animal. (A) Frequency of IFNγ+ CD4 T cells. (B) Frequency of IFNγ/IL-2/TNF-α+ CD4 T cells (C) Frequency of IL-4+ CD4 T cells. (D) Correlation of frequency of IFNγ+ with IL-4+ CD4 T cells at Day 42 (21 days post dose 2). (E) Frequency of IFNγ+ CD8 T cells.

The six rhesus macaques that had received two immunizations with 100 µg BNT162b2 and three age-matched macaques that had received saline were challenged with 1.05×10^6 plaque forming units of SARS-CoV-2 (strain USA-WA1/2020), split equally between intranasal and intratracheal routes, as previously described.[8] Three additional non-immunized, age-matched rhesus macaques (sentinels) were mock-challenged with cell culture medium. At the time of challenge, SARS-CoV-2 neutralizing titers ranged from 260 to 1,004 in the BNT162b2 (V9)-immunized animals. Neutralizing titers were undetectable in animals from the control-immunized and sentinel groups. Nasal and oropharyngeal (OP) swabs were collected and bronchoalveolar lavage (BAL) was performed at the times indicated, and samples were tested for SARS-CoV-2 RNA (genomic RNA or subgenomic transcripts) by reverse-transcription quantitative polymerase chain reaction (RT-qPCR; Figure 5). All personnel performing clinical, radiological, histopathological, or RT-qPCR evaluations were blinded to the group assignments of the macaques.

Viral RNA was detected in BAL fluid from 2 of the 3 control-immunized macaques on Day 3 after challenge and from 1 of 3 on Day 6 (Figure 5A). At no time point sampled was viral RNA detected in BAL fluid from the BNT162b2-immunized and SARS-CoV-2 challenged macaques. The difference in viral RNA detection in BAL fluid between BNT162b2-immunized and control-immunized rhesus macaques after challenge is highly statistically significant (by a nonparametric test, p=0.0014).

From control-immunized macaques, viral RNA was detected in nasal swabs obtained on Days 1, 3, and 6 after SARS-CoV-2 challenge; from BNT162b2-immunized macaques, viral RNA was detected only in nasal swabs obtained on Day 1 after challenge and not in swabs obtained on Day 3 or subsequently (Figure 5B). The pattern of viral RNA detection from OP swabs was similar to that for nasal swabs (Figure 5C). The difference in the proportion of animals with detectable viral RNA between BNT162b2-immunized and control-immunized animals, based on samples obtained after immunization, is statistically significant for OP swabs (p=0.0007) but not for nasal swabs (p=0.2622).

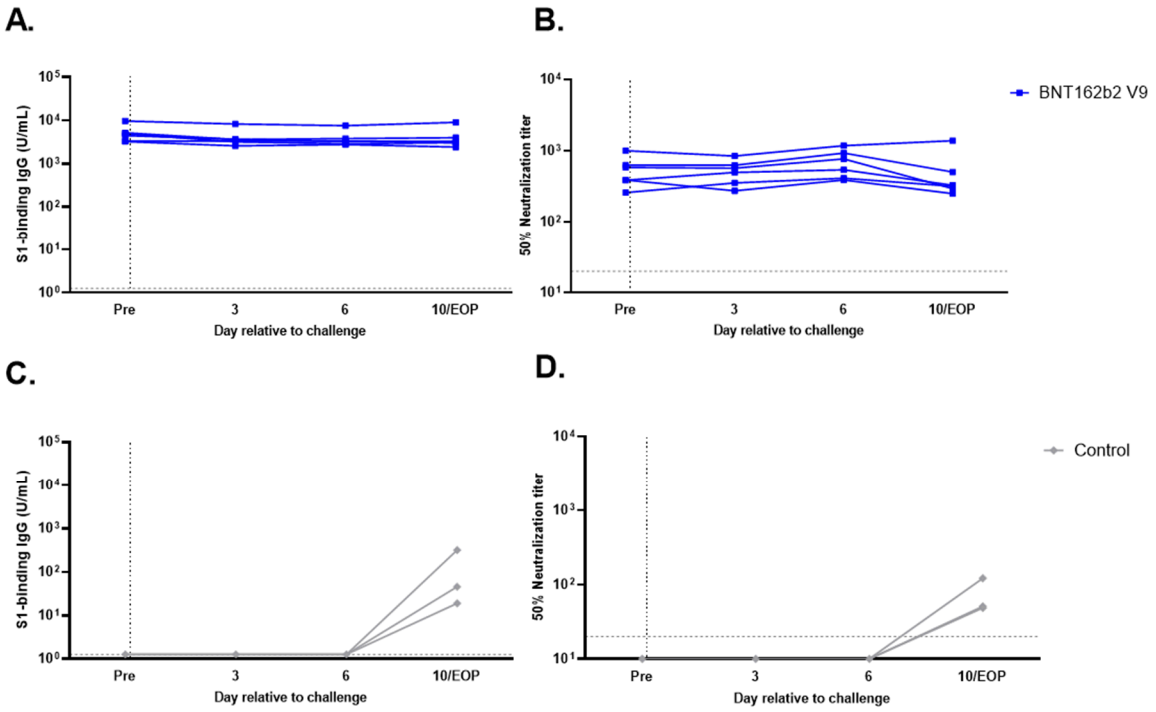
Figure 5. Viral RNA in BAL Fluid, Nasal Swabs, and Oropharyngeal Swabs of Rhesus Macaques after Infectious SARS-CoV-2 Challenge



Rhesus macaques were challenged by the intranasal and intratracheal routes with 1.05×10^6 plaque forming units of SARS-CoV-2. Viral RNA levels were detected by RT-qPCR. a, Viral RNA in bronchoalveolar lavage (BAL) fluid. b, Viral RNA in nasal swabs. c, Viral RNA in OP swabs. Ratios above data points indicate the number of viral RNA positive animals among all animals per group. Dotted lines indicate the lower limits of detection (LLOD). Values below the LLOD set to ½ the LLOD. The viral RNA levels between control-immunized and BNT162b2-immunized animals after challenge were compared by a non-parametric analysis (Friedman's test), and the p-values are 0.0014 for BAL fluid, 0.2622 for nasal swabs, and 0.0007 for OP swabs. The Friedman's test is a non-parametric analysis based on the ranking of viral RNA shedding data within each day. PROC RANK and PROC GLM from SAS® 9.4 were used to calculate the p-values.

The control animals responded to infectious virus challenge with an increase in S1-binding IgG and SARS-CoV-2 neutralizing titers; however, there was no trend toward increasing IgG levels or SARS-CoV-2 neutralizing titers in response to viral challenge in the BNT162b2-immunized animals, providing further evidence that the immunization suppressed SARS-CoV-2 infection (Figure 6).

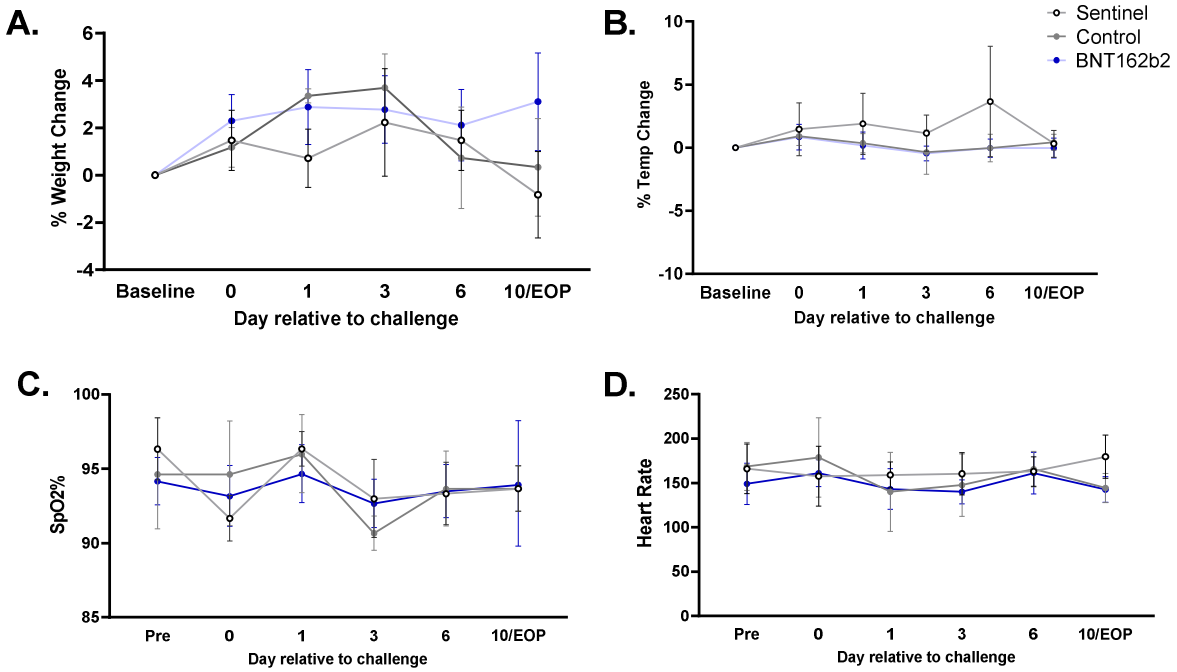
Figure 6. S1-binding IgG and 50% Serum Neutralization Responses in Rhesus Macaques after Infectious SARS-CoV-2 Challenge



S1-binding IgG concentrations (panels A & C) and 50% serum neutralization titers (panels B & D) were obtained just prior to challenge (Pre) and 3, 6, and either end of project (EOP; Days 7/8 for BNT162b2-immunized) or 10 days after challenge (Control animals). Each line represents the kinetics of the response of an individual rhesus macaque that was either immunized twice with 100 µg of BNT162b2 V9 (n=6, blue) or Control (saline) (n=3, gray). All animals were challenged by the intranasal and intratracheal routes with 1.05×10^6 plaque forming units of SARS-CoV-2. Horizontal dotted line represents the LLOQ.

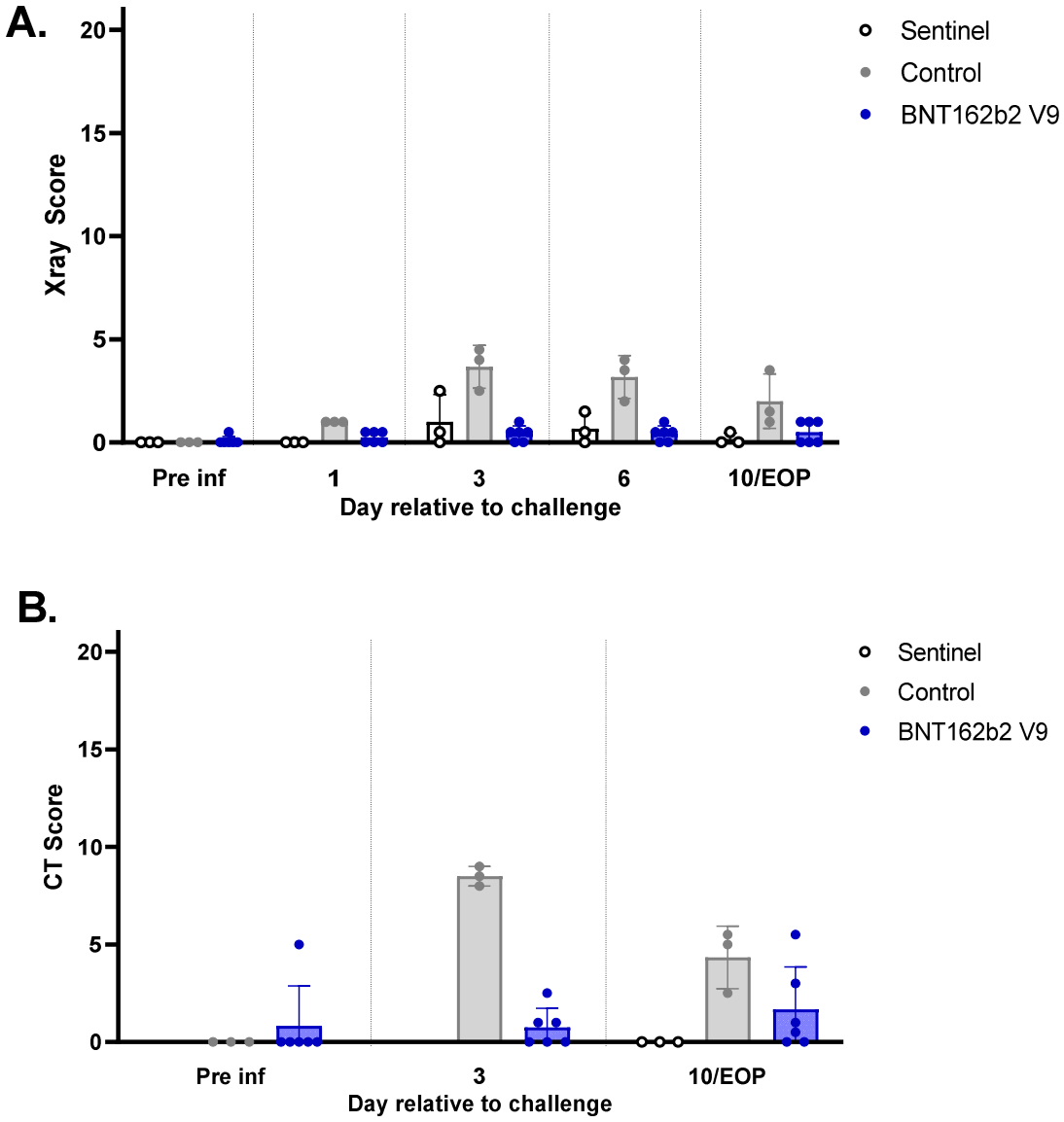
Despite the presence of viral RNA in BAL fluid from challenged control animals, none of the challenged animals, immunized or control, showed clinical signs of illness (Figure 7). Lung radiograph (Figure 8A) and computerized tomography (CT) (Figure 8B) scores were determined by two board-certified veterinary radiologist who were blinded to treatment group. Data in Figure 8 represent the average of the two scores. Radiographic evidence of pulmonary abnormality was observed in challenged controls but not in challenged BNT162b2-immunized animals nor in unchallenged sentinels. No radiographic evidence of vaccine-elicited enhanced disease was observed.

Figure 7. Clinical Signs in Rhesus Macaques after Immunization with BNT162b2 and Challenge with Infectious SARS-CoV-2.



Rhesus macaques were immunised with BNT162b2 (V9), or saline, and challenged with SARS-CoV-2 or cell culture medium as described in the Figure 5 legend. Clinical signs were recorded on the days indicated. EOP, end of project. BNT162b2-immunized (n=6), control (n=3), and sentinel (n=3) macaques. A, Body weight change. B, Temperature change. C, Oxygen saturation (SpO_2). D, Heart rate.

Figure 8. Radiograph and CT Scores of Rhesus Macaque Lungs after Infectious SARS-CoV-2 Challenge

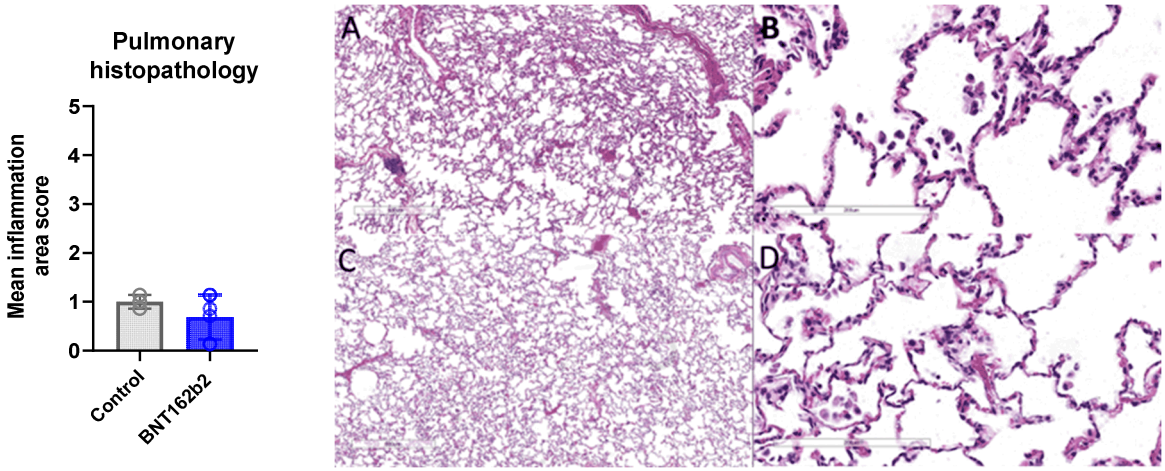


Fifty-five days after the second immunization, BNT162b2 or Control (saline)-immunized animals were challenged with 1.05×10^6 pfu of SARS-CoV-2 split equally between the IN and IT routes. Three age-matched unimmunized rhesus macaques were challenged with cell culture medium only (Sentinel). Chest X-rays and CT scans were performed prior to challenge and at the times indicated on the x-axis. EOP, end of project. Radiograph (A) and CT (B) scores were assigned to a total of 7 regions on a scale of 1-20. Images were evaluated by two board-certified veterinary radiologists blinded to treatment group. Individual data points represent the average of the two scores. The height of the bars indicates the mean score. Error bars indicate the standard deviation.

At necropsy on Day 7 or 8 after virus challenge, there were no significant gross pathology findings in any organs. Microscopically, the main 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.

Figure 9. Lung Inflammation Area Score after IN/IT SARS-CoV-2 Challenge



Graph (left panel): Lung inflammation area score on Day 7 or 8 after IN/IT SARS-CoV-2 challenge. Each data point represents the mean lung inflammation area score of a single animal (mean score of the 7 lung lobes). Saline-immunized and challenged animals (Control; n=3) are shown in grey and BNT162b2-vaccinated and challenged animals (BNT162b2; n=6) are shown in blue. Each dot represents the inflammation mean area score for an individual animal. Bars indicate the geometric mean area scores within each group. Photomicrographs (right panel; 2.5x objective, A and C; 20x objective, B and D) of hematoxylin and eosin-stained lung sections from Control animals (A and B) and lungs from BNT162b2-immunized and challenged animals (C and D).

5. CONCLUSION

We demonstrate that BNT162b2 (V9), an LNP-formulated, m1Ψ nucleoside-modified mRNA encoding SARS-CoV-2 S captured in a prefusion conformation is highly immunogenic in rhesus macaques.

The immunogenicity of BNT162b2 in rhesus macaques paralleled its immunogenicity in mice. Seven days after Dose 2 of 100 µg, the neutralizing GMT reached 18-times that of a human SARS-CoV-2 convalescent serum panel remained 3.3-times higher than this benchmark five weeks after the last immunization. The strongly Th1-biased $CD4^+$ T cell response and $IFN\gamma^+$ $CD8^+$ T-cell response to BNT162b2 is a pattern favoured for vaccine safety and efficacy, providing added reassurance for clinical translation.[13] 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. Strong RT-qPCR

evidence for lower respiratory tract protection was demonstrated by the absence of detectable SARS-CoV-2 RNA in serial BAL samples obtained starting 3 days after challenge of BNT162b2-immunized rhesus macaques.

6. DEVIATIONS

Not applicable.

7. REFERENCES

1. Pallesen J, Wang N, Corbett KS, et al. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. Proc Natl Acad Sci USA 2017;114(35):E7348-57.

2. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020;367(6483):1260-3.

3. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 2020;382(12)(03):1177-9.

4. Kim JY, Ko JH, Kim Y, et al. Viral load kinetics of SARS-CoV-2 infection in first two patients in Korea. J Korean Med Sci 2020;35(7)(Feb):e86.

5. Zhou M, Zhang X, Qu J. Coronavirus disease 2019 (COVID-19): a clinical update. Front Med 2020(Apr):1-10.

6. Muruato AE, Fontes-Garfias CR, Ren P, et al. A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation. Nat Commun 2020;11(1):4059.

7. Xie X, Muruato A, Lokugamage KG, et al. An infectious cDNA clone of SARS-CoV-2. Cell Host Microbe 2020; 27(5):841-848.e3.

8. Singh DK, Ganatra SR, Singh B, et al. SARS-CoV-2 infection leads to acute infection with dynamic cellular and inflammatory flux in the lung that varies across nonhuman primate species. Available from: https://doi.org/10.1101/2020.06.05.136481. Accessed: 24 Sep 2020.

9. Kaushal D, Foreman TW, Gautam US, et al. Mucosal vaccination with attenuated Mycobacterium tuberculosis induces strong central memory responses and protects against tuberculosis. Nat Commun 2015; 6:8533.

10. Gautam US, Forman TW, Buscan AN, et al. In vivo inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of Mycobacterium tuberculosis. Proc Natl Acad Sci USA 2018;115(1)(01):E62-E71.

11. Joosten SA, van Meijgaarden KE, van Weeren PC, et al. Mycobacterium tuberculosis peptides presented by HLA-E molecules are targets for human CD8 T-cells with cytotoxic as well as regulatory activity. PLoS Pathog 2010;6(2)(Feb):e1000782.

12. Mehra S, Alvarez X, Didier PJ, et al. Granuloma correlates of protection against tuberculosis and mechanisms of immune modulation by Mycobacterium tuberculosis. J Infect Dis 2013;207(7)(Apr):1115-27.

13. Lambert PH, Ambrosino DM, Andersen SR, et al. Consensus summary report for CEPI/BC March 12-13, 2020 meeting: assessment of risk of disease enhancement with COVID-19 vaccines. Vaccine 2020;38(31)(06):4783-91.

8. APPENDIX

8.1. SARS-CoV-2 Neutralizing Titers and Anti-S1 IgG Levels Elicited by BNT162b2 (V9) Immunization of Rhesus Macaques

	NT50 Geometric Mean Titer (GMT) (95% CI)			Anti S1 IgG Geometric Mean Concentration (GMC) U/mL (95% CI)		
Day	**Control**	**30 µg BNT162b2**	**100 µg BNT162b2**	**Control**	**30 µg BNT162b2**	**100 µg BNT162b2**
0	10 (10, 10)	10 (10, 10)	10 (10, 10)	0.8 (0.5, 1.0)	0.9 (0.4, 2)	1.0 (0.4, 5)
14	10 (10, 10)	47 (31, 73)	54 (35, 82)	0.8 (0.5, 1.0)	2,143 (1186, 3874)	3,917 (2190, 7006)
21	11.3 (8.2, 15.6)	65 (40, 104)	81 (56, 118)	0.6 (0.6, 0.6)	1,921 (1180, 3126)	2,698 (1475, 4936)
28	10 (10,10)	809 (462, 1415)	1689 (1068, 2673)	0.6 (0.6, 0.6)	30,339 (15690, 58665)	34,668 (21650, 55514)
35	10 (10,10)	962 (529, 1750)	1277 (821, 1986)	0.8 (0.5, 1)	14,978 (6975, 32163)	18,603 (11624, 29775)
42	10 (10,10)	637 (356, 1141)	1007 (675, 1504)	0.8 (0.5, 1)	10,288 (5418, 19533)	12,879 (7840, 21155)
56	10 (10,10)	285 (136, 598)	310 (175, 549)	No data available	4,236 (1380, 13003)	6,317 (3877, 10291)
HCS	94			631		

8.2. Frequencies of Cytokine Expressing CD4 and CD8 T cells Measured by ICS

	CD4+ IFN-γ (% of CD4 T cells)			CD4+ IL-4 (% of CD4 T cells)			CD4+ IFN-γ/IL-2/TNF-α (% of CD4 T cells)			CD8+ IFN-γ (% of CD8 T cells)		
Day	**Control**	**30 µg BNT162b2**	**100 µg BNT162b2**	**Control**	**30 µg BNT162b2**	**100 µg BNT162b2**	**Control**	**30 µg BNT162b2**	**100 µg BNT162b2**	**Control**	**30 µg BNT162b2**	**100 µg BNT162b2**
0	0.001 ± 0.0006	0.001 ± 0.0011	0.000 ± 0.0004	0.001 ± 0.0004	0.000 ± 0.0001	0.000 ± 0.0000	0.013 ± 0.0053	0.013 ± 0.0017	0.003 ± 0.0023	0.005 ± 0.0023	0.003 ± 0.0015	0.002 ± 0.0014
14	0.001 ± 0.0004	0.006 ± 0.0034	0.010 ± 0.0036	0.000 ± 0.0001	0.001 ± 0.0006	0.004 ± 0.0012	0.011 ± 0.0015	0.128 ± 0.0289	0.137 ± 0.0416	0.004 ± 0.0023	0.009 ± 0.0072	0.028 ± 0.0179
28	NT	0.078 ± 0.0144	0.110 ± 0.0287	NT	0.017 ± 0.0033	0.036 ± 0.0070	NT	0.470 ± 0.0808	0.529 ± 0.1107	NT	0.033 ± 0.0101	0.032 ± 0.0156
42	0.001 ± 0.0007	0.045 ± 0.0092	0.080 ± 0.0183	0.001 ± 0.0005	0.011 ± 0.0031	0.020 ± 0.0051	0.014 ± 0.0038	0.262 ± 0.0443	0.339 ± 0.0528	0.009 ± 0.0038	0.023 ± 0.0103	0.047 ± 0.0257

NT, not tested
Values reported are mean ± standard error of the mean (SEM) of 6 animals within each group

8.3. Frequencies of Cytokine Secreting Cells Measured by ELISpot

	IFNγ SFC/10^6 PBMCs (Mean±SEM)			IL-4 SFC/10^6 PBMCs (Mean±SEM)		
Day	**Control**	**30µg BNT162b2**	**100µg BNT162b2**	**Control**	**30µg BNT162b2**	**100µg BNT162b2**
0	41±6	35±0	35±0	5±1	5±1	4±0
14	35±0	159±92	88±27	4±0	16±6	7±2
28	NT	947±472	765±151	NT	202±90	179±32
42	35±0	710±227	850±202	4±0	154±54	121±32

PBMCs, peripheral blood mononuclear cells; SEM, standard error of the mean; NT, not tested

8.4. Frequencies of Cytokine Expressing CD4 and CD8 T cells Measured by ICS

	CD4+ IFN-γ (% of CD4 T cells)			CD4+ IL-4 (% of CD4 T cells)			CD4+ IFN-γ/IL-2/TNF-α (% of CD4 T cells)			CD8+ IFN-γ (% of CD8 T cells)		
Day	**Control**	**30 µg BNT162b2**	**100 µg BNT162b2**	**Control**	**30 µg BNT162b2**	**100 µg BNT162b2**	**Control**	**30 µg BNT162b2**	**100 µg BNT162b2**	**Control**	**30 µg BNT162b2**	**100 µg BNT162b2**
0	0.001 ± 0.0006	0.001 ± 0.0011	0.000 ± 0.0004	0.001 ± 0.0004	0.000 ± 0.0001	0.000 ± 0.0000	0.013 ± 0.0053	0.013 ± 0.0017	0.003 ± 0.0023	0.005 ± 0.0023	0.003 ± 0.0015	0.002 ± 0.0014
14	0.001 ± 0.0004	0.006 ± 0.0034	0.010 ± 0.0036	0.000 ± 0.0001	0.001 ± 0.0006	0.004 ± 0.0012	0.011 ± 0.0015	0.128 ± 0.0289	0.137 ± 0.0416	0.004 ± 0.0023	0.009 ± 0.0072	0.028 ± 0.0179
28	NT	0.078 ± 0.0144	0.110 ± 0.0287	NT	0.017 ± 0.0033	0.036 ± 0.0070	NT	0.470 ± 0.0808	0.529 ± 0.1107	NT	0.033 ± 0.0101	0.032 ± 0.0156
42	0.001 ± 0.0007	0.045 ± 0.0092	0.080 ± 0.0183	0.001 ± 0.0005	0.011 ± 0.0031	0.020 ± 0.0051	0.014 ± 0.0038	0.262 ± 0.0443	0.339 ± 0.0528	0.009 ± 0.0038	0.023 ± 0.0103	0.047 ± 0.0257

NT, not tested
Values reported are mean ± standard error of the mean (SEM) of 6 animals within each group

8.5. Challenge Study Design

Challenge Group	Animal ID	Immunization	DOB	Serum collection relative to immunization	Pre challenge serum collection week relative to first immunization	Sample collections relative to challenge					Necropsy Day (post challenge)
						Nasal, Oral, Rectal Swab	Chest X-ray	Chest CT	BAL	Serum	
BNT162b2	A17N114	BNT162b2 100 µg	5/19/2017	Pre, 6h, 24h, W1, 2, 3, 4, 5, 6, 8	10	pre/1/3/6/7	pre/1/3/6/7	pre/3/7	pre/3/6/7	pre/3/6/7	7
	A17N113	BNT162b2 100 µg	5/19/2017	Pre, 6h, 24h, W1, 2, 3, 4, 5, 6, 8	10	pre/1/3/6/7	pre/1/3/6/7	pre/3/7	pre/3/6/7	pre/3/6/7	7
	A17N139	BNT162b2 100 µg	6/1/2017	Pre, 6h, 24h, W1, 2, 3, 4, 5, 6, 8	10	pre/1/3/6/7	pre/1/3/6/7	pre/3/7	pre/3/6/7	pre/3/6/7	7
	A17N167	BNT162b2 100 µg	6/14/2017	Pre, 6h, 24h, W1, 2, 3, 4, 5, 6, 8	10	pre/1/3/6/7	pre/1/3/6/7	pre/3/7	pre/3/6/7	pre/3/6/7	7
	A17N105	BNT162b2 100 µg	5/18/2017	Pre, 6h, 24h, W1, 2, 3, 4, 5, 6, 8	10	pre/1/3/6/8	pre/1/3/6/8	pre/3/8	pre/3/6/8	pre/3/6/8	8
	A17N109	BNT162b2 100 µg	5/19/2017	Pre, 6h, 24h, W1, 2, 3, 4, 5, 6, 8	10	pre/1/3/6/8	pre/1/3/6/8	pre/3/8	pre/3/6/8	pre/3/6/8	8
Control	A17N118	Saline	5/22/2017	Pre, 6h, 24h, W1, 2, 3	6	pre/1/3/6/10	pre/1/3/6/10	pre/3/10	pre/3/6	pre/3/6/10	not necropsied
	A17N157	Saline	6/12/2017	Pre, 6h, 24h, W1, 2, 3	6	pre/1/3/6/10	pre/1/3/6/10	pre/3/10	pre/3/6	pre/3/6/10	
	A17N128	Saline	5/29/2017	Pre, 6h, 24h, W1, 2, 3	6	pre/1/3/6/10	pre/1/3/6/10	pre/3/10	pre/3/6	pre/3/6/10	
Sentinel	A17N125	BNT162b2 30 µg	5/27/2017	Pre, 6h, 24h, W1, 2, 3, 4, 5, 6, 8	10	pre/1/3/6/10	pre/1/3/6/10	10	pre/3/6	pre/3/6/10	
	A17N107	BNT162b2 30 µg	5/18/2017	Pre, 6h, 24h, W1, 2, 3, 4, 5, 6, 8	10	pre/1/3/6/10	pre/1/3/6/10	10	pre/3/6	pre/3/6/10	

Document Approval Record

Document Name:	VR-VTR-10671
Document Title:	BNT162b2 (V9) Immunogenicity and Evaluation of Protection against SARS-CoV-2 Challenge in Rhesus Macaques

Signed By:	Date(GMT)	Signing Capacity
(b) (6)	23-Nov-2020 21:30:40	Final Approval
	23-Nov-2020 21:43:40	Final Approval
	23-Nov-2020 22:12:42	Author Approval
	23-Nov-2020 22:56:50	Scientific Review
	23-Nov-2020 23:06:48	Quality Assurance Approval